ALGAL GREEN CHEMISTRY

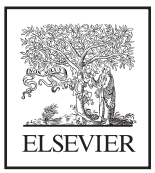
RECENT PROGRESS IN BIOTECHNOLOGY

Edited by

RAJESH PRASAD RASTOGI
Sardar Patel University, Anand, Gujarat, India

DATTA MADAMWAR
Sardar Patel University, Anand, Gujarat, India

ASHOK PANDEY
Center of Innovative and Applied Bioprocessing, Mohali, Punjab, India



elsevier.com

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-444-64041-3

Publisher: John Fedor
Acquisition Editor: Kostas Marinakis
Editorial Project Manager: Christine McElvenny
Production Project Manager: Anitha Sivaraj
Designer: Greg Harris

Typeset by TNQ Books and Journals

Contributors

C. Agrawal Banaras Hindu University, Varanasi, India

M. Arumugam National Institute for Interdisciplinary Science and Technology (NIIST), Council of Scientific and Industrial Research (CSIR), Trivandrum, Kerala, India

A. Bharti ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

H. Chakdar ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, India

A. Chatterjee Banaras Hindu University, Varanasi, India

L. Contreras-Porcia Universidad Andres Bello, Santiago, Chile

B. Fernandes University of Minho, Braga, Portugal

P. Geada University of Minho, Braga, Portugal

A. Hongsthong National Center for Genetic Engineering and Biotechnology at King Mongkut's University of Technology Thonburi, Bangkok, Thailand

S. Jantaro Chulalongkorn University, Bangkok, Thailand

H. Kageyama Meijo University, Nagoya, Japan

S. Kanwal Chulalongkorn University, Bangkok, Thailand

A.D. Kroumov The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

M. Kumar University of Technology Sydney (UTS), Sydney, NSW, Australia

U. Kuzhiumparambil University of Technology Sydney (UTS), Sydney, NSW, Australia

D. Madamwar Sardar Patel University, Anand, Gujarat, India

C.D. Miller Utah State University, Logan, UT, United States

A.N. Modenes The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

H. Najdenski The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

H. Nakamoto Saitama University, Saitama, Japan

S. Pabbi ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

A. Pandey Center of Innovative and Applied Bioprocessing, Mohali, Punjab, India

R. Prasanna ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

A. Rahman NASA Ames Research Center, Moffett Field, CA, United States

R. Rai Banaras Hindu University, Varanasi, India

L.C. Rai Banaras Hindu University, Varanasi, India

K. Rajesh CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, Telangana, India; Academy for Scientific and Industrial Research (AcSIR), India

P.J. Ralph University of Technology Sydney (UTS), Sydney, NSW, Australia

R.P. Rastogi Sardar Patel University, Anand, Gujarat, India

M.V. Rohit CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, Telangana, India; Academy for Scientific and Industrial Research (AcSIR), India

F.B. Scheufele The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

J. Senachak National Center for Genetic Engineering and Biotechnology at King Mongkut's University of Technology Thonburi, Bangkok, Thailand

S. Singh Banaras Hindu University, Varanasi, India

R.R. Sonani Sardar Patel University, Anand, Gujarat, India

T. Takabe Meijo University, Nagoya, Japan

Y. Tanaka Meijo University, Nagoya, Japan

S. Thapa ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

D.E.G. Trigueros The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

A. Udayan National Institute for Interdisciplinary Science and technology (NIIST), Council of Scientific and Industrial Research (CSIR), Trivandrum, Kerala, India

V. Vasconcelos University Porto, Porto, Portugal

S. Venkata Mohan CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, Telangana, India; Academy for Scientific and Industrial Research (AcSIR), India

A. Vicente University of Minho, Braga, Portugal

R. Waditee-Sirisattha Chulalongkorn University, Bangkok, Thailand

S. Yadav Banaras Hindu University, Varanasi, India

M. Zaharieva The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

Editor's Biography

Rajesh Prasad Rastogi, PhD
Post Graduate Department of Biosciences, Sardar Patel University, Satellite Campus, Vadtal Road, Bakrol 388 315, Anand, Gujarat, India
Phone: +91-958-669-7525, Email: raj_rastogi@rediffmail.com

Dr. Rajesh Prasad Rastogi is currently a research scientist at Post Graduate Department of Biosciences, Sardar Patel University, Gujarat, India. He obtained his PhD in photobiology and molecular biology of cyanobacteria at Banaras Hindu University, Varanasi, India, where he contributed to studies related to DNA damage and repair mechanisms. Dr. Rastogi had postdoctoral stints on algal/cyanobacterial biotechnology in South Korea and Thailand. He was a visiting scientist at Friedrich Alexander University, Nuremberg, Germany and served as a visiting professor of biochemistry at Chulalongkorn University, Thailand. His main research interest is on algae or cyanobacteria with main focus on the biosynthesis of various pigments and UV photoprotectants and their potential application as therapeutics or cosmeceuticals. Dr. Rastogi has explored several photoprotective biomolecules having great capacity to absorb high-energy photons. He has published a number of research papers in journals of international repute and several book chapters. He is an editorial board member for some national and international journals such as Frontiers in Microbiology, Switzerland. He is a life member of several scientific organizations such as BRSI, AMI, and ISEB and has been conferred with BRSI-Malviya Memorial Award for his outstanding research performance and significant contributions in the field of microbial biotechnology.

Datta Madamwar, PhD, FBRS, FAMI, FABAP, FGSA
Post Graduate Department of Biosciences, Sardar Patel University, Satellite Campus, Vadtal Road, Bakrol 388 315, Anand, Gujarat, India
Phone: +91-982-568-6025
Email: datta_madamwar@yahoo.com, d_madamwar@spuvvn.edu

Dr. Datta Madamwar, currently Professor, Post Graduate Department of Biosciences and Dean, Faculty of Science at Sardar Patel University, Vallabh Vidyanagar, Gujarat, India, got his PhD from BITS, Pilani. He has a vast research experience as a postdoctoral fellow at TIFR,

Mumbai, Universistat Frankfurt, Germany, Universitst at Konstanz, Germany, and also served at BITS, Pilani. Professor Madamwar is a Microbial Biotechnologist with diverse research interest. His current main focus is on nonaqueous enzymology, industrial liquid waste management, and cyanobacterial phybiliproteins. Dr. Madamwar has provided a concept for the enzyme catalysis in apolar organic solvents without the loss of enzyme activity. He has reported various novel, efficient, and rapid methods of purification of phycobiliproteins. The phycoerythrin has been purified to the highest purity level 5:70 ever achieved so far. This has laid to crystallization and structure determination of α-subunit of phycoerythrin. He is a recipient of European Commission Visiting Scientist Fellowship, a Fellow of Biotech Research Society of India, Fellow of Association of Microbiologists of India, Fellow of Association of Biotechnology and Pharmacy and Gujarat Science Academy, and member of several academic bodies. Dr. Madamwar worked as Visiting Professor at Swiss Federal Institute of Technology of Lausanne, EPFL-ENAC-SGC, Lausanne, Switzerland in 2009 and University of Blaise Pascal, Clermont-Ferrand, France in 2016. Dr. Madamwar is a member of several taskforce and advisory committees of the National funding agencies like DBT, DST, GSBTM. He is also a member of editorial board of several national and international journals such as Bioresource Technology, Elsevier, and Current Biotechnology. Professor Madamwar has more than 230 research publications and several book chapters and one US patent to his credit.

Ashok Pandey, PhD, FBRS, FRSB, FNASc, FIOBB, FAMI, FISEES

Eminent Scientist

Center of Innovative and Applied Bioprocessing

(A national institute under Department of Biotechnology, Ministry of S&T, Govt of India)

C-127, 2nd Floor, Phase 8 Industrial Area, SAS Nagar, Mohali-160 071, Punjab, India

Tel: +91-172-499 0214, Email: pandey@ciab.res.in, ashokpandey1956@gmail.com

Professor Ashok Pandey is an eminent scientist at the Center of Innovative and Applied Bioprocessing, Mohali (a national institute under Department of Biotechnology, Ministry of Science and Technology, Government of India) and former Chief Scientist and Head of Biotechnology Division at CSIR's National Institute for Interdisciplinary Science and Technology at Trivandrum. He is the adjunct Professor at MACFAST, Thiruvalla, Kerala and Kalaslingam University, Krishnan Koil, Tamil Nadu. His major research interests are in the areas of microbial, enzyme, and bioprocess technology, which span over various programs, including biomass to fuels and chemicals, probiotics and nutraceuticals, industrial enzymes, solid-state fermentation, etc. He has more than 1100 publications/communications, which include 16 patents, more than 50 books, 125 book chapters, 425 original and review papers, etc. with *h* index of 79 and more than 25,000 citations (Goggle scholar). He has transferred four

technologies to industries and has done industrial consultancy for about a dozen projects for Indian/international industries. He is the editor-in-chief of a book series on *Current Developments in Biotechnology and Bioengineering*, comprising nine books published by Elsevier.

Professor Pandey is the recipient of many national and international awards and fellowships, which include Fellow, Royal Society of Biology, UK; Elected Member of European Academy of Sciences and Arts, Germany; Fellow of International Society for Energy, Environment and Sustainability; Fellow of National Academy of Science (India); Fellow of the Biotech Research Society, India; Fellow of International Organization of Biotechnology and Bioengineering; Fellow of Association of Microbiologists of India; Honorary Doctorate degree from Univesite Blaise Pascal, France; Thomson Scientific India Citation Laureate Award, USA; Lupin Visiting Fellowship, Visiting Professor in the University Blaise Pascal, France; Federal University of Parana, Brazil and EPFL, Switzerland, Best Scientific Work Achievement award, Govt of Cuba; UNESCO Professor; Raman Research Fellowship Award, CSIR; GBF, Germany and CNRS, France Fellowship; Young Scientist Award, etc. He was the Chairman of the International Society of Food, Agriculture and Environment, Finland (Food & Health) during 2003–2004. He is the Founder President of the Biotech Research Society, India (www.brsi.in); International Coordinator of International Forum on Industrial Bioprocesses, France (www.ifibiop.org), Chairman of the International Society for Energy, Environment & Sustainability (www.isees.org), and Vice-President of All India Biotech Association (www.aibaonline.com). Prof Pandey is Editor-in-chief of *Bioresource Technology*, Honorary Executive Advisors of *Journal of Water Sustainability* and *Journal of Energy and Environmental Sustainability*, Subject editor of *Proceedings of National Academy of Sciences (India)* and editorial board member of several international and Indian journals, and also member of several national and international committees.

Preface

Algae, including cyanobacteria, are the most primitive and dominant photosynthetic life over the planet, which play a crucial role for sustainability and development of entire ecosystems. They are ubiquitous in freshwater and marine habitats, and considered as major biomass producers, maintaining the trophic energy dynamics of both aquatic and terrestrial ecosystems. It has been estimated that prokaryotic and eukaryotic microalgae account for more than 40% of the Earth's net primary photosynthetic productivity and convert solar energy into biomass-stored chemical energy. Owing to obstinate survival in assorted environments, these organisms evolved a range of chemicals or secondary compounds, each with specialized functions to compete successfully on the planet. Moreover, algae are immense sources of several valuable natural products of ecological and economic importance. During the past few years, there is growing interest in fresh and marine algal biochemistry to explore the important chemicals or metabolic processes or pathways for the competent progress in metabolic engineering and future biotechnological mission at global level. The development of green algal technology for bioremediation, ecofriendly and alternative renewable energy or biofuels, biofertilizers, biogenic biocides, cosmeceuticals, sunscreens, antibiotics, antiaging, and an array of other biotechnologically important chemicals may prove a prodigious boon for human life and their contiguous environment. In recent times, a number of novel algal products of potential commercial values ensued from advances in algal green chemistry, which may be exploited as drug leads. In the past few decades, numerous industries have been established worldwide for the production of algae-based value-added green products with marked applications in the food, pharmaceutical, cosmetics, agriculture, and energy sectors for the benefit of human welfare and sustainable future.

The present book "Algal Green Chemistry: Recent Progress in Biotechnology" presents state-of-the-art information on various eco-friendly products or processes from algae/cyanobacteria by the internationally recognized experts and subject matter experts. It is certainly not possible to consider all aspects of algal biology as mentioned above in a single volume book but efforts have been made here to provide most comprehensive and related information. Accordingly, the book contains 14 chapters with macro-level attempt to address the key concepts of knowledge associated with recent advances on promising algal biotechnology. Recent progress on the research of osmoprotectant molecules in halophilic algae/cyanobacteria with their possible biotechnological application is discussed. Some chemical compounds such as mycosporine-like amino acids and scytonemin (Scy) are recognized as strong UV-absorbing/screening biomolecules that can be used in cosmetic and pharmaceutical industries for development of novel drugs. Recent advances in synthesis and biofunctionalities of some UV-sunscreens from algae are discussed with special emphasis on their potential use as cosmeceuticals.

Algae and cyanobacteria have great ability to absorb greenhouse gas (CO_2) and can be grown at large-scale outdoor cultures for production of bioproduct. Genome- and proteome-wide analyses for targeted manipulation and enhancement of bioproducts in cyanobacteria is discussed in a chapter. Microalgae are rich source of several nutritionally important compounds such as proteins, pigments, carbohydrates, poly unsaturated fatty acids, dietary fibers, and bioactive compounds with wide range of health benefits. A chapter is focused on the production of different nutraceuticals of micro- or macroalgal origin with their biochemical properties and health benefits. Nature have devised inherent defense system comprises of several antioxidants to fight against oxidative stress in various organisms. A chapter summarizes an overall update in the field of "algal antioxidants" and their promising applications in pharmaceutical and biomedical research in therapeutics of various physiological anomalies, including aging, neurodegeneration, and cancer. Microalgae-based carotenoid production is of great interest in the recent times owing to their high commercial values. A chapter tends to provide an overview of carotenogenesis from microalgae. Health-promoting properties of various algal pigments are also provided in some details. There is worldwide increasing demand for bioplastics. Microalgae-derived bioplastics are biodegradable, which also makes them eco-friendly. A chapter discusses both direct usage of microalgal biomass and derivatized microalgae biomass for bioplastic production.

Recent advances and up-to-date knowledge on low-molecular-weight nitrogenous compounds such as GABA (γ-aminobutyric acid) and polyamines (PAs) derived from microalgae are focused in a chapter. Production of PAs in marine macrophytes in response to abiotic stress conditions is also conferred. Sustainable agriculture is advantageous over conventional agriculture for its capacity to accomplish food demand by utilizing environmental resources without negatively affecting it. An overview of the role of algae as biofertilizers is well documented in a chapter. A part of the book combines the technoeconomic analysis as well as innovative approaches and achievements in modeling of microalgal process for the production of bioenergy and high-value coproducts. Optimizing large-scale culture cultivation arises as a permanent need at industrial scale to increase the cost-effective production of algal biomass. This is discussed in a chapter addressing several important issues occurring during microalgal biomass cultivation. Finally, a chapter evaluates the algal biofilms and their significance in agriculture and environmental biology for bioremediation and nutrient sequestration. Moreover, prodigious research in the field of algal green chemistry will certainly be a windfall in the field of environmental biotechnology, green energy, and various aspects of agricultural as well as biomedical research and biochemical industries for sustainable development of current and future populations.

We strongly feel that the contents of the book would be of special interest to the graduate/postgraduate students, teachers, biochemists, researchers in the fields of applied and environmental microbiology, medical microbiology, microbial biotechnology, and metabolic engineers engaged in

the development of algae-based bioproducts. As it is expected, the current context and discourse on algal green chemistry will be highly promising for facilitating the readers toward front-line knowledge of algal biology and biotechnology for process and product development.

We thank authors of all the articles for their kind cooperation and also for their readiness in revising the manuscripts in a specified time frame. We also appreciate the consistent support from the reviewers of particular chapters for critical inputs to improve the articles. We are extremely thankful to Dr. Marinakis Kostas, Dr. Christine McElvenny, and the entire team of Elsevier for their cooperation and efforts in producing this book.

Editors
Rajesh Prasad Rastogi
Datta Madamwar
Ashok Pandey

CHAPTER

1

Osmoprotectant and Sunscreen Molecules From Halophilic Algae and Cyanobacteria

H. Kageyama[1], *R. Waditee-Sirisattha*[2], *Y. Tanaka*[1], *T. Takabe*[1]

[1]**Meijo University, Nagoya, Japan;** [2]**Chulalongkorn University, Bangkok, Thailand**

OUTLINE

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00001-1

1. INTRODUCTION

To survive under halophilic environments, halophilic microorganisms must have developed the special systems such as synthesis of osmoprotectant and sunscreen molecules. Osmoprotectants are small molecules that act as osmolytes and help organisms survive under extreme saline conditions. Examples of compatible solutes include betaines, amino acids, dimethylsulfoniopropionate (DMSP), and sugars. These molecules accumulate in cells and balance the osmotic difference between the cell's surroundings and the cytosol. Compatible solutes have also been shown to play a protective role by maintaining enzyme activity under abiotic stress conditions. Their specific action is unknown but is thought that they are preferentially excluded from the proteins interface due to their propensity to form water structures. Here, we summarize recent progress on the research of osmoprotectant and sunscreen molecules in halophilic algae/cyanobacteria. Their possible biotechnological application in the field of green energy, biomedical research, and various biochemical industries were described.

2. OSMOPROTECTANTS AND SUNSCREEN MOLECULES (MAA)

2.1 Basic Features of Osmoprotectants in Cyanobacteria and Algae

Cyanobacteria and algae, as primary producers of ecosystems, have wide range of habitats from freshwater to hypersaline environments [1,2]. To survive under high salt conditions, special mechanisms are required to cope ionic/osmotic imbalance. Since salt stress is a major factor to decrease crop yield, extensive studies have been carried out on salt stress on plants. Unique systems and unique genes in halophilic algae and cyanobacteria could be applied to increase the crop yield of plants [3]. For the ionic regulation under high salinity conditions, the capacity of plants to maintain a high cytosolic K^+/Na^+ ratio is the key determinant of plant-salt-tolerance [4]. Besides the ionic regulation, the accumulations of alternative solutes without inhibiting metabolic activities inside the cells are necessary [2–4]. Such solutes are termed "compatible solutes," which are organic molecules with low molecular weight, highly soluble in water, and usually without net charge. Based on their chemical structure, compatible solutes can be classified into several groups. The main groups are (1) disaccharides, (2) polyols, (3) heterosides, (4) zwitterionic quaternary ammonium and tertiary sulfonium compounds, and (5) amino acids [1–3]. In addition to their osmotic functions, compatible solutes

have protective effect on proteins and membranes against denaturation under various abiotic stresses.

Cyanobacteria can be divided into three groups according to their salt tolerance, freshwater cyanobacteria (sensitive to salinity), marine type cyanobacteria (tolerant up to near 1 M NaCl), and halophilic cyanobacteria. Freshwater strains tend to accumulate disaccharides, marine strains generally use glucosylglycerol (GG), and halophilic strains accumulate glycine betaine (GB) [2]. In algae, because of the phylogenetic diversity, there seems to be a great variety of compatible solutes. The compatible solutes such as disaccharides in green algae, several kinds of heterosides in red algae, and polyols in brown algae have been reported. In marine micro- and macroalgae, accumulation of DMSP, GB, and proline has been reported [5]. The genus *Dunaliella* contains species whose normal habitats range from seawater of around 0.4 M NaCl to 5 M NaCl [1]. *Dunaliella salina* has adapted to survive in high salinity environments by accumulating glycerol to balance osmotic pressure.

In this chapter, we focus on saccharides, GB, and DMSP as osmoprotectants in cyanobacteria and algae.

2.2 Saccharides and Their Derivatives

2.2.1 *Glucosylglycerol and Glucosylglycerate*

Moderately salt-tolerant and marine cyanobacteria often accumulate GG as a compatible solute. GG-accumulating cyanobacteria *Synechocystis* sp. PCC 6803 can grow in freshwater and media with salt concentration higher than seawater [6]. Glucosylglycerate (GGA) is an uncommon compatible solute because it carries a net charge at physiological pH. GGA accumulation was found in marine picoplanktonic cyanobacteria, *Prochlorococcus* and *Synechococcus* spp., and in *Synechococcus* sp. PCC 7002. The amount of GGA was dependent on the extent of salinity. It has been hypothesized that GGA replaces glutamate under N-limiting conditions as alternative organic anion to counteract cations, such as K^+, inside cyanobacterial cells [7].

2.2.2 *Biosynthetic Pathway*

The biosynthetic pathways of GG and GGA show similar two-step reactions. In many organisms, firstly glucosyltransferase reaction produces phosphorylated intermediates, and then this is hydrolyzed by phosphatase to the final saccharide and derivatives [8]. The initial step of GG synthesis is catalyzed by GG-phosphate synthase (GGPS).

$$\text{ADP-glucose} + \text{glycerol 3-phosphate} \rightarrow \text{glucosylglycerol-phosphate} + \text{ADP}$$

The next step is hydrolysis of phospho-moiety of the intermediate by GG-phosphate phosphatase (GGPP).

$$\text{Glucosylglycerol-phosphate} + H_2O \rightarrow \text{GG} + \text{Pi}$$

GGPS and GGPP were firstly identified in salt-sensitive mutant of *Synechocystis* sp. PCC 6803 [8]. GGPS shows considerable similarity to the trehalose-phosphate synthase (OtsA) from heterotrophic bacteria. GGPS of *Synechocystis* sp. PCC 6803 was activated by addition of NaCl into the assay solution. The result indicated that GGPS in *Synechocystis* was

posttranslationally regulated by NaCl. GGPP as well as GGPS become activated when NaCl was added at concentration of 100 mM [9].

In GGA synthetic pathway, GGA-phosphate synthase (GGAPS) catalyzes the first step [7,10].

$$\text{NDP-glucose} + \text{glycerate 3-phosphate} \rightarrow \text{GGA-phosphate} + \text{NDP}$$

In the second step, GGA is produced by GGA-phosphate phosphatase (GGAPP).

$$\text{GGA-phosphate} \rightarrow \text{GGA} + \text{Pi}$$

Genes encoding proteins similar to GGAPS and GGAPP of the heterotrophic bacteria were identified in the genomes of many marine *Prochlorococcus* and *Synechococcus* strains [7,11]. GGA synthesis genes were not found in the genomes of marine N_2-fixing strains [11]. Cyanobacterial genes encoding GGAPS are usually found in an operon with two other genes coding GGAPP and GGA hydrolase as in heterotrophic bacterial genome. Recently, homologous genes involved in the biosynthesis of galactosylglycerol were identified in the red alga [12].

2.3 Glycine Betaine

2.3.1 *Accumulation and Response to Environment*

GB (*N,N,N*-trimethylglycine) is one of the most predominant compatible solutes to protect organisms thriving under very high salinity [13–15]. It has been shown that the highly salt-tolerant cyanobacteria such as *Aphanothece halophytica* accumulates high amount of GB (near 1 M) under salt stress condition [16,17]. Since this cyanobacterium was originally isolated from the Dead Sea, high accumulation level of GB would be an advantage for thriving under this extreme environment. In addition to de novo biosynthesis of GB, the betaine transporter gene (*betT*) derived from *A. halophytica*, which specifically transported GB, has been isolated and functionally characterized [18]. BetT is classified as the member of the betaine-choline-carnitine transporter family. Functional characterization of BetT revealed that this transporter had high activity under alkaline pH conditions.

2.3.2 *Biosynthetic Pathway*

GB is synthesized either by choline oxidation or glycine methylation (Fig. 1.1). For the case of choline oxidation, the first step is catalyzed by choline monooxygenase (CMO) in plants [19], choline dehydrogenase (CDH) in animals and bacteria [20], and choline oxidase (COX) in some bacteria [21]. The second step is catalyzed by betaine aldehyde dehydrogenase in all organisms [20,22]. Unlike other GB synthesizing organisms, the halotolerant cyanobacterium *A. halophytica* possesses a novel biosynthetic pathway for GB via three subsequent methylation reactions of glycine. The methylation reactions are catalyzed by two enzymes, glycine/sarcosine-N-methyltransferase (GSMT) and dimethylglycine-N-methyltransferase (DMT), with S-adenosyl-methonine (SAM) acting as the methyl donor [23].

Since many crop plants do not have a GB synthetic pathway, genetic engineering of GB biosynthesis pathways represents a potential way to improve crop plant stress tolerance. Choline oxidation enzymes such as CMO, CDH, and COX have been introduced into non-GB-accumulating plants, and this has often improved stress tolerance. However, the engineered levels of GB are generally low, and the increases in tolerance are commensurately

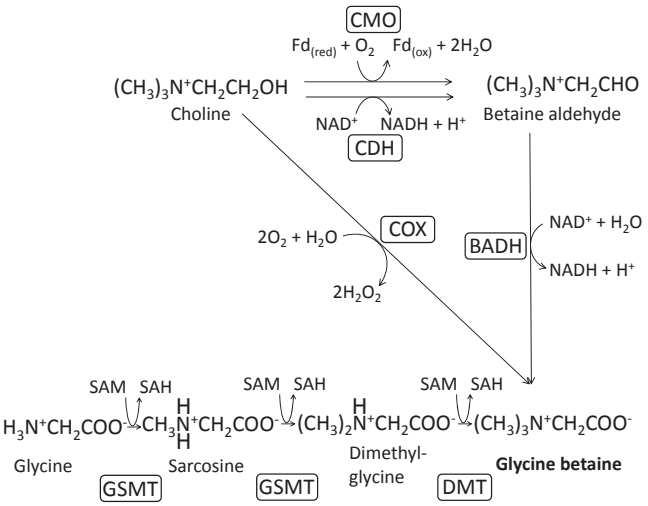


FIGURE 1.1 Biosynthetic pathways of glycine betaine. *BADH*, bataine aldehyde dehydrogenase; *CDH*, choline dehydrogenase; *CMO*, choline monooxygenase; *COX*, choline oxidase; *DMT*, dimethylglycine methyltransferase; *Fd*, ferredoxin; *GSMT*, glycine sarcosine methyltransferase; *SAH*, S-adenosylhomocysteine; *SAM*, S-adenosylmethionine.

small. Interestingly, the transfer of genes for the three-step methylation of glycine yielded much higher accumulation of GB and also enhanced halotolerance for transformed cells in both the freshwater cyanobacterium *Synechococcus* sp. PCC 7942 and the higher plant *Arabidopsis thaliana*. Halotolerance of these transformed *Synechococcus* and *Arabidopsis* cells correlated to the accumulation of elevated levels of GB [15].

It has been shown that provision of substrate for GB synthesis via choline oxidation pathway could enhance GB [24]. Supplementation of glycine could also enhance the GB level significantly via glycine methylation [17]. These results suggest that provision of substrate is crucial for boosting GB accumulation. In *A. halophytica*, GB is synthesized using glycine as substrate. Serine and glycine are interconvertible through the catalysis of serine hydroxymethyltransferase (SHMT) [25]. Choline is synthesized from ethanolamine, which is derived from serine. Therefore, two routes for the biosynthesis of GB can utilize serine as an upstream precursor. Overexpression of the *A. halophytica* SHMT in *Escherichia coli* (*E. coli*) resulted in higher GB accumulation, despite the fact that *E. coli* synthesizes GB via choline oxidation [25]. The similar result was observed in the case of transfer 3-phosphoglycerate dehydrogenase gene (*ApPGDH*), which encodes the first step of the phosphorylated pathway of serine biosynthesis into *E. coli* and *A. thaliana* [17]. These data showed the importance of provision of upstream precursor for the enhancement of GB accumulation through both the choline oxidation and the glycine methylation routes.

2.3.3 *Regulation of Related Enzyme Activity and Gene Expression*

The activities of GSMT and DMT in *A. halophytica* increased about 1.6- to 2.5-fold upon the increase of NaCl from 0.5 to 2.5 M [23]. Glycine can be synthesized by two biosynthetic routes in photoautotrophic organisms. One starts from 2-phosphoglycolate in photorespiratory pathway [26], and another starts from 3-phosphoglycerate in phosphoserine pathway [27]. The gene expression of 3-phosphoglycerate dehydrogenase was induced by salt-upshock in

A. halophytica [17]. GB biosynthesis by three sequential methylations generates S-adenosylhomocysteine (SAH), which is known as transmethylation inhibitor. Continuous GB synthesis needs for the regeneration of SAM from SAH not only to supply methyl donor but also to remove the inhibitor. In *A. halophytica*, SAH hydrolase (SAHH) catalyzed the reversible reaction of hydrolysis and synthesis of SAH [28]. It was shown that the synthetic activity of SAHH was inhibited by 0.4 M KCl but the hydrolytic activity was not affected by KCl. Moreover, the addition of GB increased the synthetic activity in the presence of 0.4 M KCl, but it had no effect on the hydrolytic activity. These results suggested that the GB biosynthesis is regulated by the ratio of K^+ and GB in *A. halophytica* cells.

CMO and BADH are localized in chloroplasts in *Amaranthus* plants such as spinach [19]; however, barley BADH was localized in peroxisomes [29]. The evidence of choline oxidation enzymes, other than Amaranthus plants, especially in monocots, largely remains to clarify [29,30].

2.4 Glycerol

2.4.1 Accumulation and Response to Environment

Unicellular green alga *Dunaliella* species, the most salt-tolerant photoautotropic organism, accumulates glycerol as a compatible solute [31,32]. Glycerol concentration in *Dunaliella parva* reached above 7 M in the growth medium containing 4 M NaCl [32]. The energetic cost of its biosynthesis is notably inexpensive than the production of other compatible solutes [33], and it can be mixed infinitely with water. Although these merits are thought to be a favor for compatible solute, it was reported that glycerol is chaotropic at high concentration [34]. It has been reported that the ATP synthesis activity of spinach thylakoid was inhibited 50% by 2.75 M glycerol, while that of *Dunaliella bardawil* was twofold stimulated under the same concentration of glycerol [35]. The results showed ATPase of *D. bardawil* had been adapted to high concentration of glycerol. Unlike other green algae, *Dunaliella* cells lack a cell wall [36] but have an elastic plasma membrane to enable flexible change in cell volume [37]. Usually, biological membranes are permeable to glycerol. However, it has been shown that the permeability for glycerol of membranes from *Dunaliella* was exceptionally low [38]. This enables *Dunaliella* cells to keep high concentration of glycerol inside the cell.

2.4.2 Biosynthesis Pathway

Glycerol is synthesized by the pathway in which glycerol 3-P dehydrogenase (G3PDH) and glycerol 3-P phosphatase (G3PP) convert dihydroxyacetone phosphate to glycerol [36].

$$\text{(G3PDH) dihydroxyacetone phosphate} + \text{NAD(P)H} \rightarrow \text{glycerol-3-phosphate} + \text{NAD(P)}^+$$

$$\text{(G3PP) glycerol-3-phosphate} \rightarrow \text{glycerol} + \text{Pi}$$

The activities of G3PDH and G3PP in *Dunaliella* cells were increased under high salinity [39]. Upon hypoosmotic shock, glycerol content in *Dunaliella* cells decreased with a parallel increase in starch content, indicating metabolic conversion of glycerol to starch [36]. Dynamic interconversion between glycerol and starch may occur in *Dunaliella* cells.

2.5 Dimethylsulfoniopropionate

2.5.1 Accumulation and Response to Environment

DMSP is a zwitterionic tertiary sulfonium compound and a widespread compatible solute in marine micro- and macroalgae [40,41] and some species of higher plants [42]. In algae, it was reported that intracellular DMSP concentrations increase in response to high salinity [43], low temperatures [44], variations in light [45], and nutrient limitation [46–49]. DMSP and its metabolite, acrylate, are shown to act as scavengers for reactive oxygen species [48]. Besides, DMSP has a role in defense against grazers [50]. Moreover, DMSP is known as a central molecule in the marine sulfur cycle and as the precursor of dimethylsulfide (DMS) that has an impact on the global climate [51].

2.5.2 Biosynthetic Pathway

In algae, it has been proposed that DMSP is synthesized from methionine by four-step reactions [52]. First, methionine is converted to 4-(methylthio)-2-oxobutanoic acid (MTOB) by aminotransferase. MTOB is next reduced to 2-hydroxy-4-(methylthio) butanoic acid (MTHB) by reductase. Then, S-methyltransferase converts MTHB to 4-(dimethylsulfonio)-2-hydroxy-butanoate (DMSHB). Finally DMSP is produced by oxidative decarboxylase from DMSHB. Although the enzymatic activity of each step of DMSP biosynthesis was examined, putative genes encoding the enzymes have yet to be identified in any organisms. But, by proteomic and transcriptomic approaches using diatom, some candidate proteins or genes responsible for DMSP synthesis have been proposed so far [53–55]. The conversion of MTHB to DMSHB is believed to be a committing step because the reaction is unidirectional while the reactions from methionine to MTHB are reversible. It was found recently that MTHB S-methyltransferase activity increased upon the increase of salinity and decreased upon S deficiency in *Ulva pertusa* and was inhibited by high concentration of DMSP [52]. Although *U. pertusa* does not synthesize GB, the level of DMSP decreased significantly upon the uptake of exogenously supplied GB under S-deficient condition. In contrast, the level of proline in *Ulva* was not affected by GB supply. Besides the synthesis of DMSP, DMSP levels may be regulated by its catabolic reactions or by the balance of its release and uptake.

2.5.3 Omics Approaches to Identify DMSP Biosynthetic Enzymes and Genes

The synthesis of DMSP by diatoms has been shown to be regulated by light intensity or availability of nutrients [53]. The investigation of protein changes associated with salinity-induced DMSP increases in the model sea-ice diatom *Fragilariopsis cylindrus* (CCMP 1102) revealed that SAH hydrolases, SAM synthetases, and SAM-dependent methyltransferase, those are involved in the cycle of SAM synthesis, increased significantly, suggesting the flux of the active methyl cycle is regulated for the synthesis of DMSP and GB [53]. In addition, candidate proteins involved in DMSP biosynthesis in marine algae (an aminotransferase, an NADPH-dependent flavinoid reductase, two putative SAM-dependent methyltransferases, two putative decarboxylases) were nominated among proteins induced by the increase in salinity. As the genome of *Thalassiosira pseudonana* has been sequenced, transcriptomic and proteomic analyses were conducted to elucidate DMSP biosynthetic genes [54,55]. However, homologs of the candidate proteins were not induced by abiotic stresses that increased DMSP

content in the algal cells. The authors discussed that there is no individual limiting step to control DMSP synthesis. Instead, different components could be limiting under different conditions. In general, compatible solutes are thought to be final metabolites. Indeed, turnover of compatible solutes was often found to be low, e.g., for glycerol [37] and glucosylglycerol [5]. A clear exception to this rule is amino acid proline, which is actively metabolized like other amino acids.

2.6 Basic Features of Mycosporines and MAAs

Mycosporines and mycosporine-like amino acids (MAAs) are water-soluble small (<400 Da) secondary metabolites known as a sunscreen molecule. These metabolites are characterized by maximum absorbance in the UV range of 310−362 nm with high molar extinction coefficients ($\varepsilon = 28{,}100-50{,}000\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$) [56]. Mycosporines and MAAs were originally discovered as fungal metabolites, and its chemical structure was determined in 1970s [57]. Structures of mycosporines and MAAs consist of a cyclohexenone ring or imino cyclohexene rings, on which one or two amino acids are substituted, respectively [58]. For instance, mycosporine-glycine contains a glycine at C3 position (Fig. 1.2). Shinorine contains glycine and serine at C3 and C1, respectively. The nitrogen atom at the imine group is protonated in MAAs, and the positive charge on a nitrogen atom is delocalized. Extensive conjugation due to resonance tautomers facilitates the unique absorption maximum and higher extinction coefficient of MAAs. To date, more than 20 different MAAs have been identified. Chemical structures of some mycosporines, MAAs, and their precursor compound 4-deoxygadusol are shown in Fig. 1.2B. Mycosporines and MAAs are synthesized in cyanobacteria, fungi, and algae [59]. Although MAAs can be detected in animals, it is believed that these MAAs are through the food chain or derived from symbiotic microorganisms [60]. However, it was found that coral and sea anemones possess the gene cluster homologs to cyanobacterial MAAs, suggesting the de novo synthesis of MAAs in these animals [61]. It was also shown that fish can synthesize MAAs-related compound, gadusol, de novo [62]. Biological function of mycosporines and MAAs was anticipated as a sunscreen molecule because of their UV absorbing capacity, but other functions such as reactive oxygen species (ROS) scavenger and compatible solutes have also been reported.

2.7 Biosynthetic Pathway of MAAs

2.7.1 Genes and Proteins Responsible for Biosynthesis of MAAs

4-Deoxygadusol (4-DG) is the common precursor for the synthesis of mycosporines and MAAs. Two possible pathways for the synthesis of 4-DG have been proposed. One is the shikimate pathway. Synthesis of mycosporines and MAAs from 3-dehydroquinate (3-DHQ), an intermediate of shikimate pathway, through 4-DG was shown in fungus *Trichothecium roseum* [63]. This pathway was supported by the finding that synthesis of MAAs in the coral *Stylophora pistillata* was inhibited by glyphosate, a shikimate pathway-specific inhibitor [64]. Based on genome mining, Singh et al. found that two genes in cyanobacterium *Anabaena variabilis* ATCC29413, Ava_3858 (demethyl 4-deoxygadusol (DDG) synthase) and Ava_3857 (O-methyltransferase), might be responsible for the production of 4-DG from 3-DHQ [65].

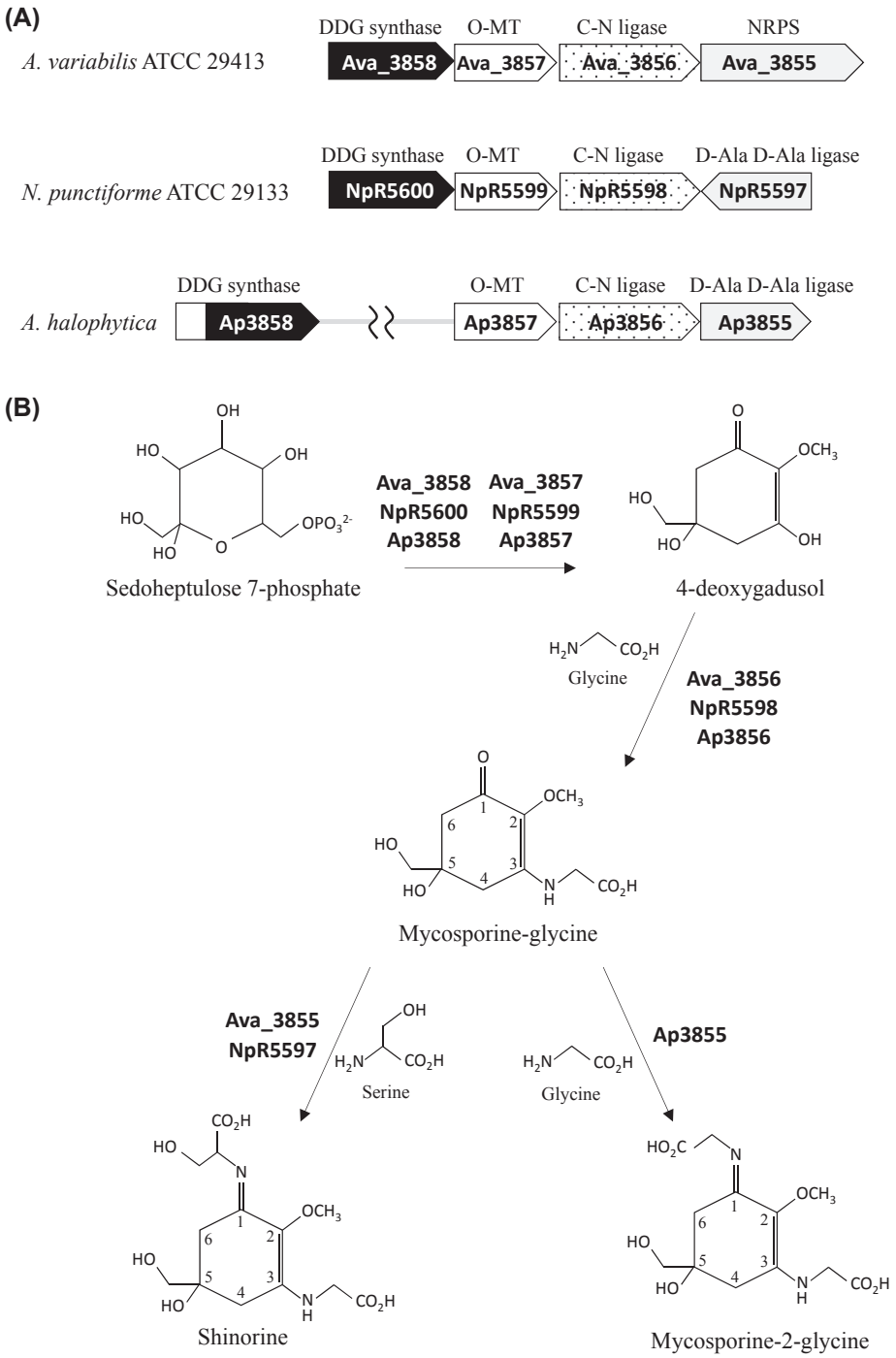


FIGURE 1.2 Cyanobacterial MAAs biosynthesis. (A) MAA synthetic gene clusters from *Anabaena variabilis* ATCC 29413, *Nostoc punctiforme* ATCC 29133, and *Aphanothece halophytica*. (B) Biosynthetic pathway of shinorine and mycosporine-2-glycine.

The genes homologs, NpR5600 and NpR5599, to Ava_3858 and Ava_3857 were found in another cyanobacterium *Nostoc punctiforme* ATCC29133. It was shown that NpR5600 and NpR5599 produced 4-DG from sedoheptulose-7-phosphate (SHP), but not from 3-DHQ, in the presence of S-adenosylmethionine (SAM), nicotine amide adenine dinucliotide (NAD^+), and Co^{2+} in vitro [66]. Furthermore, it was shown that Ava_3855 and Ava_3856 are involved in shinorine biosynthesis [66]. Ava_3856, a C-N ligase, produces mycosporine-glycine from glycine and 4-DG. Ava_3855, a nonribosomal peptide synthetase, produces shinorine from serine and mycosporine-glycine. A similar cluster of four genes in *Nostoc punctiforme* ATCC29133 (NpR5600, NpR5599, NpR5598, and Npr5597) has also been shown to catalyze the same reaction, although in this case, NpF5597 encodes D-Ala D-Ala ligase and the direction of transcription is opposite to that of NpR5600 and NpR5598 [66,67].

In a halotolerant cyanobacterium *A. halophytica*, closely located three genes, Ap3857, Ap3856, and Ap3855, homologous to Ava_3857/NpR5599, Ava_3856/NpR5598, and NpR5597, respectively, were found [68]. A gene Ap3858, homologous to Ava3858/NpR5600, was not found in the upper region of Ap3857, but found at the distant end. Ap3858 protein contained an additional functionally unknown N-terminal domain (Fig. 1.2A) [68]. The *E. coli* cells transformed with four genes from *A. halophytica* produced mycosporine-2-glycine (M2G) [68].

Cyanobacterial MAAs synthetic gene homologs have also been found in bacteria, fungi, dinoflagellates, sea anemones, and coral [61,66,69]. Phylogenetic analysis suggested that the genes Ava_3858 and Ava_3857 were horizontally transferred from cyanobacteria to dinoflagellates [65]. To date, detailed molecular analyses of genes associated with MAAs have only been conducted in cyanobacteria. Molecular investigation of MAAs synthetic pathway with other organisms will be an important subject.

2.7.2 Regulation of Biosynthesis of MAAs

2.7.2.1 UNDER UV RADIATION

Biosynthesis of MAAs is enhanced by UV light. In cyanobacteria, intracellular accumulation of MAAs was significantly increased by photosynthetically active radiation (PAR), UV-A (315–400 nm) radiation, and UV-B (280–315 nm) radiation [70–72]. The most effective induction by UV-B radiation was demonstrated in *Anabaena variabilis*, *Nostoc commune*, *Scytonema* sp., and *Arthrospira* sp. Induction of MAAs by UV radiation was also observed in yeast, macroalgae, and marine microalgae such as prymnesiophyte, diatoms, and dinoflagellate [73–76].

As a molecular mechanism of MAAs induction by UV radiation in cyanobacteria, an evidence of UV-B photoreceptor was proposed [77]. The possibility of MAAs induction without specific photoreceptors was also presented. The induction of MAAs via ROS was demonstrated [78]. In this case, ROS probably acts as signal to enhance MAAs bioproduction.

2.7.2.2 UNDER ABIOTIC STRESSES

Induction of MAAs by salt stress, without PAR or UV radiation, was reported in cyanobacteria *Chlorogloeopsis*, *A. variabilis*, and *A. halophytica* [68,70,77], and in marine dinoflagellate *Gymnodinium catenatum* [79]. A significant salt-induced increase of mRNA for four M2G biosynthetic genes was observed in a halotolerant cyanobacterium *A. halophytica* [68]. Heat stress also increased the accumulation level of MAAs in corals *Lobophytum compactum* and

Sinularia flexibilis [80]. However, in cyanobacteria *Chlorogloeopsis* PCC6912 and *A. variabilis* PCC7973, high temperature did not enhance MAA accumulation [70,81]. Further investigation is required to clarify the mechanisms of abiotic-induced MAAs accumulation.

In addition to abiotic stresses, nitrogen supply also induced MAAs biosynthesis. Increase of shinorine by addition of ammonium to the medium was observed in cyanobacterium *A. variabilis* PCC7937 [70]. Ammonium supply with UV radiation induced accumulation of MAAs including shinorine and porphyra-334 in marine red macroalga *Porphyra columbina* [82]. MAAs act as intracellular nitrogen storage molecules due to their nitrogenous compounds [83].

2.8 Biological Function of Mycosporines and MAAs

2.8.1 Sunscreen Role

Mycosporines and MAAs could absorb UV-A and UV-B without generating ROS. A correlation between MAAs content and irradiance level supports the role of MAAs in UV protection. MAAs localize in the cytoplasm of cell and have a highly water-soluble property [83]. MAAs are commercialized as Helioguard 365, which contains shinorine and porphyra-334 isolated from the red alga *Porphyra umbilicalis* [66]. It is believed that mycosporines and MAAs produced in marine organisms such as cyanobacteria play an important role to reduce the damage by UV radiation in their cells [84]. However, in a cyanobacterium *Microcystis aeruginosa*, shinorine, as the sole MAA type of this strain, did not contribute to the protection against UV radiation [85]. In this case, shinorine was located in extracellular polymeric substances. Further investigations are required to assess the real function of MAAs.

2.8.2 Osmoprotectant Role

In addition to sunscreen property of mycosporines and MAAs, their roles to keep the balance between intracellular osmotic pressure and outer environment were presented. Mycosporines and MAAs are small, uncharged, and water-soluble organic compounds like osmoprotectants [83]. Very high concentration of MAAs were found in community of cyanobacteria inhabiting a gypsum crust developing on the bottom of a hypersaline saltern pond in Eilat, Israel in which cyanobacterium *A. halophytica* was detected [86,87]. Dilution of the medium by freshwater to reduce salinity resulted in excretion of MAAs [87]. This fact supported osmoprotectant property of MAAs. However, it should be noted that additional compounds such as glycine betaine also contribute to balance osmotic condition in these organisms [83].

2.8.3 Antioxidant Role

Dissipation of light energy by MAAs as heat without the generation of ROS has been demonstrated [88]. It was also shown that MAAs have a property to scavenge ROS such as hydroxyl radicals, hydroperoxyl radicals, singlet oxygen, and superoxide anions [83]. Thus MAAs act as an antioxidant role under photooxidative stress condition caused by ROS [84]. Antioxidant activity of mycosporine-glycine was demonstrated in vitro [83] and in vivo in *Stylophora pistillata* and dinoflagellates [89]. 4-Deoxygadual, the precursor compound of MAAs, also has antioxidant activity. Thus MAAs act as antioxidant in cyanobacteria and marine algae. However, MAAs, such as shinorine and porphyra-334, which consist of

an aminocyclohexene imine structure, did not show strong antioxidant activity like mycosporine-glycine or 4-deoxygadusol [60].

2.8.4 Roles of MAAs in Halotolerant Cyanobacteria

Halotolerant cyanobacterium accumulates M2G, and the combination of UV-B radiation and high salinity stresses further enhanced the M2G level significantly. This suggests the role of M2G as both sunscreen and osmoprotectant. Analysis of cyanobacteria in hypersaline saltern pond also supports osmoprotectant property of MAAs in halophilic cyanobacteria [87]. In a halophilic cyanobacterium possessing GB, its level was much higher than M2G under high salinity condition [90]. However, the accumulation rate of M2G was significantly faster than that of GB when the cells were transferred from low to high salinity condition, suggesting the role of M2G as osmoprotectant during early stage acclimation to salt stress.

3. CONCLUSIONS AND PERSPECTIVES

Halophilic microorganisms have evolved unique adaptations to thrive in hypersaline environments. The intracellular accumulation of osmoprotectant via uptake and/or biosynthesis is of central importance for the cellular defense under harsh conditions. The accumulating findings revealed the biosynthetic regulations of various osmoprotectant molecules, for example DMSP, GB, and sunscreen compound MAA. The present review is designed to address important aspects of these molecules through accumulation, response to environment stress, biosynthesis, and regulation. We also highlight the areas that have seen substantial progress in recent years. However, for the comprehensive picture and understanding of their features, further investigations are required. Comprehensive analysis including not only osmoprotectant functions but also other physiological roles/significances should be done because they are possibly multifunctional. In addition, MAAs might also be multifunctional as mentioned above. Further investigations of these multifunctional molecules would be useful not only to understand the molecular mechanisms for acclimation toward various environmental stresses but also could be applied to biotechnological field such as generating stress-tolerant organisms.

References

[1] A. Oren, Diversity of organic osmotic compounds and osmotic adaptation in cyanobacteria and algae, in: J. Seckbach (Ed.), Algae and Cyanobacteria in Extreme Environments, Springer, Netherland, 2007, pp. 641–655.
[2] N. Pade, M. Hagemann, Salt acclimation of cyanobacteria and their application in biotechnology, Life 5 (2015) 25–49.
[3] A.K. Rai, T. Takabe, Abiotic Stress Tolerance in Plants – toward the Improvement of Global Environment and Food, Springer, Dordrecht, the Netherlands, 2006.
[4] T.J. Flowers, R. Munns, T.D. Colmer, Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes, Ann. Bot. 115 (2015) 419–431.
[5] N. Erdmann, M. Hagemann, Salt acclimation of algae and cyanobacteria: a comparison, in: L.C. Rai (Ed.), Algal Adaptation to Environmental Stresses, Springer-Verlag, Berlin Heidelberg, 2001, pp. 323–361.
[6] K. Marin, E. Zuther, T. Kerstan, A. Kunert, M. Hagemann, The *ggpS* gene from *Synechocystis* sp. strain PCC 6803 encoding glucosyl-glycerol-phosphate synthase is involved in osmolyte synthesis, J. Bacteriol. 180 (1998) 4843–4849.

[7] S. Klähn, C. Steglich, W.R. Hess, M. Hagemann, Glucosylglycerate: a secondary compatible solute common to marine cyanobacteria from nitrogen-poor environments, Environ. Microbiol. 12 (2010) 83–94.
[8] S. Klähn, M. Hagemann, Compatible solute biosynthesis in cyanobacteria, Environ. Microbiol. 13 (2011) 551–562.
[9] M. Hagemann, A. Schoor, N. Erdmann, NaCl acts as a direct modulator in the salt adaptive response: salt-dependent activation of glucosylglycerol synthesis in vivo and in vitro, J. Plant Physiol. 149 (1996) 746–752.
[10] C. Luley-Goedel, B. Nidetzky, Glycosides as compatible solutes: biosynthesis and applications, Nat. Prod. Rep. 28 (2011) 875–896.
[11] M. Hagemann, Molecular biology of cyanobacterial salt acclimation, FEMS Microbiol. Rev. 35 (2011) 87–123.
[12] M. Hagemann, N. Pade, Heterosides – compatible solutes occurring in prokaryotic and eukaryotic phototrophs, Plant Biol. 17 (2015) 927–934.
[13] D. Rhodes, A.D. Hanson, Quaternary ammonium and tertiary sulfonium compounds in higher plants, Annu. Rev. Plant Physiol. Mol. Biol. 44 (1993) 357–384.
[14] T. Takabe, T. Nakamura, M. Nomura, Y. Hayashi, M. Ishitani, Y. Muramoto, A. Tanaka, T. Takabe, Glycinebetaine and the genetic engineering of salinity tolerance in plants, in: K. Satoh, N. Murata (Eds.), Stress Responses of Photosynthetic Organisms, Elsevier Science, Amsterdam, the Netherlands, 1997, pp. 115–132.
[15] R. Waditee, M.N.H. Bhuiyan, V. Rai, K. Aoki, Y. Tanaka, T. Hibino, S. Suzuki, J. Takano, A.T. Jagendorf, T. Takabe, T. Takabe, Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis*, Proc. Natl. Acad. Sci. USA 102 (2005) 1318–1323.
[16] R.H. Reed, J.A. Chudek, R. Foster, W.D.P. Stewart, Osmotic adjustment in cyanobacteria from hypersaline environments, Arch. Microbiol. 138 (1984) 333–337.
[17] R. Waditee, N.H. Bhuiyan, E. Hirata, T. Hibino, Y. Tanaka, M. Shikata, T. Takabe, Metabolic engineering for betaine accumulation in microbes and plants, J. Biol. Chem. 282 (2007) 34185–34193.
[18] S. Laloknam, K. Tanaka, T. Buaboocha, R. Waditee, A. Incharoensakdi, T. Hibino, Y. Tanaka, T. Takabe, Halotolerant cyanobacterium *Aphanothece halophytica* contains a betaine transporter active at alkaline pH and high salinity, Appl. Environ. Microbiol. 72 (2006) 6018–6026.
[19] B. Rathinasabapathi, M. Burnet, B.L. Russell, D.A. Gage, P.C. Liao, G.J. Nye, P. Scott, J.H. Golbeck, A.D. Hanson, Choline monooxygenase, an unusual iron-sulfur enzyme catalyzing the first step of glycine betaine synthesis in plants: prosthetic group characterization and cDNA cloning, Proc. Natl. Acad. Sci. USA 94 (1997) 3454–3458.
[20] T. Lamark, I. Kaasen, M.W. Eshoo, J. McDougall, A.R. Strom, DNA sequence and analysis of the *bet* genes encoding the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*, Mol. Microbiol. 5 (1991) 1049–1064.
[21] J. Boch, B. Kempf, R. Schmid, E. Bremer, Synthesis of the osmoprotectant glycine betaine in *Bacillus subtilis*. Characterization of the *gbsAB* genes, J. Bacteriol. 178 (1996) 5121–5129.
[22] E.A. Weretilnyk, A.D. Hanson, Molecular cloning of a plant betaine-aldehyde dehydrogenase, an enzyme implicated in adaptation to salinity and drought, Proc. Natl. Acad. Sci. USA 87 (1990) 2745–2749.
[23] R. Waditee, Y. Tanaka, K. Aoki, T. Hibino, H. Jikuya, J. Takano, T. Takabe, T. Takabe, Isolation and functional characterization of *N*-methyltransferases that catalyze betaine synthesis from glycine in a halotolerant photosynthetic organism *Aphanothece halophytica*, J. Biol. Chem. 278 (2003) 4932–4942.
[24] M. Nomura, M. Ishitani, T. Takabe, A.K. Rai, T. Takabe, *Synechococcus* sp. PCC7942 transformed with *Escherichia coli* bet genes produces glycine betaine from choline and acquires resistance to salt stress, Plant Physiol. 107 (1995) 703–708.
[25] R. Waditee-Sirisattha, D. Sittipol, Y. Tanaka, T. Takabe, Overexpression of serine hydroxymethyltransferase from halotolerant cyanobacterium in *Escherichia coli* results in increased accumulation of choline precursors and enhanced salinity tolerance, FEMS Microbiol. Lett. 333 (2012) 46–53.
[26] H. Knoop, Y. Zilliges, W. Lockau, R. Steuer, The metabolic network of *Synechocystis* sp. PCC 6803: systemic properties of autotrophic growth, Plant Physiol. 154 (2010) 410–422.
[27] F. Klemke, A. Baier, H. Knoop, R. Kern, J. Jablonsky, G. Beyer, T. Volkmer, R. Steuer, W. Lockau, M. Hagemann, Identification of the light-independent phosphoserine pathway as an additional source of serine in the cyanobacterium *Synechocystis* sp. PCC 6803, Microbiology 161 (2015) 1050–1060.
[28] M.H. Sibley, J.H. Yopp, Regulation of S-adenosylhomoeysteine hydrolase in the halophilic cyanobacterium *Aphanothece halophytica*: a possible role in glycinebetaine biosynthesis, Arch. Microbiol. 149 (1987) 43–46.
[29] T. Nakamura, M. Nomura, H. Mori, A.T. Jagendorf, A. Ueda, T. Takabe, An isozyme of betaine aldehyde dehydrogenase in barley, Plant Cell Physiol. 42 (2001) 1088–1092.

[30] N.H. Bhuiyan, A. Hamada, N. Yamada, V. Rai, T. Hibino, T. Takabe, Regulation of betaine synthesis by precursor supply and choline monooxygenase expression in *Amaranthus tricolor*, J. Exp. Bot. 58 (2007) 4203–4212.
[31] L.J. Borowitzka, A.D. Brown, The salt relations of marine and halophilic species of the unicellular green alga, *Dunaliella*. The role of glycerol as a compatible solute, Arch. Microbiol. 96 (1974) 37–52.
[32] M. Avron, The osmotic components of halotolerant algae, Trends Biochem. Sci. 11 (1986) 5–6.
[33] A. Oren, Bioenergetic aspects of halophilism, Microbiol. Mol. Biol. Rev. 63 (1999) 334–348.
[34] J.A. Cray, J.T. Russell, D.J. Timson, R.S. Singhal, J.E. Hallsworth, A universal measure of chaotropicity and kosmotropicity, Environ. Microbiol. 15 (2013) 287–296.
[35] M. Finel, U. Pick, S. Selman-Reimer, B.R. Selman, Purification and characterization of a glycerol-resistant CF_0–CF_1 and CF_1 ATPase from the halotolerant alga *Dunaliella bardawil*, Plant Physiol. 74 (1984) 766–772.
[36] M. Avron, A. Ben-Amotz, *Dunaliella*: Physiology, Biochemistry, and Biotechnology, CRC Press, Boca Raton, 1992.
[37] M. Shariati, R.M. Lilley, Loss of intracellular glycerol from *Dunaliella* by electroporation at constant osmotic pressure: subsequent restoration of glycerol content and associated volume changes, Plant Cell Environ. 17 (1994) 1295–1304.
[38] H. Gimmler, W. Hartung, Low permeability of the plasma membrane of *Dunaliella parva* for solutes, J. Plant Physiol. 133 (1988) 165–172.
[39] H. Chen, Y. Lu, J.-G. Jiang, Comparative analysis on the key enzymes of the glycerol cycle metabolic pathway in *Dunaliella salina* under osmotic stresses, PLoS One 7 (2012) e37578.
[40] R.H. Reed, Measurement and osmotic significance of β-dimethylsulfoniopropionate in marine macroalgae, Mar. Biol. Lett. 4 (1983) 173–181.
[41] A.M.N. Caruana, M. Steinke, S.M. Turner, G. Malin, Concentrations of dimethylsulphoniopropionate and activities of dimethylsulphide-producing enzymes in batch cultures of nine dinoflagellate species, Biogeochemistry 110 (2012) 87–107.
[42] M.L. Otte, G. Wilson, J.T. Morris, B.M. Moran, Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants, J. Exp. Bot. 55 (2004) 1919–1925.
[43] U. Karsten, C. Wiencke, G.O. Kirst, The effect of light intensity and daylength of the β-dimethylsulphoniopropionate (DMSP) content of marine green macroalgae from Antarctica, Plant Cell Environ. 13 (1990) 989–993.
[44] A. Vairavamurthy, M.O. Andreae, R.L. Iverson, Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations, Limnol. Oceanogr. 30 (1985) 59–70.
[45] U. Karsten, C. Wiencke, G.O. Kisrt, Dimethylsuiphoniopropionate (DMSP) accumulation in green macroalgae from polar to temperate regions: interactive effects of light versus salinity and light, Polar Biol. 12 (1992) 603–607.
[46] D. Slezak, G.J. Herndl, Effects of ultraviolet and visible radiation on the cellular concentrations of dimethylsulfoniopropionate (DMSP) in *Emiliania huxleyi* (strain L.), Mar. Ecol. Prog. Ser. 246 (2003) 61–71.
[47] T. Gröne, G.O. Kirst, The effect of nitrogen deficiency, methionine and inhibitors of methionine metabolism on the DMSP contents of *Tetraselmis subcordiformis* (Stein), Mar. Biol. 112 (1992) 497–503.
[48] W. Sunda, D.J. Kieber, R.P. Kiene, S. Huntsman, An antioxidant function for DMSP and DMS in marine algae, Nature 418 (2002) 317–320.
[49] W.G. Sunda, R. Hardison, R.P. Kiene, E. Bucciarelli, H. Harada, The effect of nitrogen limitation on cellular DMSP and DMS release in marine phytoplankton: climate feedback implications, Aquat. Sci. 69 (2007) 341–351.
[50] G.V. Wolfe, M. Steinke, G.O. Kirst, Grazing-activated chemical defence in a unicellular marine alga, Nature 387 (1997) 894–897.
[51] P.K. Quinn, T.S. Bates, The case against climate regulation via oceanic phytoplankton sulphur emissions, Nature 480 (2011) 51–56.
[52] T. Ito, Y. Asano, Y. Tanaka, T. Takabe, Regulation of biosynthesis of dimethylsulfoniopropionate and its uptake in sterile mutant of *Ulva pertusa* (Chlorophyta), J. Phycol. 47 (2011) 517–523.
[53] B.R. Lyon, P.A. Lee, J.M. Bennett, G.R. DiTullio, M.G. Janech, Proteomic analysis of a sea-ice diatom: salinity acclimation provides new insight into the dimethylsulfoniopropionate production pathway, Plant Physiol. 157 (2011) 1926–1941.
[54] N.L. Hockin, T. Mock, F. Mulholland, S. Kopriva, G. Malin, The response of diatom central carbon metabolism to nitrogen starvation is different from that of green algae and higher plants, Plant Physiol. 158 (2012) 299–312.

[55] N.L. Kettles, S. Kopriva, G. Malin, Insights into the regulation of DMSP synthesis in the diatom *Thalassiosira pseudonana* through APR activity, proteomics and gene expression analyses on cells acclimating to changes in salinity, light and nitrogen, PLoS One 9 (2014) e94795.
[56] W.C. Dunlap, J.M. Shick, Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective, J. Phycol. 34 (1998) 418–430.
[57] J. Favre-Bonvin, N. Arpin, C. Brevard, Structure de la mycosporine (P310), Can. J. Chem. 54 (1976) 1105–1113.
[58] J.I. Carreto, M.O. Carignan, Mycosporine-like amino acids: relevant secondary metabolites. Chemical and ecological aspects, Mar. Drugs 9 (2011) 387–446.
[59] P.N. Leão, N. Engene, A. Antunes, W.H. Gerwick, V. Vasconcelos, The chemical ecology of cyanobacteria, Nat. Prod. Rep. 29 (2012) 372–391.
[60] N. Wada, T. Sakamoto, S. Matsugo, Multiple roles of photosynthetic and sunscreen pigments in cyanobacteria on the oxidative stress, Metabolites 3 (2013) 463–483.
[61] C. Shinzato, E. Shoguchi, T. Kawashima, M. Hamada, K. Hisata, M. Tanaka, M. Fujie, M. Fujiwara, R. Koyanagi, T. Ikuta, A. Fujiyama, D.J. Miller, N. Satoh, Using the *Acropora digitifera* genome to understand coral responses to environmental change, Nature 476 (2011) 320–323.
[62] A.R. Osborn, K.H. Almabruk, G. Holzwarth, S. Asamizu, J. LaDu, K.M. Kean, P.A. Karplus, R.L. Tanguay, A.T. Bakalinsky, T. Mahmud, De novo synthesis of a sunscreen compound in vertebrates, Elife (2015) e05919.
[63] J. Favre-Bonvin, J. Bernillon, N. Salin, N. Arpin, Biosynthesis of mycosporines: mycosporine glutaminol in *Trichothecium roseum*, Phytochemistry 26 (1987) 2509–2514.
[64] J.M. Shick, S. Romaine-Lioud, C. Ferrier-Pagès, J.P. Gattuso, Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreased in its population of symbiotic dinoflagellates, Limnol. Oceanogr. 44 (2002) 1667–1682.
[65] S.P. Singh, M. Klisch, R.P. Sinha, D.P. Häder, Genome mining of mycosporine-like amino acid (MAA) synthesizing and non-synthesizung cyanobacteria: a bioinformatics study, Genomics 95 (2010) 120–128.
[66] E.P. Balskus, C.T. Walsh, The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria, Science 329 (2010) 1653–1656.
[67] Q. Gao, F. Garcia-Pichel, An ATP-grasp ligase involved in the last biosynthetic step of the iminomycosporine shinorine in *Nostoc punctiforme* ATCC 29133, J. Bacteriol. 193 (2011) 5923–5928.
[68] R. Waditee-Sirisattha, H. Kageyama, W. Sopun, Y. Tanaka, T. Takabe, Identification and upregulation of biosynthetic genes required for accumulation of mycosporine-2-glycine under salt stress conditions in the halotolerant cyanobacterium *Aphanothece halophytica*, Appl. Environ. Microbiol. 80 (2014) 1763–1769.
[69] M.L. Micallef, P.M. D'Agostino, D. Sharma, R. Viswanathan, M.C. Moffitt, Genome mining for natural product biosynthetic gene clusters in the subsection V cyanobacteria, BMC Genomics 16 (2015) 669–678.
[70] S.P. Singh, M. Klisch, R.P. Sinha, D.P. Häder, Effects of abiotic stressors on synthesis of the mycosporine-like amino acid shinorine in the cyanobacterium *Anabaena variabilis* PCC 7937, Photochem. Photobiol. 84 (2008) 1500–1505.
[71] R.P. Rastogi, A. Incharoensakdi, Analysis of UV-absorbing photoprotectant mycosporine-like amino acid (MAA) in the cyanobacterium *Arthrospira* sp. CU2556, Photochem. Photobiol. Sci. 13 (2014) 1016–1024.
[72] R.P. Sinha, M. Klisch, E.W. Helbling, D.P. Häder, Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation, J. Photochem. Photobiol. B 60 (2001) 129–135.
[73] D. Libkind, P.A. Perez, R. Sommaruga, M.C. Díeguez, M. Ferraro, S. Brizzio, H. Zagarese, M. van Broock, Constitutive and UV-inducible synthesis of photoprotective compounds (carotenoids and mycosporines) by freshwater yeasts, Photochem. Photobiol. Sci. 3 (2004) 281–286.
[74] L. Riegger, D. Robinson, Photoinduction of UV-absorbing compounds in Antarctic diatoms and *Phaeocystis antarctica*, Mar. Ecol. Prog. Ser. 160 (1997) 13–25.
[75] M. Klisch, D.P. Häder, Mycosporine-like amino acids in the marine dinoflagellate *Gyrodinium dorsum*: induction by ultraviolet irradiation, J. Photochem. Photobiol. B 55 (2000) 178–182.
[76] M. Arróniz-Crespo, R.P. Sinha, J. Martínez-Abaigar, E. Núñez-Olivera, D.P. Häder, Ultraviolet radiation-induced changes in mycosporine-like amino acids and physiological variables in the red alga *Lemanea fluviatilis*, J. Fresh. Ecol. 20 (2005) 677–687.
[77] A. Portwich, F. Garcia-Pichel, A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912, Photochem. Photobiol. 71 (2000) 493–498.

[78] J.M. Shick, The continuity and intensity of ultraviolet irradiation affect the kinetics of biosynthesis, accumulation, and conversion of mycosporine-like amino acids (MAAs) in the coral *Stylophora pistillata*, Limnol. Oceanogr. 49 (2004) 442–458.
[79] P. Vale, Effects of light and salinity stresses in production of mycosporine-like amino acids by *Gymnodinium catenatum* (dinophyceae), Photochem. Photobiol. 91 (2015) 1112–1122.
[80] J.M. Shick, W.C. Dunlap, Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms, Annu. Rev. Physiol. 64 (2002) 223–262.
[81] A. Portwich, F. Garcia-Pichel, Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912, Arch. Microbiol. 172 (1999) 187–192.
[82] N. Korbee Peinado, R.T. Abdala Díaz, F.L. Figueroa, E.W. Helbling, Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina, J. Phycol. 40 (2004) 248–259.
[83] A. Oren, N. Gunde-Cimerman, Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol. Lett. 269 (2007) 1–10.
[84] N.N. Rosic, S. Dove, Mycosporine-like amino acids from coral dinoflagellates, Appl. Environ. Microbiol. 77 (2011) 8478–8486.
[85] C. Hu, G. Völler, R. Süβmuth, E. Dittmann, J.C. Kehr, Functional assessment of mycosporine-like amino acids in *Mycrocystis aeruginosa* strain PCC 7806, Environ. Microbiol. 17 (2015) 1548–1559.
[86] A. Oren, M. Kuhl, U. Karsten, An endevaporitic microbial mat within a gypsum crust: zonation of phototrophs, photopigments, and light penetration, Mar. Ecol. Prog. Ser. 128 (1995) 151–159.
[87] A. Oren, Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria, Geomicrobiol. J. 14 (1997) 231–240.
[88] F.R. Conde, M.S. Churio, C.M. Previtali, The deactivation pathways of the excited-states of the mycosporine-like amino acids shinorine and porphyra-334 in aqueous solution, Photochem. Photobiol. Sci. 3 (2004) 960–967.
[89] I. Yakovleva, R. Bhagooli, A. Takemura, M. Hidaka, Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 139 (2004) 721–730.
[90] R. Waditee-Sirisattha, H. Kageyama, M. Fukaya, V. Rai, T. Takabe, Nitrate and amino acid availability affects glycine betaine and mycosporine-2-glycine in response to changes of salinity in a halotolerant cyanobacterium *Aphanothece halophytica*, FEMS Microbiol. Lett. 362 (2015) fnv198.

CHAPTER

2

UV Photoprotectants From Algae—Synthesis and Bio-Functionalities

R.P. Rastogi, R.R. Sonani, D. Madamwar

Sardar Patel University, Anand, Gujarat, India

OUTLINE

1. INTRODUCTION

Algae including cyanobacteria are the largest group of photosynthetic organisms in both aquatic and terrestrial ecosystems. They are immense source of several secondary compounds,

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00002-3

each with specialized functions and high economic values. Increased solar ultraviolet (UV: 280−400 nm) radiation on the Earth's surface due to depletion or thinning of ozone layer by means of natural and/or anthropogenically released ozone-depleting substances has generated tremendous concern about their harmful effects on aquatic and terrestrial organisms [1−3]. Increased solar UV radiation may alter normal physiology and biochemistry of all photosynthetic life either directly by affecting the key cellular machinery such as lipids, proteins, and DNA or indirectly by the generation of reactive oxygen species (ROS) or other free radicals [4−6]. Incidence of solar UV-B (280−315 nm) radiation may cause drastic effects to all life forms including human beings. Solar UV (mainly UV-B) radiation may induce skin disorders such as edema/erythema, premature skin aging, and carcinogenesis. UV-induced oxidative stress may also alter the expression of certain genes leading to induction of a sequence of collagen and elastin-degrading enzymes, such as metalloproteinase, and cause photoaging. ROS-mediated lipid-peroxidation, protein modification, DNA damages such as strand breaks, and formation of purine/pyrimidine dimers may cease the vital functionality of the cell.

Moreover, increased incidence of solar ultraviolet (UV) radiation on the Earth's surface is supposed to be a major physiological stress factor to all the photosynthetic or nonphotosynthetic life forms. Several organisms have developed a number of defense mechanisms to overcome the detrimental effects of UV radiation (Fig. 2.1).

An increase in high-energetic solar radiation (280−315 nm) has aroused interest in the search for natural photoprotectant biomolecules from various organisms. A number of UV-absorbing/-screening biomolecules, such as mycosporine-like amino acids (MAAs), scytonemin (Scy), melanins, carotenoids, flavonoids, parietin, and usnic acid, have been reported from diverse organisms (Fig. 2.2) [7].

Algae including cyanobacteria are capable of protecting themselves from harmful solar UV radiation by synthesizing the UV-absorbing secondary compounds. Some specific UV-photoprotective biomolecules have been reported in different algae. MAAs and Scy are the most important photoprotectants that have great efficacy to protect from harmful UV radiation [8−10]. Since application of synthetic UV filters might be harmful due to endocrine side effects [11], the photoprotectants such as MAAs and Scy may be a great substitute in pharmaceutical and cosmetic industries for the development of novel cosmeceuticals and natural suncare products against photoaging and other ROS-associated disorder. This article summarizes the occurrence of UV protective compounds MAAs and Scy in algae, their synthesis, and bio-functionalities with special emphasis on their potential use as cosmeceuticals.

2. PHOTOPROTECTANTS FROM ALGAE

A number of photoprotectants have been derived from different taxonomic groups of algae. Biosynthesis or accumulation of some photoprotective compounds such as MAAs and Scy has been documented as important phenomena against short wavelength UV-A or UV-B radiation in several micro-/macroalgae including cyanobacteria. Herein, we have discussed about the photoprotectant small molecules MAAs and Scy pigment.

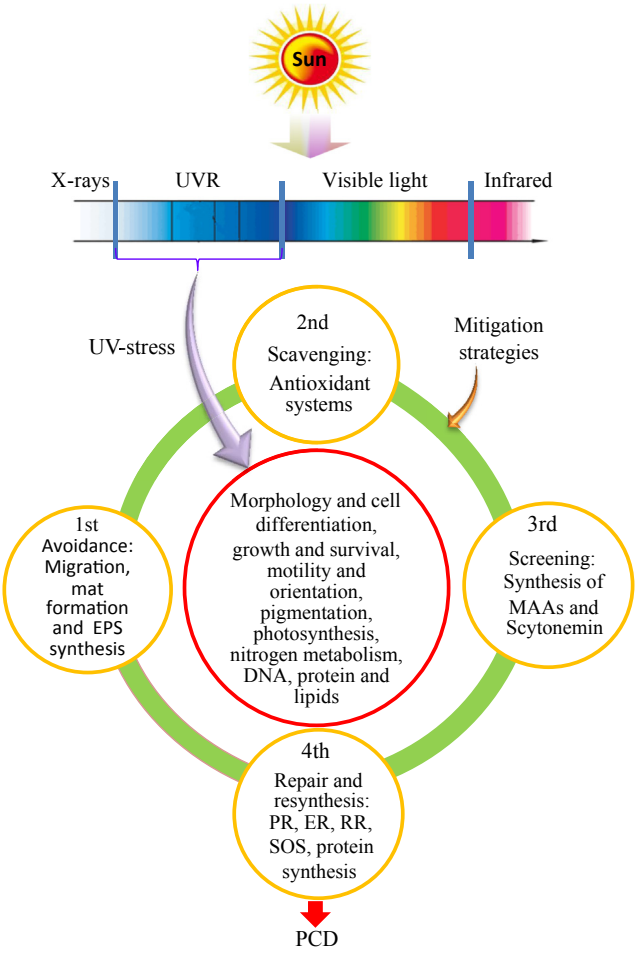


FIGURE 2.1 Defense mechanisms adopted by the organisms to overcome the detrimental effects of UV radiation.

2.1 Mycosporine-like Amino Acids

MAAs are small, hydrophilic, colorless, intracellular secondary compounds composed of cyclohexenone or cyclohexenimine chromophores conjugated with the nitrogen substituent of an amino acid or its imino alcohol. In general, the ring system of MAAs includes a glycine subunit at the third carbon atom; however, some MAAs also contain sulfate esters or glycosidic linkages through the imine substituents [12]. The extreme hydrophilicity of MAAs are due to their zwitter ionic form derived from the amino acid substitution. Moreover, the hydrophilicity can also be increased by modification with sulfonic acids or sugar molecules [12,13]. The MAAs have high molar extinction coefficients, UV absorption maxima, and stability under different physicochemical factors. The UV absorption properties of different MAAs differ due to variations in the attached side groups and nitrogen substituents [12,13]. Moreover, the specific stereo structure of MAAs (except palythine and

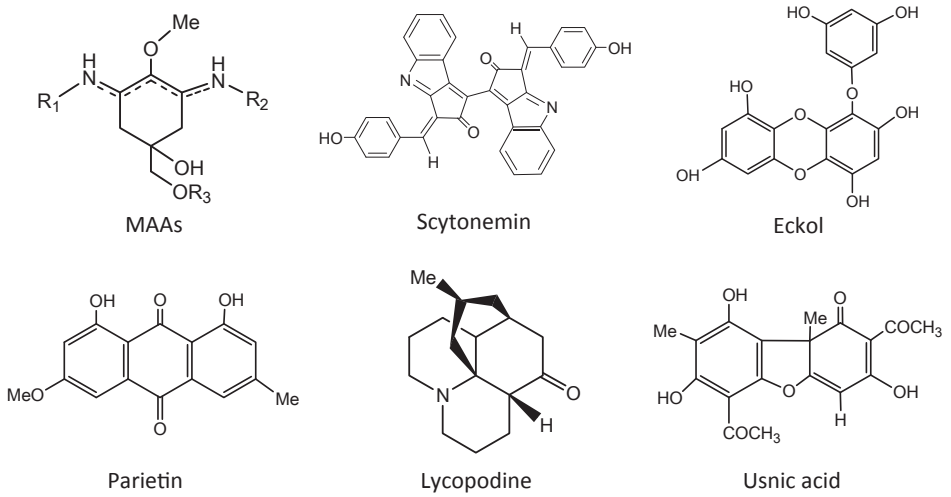


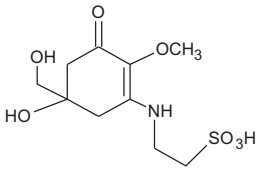
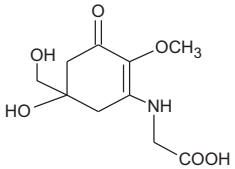
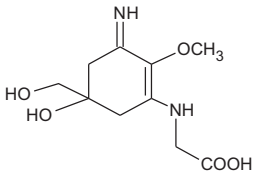
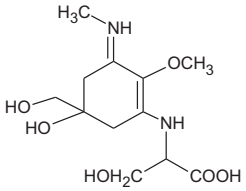
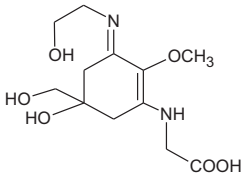
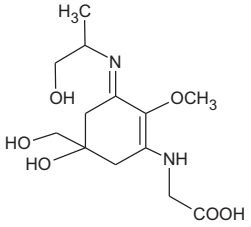
FIGURE 2.2 Chemical structure of some common UV-absorbing compounds from different taxonomic groups.

palythene), including amino acids substituents is not completely interpreted. Currently, more than 25 MAAs have been reported from diverse organisms, among which several MAAs (Table 2.1) have been isolated from different species/strains of algae or cyanobacteria.

2.2 The Glycosylated MAAs

Some MAAs are covalently linked to different oligosaccharides comprising of galactose, glucose, xylose, glucoronic acid, or glucosamine [13,14] and known as glycosylated MAAs. The UV-absorbing compounds having absorption maxima at 335 nm and 312 nm is supposed to be first glycosylated MAA (G-MAAs) isolated from the extracellular glycan sheath of *Nostoc* sp. [13]. The G-MAAs has extremely high molecular weight due to attached oligosaccharides. The G-MAA pentose-bound porphyra-334 (λ_{max}: 335 nm; 478 Da) was identified from the cyanobacterium *Nostoc commune* [14]. Another G-MAA with double absorption maxima at 312 and 340 nm with a molecular mass of 1050 Da was also found in the cyanobacterium *Nostoc commune*. The unique structure of 1050-Da G-MAA consisted of two distinct chromophores of 3-aminocyclohexen-1-one and 1,3-diaminocyclohexen and two pentose and hexose sugars [14]. Recently, two novel glycosylated MAAs such as a hexose-bound porphyra-334 (λ_{max}: 334 nm) and a two hexose-bound palythine-threonine (λ_{max}: 322 nm) with a molecular mass of 508 Da and 612 Da, respectively, were found in the terrestrial cyanobacterium *Nostoc commune* [15]. Furthermore, a number of G-MAAs such as 7-O-(b-arabinopyranosyl)-porphyra-334 (478 Da), pentose-bound shinorine (464 Da), hexose-bound porphyra-334 (508 Da), and some other G-MAAs such as 273-Da MAA were isolated from different strains of *Nostoc commune* [16]. Moreover, the occurrence of G-MAAs has been reported only in some cyanobacteria like *Nostoc* spp., and needs an extensive study to explore these MAAs in diverse organisms.

TABLE 2.1 Chemical Structure of Some Common MAAs Isolated From Cyanobacteria (Blue Green Algae) and Eukaryotic Algae

No.	MAAs	Chemical Structure	Abs. Maxima (nm)
01.	Mycosporine-tau		309
02.	Mycosporine-glycine		310
03.	Palythine		320
04.	Mycosporine-methylamine-serine		327
05.	Asterina-330		330
06.	Palythinol		332

(*Continued*)

TABLE 2.1 Chemical Structure of Some Common MAAs Isolated From Cyanobacteria (Blue Green Algae) and Eukaryotic Algae—cont'd

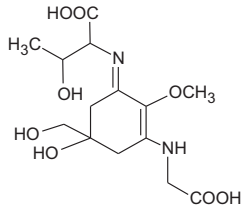
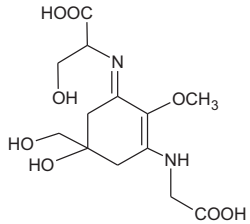
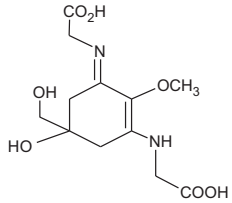
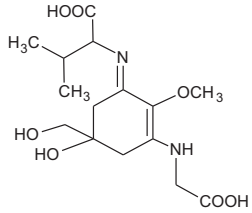
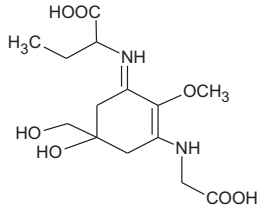
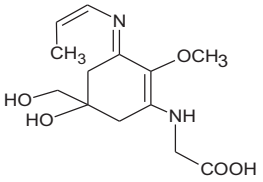
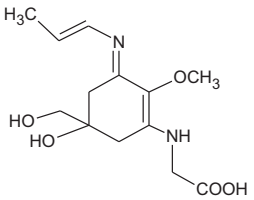
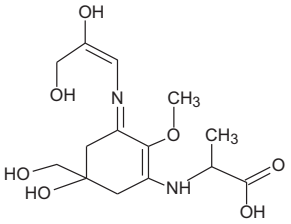
No.	MAAs	Chemical Structure	Abs. Maxima (nm)
07.	Porphyra-334		334
08.	Shinorine		334
09.	Mycosporine-2-glycine		334
10.	Mycosporine-glycine-valine		335
11.	Palythenic acid		337

TABLE 2.1 Chemical Structure of Some Common MAAs Isolated From Cyanobacteria (Blue Green Algae) and Eukaryotic Algae—cont'd

No.	MAAs	Chemical Structure	Abs. Maxima (nm)
12.	Usujirene		357
13.	Palythene		360
14.	Euhalothece-362		362

3. OCCURRENCE OF MAAs

MAAs have been reported in diverse organisms such as micro-/macroalgae, cyanobacteria and several aquatic invertebrates such as sea anemones, limpets, shrimp, sea urchins, and vertebrates [17]. It has been assumed that in higher animals occurrence of MAAs can be attributed either to their ingestion through food chain or their synthesis by symbiotic algal partners. A number of MAAs reported to be from different taxonomic groups of algae belong to cyanophyceae, chlorophyceae, rhodophyceae, phaeophyceae, and bacillariophyceae (Table 2.2).

3.1 MAAs From Cyanobacteria (Blue Green Algae)

Cyanobacteria are the most dominant photoautotrophs that can synthesize a range of different MAAs [18] (Table 2.2). Besides the most dominant MAAs, i.e., porphyra-334, shinorine, and mycosporine-glycine [7,19], some other MAAs such as asterina-330, palythine, palythinol, euhalothece-362, and mycosporine-2-glycine are considered as common MAAs found in cyanobacteria. The occurrence of G-MAAs has also been reported in certain species of cyanobacteria as mentioned above. Recently, some novel MAAs such as palythine (λ_{max}: 319 nm;

TABLE 2.2 Occurrence of Some Common MAAs in Cyanobacteria and Eukaryotic Algae

	Mycosporine-Like Amino Acids													
Algal Groups	**MT**	**MG**	**PT**	**MMS**	**AS**	**PL**	**PR**	**SH**	**M2G**	**MGV**	**PA**	**US**	**PE**	**E−362**
Cyanobacteria	+	+	+		+	+	+	+	+			+	+	+
Green algae		+	+		+	+	+	+						
Brown algae		+	+		+	+	+	+						
Red algae		+	+		+	+	+	+				+	+	
Diatoms	+	+	+		+	+	+	+	+		+		+	
Dinoflagellate		+	+	+		+	+	+		+	+	+	+	
Haptophyte		+	+				+	+		+				

AS, asterina-330; *E-362*, euhalothece-362; *M2G*, mycosporine-2-glycine; *MG*, mycosporine-glycine; *MGV*, mycosporine-glycine valine; *MMS*, mycosporine-methylamine-serine; *MT*, mycosporine-taurine; *PA*, palythenic acid; *PE*, palythene; *PL*, palythinol; *PR*, porphyra-334; *PT*, palythine; *SH*, shinorine; *US*, usurijene.

m/z: 245), asterina (λ_{max}: 330 nm; *m/z*: 289), and an unknown MAA M-312 (λ_{max}: 312 nm) were found in the cyanobacterium *Lyngbya* sp. [20]. The MAAs shinorine (λ_{max}: 333 nm) and M-307 (λ_{max}: 307 nm) were reported from the unicellular cyanobacterium *Gloeocapsa* sp. [21]. A primary MAA mycosporine-glycine (λ_{max}: 310 nm) was first time reported and chemically characterized in the cyanobacterium *Arthrospira* sp. studied so far [22]. Sinha et al. [23] have reported the MAAs porphyra-334 and shinorine from three different species of *Nodularia* upon UV-B irradiation. MAA-producing cyanobacteria are abundant in hot spring [19] and hypersaline environments [24,25]. Moreover, an extensive study is needed to explore the occurrence of some novel MAAs in different taxonomic groups of cyanobacteria.

3.2 MAAs From Eukaryotic Micro-/Macroalgae

Eukaryotic algae are capable of protecting themselves from harmful solar UV radiation by synthesizing a range of UV-absorbing biomolecules. A number of MAAs have been reported from eukaryotic micro-/macroalgae (Table 2.2) [26−30]. MAAs have been reported to occur in the members of the chlorophyceae (green algae), phaeophyceae (brown algae), rhodophyceae (red algae), dinophyceae (dinoflagellate), bacillariophyceae (diatom), and haptophyceae (or prymnesiophyceae). The occurrence of MAAs in green or brown algae is very limited.

3.2.1 Chlorophyceae

Some common MAAs such as porphyra-334, shinorine, palythinol, mycosporine-glycine, and asterina-330 were found in different green alga such as *Acrosiphonia* sp., *Boodle* sp., *Caulerpa* sp., *Chaetomorpha* sp., *Codium* sp., and *Ulva* sp. [27,31−34]. The presence of an UV-absorbing compound with an absorption maximum at 324 nm was reported in the subaerial green macroalga *Prasiola crispa* spp. [35] and *Prasiola stipitata* [36]. Karsten et al. [37] also reported the presence of two MAAs from *Prasiola crispa* with identical absorption spectra and a maximum at 324 nm. A 322 nm-MAA (λ_{max}: 322 nm) was also found in a green microalga

Myrmecia incise [38]. Two UV-absorbing compounds with absorption maximum at 324 nm and 322 nm were also found in the green microalga *Tetraspora* sp. [30]. Jeffrey et al. [39] have investigated more than 200 strains of microalgae including several green algae for the presence of UV-absorbing compounds and found the sunscreening compounds with absorption maxima between 330 and 340 nm. Karsten et al. [33] have found a significant concentration of photoprotective mycosporine-glycine and porphyra-334 in the green algae *Boodlea composita* and *Caulerpa racemosa*, respectively. Recently, some common MAAs such as porphyra-334, shinorine, and mycosporine-glycine were isolated from the extracts of marine green alga *Chlamydomonas hedleyi* [40].

3.2.2 Phaeophyceae (Brown Algae)

Some UV-absorbing compounds were found in the member of phaeophyceae [33]. A single MAA porphyra-334 was found in the brown algae *Dictyota bartayresii*, *Hydroclathrus clathratus*, *Chorda tomentosa*, *Dictyosiphon foeniculaceus*, and *Pilayella littoralis* [33,34]. The brown alga *Sargassum oligocystum* was found to synthesize three different MAAs such as shinorine, porphyra-334, and palythine [33]. The MAAs porphyra-334 and shinorine were isolated from the brown alga *Desmarestia aculeata* [27]. The brown alga *Halopteris scoparia* was found to produce palythine, porphyra-334, and shinorine [27,33,34]. A number of MAAs such as palythine, asterina-330, palythinol, porphyra-334, and shinorine was found in a brown alga *Padina crassa* [31].

3.2.3 Rhodophyceae (Red Algae)

The red algae are able to synthesize or accumulate several MAAs in high concentrations [27,35]. Several species of red algae, such as *Acanthophora*, *Bangia*, *Bostrychia*, *Caloglossa*, *Catenella*, *Devaleraea*, *Ceramium*, *Chondrus*, *Corallina*, *Curdiea*, *Cystoclonium*, *Devaleraea*, *Dumontia*, *Galaxaura*, *Gelidiella*, *Gelidium*, *Gracilaria*, *Iridea*, *Palmaria*, *Phyllophora*, *Polysiphonia*, *Porphyra*, *Stictosiphonia*, etc., synthesize high concentrations of different MAAs [27,33,34]. The MAAs such as palythine, asterina-330, palythinol, porphyra-334, and shinorine are widely distributed MAAs in different species of red algae. Moreover, the red alga *Gracilaria changii* was found to produce seven different MAAs [33]. The red alga *Porphyra umbilicalis* synthesizes three main MAAs such as palythine, shinorine, and porphyra-334 [37]. The MAAs porphyra-334 + shinorine and asterina-330 + palythine were found in the red alge *Porphyra rosengurttii* and *Gelidium corneum*, respectively [41]. Six different MAAs, palythine, shinorine, asterina-330, porphyra-334, palythinol, and the low-polarity usujirene, have been reported in the edible red alga, *Palmaria palmata* (dulse) [42]. The MAA porphyra-334 was found in high concentration in some algae, particularly *Porphyra* spp. and *Bangia atropurpurea* [43–45]. Recently, a novel MAA catenelline was isolated and chemically characterized from marine red seaweed *Catenella repens* [46]. Two MAAs shinorine and palythine were derived from Russian red algae *Gloiopeltis fucatas* and *Mazzaella* sp. [47].

3.2.4 Bacillariophyceae, Dinophyceae, and Haptophyceae

Several MAAs have been reported to occur predominantly in members of the bacillariophyceae (diatom), dinophyceae (dinoflagellate), and haptophyceae (or prymnesiophyceae) [27,39]. A number of MAAs have been reported from different species of diatoms [48,49]. High content of UV-absorbing compounds were detected in the chain-forming diatom

Thalassiosira gravida from Antarctica [50]. The UV-absorbing compound having absorption maximum at 334 nm was observed in a marine red-tide alga Skeletonema costatum (diatom) [51]. The presence of different MAAs such as palythine, porphyra-334, shinorine, and traces of asterina-330, palythinol, and palythinic acid were observed in the frustules of marine diatoms [52]. The MAAs porphyara-334 and shinorine were found in some Antarctic diatoms such as *Porosira glacialis*, *Porosira pseudodenticulata*, *Probiscia inemis*, *Stellarima microtrias*, *Thalassiosira antarctica*, *Thalassiosira tumida*, and other *Thalassiosira* spp. [49,53]. High concentration of some MAAs was observed in some phytoplankton community including diatoms from Kongsfjorden, Svalbard, Arctic [54]. Recently, occurrence of MAAs with photoprotective function was shown in a bipolar diatom *Porosira glacialis* [55]. The diatoms *Thalassiosira weissflogiilow* produces low concentrations of porphyra-334 [28]. Piiparinen et al. [56] have observed the group of some MAAs shinorine, palythine, porphyra-334, and an unknown compound with absorption peaks at 335 and 360 nm in diatom and dinoflagellate-dominated sea-ice algal community in the Baltic Sea [56].

Some species of dinoflagellates such as *Alexandrium excavatum* is known to produce high concentrations of MAAs [57]. It has been found that the bloom-forming dinoflagellate *Alexandrium excavatum* grown under intense light possess a complex mixture of MAAs such as shinorine, porphyra-334, asterina-330, and small proportion of palythine [57]. The dinoflagellates such as *Prorocentrum micans* was found to synthesize four different MAAs such as mycosporine-glycine, asterina-330, porphyra-334, and shinorine [58], whereas only two MAAs palythine and shinorine were found in *P. minimum* [59]. In a dinoflagellate *Amphidinium carterae* the MAA mycosporine-glycine was found [60]. In the marine dinoflagellate *Gyrodinium dorsum*, five MAAs, i.e., shinorine, porphyra-334, palythine, and two unidentified MAAs having λ_{max} at 310 and 331 nm were found [61]. High-performance liquid chromatography analysis of *Gymnodinium catenatum* extracts revealed the presence of mycosporine-glycine, shinorine, porphyra-334, and several unknown UV-absorbing compounds [39]. Several UV photoprotective compounds such as shinorine, porphyra-334, and mycosporine-glycine, palythene, and an unknown M-370 were found in *Gymnodinium catenatum* [62]. High concentrations of UV-absorbing compounds have been observed in several species of bloom-forming dinoflagellates [57,63]. Five different MAAs such as palythine, palythinol, porphyra-334, palythene, and mycosporine-glycine were found in a red-tide dinoflagellate *Lingulodinium polyedra* [64]. A range of MAAs having UV-absorption maxima between 310 and 360 nm, including the MAAs mycosporine-glycine, palythine, asterina-330, palythinol, shinorine, porphyra-334, palythenic acid, cis-usujirene, and palythene were found in the red-tide dinoflagellate *Alexandrium excavatum* [39]. In the presence of UV radiation, high concentrations of mycosporine-glycine, shinorine, and porphyra-334 were produced by *Symbiodinium microadriaticum* [65]. An unusual MAA having UV-absorption maximum at 333 nm (called MAA M-333) was reported from different dinoflagellates such as *Alexandrium tamarense* and *Heterocapsa triquetra* [66], which was tentatively identified as a shinorine methyl ester. However, recently, Carignan and Carreto [29] characterized this novel compound M-333 as mycosporine-serine-glycine methyl ester, by nuclear magnetic resonance.

Like dinoflagellates, some species of prymnesiophyte e.g., *Phaeocystis pouchetii* (haptophyceae or prymnesiophyceae) also produce high concentration of different MAAs [67]. In Antarctica, high UV-absorption maxima, an indicative of MAAs was characteristic of assemblages dominated by prymnesiophytes [68]. Mycosporine-glycine, shinorine, and

mycosporine-glycine valine were the major MAAs responsible for strong in vivo UV-absorption in *Phaeocystis antarctica* [69]. Moreover, several UV-absorbing compounds were observed in some members of haptophyceae such as *Emiliania huxleyi*, *Isochrysis galbana*, and *Phaeocystis globosa* but chemically not quantified [27,28].

4. GENETIC AND ENVIRONMENTAL REGULATION OF MAAs BIOSYNTHESIS

The biosynthesis of MAAs is supposed to occur via first part of the shikimate pathway, where 3-dehydroquinate formed during the early stages of this pathway and serves as a precursor for the synthesis of primary MAAs via gadusols or 4-deoxygadusol (4-DG) [70–72] (Fig. 2.3). The shikimate pathway for MAAs synthesis was supported by the fact that in the reef-building coral *Stylophora pistillata*, MAA synthesis was blocked by the use of glyphosate, which is a specific shikimate pathway inhibitor [73]. However, genome-mining for MAA synthesis revealed the occurrence of specific genes responsible for MAA synthesis in some cyanobacteria [74–76]. The genetic basis of MAA biosynthetic pathway has recently been elucidated [74]. It has been suggested that the MAAs originate from the pentose phosphate pathway intermediate sedoheptulose-7-phosphate via 4-DG [74] (Fig. 2.3). A cluster of four genes i.e., dehydroquinate synthase (DHQS) homolog Ava_3858, O-methyltransferase (O-MT) Ava_3857, ATP-grasp Ava_3856, and nonribosomal peptide synthetase (NRPS) homolog Ava_3855 was found in *Anabaena variabilis* responsible for MAA (shinorine) synthesis [74]. It has been proposed that the DHQS and O-MT enzymes convert the precursor into 4-DG, and ATP-grasp catalyzes the addition of glycine to 4-DG to form mycosporine-glycine (M-Gly), while NRPS catalyzes the addition of serine to M-Gly and form shinorine. A cluster of four genes (NpR5600, NpR5599, NpR5598, and NpF5597) responsible for MAAs synthesis has also been reported in the cyanobacterium *Nostoc punctiforme* ATCC 29133 [74,76]. The gene organization for MAA synthesis has been investigated in the halotolerant cyanobacterium *Aphanothece halophytica* under salt stress conditions [77]. Recently, Pope et al. [78] have disclosed that pentose phosphate pathway and shikimate pathways are inextricably linked to MAA biosynthesis in the cyanobacterium *Anabaena variabilis* ATCC 29413. The shikimate pathway is supposed to be a more predominate route for UV-induced MAA biosynthesis in *A. variabilis* ATCC 29413 [78]. Moreover, the genetic regulation of MAA synthesis in different organisms is still ambiguous and needs more extensive study to reveal the complete pathway.

Biosynthesis of MAAs is regulated by a number of environmental factors such as different wavelengths of PAR and UV radiation, desiccation, nutrients, and salt concentrations [7]. Synthesis or accumulation of MAAs is highly responsive to UV-B radiation; however, PAR and UV-A radiation has also been found to increase the synthesis of some MAAs to a certain extent [79,80]. PAR-induced synthesis of MAAs was shown in a marine macroalga *Chondrus crispus* [81] and in the dinoflagellate *Alexandrium excavatum* [57]. Synthesis of some MAAs such as porphyra- 334, palythine, and asterina-330 was stimulated under blue light, while shinorine was found to accumulate under white, green, yellow, and red light in a red alga *Porphyra leucosticta* [82]. UV-induced synthesis of MAAs (Fig. 2.4) has been observed in several species of cyanobacteria [20,21,83] and eukaryotic algae [30,84]. The UV-A radiation

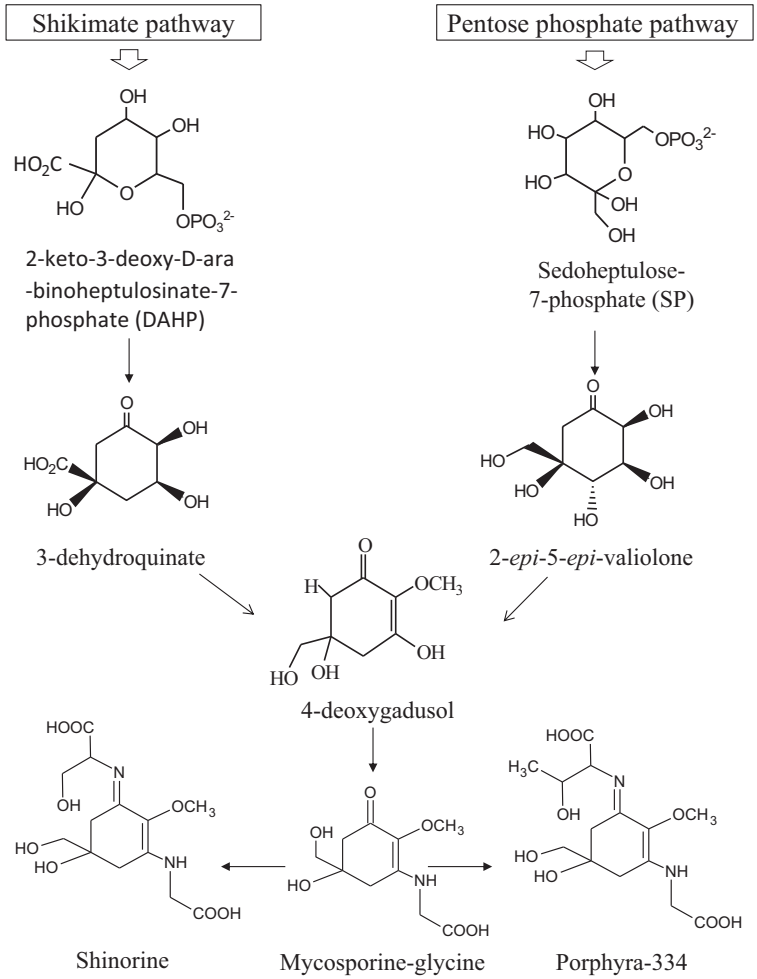


FIGURE 2.3 A proposed pathway of MAAs biosynthesis.

as well as availability of certain nutrients was found to affect the accumulation of some MAAs in a dinoflagellate *Gymnodinium catenatum* [62]. The bloom-forming prymnesiophyte *Phaeocystis pouchetii* was found to produce UV-absorbing compounds in response to exposure under UV-B radiation [67]. A circadian induction of MAAs was observed in the cyanobacteria *Scytonema* sp. HKAR-3 [80] and *Fischerella muscicola* TISTR8215 [83].

A significant decrease in MAAs synthesis was observed in the marine dinoflagellates *Akashiwo sanguinea* (syn. *Gymnodinium sanguineum*) and *Gymnodinium cf. instriatum* growing under nitrogen-limited condition [85]. Increased content of MAAs was observed in *Porphyra* spp. growing under ammonium-rich medium [86,87]. The cyanobacterium *A. variabilis* PCC 7937 growing under sulfur deficiency showed the bioconversion of a primary MAA shinorine into a secondary MAA palythine-serine [88]. MAAs were also shown to respond to elevated osmotic stress in some cyanobacteria [89,90]. Portwich and Garcia-Pichel [91] have reported

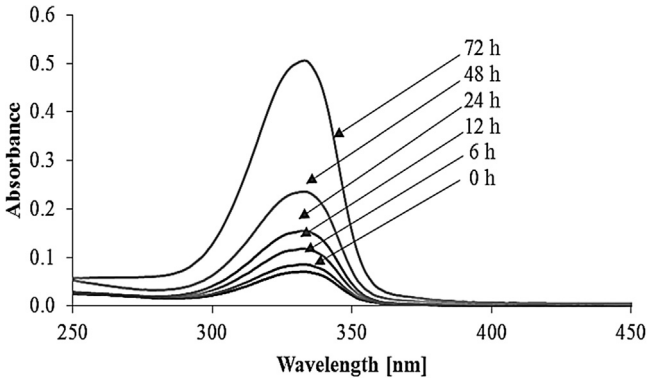


FIGURE 2.4 Induction of shinorine after different durations of UV-B irradiation (under 295 cut-off filter) in the cyanobacterium *Gloeocapsa* sp. [21].

the induction of the synthesis of MAA under salt stress without PAR or UV radiation in the cyanobacterium *Chlorogloeopsis* sp. PCC 6912. Moreover, several cyanobacteria do not synthesize or accumulate sufficient MAAs for protection against harmful UV radiation [92,93]; hence the role of MAAs other than photoprotection cannot be ignored. Overall, the mechanisms behind the environmentally regulated synthesis or accumulation of MAAs have not been elucidated.

5. SCYTONEMIN

Scy is a small, yellow-brown colored, lipophilic, dimeric compound exclusively produced by some species of cyanobacteria [9]. It is a highly polar pigment molecule accumulated in the extracellular sheath of extremophilic cyanobacteria (Fig. 2.5A). The UV-absorption maximum of purified Scy is 386; however, it also absorbs significantly at 252 ± 2, 278 ± 2, and 300 ± 2 nm (Fig. 2.5B) [8,20]. In general, Scy exists in oxidized (green) or reduced (red) form (Fig. 2.5B) [20,94]. However, some other forms of Scy have also been reported [9].

The genes responsible for Scy synthesis have been identified in some cyanobacteria [95,96]. The biosynthesis of Scy is supposed to be regulated by a cluster of 18 genes (ORFs: NpR1276–NpR1259) [95]. Moreover, a total eight genes have been identified, which are involved in the biosynthesis of tryptophan and tyrosine, while the function of other genes do not show any significant homology with functionally characterized proteins (Fig. 2.6) [7,95]. Some genetic variations was observed in the genome clusters of different cyanobacteria, but majority of the Scy-synthesizing genes showed high degree of amino acid sequence similarity [95], indicating that the Scy biosynthesis in cyanobacteria is a highly conserved process.

The biosynthesis of Scy is greatly affected by a number of environmental factors. The expression of Scy gene cluster was observed in the cyanobacteria exposed under UV-A radiation [95]. Induction of the synthesis of Scy was observed in the cyanobacterium *Chroococcidiopsis* sp. [97] and *Scytonema* sp. R77DM [98] under increased temperature and oxidative stress conditions. An increase in Scy synthesis was shown in the cyanobacterium *Nostoc punctiforme* PCC 73102 grown under nitrogen-limited condition [99]. Salinity-induced synthesis of

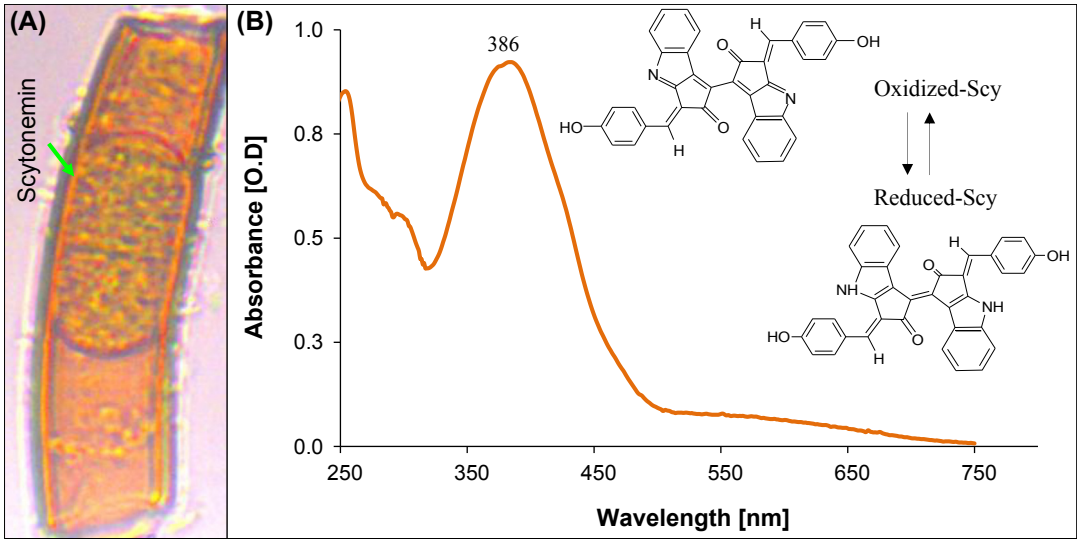


FIGURE 2.5 Synthesis of scytonemin in the extracellular sheath of *Lyngbya* sp. (A) having the UV-absorption maximum at 386 nm (B) [20].

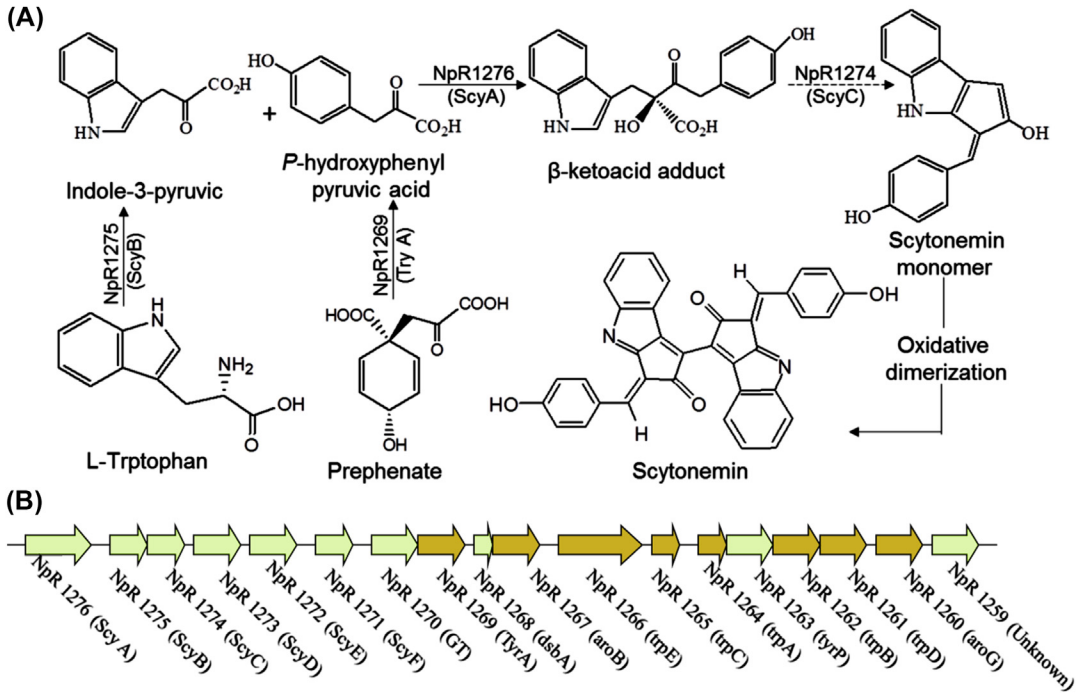


FIGURE 2.6 A generalized pathway of scytonemin biosynthesis (A) and genes associated with its biosynthesis (B) in the cyanobacterium *Nostoc punctiforme* [7,95].

Scy was also observed in the cyanobacterium *Lyngbya aestuarii* [100]. It is a highly stable compound under different abiotic stressors such as UV, heat, and oxidation stresses [98].

6. BIO-FUNCTIONALITIES OF MAAs AND SCYTONEMIN

MAAs exhibit strong UV absorption maxima (310−362 nm), high molar extinction coefficients (e = 28,100−50,000 M^{-1} cm^{-1}), UV-induction, photostability, and resistance to several abiotic stressors such as heat, UV radiations, pH, oxidizing agents, and different organic solvents [21,22,101−103], and play an important role in photoprotection of organisms residing under intense solar light. Biosynthesis or accumulation of MAAs in diverse organisms in response to UV-A/B radiations strongly favors their role in diminishing the harmful effects of short wavelength UV radiation. MAAs can dissipate absorbed radiation efficiently as heat without producing ROS [104]. It has been proposed that MAAs provide protection from UV radiation not only to their producers, but also to primary and secondary consumers through the food chain [105]. Recently, Rastogi and Incharoensakdi [30] reported the UV protective function of 324 nm-MAA and 322 nm-MAA isolated from a green microalga, *Tetraspora* sp. The MAAs such as porphyra-334 and M-gly were found to protect eggs of the sea hare *Aplysia dactylomela* from UV radiation [106]. MAAs can prevent three of every ten photons from striking cytoplasmic targets in cyanobacteria [92]. The production of MAAs in microalgae and various other organisms may serve as passive defense mechanisms that allow them to capture photons preventing their interaction with key cellular machinery, such as proteins and DNA. Moreover, the exact biological functions of different MAAs synthesizing in diverse taxonomic groups is still ambiguous.

MAAs can detoxify the harmful effects of UV radiation with their great efficacy as strong antioxidants. Some MAAs such as porphyra-334 and shinorine has great antioxidant potential [107−110]. The MAA M-gly has also been found to protect the biological systems against photodynamic damage by quenching single oxygen [111]. The G-MAAs isolated from *Nostoc commune* showed efficient free radical scavenging activity [14]. A mixture of MAAs such as shinorine + M-307 and palythine + asterina + M-312 isolated from *Gloeocapsa* sp. [21] and *Lyngbya* sp. [20], respectively was found to act as strong free radical scavenger (Fig. 2.7).

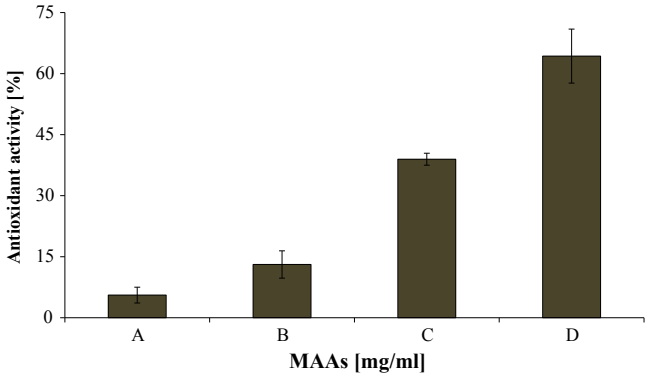


FIGURE 2.7 DPPH free radical scavenging activity of MAAs (shinorine + M-307) isolated from *Gloeocapsa* sp. CU2556 [0.2 (A), 0.4 (B), 0.83 (C) and 1.6 (D) mg/mL [21].

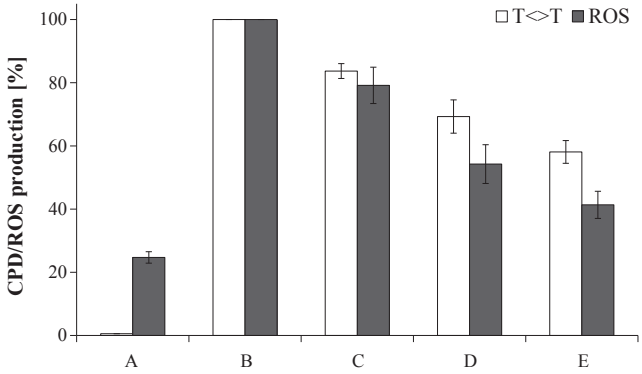


FIGURE 2.8 Reduction in ROS generation and thymine dimer formation in *Scytonema* R77DM under UV-induced oxidative stress. (A, control; B, UV-irradiated; C, UV + Scytonemin; D, UV + ascorbic acid; and E, UV + Scytonemin + ascorbic acid) [98].

MAAs can maintain the genome integrity by minimizing the UV-induced formation of thymine dimers [112,113], the most genotoxic and cytotoxic DNA lesions within the cell [4]. MAAs may be considered as broad-spectrum UV absorbers/protectors and can be used as potential ingredients in cosmetics and other cosmeceutical industries. MAAs can prevent the occurrence of UV-induced skin cancer [114]. The MAAs (porphyra-334 + shinorine) isolated from red algae was found to protect against premature skin aging [115]. Oyamada et al. [116] have reported the role of MAAs in protecting the human fibroblast cells from UV-induced cell death. Some synthetic analogues of MAAs, such as tetrahydropyridine derivatives, have been developed for commercial application as suncare products [109,117,118].

Like MAAs, Scy also play an important role in photoprotection. The UV screening function of Scy is well established in cyanobacteria [9,119,120]. It can prevent up to 90% of the incident solar UV radiation from reaching the potential targets of a cell [94]. Scy can also minimize the production of cellular ROS and thymine dimers [8,98] (Fig. 2.8). Scy has great pharmacological potential with interesting anti-inflammatory and anti-proliferative activities [121]. Moreover, due to potent UV protecting capacity [7,122–124], MAAs and Scy can be used in the field of biotechnology and biomedical research [9,125] for the commercial development of suncare and other cosmeceuticals products.

7. CONCLUSION AND FUTURE PERSPECTIVES

The algae including cyanobacteria are capable of protecting themselves from harmful solar UV radiation by synthesizing the UV-absorbing biomolecules, such as the MAAs and Scy. Although a number of MAAs has been reported from diverse organisms, several other UV photoprotectants remain to be explored from the nature. The complete pathway for genetic and ecological regulation of MAAs and Scy biosynthesis is still obscure. These compounds are highly stable against different physicochemical factors and act as strong antioxidants. MAAs and Scy are able to prevent cellular as well as genomic damage resulting from UV-induced ROS. It has been shown that the biosynthesis of these photoprotectants greatly induced under UV exposure and can be established as the key biomarkers of UV-stressed

environment. Natural photoprotectants may be of great interest in pharmaceutical and other biotechnological research for the development of novel sunscreens and other commercial products against ROS-associated disorders.

Acknowledgments

Rajesh P. Rastogi is thankful to the University Grant Commission (UGC), New Delhi, India, for financial support in the form of Dr. D.S. Kothari Postdoctoral grant.

References

[1] C.E. Williamson, R.G. Zepp, R.M. Lucas, S. Madronich, A.T. Austin, C.L. Ballare, M. Norval, B. Sulzberger, et al., Solar ultraviolet radiation in a changing climate, Nat. Clim. Chang. 4 (2014) 434–441.
[2] R.P. Rastogi, R.P. Sinha, S.H. Moh, T.K. Lee, S. Kottuparambil, Y.-J. Kim, J.-S. Rhee, E.-M. Choi, M.T. Brown, D.-P. Häder, T. Han, Ultraviolet radiation and cyanobacteria, J. Photochem. Photobiol. B 141 (2014) 154–169.
[3] D.-P. Häder, C.E. Williamson, S.A. Wängberg, M. Rautio, K.C. Rose, K. Gao, E.W. Helbling, R.P. Sinha, et al., Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors, Photochem. Photobiol. Sci. 14 (2015) 108–126.
[4] R.P. Rastogi, Richa, A. Kumar, M.B. Tyagi, R.P. Sinha, Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair, J. Nucleic Acids 2010 (2010) 592980.
[5] R.P. Rastogi, S.P. Singh, D.-P. Häder, R.P. Sinha, Ultraviolet-B-induced DNA damage and photorepair in the cyanobacterium *Anabaena variabilis* PCC 7937, Environ. Exp. Bot. 74 (2011) 280–288.
[6] R.P. Rastogi, A. Incharoensakdi, D. Madamwar, Responses of a rice field cyanobacterium *Anabaena siamensis* TISTR-8012 upon exposure to PAR and UV radiation, J. Plant Physiol. 171 (2014) 1545–1553.
[7] R.P. Rastogi, Richa, R.P. Sinha, S.P. Singh, D.-P. Häder, Photoprotective compounds from marine organisms, J. Ind. Microbiol. Biotechnol. 37 (2010a) 537–558.
[8] R.P. Rastogi, R.P. Sinha, A. Incharoensakdi, Partial characterization, UV-induction and photoprotective function of sunscreen pigment, scytonemin from *Rivularia* sp. HKAR-4, Chemosphere 93 (2013) 1874–1878.
[9] R.P. Rastogi, R.R. Sonani, D. Madamwar, Cyanobacterial sunscreen scytonemin: role in photoprotection and biomedical research, Appl. Biochem. Biotechnol. 176 (2015) 1551–1563.
[10] R.P. Rastogi, R.R. Sonani, D. Madamwar, A. Incharoensakdi, Characterization and antioxidant functions of mycosporine-like amino acids in the cyanobacterium *Nostoc* sp. R76DM, Algal Res. 16 (2016) 110–118.
[11] M. Schlumpf, B. Cotton, M. Conscience, V. Haller, B. Steinmann, W. Lichtensteiger, In vitro and in vivo estrogenicity of UV screens, Environ. Health Perspect. 109 (2001) 239–244.
[12] J.J. Wu Won, B.E. Chalker, J.A. Rideout, Two new UV absorbing compounds from *Stylophora pistillata*: sulfate esters of mycosporine-like amino acids, Tetrahedron Lett. 38 (1997) 2525–2526.
[13] G.A. Böhm, W. Pfleiderer, P. Böger, S. Scherer, Structure of a novel oligosaccharide-mycosporine-amino acid ultraviolet A/B sunscreen pigment from the terrestrial cyanobacterium Nostoc commune, J. Biol. Chem. 270 (1995) 8536–8539.
[14] K. Matsui, E. Nazifi, S. Kunita, N. Wada, S. Matsugo, T. Sakamoto, Novel glycosylated mycosporine-like amino acids with radical scavenging activity from the cyanobacterium Nostoc commune, J. Photochem. Photobiol. B 105 (2011) 81–89.
[15] E. Nazifi, N. Wada, M. Yamaba, T. Asano, T. Nishiuchi, S. Matsugo, T. Sakamoto, Glycosylated porphyra-334 and palythine-threonine from the terrestrial cyanobacterium Nostoc commune, Mar. Drugs 11 (2013) 3124–3154.
[16] E. Nazifi, N. Wada, T. Asano, T. Nishiuchi, Y. Iwamuro, S. Chinaka, S. Matsugo, T. Sakamoto, Characterization of the chemical diversity of glycosylated mycosporine-like amino acids in the terrestrial cyanobacterium Nostoc commune, J. Photochem. Photobiol. B 142 (2015) 154–168.
[17] R.P. Sinha, S.P. Singh, D.-P. Häder, Database on mycosporines and mycosporine-like amino acids (MAAs) in fungi, cyanobacteria, macroalgae, phytoplankton and animals, J. Photochem. Photobiol. B 89 (2007) 29–35.
[18] K. Shibata, Pigments and a UV-absorbing substance in coral and a blue-green alga living on the Great Barrier Reef, Plant Cell Physiol. 10 (1969) 325–335.

[19] R.P. Rastogi, S. Kumari, Richa, T. Han, R.P. Sinha, Molecular characterization of hot spring cyanobacteria and evaluation of their photoprotective compounds, Can. J. Microbiol. 58 (2012) 719–727.
[20] R.P. Rastogi, A. Incharoensakdi, Characterization of UV-screening compounds, mycosporine-like amino acids, and scytonemin in the cyanobacterium *Lyngbya* sp. CU2555, FEMS Microbiol. Ecol. 87 (2014c) 244–256.
[21] R.P. Rastogi, A. Incharoensakdi, UV radiation-induced biosynthesis, stability and antioxidant activity of mycosporine-like amino acids (MAAs) in a unicellular cyanobacterium *Gloeocapsa* sp. CU2556, J. Photochem. Photobiol. B 130 (2014a) 287–292.
[22] R.P. Rastogi, A. Incharoensakdi, Analysis of UV-absorbing photoprotectant mycosporine-like amino acid (MAA) in the cyanobacterium *Arthrospira* sp. CU2556, Photochem. Photobiol. Sci. 13 (2014b) 1016–1024.
[23] R.P. Sinha, N.K. Ambasht, J.P. Sinha, M. Klisch, D.-P. Häder, UVB-induced synthesis of mycosporine-like amino acids in three strains of *Nodularia* (cyanobacteria), J. Photochem. Photobiol. B 71 (2003) 51–58.
[24] A. Oren, Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria, Geomicrobiol. J. 14 (1997) 231–240.
[25] F. Garcia-Pichel, U. Nübel, G. Muyzer, The phylogeny of unicellular, extremely halotolerant cyanobacteria, Arch. Microbiol. 169 (1998) 469–482.
[26] F. Xiong, J. Kopecky, L. Nedbal, The occurrence of UV-B absorbing mycosporine-like amino acids in freshwater and terrestrial microalgae (Chlorophyta), Aquat. Bot. 63 (1999) 37–49.
[27] A. Gröniger, R.P. Sinha, M. Klisch, D.-P. Häder, Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – a database, J. Photochem. Photobiol. B 58 (2000) 115–122.
[28] C.A. Llewellyn, R.L. Airs, Distribution and abundance of MAAs in 33 species of microalgae across 13 classes reported in several microalgae, Mar. Drugs 8 (2010) 1273–1291.
[29] M.O. Carignan, J.I. Carreto, Characterization of mycosporine-serine-glycine methyl ester, a major mycosporine-like amino acid from dinoflagellates: a mass spectrometry study, J. Phycol. 49 (2013) 680–688.
[30] R.P. Rastogi, A. Incharoensakdi, UV radiation-induced accumulation of photoprotective compounds in the green alga *Tetraspora* sp. CU2551, Plant Physiol. Biochem. 70 (2013) 7–13.
[31] H. Nakamura, J. Kobayashi, Y. Hirata, Separation of mycosporinelike amino acids in marine organisms using reverse-phase high performance liquid chromatography, J. Chromatogr. 250 (1982) 113–118.
[32] D. Karentz, F.S. McEuen, M.C. Land, W.C. Dunlap, A survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure, Mar. Biol. 108 (1991) 157–166.
[33] U. Karsten, T. Sawall, C. Wiencke, A survey of the distribution of UV-absorbing substances in tropical macroalgae, Phycol. Res. 46 (1998a) 271–279.
[34] U. Karsten, T. Sawall, D. Hanelt, K. Bischof, F.L. Figueroa, A. Flores-Moya, C. Wiencke, An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions, Bot. Mar. 41 (1998b) 443–453.
[35] K. Hoyer, U. Karsten, C. Wiencke, Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions, Mar. Biol. 141 (2002) 619–627.
[36] A. Gröniger, D.-P. Häder, Induction of the synthesis of an UV absorbing substance in the green alga Prasiola stipitata, J. Photochem. Photobiol. B 66 (2002) 54–59.
[37] U. Karsten, K. Escoubeyrou, F. Charles, The effect of redissolution solvents and HPLC columns on the analysis of mycosporine-like amino acids in the eulittoral macroalgae *Prasiola crispa* and Porphyra umbilicalis, Helgol. Mar. Res. 63 (2009) 231–238.
[38] U. Karsten, T. Friedl, R. Schumann, K. Hoyer, S. Lembcke, Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta), J. Phycol. 41 (2005) 557–566.
[39] S.W. Jeffrey, H.S. MacTavish, W.C. Dunlap, M. Vesk, K. Groenewoud, Occurrence of UVA- and UVB absorbing compounds in 152 species (206 strains) of marine microalgae, Mar. Ecol. Prog. Ser. 189 (1999) 35–51.
[40] S.-S. Suh, J. Hwang, M. Park, H.H. Seo, H.-S. Kim, J.H. Lee, S.H. Moh, T.-K. Lee, Anti-inflammation activities of mycosporine-like amino acids (MAAs) in response to UV radiation suggest potential anti-skin aging activity, Mar. Drugs 12 (2014) 5174–5187.
[41] F.D.L. Coba, J. Aguilera, F.L. Figueroa, M.V. de Gàlvez, E. Herrera, Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen, J. Appl. Phycol. 21 (2009) 161–169.
[42] Y.V. Yuan, N.D. Westcott, C. Hu, D.D. Kitts, Mycosporine-like amino acid composition of the edible red alga, *Palmaria palmata* (dulse) harvested from the west and east coasts of Grand Manan Island, New Brunswick, Food Chem. 112 (2009) 321–328.

[43] K. Hoyer, U. Karsten, T. Sawall, C. Wiencke, Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages, Mar. Ecol. Prog. Ser. 211 (2001) 117–129.
[44] P. Huovinen, I. Gómez, F.L. Figueroa, N. Ulloa, V. Morales, C. Lovengreen, Ultraviolet-absorbing mycosporine-like amino acids in red macroalgae from Chile, Bot. Mar. 47 (2004) 21–29.
[45] L.-F. Chuang, H.-N. Chou, P.-J. Sung, Porphyra-334 isolated from the marine algae *Bangia atropurpurea*: conformational performance for energy conversion, Mar. Drugs 12 (2014) 4732–4740.
[46] A. Hartmann, K. Becker, U. Karsten, D. Remias, M. Ganzera, Analysis of mycosporine-like amino acids in selected algae and cyanobacteria by hydrophilic interaction liquid chromatography and a novel MAA from the red alga Catenella repens, Mar. Drugs 13 (2015) 6291–6305.
[47] M.-H. Lee, Y.-K. Kim, N.Y. Yoon, K.B. Shim, N.M. Aminina, I.A. Kadnikova, C.-W. Lim, Study on UV absorption materials derived from red algae *Gloiopeltis fucatas* and *Mazzaella* sp. in Russia, Fish Aquat. Sci. 15 (2012) 361–363.
[48] A.T. Davidson, D. Brarnich, H.J. Marchant, A. McMinn, Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms, Mar. Biol. 119 (1994) 507–515.
[49] L. Riegger, D. Robinson, Photoinduction of UV-absorbing compounds in antarctic diatoms and Phaeocystis Antarctica, Mar. Ecol. Prog. Ser. 160 (1997) 13–25.
[50] G.A. Ferreyra, I. Schloss, S. Demers, P.J. Neale, Phytoplankton responses to natural ultraviolet irradiance during early spring in the Weddell-Scotia Confluence: an experimental approach, Antarct. J. US 29 (1994) 268–270.
[51] H. Wu, K. Gao, H. Wu, Responses of a marine red tide alga *Skeletonema costatum* (Bacillariophyceae) to long-term UV radiation exposures, J. Photochem. Photobiol. B 94 (2009) 82–86.
[52] A.E. Ingalls, K. Whitehead, M.C. Bridoux, Tinted windows: the presence of the UV absorbing compounds called mycosporine-like amino acids embedded in the frustules of marine diatoms, Geochim. Cosmochim. Acta 74 (2010) 104–115.
[53] E.W. Helbling, B.E. Chalker, W.C. Dunlap, O. Holm-Hansen, V.E. Villafañe, Photoacclimation of Antarctic diatoms to solar ultraviolet radiation, J. Exp. Mar. Biol. Ecol. 204 (1996) 85–101.
[54] S.-Y. Ha, Y.-N. Kim, M.-O. Park, S.-H. Kang, H.-C. Kim, K.-H. Shin, Production of mycosporine-like amino acids of in situ phytoplankton community in Kongsfjorden, Svalbard, Arctic, J. Photochem. Photobiol. B 114 (2012) 1–14.
[55] S. La, J.-O. Min, K.-H. Chung, S.-H. Kang, K.-H. Shin, Photoprotective function of mycosporine-like amino acids in a bipolar diatom (*Porosira glacialis*): evidence from ultraviolet radiation and stable isotope probing, Diatom Res. 29 (2014) 399–409.
[56] J. Piiparinen, S. Enberg, J.-M. Rintala, R. Sommaruga, M. Majaneva, R. Autioa, A.V. Vähätaloe, The contribution of mycosporine-like amino acids, chromophoric dissolved organic matter and particles to the UV protection of sea-ice organisms in the Baltic Sea, Photochem. Photobiol. Sci. 14 (2015) 1025–1038.
[57] J.I. Carreto, M.O. Carignan, G. Daleo, S.G. De Marco, Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J. Plankton Res. 12 (1990) 909–921.
[58] M.P. Lesser, Acclimation of phytoplankton to UV-B radiation: oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate Prorocentrum micans, Mar. Ecol. Prog. Ser. 132 (1996) 287–297.
[59] R.P. Sinha, M. Klisch, A. Gröniger, D.-P. Häder, Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae, J. Photochem. Photobiol. B 47 (1998) 83–94.
[60] G. Hannach, A.C. Sigleo, Photoinduction of UV-absorbing compounds in six species of marine phytoplankton, Mar. Ecol. Prog. Ser. 174 (1998) 207–222.
[61] M. Klisch, R.P. Sinha, P.E. Richter, D.-P. Häder, Mycosporine-like amino acids (MAAs) protect against UV damage in *Gyrodinium dorsum Kofoid*, J. Plant Physiol. 158 (2001) 1449–1454.
[62] P. Vale, Effects of light quality and nutrient availability on accumulation of mycosporine-like amino acids in Gymnodinium catenatum (Dinophycea), J. Photochem. Photobiol. B 143 (2015) 20–29.
[63] M. Vernet, A. Neori, F.T. Haxo, Spectral properties and photosynthetic action in red-tide populations of *Prorocentrum micans* and Gonyaulax polyedra, Mar. Biol. 103 (1989) 365–371.
[64] M. Vernet, K. Whitehead, Release of ultraviolet-absorbing compounds by the red-tide dinoflagellate Lingulodinium polyedra, Mar. Biol. 127 (1996) 35–44.
[65] P.J. Neale, A.T. Banaszak, C.R. Jarriel, Ultraviolet sunscreens in Gymnodinium sanguineum (dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis, J. Phycol. 34 (1998) 928–938.

[66] I. Laurion, S. Roy, Growth and photoprotection in three dinoflagellates (including two strains of *Alexandrium tamarense*) and one diatom exposed to four weeks of natural and enhanced UVB radiation, J. Phycol. 45 (2009) 16–33.
[67] H.J. Marchant, A.T. Davidson, G.J. Kelly, UV-B protecting compounds in the marine alga *Phaeocystis pouchetji* from Antarctica, Mar. Biol. 109 (1991) 391–395.
[68] M. Vernet, E.A. Brody, O. Holm-Hansen, B.G. Mitchell, The response of Antarctic phytoplankton to ultraviolet radiation: absorption, photosynthesis, and taxonomic composition, in: C.S. Weiler, P.A. Penhale (Eds.), Ultraviolet Radiation in Antarctica: Measurements and Biological Effects, vol. 62, Antarctic Research Series, 1994, pp. 143–158.
[69] T.A. Moisan, B.G. Mitchell, UV absorption by mycosporine-like amino acids in *Phaeocystis Antarctica* induced by photosynthetically available radiation, Mar. Biol. 138 (2001) 217–227.
[70] J. Favre-Bonvin, J. Bernillon, N. Salin, N. Arpin, Biosynthesis of mycosporines: mycosporine glutaminol in Trichothecium roseum, Phytochemistry 26 (1987) 2509–2514.
[71] A. Portwich, F. Garcia-Pichel, Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium *Chlorogloeopsis* sp. strain PCC 6912, Phycologia 42 (2003) 384–392.
[72] S.P. Singh, S. Kumari, R.P. Rastogi, K.L. Singh, R.P. Sinha, Mycosporine-like amino acids (MAAs): chemical structure, biosynthesis and significance as UV-absorbing/screening compounds, Indian J. Exp. Biol. 46 (2008) 7–17.
[73] J.M. Shick, S. Romaine-Lioud, C. Ferrier-Pagès, J.P. Gattuso, Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates, Limnol. Oceanogr. 44 (1999) 1667–1682.
[74] E.P. Balskus, C.T. Walsh, The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria, Science 329 (2010) 1653–1656.
[75] S.P. Singh, M. Klisch, R.P. Sinha, D.P. Häder, Genome mining of mycosporine-like amino acid (MAA) synthesizing and non-synthesizing cyanobacteria: a bioinformatics study, Genomics 95 (2010a) 120–128.
[76] Q. Gao, F. Garcia-Pichel, An ATP-grasp ligase involved in the last biosynthetic step of the iminomycosporine shinorine in Nostoc punctiforme ATCC 29133, J. Bacteriol. 193 (2011) 5923–5928.
[77] R. Waditee-Sirisattha, H. Kageyama, W. Sopun, Y. Tanaka, T. Takabe, Identification and upregulation of biosynthetic genes required for accumulation of mycosporine-2-glycine under salt stress conditions in the halotolerant cyanobacterium Aphanothece halophytica, Appl. Environ. Microbiol. 80 (2014) 1763–1769.
[78] M.A. Pope, E. Spence, V. Seralvo, R. Gacesa, S. Heidelberger, A.J. Weston, W.C. Dunlap, J.M. Shick, P.F. Long, O-Methyltransferase is shared between the pentose phosphate and shikimate pathways and is essential for mycosporine-like amino acid biosynthesis in Anabaena variabilis ATCC 29413, Chem. Bio. Chem. (2016), http://dx.doi.org/10.1002/cbic.201402516.
[79] R.P. Sinha, M. Klisch, E.W. Helbling, D.-P. Häder, Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation, J. Photochem. Photobiol. B 60 (2001) 129–135.
[80] R.P. Rastogi, Richa, S.P. Singh, D.-P. Häder, R.P. Sinha, Mycosporine-like amino acids profile and their activity under PAR and UVR in a hot-spring cyanobacterium *Scytonema* sp. HKAR-3, Aust. J. Bot. 58 (2010c) 286–293.
[81] U. Karsten, L.A. Franklin, K. Lüning, C. Wiencke, Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga Chondrus crispus (Rhodophyta), Planta 205 (1998d) 257–262.
[82] N. Korbee, F.L. Figueroa, F.J. Aguilera, Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red alga *Porphyra leucosticta* (Bangiales, Rhodophyta), J. Photochem. Photobiol. B 80 (2005a) 71–78.
[83] R.P. Rastogi, A. Incharoensakdi, Occurrence and induction of a ultraviolet-absorbing substance in the cyanobacterium *Fischerella muscicola* TISTR8215, Phycol. Res. 63 (2015) 51–55.
[84] R.P. Sinha, M. Klisch, A. Gröniger, D.-P. Häder, Mycosporine-like amino acids in the marine red alga *Gracilaria cornea*-effects of UV and heat, Environ. Exp. Bot. 43 (2000) 33–43.
[85] E. Litchman, P.J. Neale, A.T. Banaszak, Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: photoprotection and repair, Limnol. Oceanogr. 47 (2002) 86–94.
[86] N.K. Peinado, R.T.A. Díaz, F.L. Figueroa, E.W. Helbling, Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina, J. Phycol. 40 (2004) 248–259.

[87] N. Korbee, P. Huovinen, F.L. Figueroa, J. Aguilera, U. Karsten, Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like amino acids in two *Porphyra* species (Bangiales, Rhodophyta), Mar. Biol. 146 (2005b) 645–654.
[88] S.,P. Singh, M. Klisch, R.P. Sinha, D.-P. Häder, Sulfur deficiency changes mycosporine-like amino acid (MAA) composition of *Anabaena variabilis* PCC 7937: a possible role of sulfur in MAA bioconversion, Photochem. Photobiol. 86 (2010b) 862–870.
[89] S.,P. Singh, M. Klisch, R.P. Sinha, D.-P. Häder, Effects of abiotic stressors on synthesis of the mycosporine-like amino acid shinorine in the cyanobacterium *Anabaena variabilis* PCC 7937, Photochem. Photobiol. 84 (2008c) 1500–1505.
[90] A. Portwich, F. Garcia-Pichel, Ultraviolet and osmotic stress induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912, Arch. Microbiol. 172 (1999) 187–192.
[91] A. Portwich, F. Garcia-Pichel, A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912, Photochem. Photobiol. 71 (2000) 493–498.
[92] F. Garcia-Pichel, C.E. Wingard, R.W. Castenholz, Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp, Appl. Environ. Microbiol. 59 (1993) 170–176.
[93] C. Hu, G. Völler, R. Süßmuth, E. Dittmann, J.-C. Kehr, Functional assessment of mycosporine-like amino acids in *Microcystis aeruginosa* strain PCC 7806, Environ. Microbiol. 17 (2015) 1548–1559.
[94] F. Garcia-Pichel, R.W. Castenholz, Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment, J. Phycol. 27 (1991) 395–409.
[95] T. Soule, K. Palmer, Q. Gao, R.M. Potrafka, V. Stout, F. Garcia-Pichel, A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria, BMC Genomics 10 (2009) 336.
[96] E.P. Balskus, R.J. Case, C.T. Walsh, The biosynthesis of cyanobacterial sunscreen scytonemin in intertidal microbial mat communities, FEMS Microbiol. Ecol. 77 (2011) 322–332.
[97] J.G. Dillon, C.M. Tatsumi, P.G. Tandingan, R.W. Castenholz, Effect of environmental factors on the synthesis of scytonemin, a UV-screening pigment, in a cyanobacterium (Chroococcidiopsis sp.), Arch. Microbiol. 177 (2002) 322–331.
[98] R.P. Rastogi, R.R. Sonani, D. Madamwar, The high-energy radiation protectant extracellular sheath pigment scytonemin and its reduced counterpart in the cyanobacterium Scytonema sp. R77DM, Bioresour. Technol. 171 (2014) 396–400.
[99] E.D. Fleming, R.W. Castenholz, Effects of nitrogen source on the synthesis of the UV screening compound, scytonemin, in the cyanobacterium Nostoc punctiforme PCC 73102, FEMS Microbiol. Ecol. 63 (2008) 301–308.
[100] J. Rath, S. Mandal, S.P. Adhikary, Salinity induced synthesis of UV-screening compound scytonemin in the cyanobacterium Lyngbya aestuarii, J. Photochem. Photobiol. B 115 (2012) 5–8.
[101] A. Gröniger, D.-P. Häder, Stability of mycosporine-like amino acids, Rec. Res. Develop. Photochem. Photobiol. 4 (2000) 247–252.
[102] K. Whitehead, J.I. Hedges, Photodegradation and photosensitization of mycosporine-like amino acids, J. Photochem. Photobiol. B 80 (2005) 115–121.
[103] F.R. Conde, M.S. Churio, C.M. Previtali, Experimental study of the excited-state properties and photostability of the mycosporine-like amino acid palythine in aqueous solution, Photochem. Photobiol. Sci. 6 (2007) 669–674.
[104] F.R. Conde, M.S. Churio, C.M. Previtali, The photoprotector mechanism of mycosporine-like amino acids. excited-state properties and photostability of porphyra-334 in aqueous solution, J. Photochem. Photobiol. B 56 (2000) 139–144.
[105] E.W. Helbling, C.F. Menchi, V.E. Villafañe, Bioaccumulation and role of UV-absorbing compounds in two marine crustacean species from Patagonia, Argentina, Photochem. Photobiol. Sci. 1 (2002) 820–825.
[106] T.H. Carefoot, M. Harris, B.E. Taylor, D. Donovan, D. Karentz, Mycosporine-like amino acids: possible UV protection in eggs of the sea hare Aplysia dactylomela, Mar. Biol. 130 (1998) 389–396.
[107] R. Nakayama, Y. Tamura, H. Kikuzaki, N. Nakatani, Antioxidant effect of the constituents of susabinori (*Porphyra yezoensis*), JAOCS 76 (1999) 649–653.
[108] F.D.L. Coba, J. Aguilera, F.L. Figueroa, Use of Mycosporine-Type Amino Acid Porphyra-334 as an Antioxidant, Intl. Patent WO2007/026035 A2, 2007.
[109] F.D.L. Coba, J. Aguilera, F.L. Figueroa, Use of Mycosporine-Type Amino Acid Shinorine as an Antioxidant, Intl. Patent WO2007/026038 A2, 2007.
[110] A. Oren, N. Gunde-Cimerman, Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol. Lett. 269 (2007) 1–10.

[111] H.J. Suh, H.W. Lee, J. Jung, Mycosporine glycine protects biological systems against photodynamic damage by quenching single oxygen with a high efficiency, Photochem. Photobiol. 78 (2003) 109–113.
[112] T. Misonou, J. Saitoh, S. Oshiba, Y. Tokitomo, M. Maegawa, Y. Inoue, H. Hori, T. Sakurai, UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) block thymine dimer production, Mar. Biotechnol. 5 (2003) 194–200.
[113] R.P. Rastogi, UV-b-induced DNA Damage and Repair in Cyanobacteria (Ph.D. thesis), Banaras Hindu University, Varanasi, India, 2010.
[114] B.A. Drolet, M.J. Connor, Sunscreens and the prevention of ultraviolet radiation-induced skin cancer, J. Dermatol. Surg. Oncol. 18 (1992) 571–576.
[115] S. Daniel, S. Cornelia, Z. Fred, UV-A sunscreen from red algae for protection against premature skin aging, Cosmet. Toilet. Manuf. Worldw. 2004 (2004) 139–143.
[116] C. Oyamada, M. Kaneniwa, K. Ebitani, M. Murata, K. Ishihara, Mycosporine-like amino acids extracted from Scallop (*Patinopecten yessoensis*) ovaries: UV protection and growth stimulation activities on human cells, Mar. Biotechnol. 10 (2008) 141–150.
[117] G. Bird, N. Fitzmaurice, W.C. Dunlap, B.E. Chalker, W.M. Bandaranayake, Sunscreen Compositions and Compounds for Use Therein, International Patent Application PCT/AU87/00330, Publication No. WO 88/02251. Australian Patent 595075, ICI Australia Operations Pty Ltd. and Australian Institute of Marine Science, Townsville, 1987.
[118] P.J. Chalmers, N. Fitzmaurice, D.J. Rigg, S.H. Thang, G. Bird, UV Absorbing Compounds and Compositions, International Patent Application PCT/AU90/00078, Publication No. WO 90/09995. Australian Patent 653495, ICI Australia Operations Pty Ltd. and Australian Institute of Marine Science, Townsville, 1990.
[119] J.G. Dillon, R.W. Castenholz, Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life, J. Phycol. 35 (1999) 673–681.
[120] F. Garcia-Pichel, N.D. Sherry, R.W. Castenholz, Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium Chlorogloeopsis sp. Photochem. Photobiol. 56 (1992) 17–23.
[121] C.S. Stevenson, E.A. Capper, A.K. Roshak, B. Marquez, C. Eichman, J.R. Jackson, M. Mattern, W.H. Gerwick, R.S. Jacobs, L.A. Marshall, The identification and characterization of the marine natural product scytonemin as a novel antiproliferative pharmacophore, J. Pharmacol. Exp. Therapy 303 (2002) 858–866.
[122] R.P. Rastogi, R.P. Sinha, Biotechnological and industrial significance of cyanobacterial secondary metabolites, Biotechnol. Adv. 27 (2009) 521–539.
[123] R.P. Rastogi, R.R. Sonani, D. Madamwar, A. Incharoensadki, The potential of mycosporine-like amino acids as UV-sunscreens, in: S.H. Sharp (Ed.), Sunscreens: Properties, Role in Skin Cancer Prevention and Health Effects, NOVA Publisher, USA, 2015b, pp. 173–190.
[124] R.P. Rastogi, D. Madamwar, A. Incharoensakdi, Sun-screening bioactive compounds mycosporine-like amino acids in naturally occurring cyanobacterial biofilms: role in photoprotection, J. Appl. Microbiol. 119 (2015c) 753–762.
[125] S.P. Singh, S. Kumari, R.P. Rastogi, K.L. Singh, Richa, R.P. Sinha, Photoprotective and biotechnological potentials of cyanobacterial sheath pigment, scytonemin, Afr. J. Biotechnol. 9 (2010) 580–588.

CHAPTER

3

Genome- and Proteome-Wide Analyses for Targeted Manipulation and Enhancement of Bioproducts in Cyanobacteria

A. Hongsthong[1], *J. Senachak*[1], *H. Nakamoto*[2]

[1]National Center for Genetic Engineering and Biotechnology at King Mongkut's University of Technology Thonburi, Bangkok, Thailand; [2]Saitama University, Saitama, Japan

OUTLINE

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00003-5

1. INTRODUCTION

In 1979, Rippka et al. classified cyanobacteria into five sections based on their morphology and reproduction: Section I: unicellular cyanobacteria that reproduce by binary fission or by budding; Section II: unicellular cyanobacteria that reproduce by multiple fission; Section III: filamentous nonheterocyst cyanobacteria that divide in only one plane trichome helical; Section IV: filamentous heterocyst cyanobacteria that divide in only one plane; and Section V: filamentous heterocystous cyanobacteria that divide in more than one plane [1]. *Spirulina* is a cyanobacterium that is currently classified into the genus *Arthrospira* and belonged to Section III of the cyanobacteria (Fig. 3.1A and B).

Spirulina was discovered in the mid-1960s by Jean Leonard, a member of a French-Belgian expedition to Africa, leading to the start of extensive studies on its physiology, biochemistry, and mass cultivation techniques [2]. Cell contents of *Spirulina* are highlighted for nutraceutical potentials, for example, phycocyanin, an antioxidant; polyunsaturated fatty acids, for example, gamma-linolenic acid that is a precursor for prostaglandin biosynthesis; and sulfolipid, an antiviral agent (*Herpes simplex* type I). In addition, *Spirulina* is a good source of protein due to high amount of protein in a cell. Proteins contribute 65–70% of total dry weight of *Spirulina* cells [3], whereas other cyanobacteria contain protein at approximately 30–50% of dry weight [4]. Thus, this organism is an important source of value-added biochemicals and proteins. There are some compounds from *Spirulina* that are commercially available, e.g., proteins including phycocyanins, lipids, and carbohydrates [5]. Moreover, Spirulina biomass is well known to be used as a food supplement. These facts draw a lot of attention to this cyanobacterium, and thus large-scale mass cultivation has been developed in outdoor ponds or culture tanks (Fig. 3.1C) to supply for the market demand of the cell biomass used as a food supplement for humans and a source for value-added biochemicals (Fig. 3.1D). The availability of outdoor mass cultivation system of *Spirulina* causes a great advantage of this cyanobacterium over others whose outdoor mass cultivation is not developed. In contrast to other bacteria including cyanobacteria, *Spirulina* is tolerant to alkaline growth media [5], which allows us to cultivate *Spirulina* under nonsterilized conditions. However, the disadvantage of *Spirulina* is lack of gene transformation system, which causes difficulties for strain improvement by using gene manipulation.

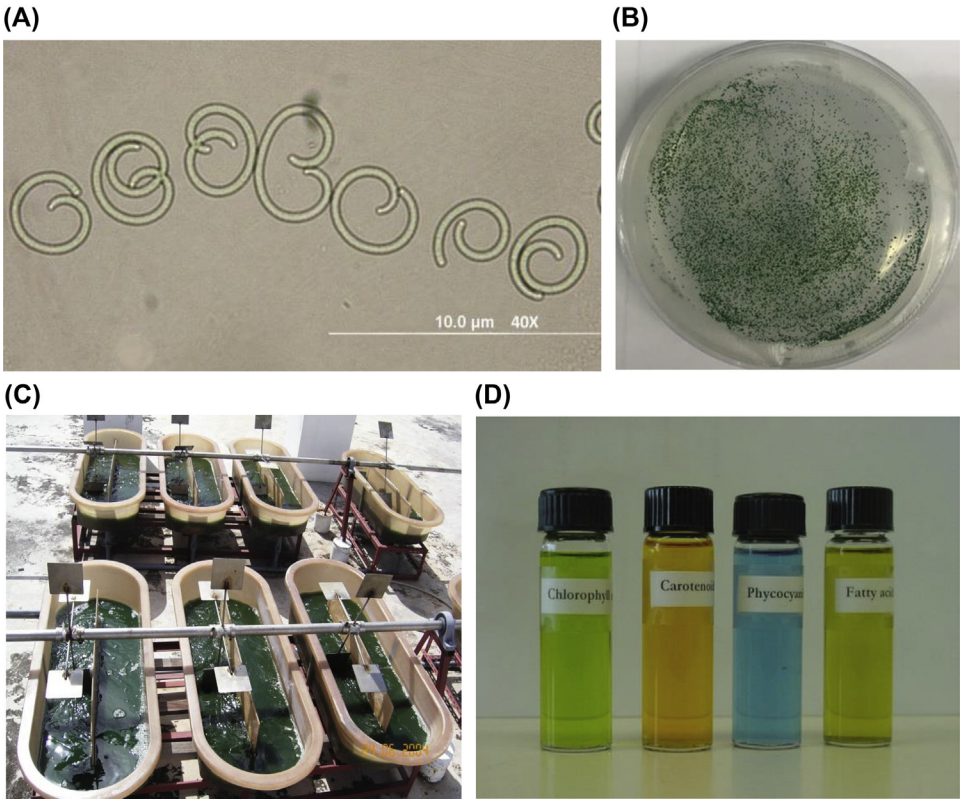


FIGURE 3.1 *Spirulina* (A) cells (strain C1) under microscope (B) strain C1 grown on a plate (C) culture grown in outdoor-pond and (D) bioproduct extracts; (from left to right) chlorophyll, carotenoid, phycocyanin, and fatty acids.

2. THE *SPIRULINA* GENOMES

Genomes of five *Spirulina* strains, *Arthrospira platensis* C1 (Fig. 3.2), *A. platensis* NIES-39, *A. platensis* Paraca, *A. maxima* CS-328, and *Arthrospira* sp. 8005 have been sequenced [6] and the statistical comparison of the five genomes (Table 3.1) shows that *A. platensis* NIES-39 has the biggest genome size of 6,788,435 bp, whereas the *A. platensis* C1 genome size is 6,089,210 bp, taking third place in the size [7].

Similar GC-content, 43.55–44.76% is found among the strains. While the genome size of *A. platensis* C1 is ranked in the third place among the five *Arthrospira* genomes, it has the largest number of protein coding genes with function prediction. Moreover, it is ranked in the second for the number of protein coding genes with enzyme function, and genes connected to KEGG-pathway and KEGG–orthology (Table 3.1). A distinct property of the strain C1 (*A. platensis* C1) is nongliding property that enables single colonies formation (Fig. 3.1B). Therefore, it makes them suitable to use as a laboratory strain for physiological and molecular-level studies including proteome analyses.

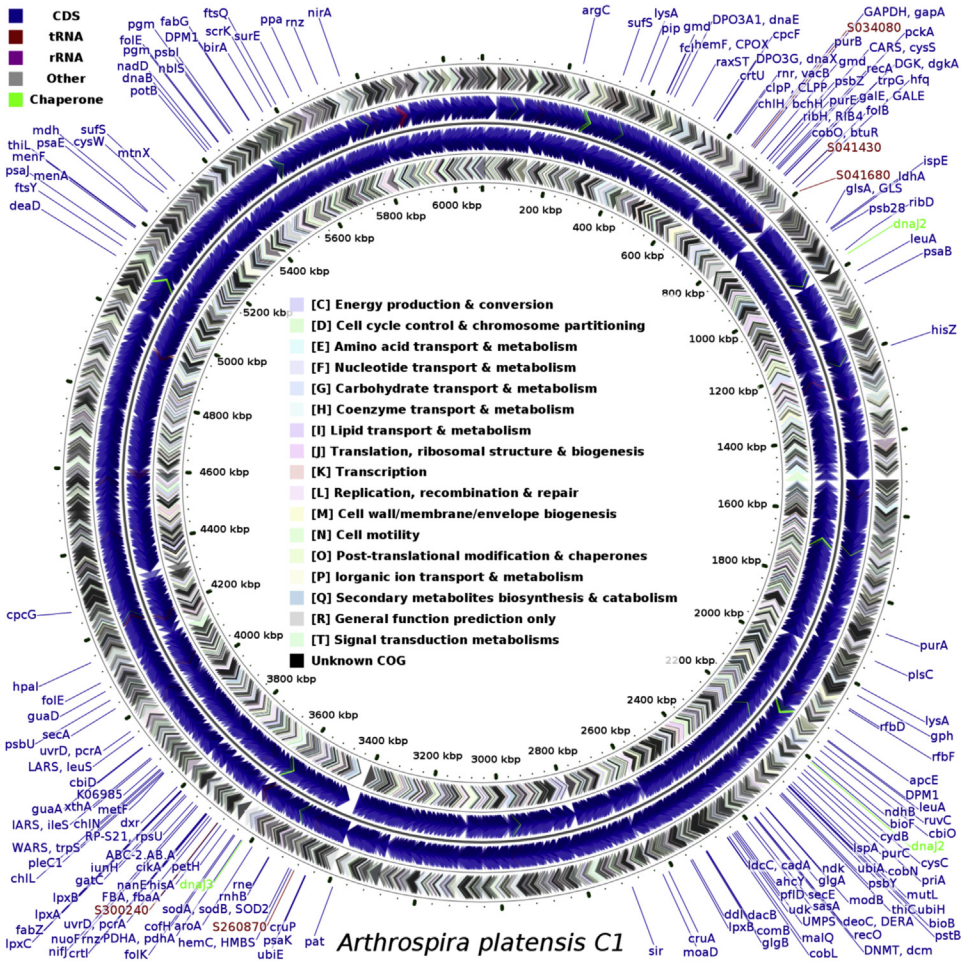


FIGURE 3.2 Circular genome map of *Spirulina* with locations of genes mentioned in the review. Note: COG category of proteins is presented in different colors shown in the middle of the circular genome.

3. TRANSCRIPTIONAL REGULATION OF GENES INVOLVED IN PRODUCTION OF VALUE-ADDED COMPOUNDS

In order to enhance the production of beneficial products by a microorganism, it is essential to know how the production is controlled in vivo. The gene regulation of the desaturation process of fatty acids in *Spirulina* has been studied to gain knowledge that would lead to an increase in the GLA (C18:3$\Delta^{9,12,6}$) production. GLA, a precursor for prostaglandin biosynthesis, is an essential unsaturated fatty acid for human and its blood-cholesterol reducing property has been detected [3]. According to the previous study, at low temperature (22°C), the cellular GLA level is increased by approximately 30% compared to its level in cells grown at the optimal growth temperature (35°C) [8,9]. The Δ^9, Δ^{12}, and Δ^6 desaturases

TABLE 3.1 Statistical Comparison of Five *Arthrospira* Genomes

Genome	*A. platensis C1*	*A. platensis NIES-39*	*A. maxima CS-328*	*A. platensis paraca*	*A.* sp. *PCC 8005*
Genome size (bp)	6,089,210	6,788,435	6,003,314	4,997,563	6,145,553
Number of scaffolds	1	1	129	1820	119
G + C content (%)	43.55	43.65	44.76	44.30	44.72
Protein coding genes	6108	6630	5690	5370	5675
RNA genes	45	46	40	31	43
tRNA genes	39	40	36	28	37
rRNA genes	6	6	4	3	6
5S	2	2	2	0	2
16S	2	2	1	1	1
23S	2	2	1	2	3
Protein coding genes with function prediction	3759	2589	3329	3052	3050
Protein coding genes with enzyme function	886	908	866	822	867
Protein coding genes connected to KEGG pathways	949	987	917	910	942
Protein coding genes connected to KEGG orthology (KO)	1609	1686	1557	1507	1603
Protein coding genes with COG	2433	2428	2433	2090	2461
Protein coding genes with Pfam	3832	4005	3939	3705	3871
Protein coding genes with TIGRfam	1189	1223	1168	1192	1211

The data were obtained from JGI Website (IMG: the integrated microbial genomes database and comparative analysis system (7)) as of December 2015.

encoded by the *desC*, *desA*, and *desD* genes, respectively, are responsible for the desaturation process. Thus, the regulation of the rate-limiting step catalyzed by Δ^6 desaturase was elucidated by using heterologous hosts, *Escherichia coli* and *Saccharomyces cerevisiae* [10,11]. The results obtained from the temperature response analyses of the 5′ upstream region of *desD* gene showed that the regulatory DNA elements and the corresponding regulatory binding proteins are distinct for each particular stress condition. In response to temperature downshift, the "AT-rich inverted repeat" (−192 to −164) served as a target-binding site for a transcriptional regulator in GntR family [12], whereas under high-temperature condition at 40°C, the AT-rich region (−98 to −80) serves as a binding site for its transcriptional regulator [13]. Moreover, the analysis of high-temperature−responsive DNA-binding protein complex by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed the presence of HtpG (Hsp90), GroEL (Hsp60 or chaperonin) and various protein kinases in the complex

[14]. These results provide evidence that molecular chaperones such as HtpG and GroEL are involved in transcriptional regulatory networks in cyanobacteria. Whereas thioredoxin (trx) was detected in the low-temperature–responsive protein complex and it was shown to play a critical role as a reducing agent that inactivates the *desD* repressor, GntR, consequently, the *desD* gene expression is induced after low-temperature exposure [14].

Moreover, the *desD* gene manipulation was carried out in heterologous hosts, *E. coli* and *S. cerevisiae*, to explore the possibility of the enhancement of the GLA production [10,11]. The data demonstrated the role of histidine residue 313 (His313) in the regioselectivity of the enzyme; the site-directed mutagenesis of this residue caused the change in the double bond position from Δ^6 to Δ^{15} [15]. In addition, the immediate electron donor ferredoxin, and intermediate electron donors, NADPH and $FADH_2$ were found to play an important role in the GLA biosynthesis in the two heterologous hosts [10,11]. Thus the classical molecular biology approaches and the *Spirulina* genome sequencing mentioned above lay the foundation for OMICS studies, which will be discussed further.

4. PROTEOME ANALYSIS

4.1 *Spirulina* Proteome

Proteome coverage of the three subcellular fractions of *A. platensis* C1 grown under optimal temperature, 35°C was carried out by using gel-based [two-dimensional differential gel electrophoresis (2D-DIGE)/matrix-assisted laser desorption ionization time of flight (MALDI-TOF)] and nongel based (LC-MS/MS) techniques. By using the two techniques, the degree of proteome coverage would be higher than using single technique because the disadvantages of one technique can be complemented by the other. For example, the proteins with pI less than 3 and higher than 10 will not be detected by 2D-DIGE due to the limitation of the range of pH gradients in the first dimension; however, this limitation is overcomed by using LC-MS/MS. The data were collected in the *A. platensis* C1 proteome database and details on the identified protein spots can be visualized individually via a bioinformatics tool SpirPro (http://spirpro.sbi.kmutt.ac.th, see Section 4.4). The number of proteins identified in strain C1 at the optimal growth temperature by using proteomics techniques is 4804, which is calculated to be 79% of the total open reading frames; however, 55% of them were hypothetical proteins. It is also noteworthy that majority of the proteins identified by using LC-MS/MS are soluble proteins, whereas most of the membrane-bound proteins were detected by 2D-DIGE. This might be due to the poor solubility of membrane proteins during the sample preparation step prior to LC-MS/MS analysis.

4.1.1 Bioinformatics Tools for Screening of Bioproduct Synthetic Capability

Bioinformatics approach plays a critical role in data mining and integration for in-depth biological analyses. Screening for bioproducts from organisms is a time- and labor-consuming process, thus genome-wide screening by using bioinformatics tool can effectively shorten such process. In terms of genome-scale screening for bioproduct synthetic capability from cyanobacteria including *Spirulina*, a bioinformatics tool named “CyanoCOG” (http://www2.sbi.kmutt.ac.th/orthoCOG/cyanoCOGnew/summary) developed by our group at

King Mongkut's University of Technology Thonburi can support not only the genome-wide screening for a gene of interest by using "search" menu but also the comparative studies of the gene among cyanobacterial genomes by using "pattern" menu. The user-guide can be found on the web-page.

Briefly on how to use CyanoCOG: Fifty-six cyanobacterial genomes were collected into the database. These cyanobacteria are categorized into four groups: unicellular (freshwater and others), filamentous (high alkaline and others), marine picocyanobacteria, and others (referred to cyanobacteria excluded from the three groups mentioned) in the "search" menu. Furthermore, subcategories are listed in the "pattern" menu: unicellular (freshwater), unicellular (others), filamentous (high alkaline), filamentous (others), extreme environment, obligate photoautotrophs, marine picocyanobacteria, uncategorized, and *Spirulina platensis*. When a given protein of interest, e.g., gene/protein name and function, is used as a keyword in the query box, the tool can perform matching using protein names, functions/properties, and functional category. On the output page of "search" menu, multisequence alignment of protein homologs among cyanobacteria can be viewed by clicking on <msa> under ortholog-id [16]. Whereas the output page of "pattern" menu provides number of genes present in each cyanobacterium in the comparative Table-format, and the corresponding nucleotide/protein sequences can be viewed by clicking on <msa> under ortholog-id. By using this tool, the target gene that is involved in the synthesis of bioproduct of interest can be screened in various cyanobacteria.

4.2 Quantitative Proteome Analysis

Temperature stress affects production of value-added biochemicals in a cell. As described above, *Spirulina* is mass-cultivated in outdoor ponds as a source for production of value-added bioproduct(s). Cells grown in outdoor cultivation system routinely encounter temperature fluctuations. For biomass production and quality control of bioproducts, it is important to elucidate how *Spirulina* respond/tolerate to temperature stress. Cells respond to stress by changing expression levels of various proteins. Thus, cellular stress response can be explored by quantitative proteome analysis, using high throughput techniques, including multidisciplinary approaches such as proteomics and bioinformatics.

Quantitative proteome analysis provides information on levels of protein expression under one experimental condition as compared with those under the other condition. Differentially expressed proteins, which include upregulated and downregulated proteins, are identified by using this approach.

In case of *A. platensis* C1, proteins in the three subcellular compartments, soluble, thylakoid membrane (TM), and plasma membrane (PM), were examined by using 2D-DIGE and iTRAQ-multidimensional-LC-MS/MS to study changes in protein expression levels and differential expression patterns upon immediate exposure to low (22°C)- and high (40°C)-temperatures [17–19]. In response to temperature downshift, 69, 436, and 369 proteins with differential expression levels were identified in PM, soluble fraction, and TM, respectively [17], whereas temperature upshift caused changes in the protein expression level of 40, 52, and 39 proteins in PM, soluble, and TM fractions, respectively [18].

Based on functional classification by COGs (Clusters of Orthologous Groups) category, the data obtained from low-temperature stress condition revealed that during the first 3 h after

the temperature reduction, the most abundant proteins are those involved in protein quality control (i.e., molecular chaperones), signal transduction systems (e.g., Ser/Thr kinase), photosynthesis, DNA repair/transcription/translation, and lipid biosynthesis [19], whereas the differentially expressed proteins identified in response to high temperature stress can be functionally classified into five major groups: two component systems (e.g., CheA/CheY), stress-related proteins (e.g., DEAD/DEAH-box DNA helicase), DNA damage/DNA repair system (e.g., exonuclease), and translational machinery (e.g., ribosomal proteins) [19].Therefore, these proteins may be important for acclimation during the initial period of the temperature down-shift.

The quantitative proteome data with cells exposed to the temperature stresses demonstrates that both low and high temperature stresses have a strong effect on photosynthesis and its related process. The enzymes involved in chlorophyll biosynthesis such as magnesium chelatase and uroporphyrinogen decarboxylase were upregulated upon temperature down-shift. In addition, some proteins in photosystem I (PSI) and photosystem II (PSII) were downregulated in common between both temperature shifts. Furthermore, the most abundant light-harvesting phycobilisome proteins such as phycocyanins, were found in the soluble fraction upon temperature up-shift, suggesting its dissociation from the thylakoid membrane. The dissociation of phycobilisome may be the first step for its degradation to generate nitrogen supply for the cells during exposure to high temperature [20].

Moreover, the quantitative proteome data sets were analyzed for interprotein relation via protein−protein interaction (PPI) network construction, which is described below (see Section 4.5). The analysis demonstrates that (1) there is a connection between temperature stress and nitrogen assimilation in both cold and heat shock responses, (2) the temperature stress is tightly linked with oxidative stress as well as photosynthesis; however, no specific mechanism is revealed in the case of the high-temperature stress response, and that (3) there is a cross-talk among different signaling pathways [19]. It is known that signal transduction takes place immediately upon stress [21]. In the case of *Spirulina*, the signaling proteins, e.g., Hik14, Hik 21, Hik26, and Hik28, were shown to have potential interactions with differentially expressed proteins, e.g., SigG, GltB, and PleD, identified under low and high temperature stress conditions [17−19]. Thus, the importance of the signal transduction system(s) is highlighted and it should be further explored by integration of phosphoproteome data sets.

4.3 Phosphoproteome Analysis

Phosphorylation is a posttranslational protein modification that commonly occurs as a regulation of protein function. When cell senses a temperature change as a signal, the signal is transmitted to regulate gene expression/protein function that are involved in the stress response mechanism. Phosphorylation of proteins is one of the common means in the signal transduction pathway. Comparative quantitative proteome analyses provide information on protein expression level and pattern, which represent the regulation at the translational level. Regulation at the posttranslational level can be analyzed by comparative phosphoproteome analyses that give information on phosphoproteins and phosphorylation sites detected under the experimental conditions.

In phosphoproteome analysis, phosphoproteins are less abundant than nonphosphoproteins in a cell; thus phosphoproteins are concentrated after total protein-sample preparation.

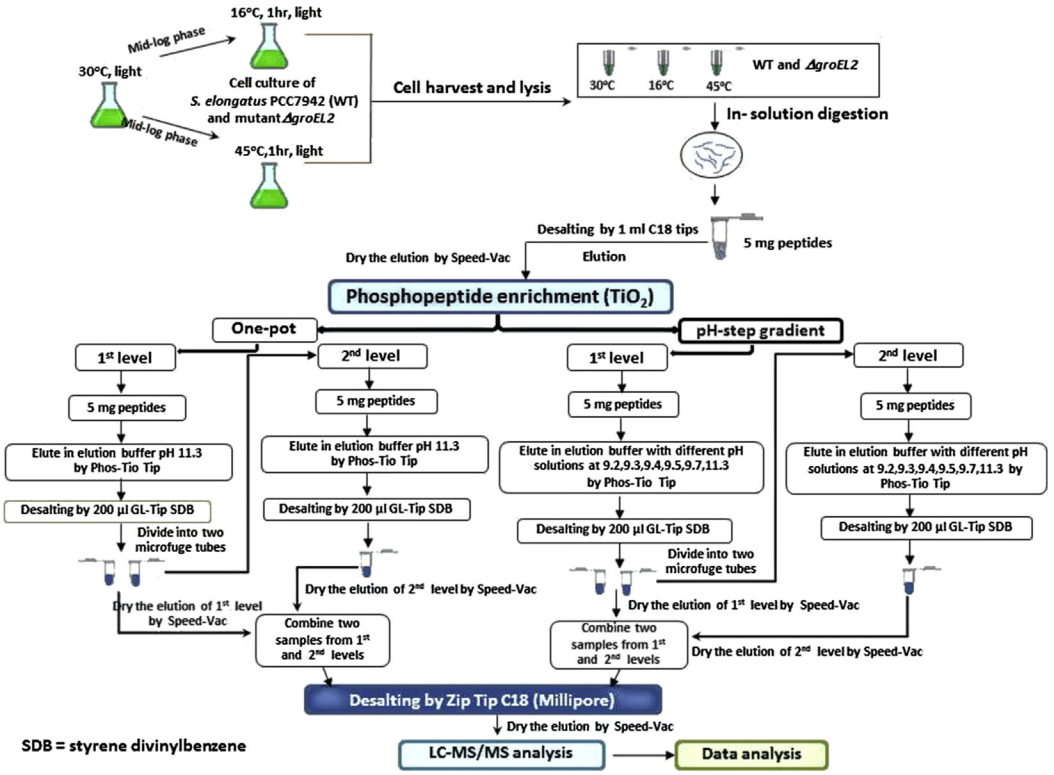


FIGURE 3.3 Workflow for phosphoproteome analysis.

As shown in Fig. 3.3, after a protein sample is digested with trypsin, the peptide mixture is subjected to the phosphopeptide enrichment step by using titanium dioxide (TiO_2).

The phosphopeptides are attached to the TiO_2 and then they can be eluted by using pH gradient. After desalting, the phosphopeptide samples are ready for the separation and analysis by LC-MS/MS. Phosphoproteins, phosphopeptides, and phosphorylation sites are identified. In Section 4.7, we will describe the phosphoproteome analysis performed with *Synechococcus elongatus* PCC7942 wild type (WT) and *groEL2*-deleted mutant.

4.4 Bioinformatic Tools in Proteome-Wide Analysis

Proteome-wide analyses for protein expression level (quantitative proteome analysis) and posttranslational modification of proteins occurred under a certain condition (e.g., phosphoproteome analysis) lead to information required for understanding cellular response to a change in the experimental condition. In terms of proteome-wide analysis tool, SpirPro is a powerful web-based tool and a database for proteome data analysis of *A. platensis* C1 [16]. Quantitative proteome and phosphoproteome data were integrated into the database. The in-depth analyses in terms of pathways, interpathway connections, metabolisms, and PPIs can be performed and visualized with this tool.

4.5 Protein–Protein Interaction Networking and Bioinformatic Tools for the Analysis

Different proteins are linked as a group or a subnetwork to perform distinct cellular functions, and these groups of proteins are interconnected in cell-wide protein networks. Understanding the structures and operational modes of these networks is one of the great challenges in biology. Furthermore, it is important to understand how the network is maintained functional during stress exposure in the production of biomass or value-added biochemicals.

PPI networking is a complicated association of proteins, and thus an effective bioinformatics tool is required in the construction and the analysis of the networking. As mentioned above that SpirPro is a web-based tool that can be used to construct PPI network [16] by using prototype (template) PPI database of *Synechocystis* [22]. The construction is based on a graph, in which the nodes and edges represent proteins and interactions, respectively. Each node is assigned by performing BLAST similarity searches to identify homologous proteins, which are mapped to their reciprocal best-hit *Synechocystis* proteins according to a significance threshold of $E < 1 \times 10^{-10}$.

4.6 Molecular Chaperones in Relation to Production of BioProducts by Bacteria

Molecular chaperones are a group of proteins that play critical roles in cellular protein homeostasis [23]. Thus, they are important for cell survival under stress conditions where proteome is changed due to protein denaturation. Among molecular chaperones, five protein families are recognized as highly conserved and ubiquitously distributed [23]. They are small Hsp, chaperonin or Hsp60 (GroEL), Hsp70 (DnaK), Hsp90 (HtpG), and Hsp104 or Hsp101 (ClpB). GroEL, DnaK, HtpG, and ClpB are terms normally used for prokaryotic members. Detailed information on various molecular chaperones in cyanobacteria can be obtained in a recently published book chapter [24].

There is good evidence that molecular chaperones play a vital role in stress management in cyanobacteria [24]. Inactivation and deletion of the single small Hsp and HtpG genes in the genomes of *Synechocystis* sp. PCC6803 and *S. elongatus* PCC7942, respectively, cause great loss of thermotolerance [25,26]. Constitutive overexpression of an exogenous small Hsp gene in *S. elongatus* PCC7942 results in enhanced cell growth/survival under heat, high salt, and oxidative stresses [27–29].

Even in the presence of multiple homologous chaperone genes in a genome, one of the genes plays a role under stress. It appears that one of them becomes specialized to take on specific cellular roles under stress. Cyanobacteria express two or more GroEL genes. We showed that a mutant strain of *Thermosynechococcus elongatus* whose gene encoding one of the two GroELs (GroEL2) is inactivated is not capable of growing at both heat and cold stresses [30]. Similarly, a *clpB1* mutant of *S. elongatus* PCC7942 is sensitive to both high and low temperatures [31,32]. GroEL1 and ClpB2 are essential [33,34], while GroEL2 and ClpB1 are not essential although they play an essential role under temperature stresses [30,31].

We are particularly interested in one of the two GroELs, GroEL2 in *S. elongatus* PCC7942. Our mutational studies indicate that GroEL2 in this cyanobacterium plays a role under

(A)

(B)

Characteristics in function and structure	*E. coli* GroEL	PCC7942 GroEL1	PCC7942 GroEL2
Essential?	Yes	Yes?	No
Stress inducible?	Yes	Yes	Yes
Complements a mutated *E. coli groEL?*	-	Yes (partially)*	No*
Suppresses aggregation of denatured proteins?	Yes	Yes	Yes
Forms an oligomer of 14 subunits?	Yes	Yes	No
Assists folding of a protein in a GroES/ATP-dependent fashion?	Yes	Not detected yet	Not detected yet

FIGURE 3.4 (A) Schematic representation of the *E. coli groESL* operon, the cyanobacterial *groESL1* operon and *groEL2* and (B) functional and structural properties of *E. coli* GroEL, *S. elongatus* PCC7942 GroEL1 and GroEL2.

various stresses including temperature stress like GroEL2 from *T. elongatus* (S. Huq and H. Nakamoto, unpublished data). The two GroELs are homologous in their amino acid sequences, but are quite different in various aspects. The most significant difference between the two paralogous *groEL* genes is that *groEL1* is arranged with an upstream co-chaperone gene, *groES*, forming a bicistronic *groESL* operon like the *E. coli groESL* operon. In contrast, the second *groEL* gene (*groEL2*) does not have an accompanying *groES* gene in its neighbor. Furthermore, we observed many differences in the structure and function of the two GroEL proteins as summarized in Fig. 3.4 [35–37]. GroEL1 appears to be similar to the *E. coli* GroEL in many aspects.

In addition to cyanobacterial genomes, other bacterial genomes also contain two or more copies of *groEL* [38]. Recent studies indicate that GroEL2 whose gene does not form an operon with *groES* in *Myxococcus xanthus* DK1622 and *Rhodococcus rhodochrous* ATCC12674 plays a vital role for the biosynthesis of a secondary metabolite (myxovirescin) and the alkane tolerance, respectively [39,40]. Those findings have important implications for the role of GroEL2 in various bioproduct/biofuel production by bacteria.

4.7 Involvement of GroEL2 in Cellular Protein Networks and *in silico* Translation of Phosphoproteome Data Obtained From One Species to the Other

Phosphoproteome data obtained from other transformable cyanobacterium, in this case *S. elongatus* PCC7942, is inferred for *Spirulina* to elucidate a role of a molecular chaperone GroEL2 under temperature stress conditions.

As described above, due to the lack of stable transformation system and specific gene manipulation system, mutants cannot be easily constructed in *Spirulina*. Thus, it is more difficult to study functions of a specific gene in *Spirulina* than transformable cyanobacteria whose

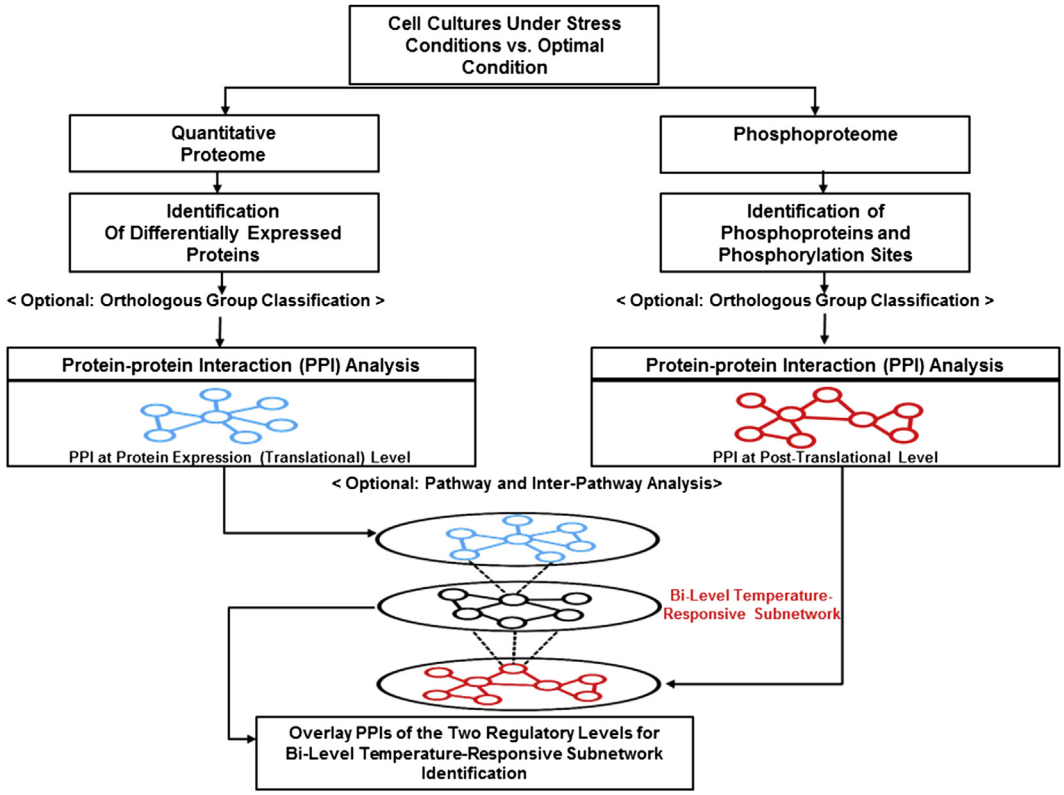


FIGURE 3.5 Schematic diagram of bi-level analysis platform for stress-response bi-level regulatory subnetwork identification.

mutants are easily constructed; however, bioinformatics may be able to solve the problems by translating results obtained with a transformable cyanobacterium to *Spirulina* (Fig. 3.5).

In silico analytical and data management platform was applied for phosphoproteome data simulation of *Spirulina* to elucidate the molecular chaperone networking under temperature stress conditions by using a transformable model cyanobacterium *S. elongatus* PCC 7942 WT and its *groEL2*-deleted mutant. The cyanobacterium is unicellular unlike the filamentous *Spirulina*.

Like *S. elongatus* PCC 7942, *A. platensis* C1 has two kinds of GroEL [6]. Quantitative proteome data of *A. platensis* C1 indicates that GroEL2 as well as other chaperones such as GroEL1, DnaK2, and DnaK3 is among those highly (more than 1.5-fold-change) upregulated proteins in response to temperature shifts [17,19]. GroEL2 as well as GroEL1 from *S. elongatus* PCC 7942 is also upregulated upon heat shock (Fig. 3.4). Moreover, similarity between GroEL1 and GroEL2 of the two cyanobacteria *A. platensis* C1 and *S. elongatus* PCC 7942 are approximately 88% and 70%, respectively, and functional domains are conserved between the two organisms (Fig. 3.6). As shown in Fig. 3.6, GroEL1 and GroEL2 can be easily distinguished by the presence of the characteristic GGM-repeating motif at the C-terminal

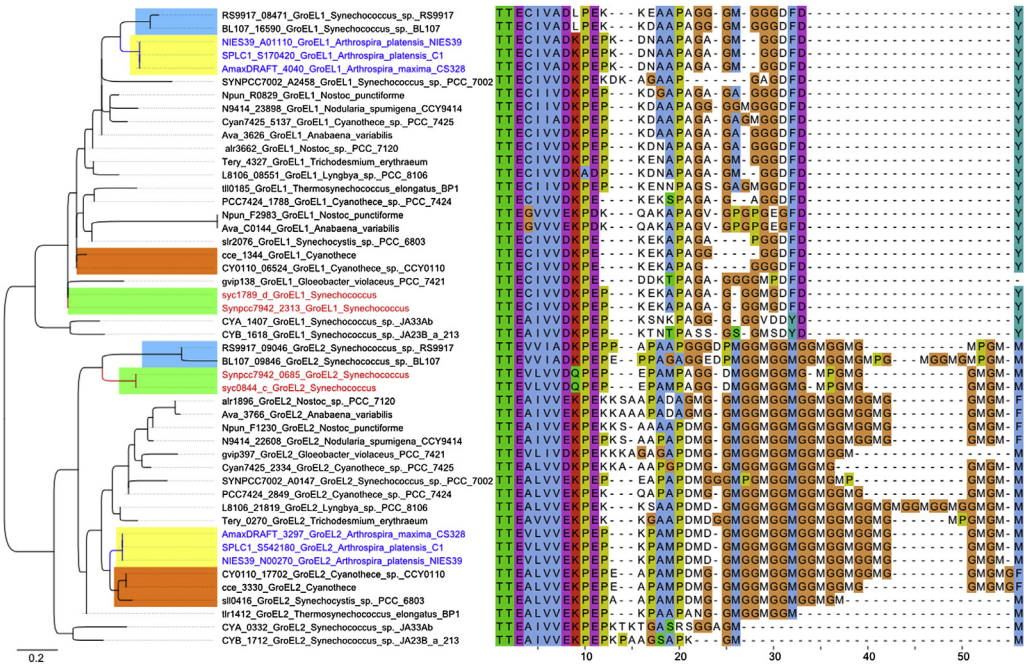


FIGURE 3.6 Phylogenetic tree of cyanobacterial GroEL1 and GroEL2 and sequence alignment of the C-terminal regions of *A. platensis* strain C1-GroEL1 (SPLC1_S170420; 545 amino acids) and -GroEL2 (SPLC1_S542180; 558 amino acids), *S. elongatus* PCC7942-GroEL1 (Synpcc7942_2313; 544 amino acids) and -GroEL2 (Synpcc7942_0685; 555 amino acids). Note: The group of GroEL1 and GroEL2 of *Arthrospira*, *Synechococcus*, marine cyanobacteria, and freshwater cyanobacteria are highlighted in *yellow (lightest gray in print version)-, green (dark gray in print version)-, cyan (gray in print version)- and orange (light gray in print version)-boxes*, respectively.

end of GroEL2. Therefore, *S. elongatus* PCC 7942 WT and the *groEL* mutant were used in the phosphoproteome analysis. However, it is worth noted that *Synechococcus* contains only 2662 protein-coding genes (KEGG genome database), thus protein networking in *Spirulina* might be much more complicated than that of the *Synechococcus*.

Phosphoproteins found in the *S. elongatus* PCC7942, WT and mutant strains (manuscript in preparation), in response to temperature stresses were computationally transformed to *Spirulina* proteins via orthologous group identification (Fig. 3.5). Then, SpirPro was applied in this step to assist in (1) integrating the data in the translational (quantitative proteome) and posttranslational (phosphoproteome) levels, (2) locating the designated phosphoproteins onto pathways obtained from KEGG, and (3) analyzing PPI networks. Up to this point, the bi-level proteome data of *Spirulina* were integrated for further in-depth analyses by using proteome-wide analysis tools described below.

4.8 Bi-Level Temperature-Responsive Subnetwork

PPI networks at the translational and posttranslational levels were constructed from the data obtained from proteome-wide analyses of protein expression level and protein

phosphorylation in response to temperature stresses. The two levels of PPI networks were aligned and compared to identify the PPI subnetwork that exists in both levels. The proteins regulated at both translation and posttranslation levels (bi-level) and their PPI networking are named as "bi-level temperature-responsive subnetwork" (Fig. 3.5) due to the hypothesis that proteins regulated at both translational and posttranslational levels and their associated proteins should be important components of the cells to respond to the temperature stresses. The bi-level–regulated proteins are shown in Table 3.2.

TABLE 3.2 Proteins Present in the Temperature-Stress-Response Bi-Level Regulatory Subnetwork

				Translational Level		Posttranslational-Level	
				Heat		Cold	
Gene Symbol	**Protein Name**	**Heat**	**Cold**	**+GroEL2**	**−GroEL2**	**+GroEL2**	**−GroEL2**
	ATPase	√			[a]		[a]
aldA	Aldehyde dehydrogenase	√		[a]		[a]	
	Hypothetical protein	√					√
	Penicillin-binding protein 1A	√					√
rpsB	30S ribosomal protein S2	√		[a]		[a]	
	30S ribosomal protein S1	√			[a]		[a]
SEM0034	Periplasmic sensor hybrid histidine kinase	√		√		√	√
	GAF sensor signal transduction histidine kinase	√				√	
mnmG	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA	√				√	
	TPR repeat-containing protein	√		[a]		[a]	
dnaK3	Molecular chaperone DnaK	√		√			√
	CheA signal transduction histidine kinase	√		[a]	√	[a]	
	Hypothetical protein	√		√	√		
	Hypothetical protein	√		√		√	√
rpoB	DNA-directed RNA polymerase subunit beta		√	√			
tilS	Hypothetical protein		√	[a]		[a]	
	Hypothetical protein		√	√			
tatA	Twin-arginine translocation protein TatA		√	[a]		[a]	

TABLE 3.2 Proteins Present in the Temperature-Stress-Response Bi-Level Regulatory Subnetwork—cont'd

				Translational Level		Posttranslational-Level	
				Heat		Cold	
Gene Symbol	**Protein Name**	**Heat**	**Cold**	**+GroEL2**	**−GroEL2**	**+GroEL2**	**−GroEL2**
groEL1	Molecular chaperone GroEL		√		√	√	
	Pyruvate kinase		√	[a]	√	[a]	√
	Methionine synthase (B12-dependent)		√		√	√	√
	Hypothetical protein		√	[a]		[a]	
	Mrr restriction system protein		√	√	√		
	Arsenite-activated ATPase ArsA		√				√
htpG	Heat shock protein 90		√	√	√		
	Cyclic nucleotide-binding protein		√	√	√		
cofH	FO synthase subunit 2		√		√		
chlN	Light-independent protochlorophyllide reductase subunit N		√		√		
	Magnesium chelatase subunit H		√	√	[a]	√	[a]
	Metal dependent phosphohydrolase		√	√	√		
	Ferredoxin-NADP oxidoreductase		√		√		√
gap2	Glyceraldehyde-3-phosphate dehydrogenase		√	√		√	√
[a]Gap2	Glyceraldehyde-3-phosphate dehydrogenase		√	√	[a]		[a]
cbbL	Ribulose 1,5 bisophosphate carboxylase (large subunit)		√	√	√	√	√
atpA	ATP synthase F0F1 subunit alpha		√	[a]		[a]	
glgB	Glycogen branching enzyme		√			√	
ccmK	Carboxysome assembly protein		√	√		√	√
ccmM	Carbonate dehydratase		√	√	√	√	√
fusA	Elongation factor G		√	[a]		[a]	
tuf	Elongation factor Tu		√	√		√	√

(*Continued*)

TABLE 3.2 Proteins Present in the Temperature-Stress-Response Bi-Level Regulatory Subnetwork—cont'd

				Translational Level		Posttranslational-Level	
				Heat		Cold	
Gene Symbol	**Protein Name**	**Heat**	**Cold**	**+GroEL2**	**−GroEL2**	**+GroEL2**	**−GroEL2**
nrtCPhl	Nitrate transport ATP-binding subunits C and D		√	[a]	√	[a]	
	D-3-phosphoglycerate dehydrogenase		√		√		
	Ribulose 1,5 bisophosphate carboxylase (small subunit)		√	√	√	√	√
ilvD	Dihydroxy-acid dehydratase		√		√	√	
argG	Argininosuccinate synthase		√	√			
	two component transcriptional regulator		√				√
	LC 7		√	√			
kdsA, CcmA	2-Dehydro-3-deoxyphosphooctonate aldolase		√	√			
	Photosystem I reaction center subunit III		√	[a]		[a]	
petA	Apocytochrome f		√				√
proS	Prolyl-tRNA synthetase		√	[a]	[a]	[a]	[a]
pyrG	CTP synthetase		√			√	
Pgk	Phosphoglycerate kinase		√		√	√	
gap1	Glyceraldehyde-3-phosphate dehydrogenase		√		√		
psbB	Photosystem II core light harvesting protein		√	√	√	√	√
	1-Cys peroxiredoxin		√		[a]		[a]
groEL2	Molecular chaperone GroEL	√	√	[a]		[a]	
secA	Preprotein translocase subunit SecA	√	√		√	√	
dnaJ	Molecular chaperone DnaJ	√	√		[a]		[a]
cynD	Nitrate transport ATP-binding subunits C and D	√	√		√		
	Glutamate synthase (ferredoxin)	√	√	√	[a]	√	[a]
clpB2	ATPase	√	√	[a]		[a]	
gyrA	DNA gyrase subunit A	√	√		[a]		[a]
	GAF sensor-containing diguanylate cyclase	√	√		√		

Note: √ represents the protein identified under the designated condition.
[a]*The protein identified under optimal temperature (35°C).*

The locus-tag of the protein in *A. platensis* C1 and *S. elongatus* PCC7942 are given in the designated Table. The complete bi-level temperature-responsive subnetwork of *A. platensis* C1 created by using SpirPro is shown in Fig. 3.7A and B, where protein nodes are labeled with locus tag, which is a systematic identifier applied to a gene in a genome.

However, in the bi-level subnetworks created by using STRING (http://string-db.org) as shown in Fig. 3.8A and B, the protein nodes are labeled with either gene symbol or locus tag of *S. elongatus* PCC7942. The PPI subnetwork generated by using STRING gives information on protein−protein relationship other than physical interactions data obtained from yeast two hybrid systems, e.g., textmining, gene neighborhood, and databases that might be beneficial for further studies.

There are 64 proteins regulated at bi-level and six molecular chaperones (GroEL1, GroEL2, ClpB2, DnaK3, DnaJ, and HtpG) are included in the group. It highlights the critical role(s) of chaperones in the stress response mechanisms. According to Fig. 3.7, these chaperones appear in the center of the bi-level subnetwork. In addition, proteins in the subnetwork are in signal transduction mechanisms, photosynthesis, energy production, CO_2 concentrating mechanism, nitrogen metabolism, Calvin cycle, protein biosynthesis, and protein transport.

When the bi-level subnetwork is considered based on the presence or absence of GroEL2, the proteins can be categorized as GroEL2-dependent and GroEL2-independent proteins based on the fact that their expression level and/or phosphorylation state are affected by the presence or absence of GroEL2. We found that expression level and/or phosphorylation state of the molecular chaperone HtpG and some proteins involved in photosynthesis such as ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) and the PSII light-harvesting protein PsbB (CP47) are not different significantly in the WT and the mutant strains (Table 3.2). Thus, these proteins are "GroEL2-independent."

Proteins can be classified as GroEL2-dependent proteins when their expression level and/or phosphorylation state are altered in the presence or absence of GroEL2. Unexpectedly, we found many GroEL2-dependent proteins (Table 3.2). In the presence of GroEL2, the proteins in the bi-level subnetwork are in the group of chaperones, such as DnaJ, DnaK3, and ClpB2; energy production, such as ATPase and ATP synthase; transcription factors, such as RpoB; and protein translation and biosynthesis, such as RpsB (Table 3.2). Whereas in the *groEL2*-deleted mutant, proteins involved in DNA repair such as GyrA, chlorophyll biosynthesis such as ChlN, and cytochrome b_6f complex which mediates the electron transfer from PS II to PS I are present in the subnetwork, suggested that photosynthesis was affected by the absence of GroEL2 (Table 3.2).

It is noteworthy that in addition to the bi-level analyses, comparative PPI network analyses of cells in the presence and absence of GroEL2 can be carried out using the PPI tool in SpirPro. As a result, GroEL2-dependent and GroEL2-independent subnetworks of phosphoproteins under the temperature stress conditions can also be constructed (manuscript in preparation). The subnetworks can tell us not only the GroEL2-networking in response to either low or high temperatures, but also the alternative responsive networking of the cells in the absence of GroEL2.

Hidden information regarding high/low temperature−specific subnetworks and common temperature-responsive subnetwork can be extracted by using the integrated platform. In case of high-temperature−responsive subnetwork, DnaK3 and a hypothetical protein

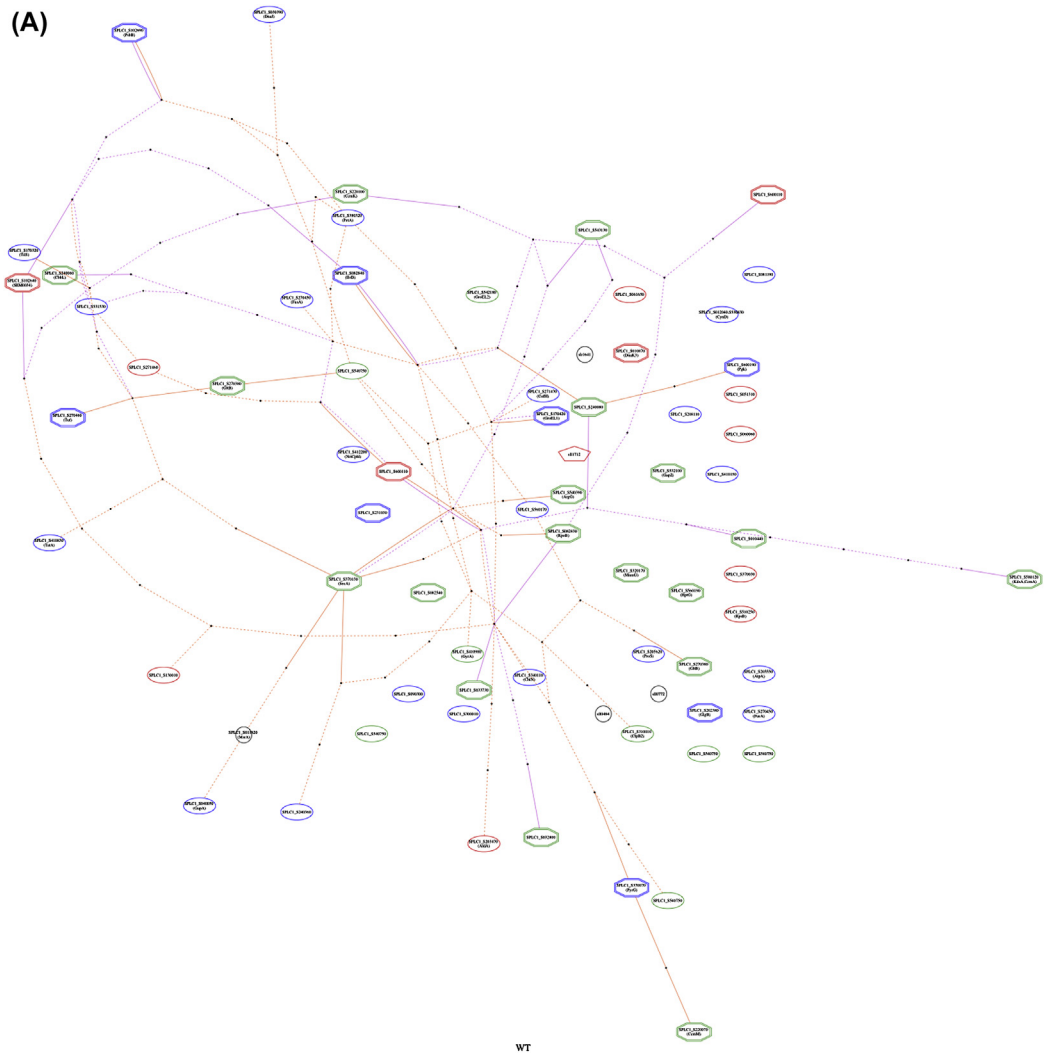


FIGURE 3.7 Protein–protein interaction (PPI) networks of the proteins, (A) in WT or in the presence of GroEL2 and (B) in the mutant or in the absence of GroEL2, involved in the temperature-stress-response bi-level regulatory subnetwork constructed by using SpirPro. Note: A protein node in *oval, pentagonal, octagonal, and circular shape represents* a differentially expressed protein, phosphoprotein, bi-level–regulated protein, and protein immediately connected to a bi-level–regulated protein (based on *Synechocystis* sp. PPI-template) that was not found in our experiments, respectively. Node color, blue (dark gray in print version), green (light gray in print version), and red (gray in print version), represents protein identified under low, optimal and high temperature conditions, respectively. An *edge represents* physical interaction verified by yeast two hybrid system of the two proteins. *Solid-edge represents* immediate (1 interaction-path) interaction of the two proteins, whereas, *dash-edge shows* nonimmediate (2 interaction-path up) interactions between the proteins. Edge color, red (dark gray in print version) and purple (gray in print version), represents interaction of proteins identified in the translational and posttranslational level, respectively.

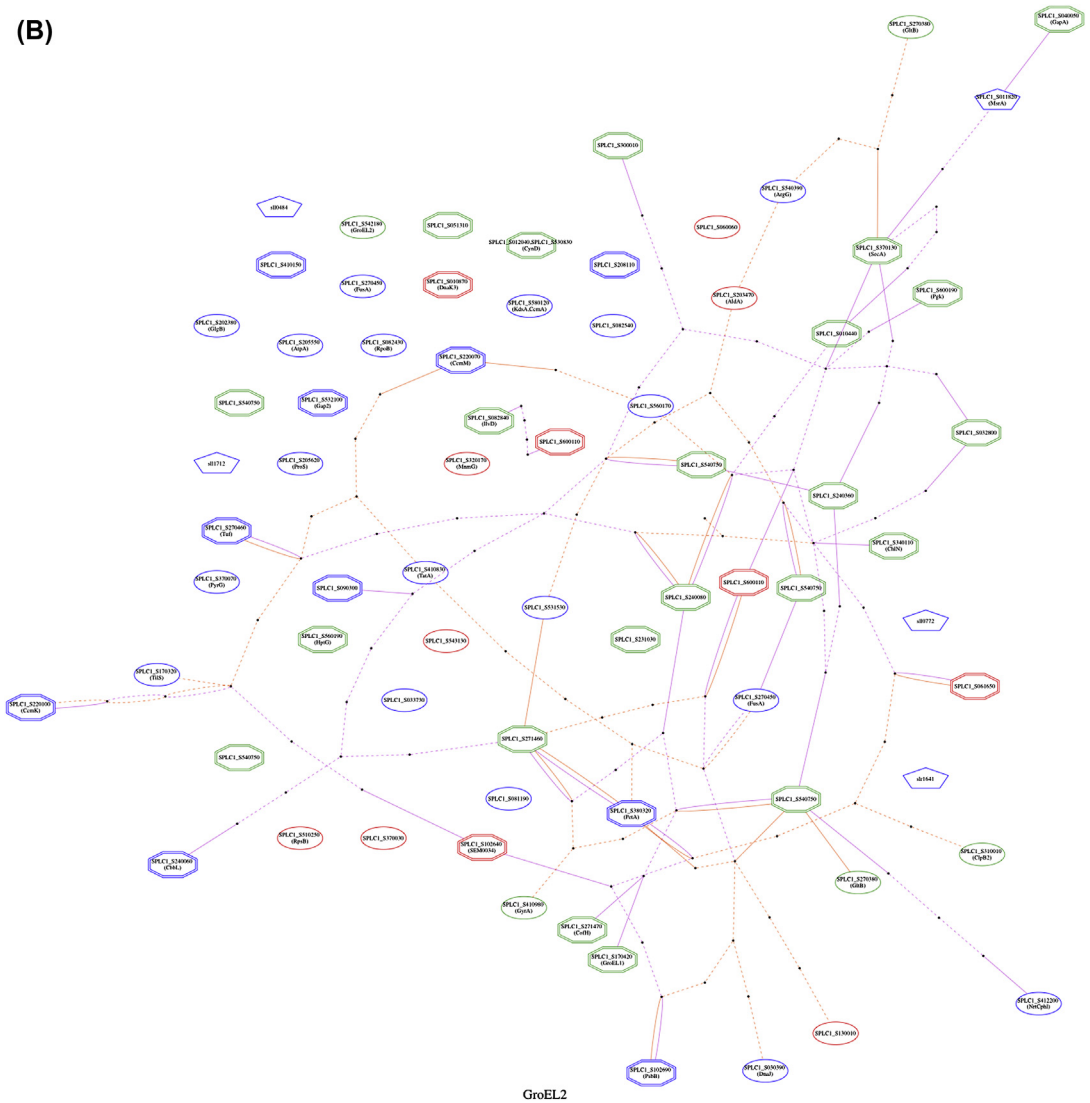


FIGURE 3.7 cont'd

(SPLC1_S600110), which contains TORPIM or topoisomerase-primase conserved domain, were the two proteins that are regulated at the translational- and posttranslational levels in the presence of GroEL2. S600110 is phosphorylated regardless of the presence of GroEL2 according to Table 3.2. However, when the GroEL2 is absent, CheA (SPLC1_S061650), a signal transduction histidine kinase in two-component regulatory system involved in chemotaxis, was revealed instead of DnaK3. The critical roles of the DnaK family in heat stress response were reported [41,42]. DnaK2 and DnaK3 are essential for cyanobacterial growth [33];

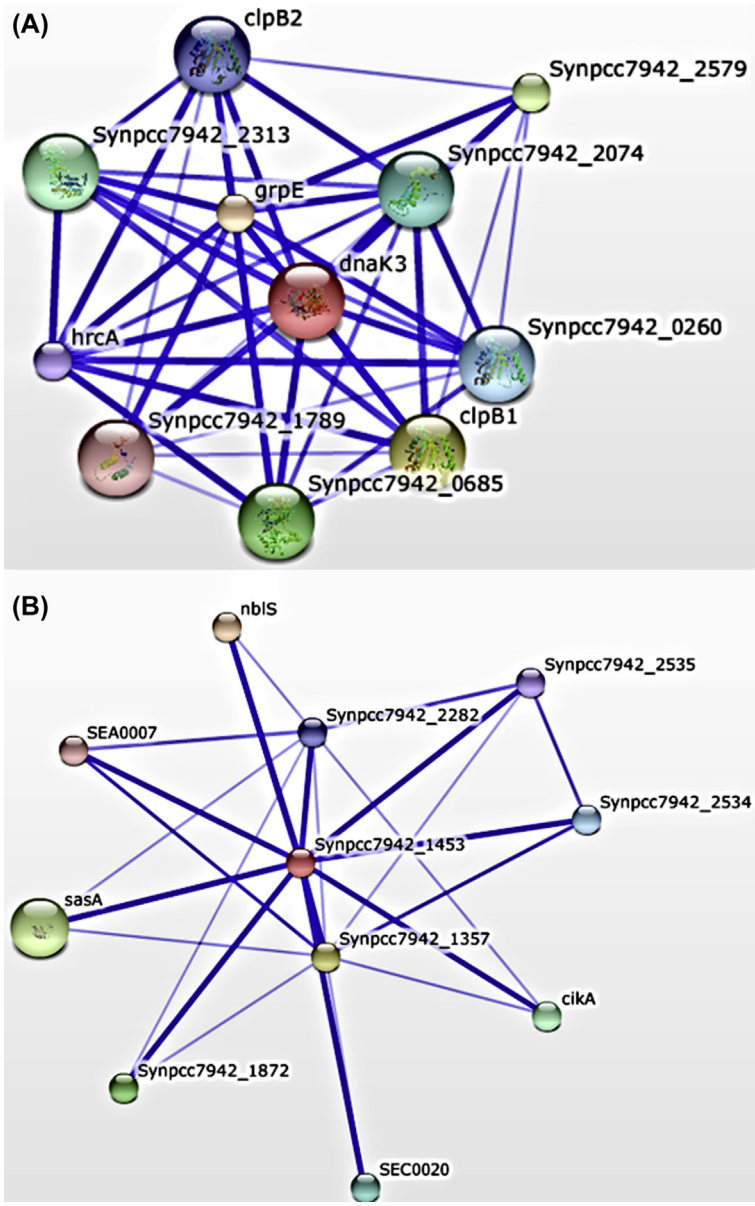


FIGURE 3.8 Protein–protein interaction (PPI) subnetwork of (A) DnaK3 (SPLC1_S010870 or Synpcc7942_2580) and (B) two-component response regulator (SPLC1_S208110 or Synpcc7942_1453) obtained by using STRING (http://string-db.org/). Note: Protein nodes in the subnetworks are labeled with gene symbol or locus tag of *S. elongatus* PCC7942.

however, the subcellular localization of the two proteins is quite different. DnaK2 is mainly detected in the cytosol, whereas the DnaK3 is associated with thylakoid membranes [43]. Thus, it might be anticipated that DnaK3 (Fig. 3.8A) might be involved in the heat-protecting mechanism of photosynthetic system in the presence of GroEL2 under high temperature stress. There are pieces of evidence to show that GroEL2 is also localized on thylakoid membranes as well as cytosol [17,19]. There are three DnaK homologs, and as shown in Fig. 3.9, DnaK3 has a characteristic C-terminal end that may be important for its localization on thylakoid membrane and its special function [42].

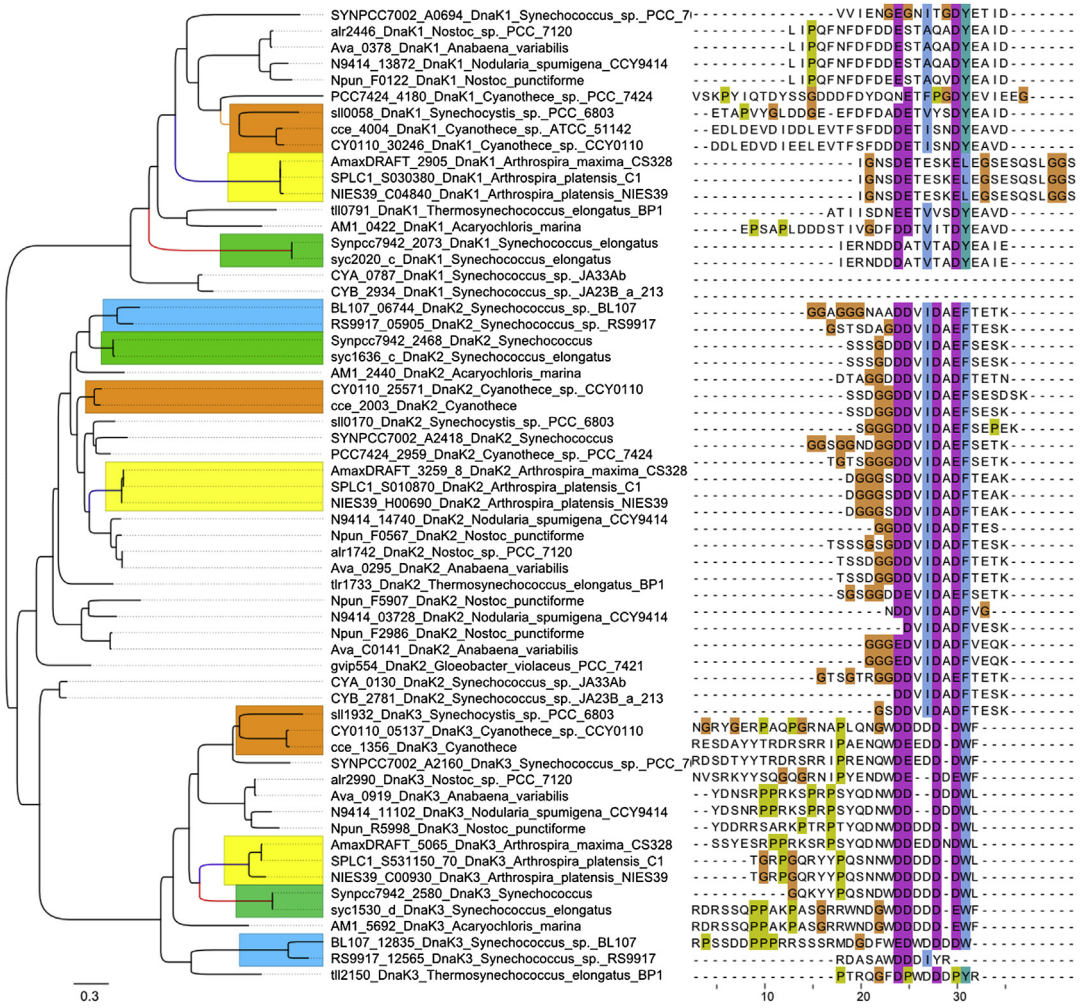


FIGURE 3.9 Phylogenetic tree of cyanobacterial DnaK1, DnaK2, and DnaK3, and sequence alignment of the C-terminal regions of *A. platensis* strain C1-DnaK1 (SPLC1_S030380; 658 amino acids), -DnaK2 (SPLC1_S010870; 637 amino acids) and -DnaK3 (SPLC1_S531170; 465 amino acids). Note: The group of DnaK1, DnaK2, and DnaK3 of *Arthrospira*, *Synechococcus*, marine cyanobacteria, and freshwater cyanobacteria are highlighted in *yellow (lightest gray in print version)-, green (dark gray in print version)-, cyan (gray in print version)-, and orange (light gray in print version)-boxes*, respectively.

Regarding bi-level low-temperature–responsive subnetwork, in the presence of GroEL2, the key chaperone, GroEL1 was one of the bi-level–regulated proteins as shown in Table 3.2. The other proteins in the subnetwork were proteins involved in carbon metabolism such as Pgk and Gap1, amino acid biosynthesis such as ProS, and nucleotide biosynthesis such as PyrG. Whereas, in the absence of GroEL2, no chaperone proteins were detected in the subnetwork, however, the proteins in the bi-level subnetwork under this condition were involved in carbon dioxide concentrating mechanism, carbon metabolism, DNA biosynthesis, photosynthesis, and two-component transcriptional regulation. It is noteworthy that the two-component transcriptional regulator, Synpcc7942_1453, which is in the same orthologous group containing winged-helix domain as SPLC1_S208110, was the only signaling protein found in the low-temperature–responsive subnetwork, whereas PPI analysis of the protein using STRING shows the relationship of the two-component response regulator with NblS (Hik33) and SasA, which are nonbleaching sensor and adaptive-response sensory kinase, respectively (Fig. 3.8B). Phosphorylated form of Hik33-dimer was reported to be induced by cold shock [44]. The phosphate is transferred to the response regulator, Rre26, and then the phosphor-Rre26 binds to regulatory region of the target cold-inducible genes to activate their expression [44]. Whereas SasA participates in the KaiABC circadian clock protein complex and it is known that resetting of the circadian clock via the phosphorylation of KaiC plays a critical role in regulation of energy storage in the form of glycogen [45]. Therefore, the data indicate direct participation of GroEL1 in the cold stress response mechanism in the presence of GroEL2. However, in the absence of GroEL2, the cells responded to cold stress by controlling their energy storage, carbon metabolism, and circadian clock, probably via two-component transcriptional regulator, SPLC1_S208110 (Synpcc7942_1453 ortholog).

Moreover, it should be noted that the protein in the bi-level subnetwork found in common between the two temperatures is GltB, which synthesizes glutamate from 2-oxoglutarate (2-OG). The 2-OG in the TCA cycle is located at the interconnection of C and N metabolism and its level is well recognized as a signal for nitrogen regulatory protein PII (GlnB) phosphorylation in response to N-limitation [46]. It indicates that the regulation of C/N ratio is important under temperature stress similar to that under N-starvation.

Interestingly, two key enzymes involved in carbon dioxide concentrating mechanism and the Calvin cycle, carbonate dehydratase (or carbonic anhydrase) and RuBisCO, were regulated at the protein expression level after cold stress exposure, whereas their phosphorylated forms were identified under high and low temperature regardless of GroEL2 (Table 3.2). The data indicate the bi-level regulation of these proteins in response to temperature downshift and the regulation at protein-modification level under elevated temperature. It suggests that carbonate dehydratase and RuBisCO have to be regulated at the bi-level for the operation of carbon fixation under temperature stresses. Taken together, the data revealed critical information on bi-level regulation of temperature-response mechanism via PPI network analysis. It should be addressed that the essential chaperones, GroEL1 and DnaK3, are present in the bi-level regulatory subnetwork, whereas GroEL2, DnaJ, and HtpG are regulated only at the protein expression level [17–19].

4.9 Pathway Visualization

4.9.1 Bioinformatics Tools for Pathway Visualization

As a part of integrated biological platform, pathway tool is required to visualize proteomics data after the analyses. Visualization of proteins in pathway and pathway interconnection based on integrated proteome-wide data of *Spirulina* under the temperature stresses can be done by using SpirPro. Pathway analysis and PPI network mapping provide biological meanings of the proteomics data, for example, overview of the cellular response and multilevel regulation via PPIs and interpathway connection. Therefore, the overall proteome data management and bioinformatics tools are integrated as a platform that can be applied for in-depth proteome data analyses obtained from other organisms.

4.9.2 An Example of the Platform Application for High Value Chemical Production

Among several bioproducts produced by *Spirulina* cells, γ-linolenic acid is an important biochemical due to its nutraceutical potential as described above. *A. platensis* can synthesize up to 23% and 13% more GLA in the plasma membrane after 24 h and thylakoid membrane after 48 h of the temperature downshift to 22°C, respectively, as compared to the level of GLA in cells maintained at the optimal growth temperature [9]. It appears that the homeoviscous adaptation comes many hours after the temperature reduction. Therefore, the present integrated proteome-wide analytical platform can be used as part of an attempt to control and manipulate conditions to maximize polyunsaturated fatty acid (PUFA) biosynthesis in this cyanobacterium. The proteome-wide data obtained in the previous cold-shock response studies of *A. platensis* [17,47] showed that several proteins involved in fatty acid biosynthesis, such as histidine kinases, (3R)-hydroxymyristoyl-[acyl-carrier-protein]-dehydratase, acyl carrier protein, and Δ^9-desaturase are differentially expressed. Moreover, the isocitrate dehydrogenase [6], the key enzyme in TCA cycle that produces 2-OG was found in its phosphorylated form. As mentioned earlier, 2-OG is positioned at the key regulatory point to control the C/N balance in the cells. The knowledge obtained can be applied to flux balanced analysis (FBA) to study the flux model and flux manipulation by controlling the enzymes of interest targeting for increasing PUFA production in *Spirulina.* The FBA model has to be validated by analysis for products of interest obtained from wild type and/or genetically manipulated mutant cells grown according to the model. Then an appropriate strain and culture conditions can be used in the industrial scale for the biochemical production.

5. CONCLUSIONS AND PERSPECTIVES

Production of bioproducts by using microorganisms including cyanobacteria requires multidisciplinary expertise. Implementation of knowledge-to-process is the key to successful biochemical production. Prior to the implementation, basic knowledge involved in biochemical syntheses and regulation in biological systems are essential. OMICS techniques including bioinformatics can facilitate the process of obtaining such basic knowledge. Thus, the linkage between knowledge and process for the synthesis of biochemicals of interest would be the

focus point. FBA based on OMICS including proteomics data is the above mentioned linkage step, where the flux model and flux manipulation by controlling the target proteins/enzymes in pathways would be simulated to achieve the maximum biomass production prior to large-scale production. Then, the FBA model has to be validated by systematic analysis of bio-product synthesis by the cells, possibly by using metabolomics approach. The appropriate strain and culture conditions can be successfully applied in the industrial scale for the biomass and/or biochemical production.

Acknowledgments

The authors would like to address that the data on *Spirulina* molecular biology and proteome analyses mentioned above were obtained from the research projects granted by National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Thailand. The proteomics and bioinformatics facilities were kindly provided by King Mongkut's University of Technology Thonburi, Thailand. They also thank financial supports by Grant-in-aids for Scientific Research (C) [No. 24580102 and 15K07349] and JSPS Bilateral Joint Research Projects to H.N. from the Ministry of Education, Science, Sports and Culture of Japan. The authors would like to thank Ms. Pavinee Kurdrid and Ms. Phuttawadee Phuengcharoen for the preparation of Figs. 3.1, 3.3 and 3.5 and Ms. Ratana Chailklahan for providing pictures of outdoor-pond mass cultivation and bioproducts extracts of *Spirulina*.

References

[1] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol. 111 (1979) 1–61.

[2] A. Hongsthong, B. Bunnag, Overview of *Spirulina*: biotechnological, biochemical and molecular biological aspects, in: P.M. Gault, H.J. Marler (Eds.), Handbook on Cyanobacteria: Biochemistry, Biotechnology and Applications, first ed., Nova Science Publishers, Inc., New York, 2009, pp. 51–103.

[3] Z. Cohen, The chemicals of *Spirulina*, in: A. Vonchak (Ed.), *Spirulina platensis* (Arthrospira): Physiology, Cell-biology and Biotechnology, Taylor & Francis Ltd, UK, 1997, pp. 175–204.

[4] C.V. Lopez, C. Garcia Mdel, F.G. Fernandez, C.S. Bustos, Y. Chisti, J.M. Sevilla, Protein measurements of microalgal and cyanobacterial biomass, Bioresour. Technol. 101 (2010) 7587–7591.

[5] A. Vonchak, Outdoor mass production of *Spirulina*: the basic concept, in: A. Vonchak (Ed.), *Spirulina platensis* (Arthrospira): Physiology, Cell-biology and Biotechnology, Taylor & Francis Ltd, UK, 1997, pp. 79–99.

[6] S. Cheevadhanarak, K. Paithoonrangsarid, P. Prommeenate, W. Kaewngam, A. Musigkain, S. Tragoonrung, S. Tabata, T. Kaneko, J. Chaijaruwanich, D. Sangsrakru, S. Tangphatsornruang, J. Chanprasert, S. Tongsima, K. Kusonmano, W. Jeamton, S. Dulsawat, A. Klanchui, T. Vorapreeda, V. Chumchua, C. Khannapho, C. Thammarongtham, V. Plengvidhya, S. Subudhi, A. Hongsthong, M. Ruengjitchatchawalya, A. Meechai, J. Senachak, M. Tanticharoen, Draft genome sequence of *Arthrospira platensis* C1 (PCC9438), Stand. Genomic Sci. 6 (2012) 43–53.

[7] V.M. Markowitz, I.M. Chen, K. Chu, E. Szeto, K. Palaniappan, Y. Grechkin, A. Ratner, B. Jacob, A. Pati, M. Huntemann, K. Liolios, I. Pagani, I. Anderson, K. Mavromatis, N.N. Ivanova, N.C. Kyrpides, IMG: the integrated metagenome data management and comparative analysis system, Nucleic Acids Res. 40 (2012) D123–D129.

[8] P. Deshnium, K. Paithoonrangsarid, A. Suphatrakul, D. Meesapyodsuk, M. Tanticharoen, S. Cheevadhanarak, Temperature-independent and -dependent expression of desaturase genes in filamentous cyanobacterium *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC 9438), FEMS Microbiol. Lett. 184 (2000) 207–213.

[9] A. Hongsthong, P. Deshnium, K. Paithoonrangsarid, S. Cheevadhanarak, M. Tanticharoen, Differential responses of three acyl-lipid desaturases to immediate temperature reduction occurring in two lipid membranes of *Spirulina platensis* strain C1, J. Biosci. Bioeng. 96 (2003) 519–524.

[10] A. Hongsthong, K. Paithoonrangsarid, P. Prapugrangkul, P. Deshnium, M. Sirijuntarat, S. Subudhi, S. Cheevadhanarak, M. Tanticharoen, The expression of three desaturase genes of *Spirulina platensis* in *Escherichia coli* DH5alpha. Heterologous expression of *Spirulina*-desaturase genes, Mol. Biol. Rep. 31 (2004) 177–189.
[11] P. Kurdrid, S. Subudhi, A. Hongsthong, M. Ruengjitchatchawalya, M. Tanticharoen, Functional expression of *Spirulina*-Delta6 desaturase gene in yeast, *Saccharomyces cerevisiae*, Mol. Biol. Rep. 32 (2005) 215–226.
[12] S. Subudhi, P. Kurdrid, A. Hongsthong, M. Sirijuntarut, S. Cheevadhanarak, M. Tanticharoen, Isolation and functional characterization of *Spirulina* D6D gene promoter: role of a putative GntR transcription factor in transcriptional regulation of D6D gene expression, Biochem. Biophys. Res. Commun. 365 (2008) 643–649.
[13] P. Kurdrid, P. Phuengcharoen, S. Cheevadhanarak, M. Tanticharoen, A. Hongsthong, Identification of a heat shock-responsive cis-acting DNA sequence and its transcriptional regulator: their roles in the expression of the *Spirulina-desD* gene in response to heat stress, J. Biosci. Bioeng. 109 (2010) 205–210.
[14] P. Kurdrid, P. Phuengcharoen, R. Yutthanasirikul, S. Roytrakul, A. Paemanee, S. Cheevadhanarak, A. Hongsthong, Identification of regulatory regions and regulatory protein complexes of the *Spirulina desD* gene under temperature stress conditions: role of thioredoxin as an inactivator of a transcriptional repressor GntR under low-temperature stress, Biochem. Cell Biol. 90 (2012) 621–635.
[15] A. Hongsthong, S. Subudhi, M. Sirijuntarat, S. Cheevadhanarak, Mutation study of conserved amino acid residues of *Spirulina* delta 6-acyl-lipid desaturase showing involvement of histidine 313 in the regioselectivity of the enzyme, Appl. Microbiol. Biotechnol. 66 (2004) 74–84.
[16] J. Senachak, S. Cheevadhanarak, A. Hongsthong, SpirPro: a *Spirulina* proteome database and web-based tools for the analysis of protein-protein interactions at the metabolic level in *Spirulina (Arthrospira) platensis* C1, BMC Bioinf. 16 (2015) 233.
[17] A. Hongsthong, M. Sirijuntarut, P. Prommeenate, K. Lertladaluck, K. Porkaew, S. Cheevadhanarak, M. Tanticharoen, Proteome analysis at the subcellular level of the cyanobacterium *Spirulina platensis* in response to low-temperature stress conditions, FEMS Microbiol. Lett. 288 (2008) 92–101.
[18] A. Hongsthong, M. Sirijuntarut, R. Yutthanasirikul, J. Senachak, P. Kurdrid, S. Cheevadhanarak, M. Tanticharoen, Subcellular proteomic characterization of the high-temperature stress response of the cyanobacterium *Spirulina platensis*, Proteome Sci. 7 (2009) 33.
[19] P. Kurdrid, J. Senachak, M. Sirijuntarut, R. Yutthanasirikul, P. Phuengcharoen, W. Jeamton, S. Roytrakul, S. Cheevadhanarak, A. Hongsthong, Comparative analysis of the *Spirulina platensis* subcellular proteome in response to low- and high-temperature stresses: uncovering cross-talk of signaling components, Proteome Sci. 9 (2011) 39.
[20] H. Nakamoto, D. Honma, Interaction of a small heat shock protein with light-harvesting cyanobacterial phycocyanins under stress conditions, FEBS Lett. 580 (2006) 3029–3034.
[21] D.A. Los, A. Zorina, M. Sinetova, S. Kryazhov, K. Mironov, V.V. Zinchenko, Stress sensors and signal transducers in cyanobacteria, Sensors (Basel) 10 (2010) 2386–2415.
[22] S. Sato, Y. Shimoda, A. Muraki, M. Kohara, Y. Nakamura, S. Tabata, A large-scale protein–protein interaction analysis in *Synechocystis* sp. PCC6803, DNA Res. 14 (2007) 207–216.
[23] F.U. Hartl, A. Bracher, M. Hayer-Hartl, Molecular chaperones in protein folding and proteostasis, Nature 475 (2011) 324–332.
[24] H. Nakamoto, Molecular chaperones and stress tolerance in cyanobacteria, in: A.K. Srivastava, A.N. Rai, B.A. Neilan (Eds.), Stress Biology of Cyanobacteria: Molecular Mechanisms to Cellular Responses, CRC Press, USA, 2013, pp. 113–144.
[25] S. Lee, D.J. Prochaska, F. Fang, S.R. Barnum, A 16.6-kilodalton protein in the cyanobacterium *Synechocystis* sp. PCC 6803 plays a role in the heat shock response, Curr. Microbiol. 37 (1998) 403–407.
[26] N. Tanaka, H. Nakamoto, HtpG is essential for the thermal stress management in cyanobacteria, FEBS Lett. 458 (1999) 117–123.
[27] H. Nakamoto, N. Suzuki, S.K. Roy, Constitutive expression of a small heat-shock protein confers cellular thermotolerance and thermal protection to the photosynthetic apparatus in cyanobacteria, FEBS Lett. 483 (2000) 169–174.
[28] Asadulghani, K. Nitta, Y. Kaneko, K. Kojima, H. Fukuzawa, H. Kosaka, H. Nakamoto, Comparative analysis of the *hspA* mutant and wild-type *Synechocystis* sp. strain PCC 6803 under salt stress: evaluation of the role of *hspA* in salt-stress management, Arch. Microbiol. 182 (2004) 487–497.
[29] K. Sakthivel, T. Watanabe, H. Nakamoto, A small heat-shock protein confers stress tolerance and stabilizes thylakoid membrane proteins in cyanobacteria under oxidative stress, Arch. Microbiol. 191 (2009) 319–328.

[30] S. Sato, M. Ikeuchi, H. Nakamoto, Expression and function of a groEL paralog in the thermophilic cyanobacterium *Thermosynechococcus elongatus* under heat and cold stress, FEBS Lett. 582 (2008) 3389–3395.
[31] M.J. Eriksson, A.K. Clarke, The heat shock protein ClpB mediates the development of thermotolerance in the cyanobacterium *Synechococcus* sp. strain PCC 7942, J. Bacteriol. 178 (1996) 4839–4846.
[32] J. Porankiewicz, A.K. Clarke, Induction of the heat shock protein ClpB affects cold acclimation in the cyanobacterium *Synechococcus* sp. strain PCC 7942, J. Bacteriol. 179 (1997) 5111–5117.
[33] M. Sato, K. Nimura-Matsune, S. Watanabe, T. Chibazakura, H. Yoshikawa, Expression analysis of multiple *dnaK* genes in the cyanobacterium *Synechococcus elongatus* PCC 7942, J. Bacteriol. 189 (2007) 3751–3758.
[34] M.J. Eriksson, J. Schelin, E. Miskiewicz, A.K. Clarke, Novel form of ClpB/HSP100 protein in the cyanobacterium *Synechococcus*, J. Bacteriol. 183 (2001) 7392–7396.
[35] M. Furuki, N. Tanaka, T. Hiyama, H. Nakamoto, Cloning, characterization and functional analysis of *groEL*-like gene from thermophilic cyanobacterium *Synechococcus vulcanus*, which does not form an operon with *groES*, Biochim. Biophys. Acta 1294 (1996) 106–110.
[36] N. Tanaka, T. Hiyama, H. Nakamoto, Cloning, characterization and functional analysis of *groESL* operon from thermophilic cyanobacterium *Synechococcus vulcanus*, Biochim. Biophys. Acta 1343 (1997) 335–348.
[37] S. Huq, K. Sueoka, S. Narumi, F. Arisaka, H. Nakamoto, Comparative biochemical characterization of two GroEL homologs from the cyanobacterium *Synechococcus elongatus* PCC 7942, Biosci. Biotechnol. Biochem. 74 (2010) 2273–2280.
[38] P.A. Lund, Multiple chaperonins in bacteria–why so many? FEMS Microbiol. Rev. 33 (2009) 785–800.
[39] Y. Wang, X. Li, W. Zhang, X. Zhou, Y.Z. Li, The *groEL2* gene, but not *groEL1*, is required for biosynthesis of the secondary metabolite myxovirescin in *Myxococcus xanthus* DK1622, Microbiology 160 (2014) 488–495.
[40] H. Takihara, C. Matsuura, J. Ogihara, N. Iwabuchi, M. Sunairi, *Rhodococcus rhodochrous* ATCC12674 becomes alkane-tolerant upon GroEL2 overexpression and survives in the *n*-octane phase in two phase culture, Microbes Environ. 29 (2014) 431–433.
[41] V. Varvasovszki, A. Glatz, N. Shigapova, K. Josvay, L. Vigh, I. Horvath, Only one *dnaK* homolog, *dnaK2*, is active transcriptionally and is essential in *Synechocystis*, Biochem. Biophys. Res. Commun. 305 (2003) 641–648.
[42] E. Rupprecht, S. Gathmann, E. Fuhrmann, D. Schneider, Three different DnaK proteins are functionally expressed in the cyanobacterium *Synechocystis* sp. PCC 6803, Microbiology 153 (2007) 1828–1841.
[43] Y. Katano, K. Nimura-Matsune, H. Yoshikawa, Involvement of DnaK3, one of the three DnaK proteins of cyanobacterium *Synechococcus* sp. PCC7942, in translational process on the surface of the thylakoid membrane, Biosci. Biotechnol. Biochem. 70 (2006) 1592–1598.
[44] S. Shivaji, J.S. Prakash, How do bacteria sense and respond to low temperature? Arch. Microbiol. 192 (2010) 85–95.
[45] G.K. Pattanayak, C. Phong, M.J. Rust, Rhythms in energy storage control the ability of the cyanobacterial circadian clock to reset, Curr. Biol. 24 (2014) 1934–1938.
[46] K. Forchhammer, The network of P_{II} signalling protein interactions in unicellular cyanobacteria, in: P.C. Hallenbeck (Ed.), Recent Advances in Phototrophic Prokaryotes, Springer, USA, 2010, pp. 71–89.
[47] W. Jeamton, S. Mungpakdee, M. Sirijuntarut, P. Prommeenate, S. Cheevadhanarak, M. Tanticharoen, A. Hongsthong, A combined stress response analysis of *Spirulina platensis* in terms of global differentially expressed proteins, and mRNA levels and stability of fatty acid biosynthesis genes, FEMS Microbiol. Lett. 281 (2008) 121–131.

CHAPTER

4

Nutraceuticals From Algae and Cyanobacteria

A. Udayan[1], M. Arumugam[1], A. Pandey[2]

[1]National Institute for Interdisciplinary Science and Technology (NIIST), Council of Scientific and Industrial Research (CSIR), Trivandrum, Kerala, India; [2]Center of Innovative and Applied Bioprocessing, Mohali, Punjab, India

OUTLINE

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00004-7

1. INTRODUCTION

As global population is increasing, there is an increased demand for sustainable food supplements. Nutraceuticals are nutrients from food or food products that not only supplement the diet, but also facilitate the prevention or treatment of a disease and/or disorder [1]. The term nutraceuticals originated from the root words "nutrition" and "pharmaceuticals." Dr. Stephen L. De Felice defines nutraceutical as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease." The major difference between pharmaceuticals and nutraceuticals is that the former is used as a drug to treat diseases and the latter as nutritional supplements that are intended to prevent diseases. Considering the current lifestyle and foodborne illness, the consumer trend has skewed toward health-promoting substances like nutraceuticals and organic food. As estimated by BCC research the global nutraceutical market was about $160.6 billion in 2013 and is estimated to grow $241.1 billion by 2019 with an estimated compound growth rate (CAGR) of 7% from 2014 to 2019 (BCC research: www.bccresearch.com).

Microalgae have been studied for decades, but in recent years a new wave of research has started as part of the search for renewable and sustainable energy sources [2–4]. The algal biomass can be cultivated in large scale using commercially available growth components like urea [5,6] and inexpensive growth media [7], to translate this potential technology commercially viable in large scale open pond cultivation. Recent reports also revealed that the microalga lipid can be enhanced adopting nitrogen starvation [4,7–9]. Microalgae are unicellular to multicellular organisms that can grow up to 60 m, one of the largest and most poorly understood group of organisms on the planet Earth. Moreover, marine microalgae, the most abundant group of living organism in the ocean, become the primary producer for many aquatic organisms [10–12]. These biological cell factories are the natural nutritional base and primary source of the aquatic food chain [10]. Algal biomass has been used for centuries as food and medicine. The health-promoting effects of algae were discovered as early as 1500 BC [13]. However, the biomass of algae gained interest as a source of chemicals and pharmaceuticals only recently. An alga contains a wide range of nutrients including proteins; lipids; carbohydrates; and trace nutrients, including vitamins, antioxidants, and trace elements [14]. These compounds have the characteristics to serve as natural nutritional supplements in human and animal feed and have many health-promoting effects. Fish meal is the protein source primarily constituted by products from processed fish traditionally used in aquaculture diets, but its increasing demand makes it limited and expensive. So, in order to overcome this problem, nutritional contents of algae are rapidly gaining importance as a renewable source to substitute the conventional ingredients in the human diet or animal feed [15].

Microalgae ensure certain exceptional advantages over other organisms, that they are photoautotrophs and therefore do not require organic substances for energy; consequently, their large-scale culture is theoretically simpler and cheaper. Solar radiation, water, CO_2, and inorganic nutrients are the basic requirement for algal growth. Furthermore, many algae also grown in saline to hypersaline waters, and thus do not compete with conventional agriculture for limited resources such as fresh water and arable land. Furthermore, algae micronutrients are immediately bioavailable and more easily digested, owing to minuscule of algal

cells that can be readily absorbed by the digestive system [16]. These distinct parameters make microalgae as a potential source for the large-scale production of nutraceuticals in an economically viable manner.

Different types of algae, specifically microalgae that could become more prevalent in food supplements and nutraceuticals are *Spirulina*, *Chlorella*, *Haematococcus*, *Nostoc*, *Botryococcus*, *Anabaena*, *Chlamydomonas*, *Scenedesmus*, *Synechococcus*, *Parietochloris*, *Crypthecodinium*, *Porphyridium*, etc. due to the capability of producing necessary vitamins. In addition, these edible microalgae are rich source of major (phosphorus, sodium, sulfur, nitrogen, magnesium, and calcium), minor, and trace elements (zinc, iodine, manganese, copper, selenium, cobalt, and molybdenum). Algae are also high producers of essential amino acids, omega-3 [docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA)], and omega-6 (arachidonic acid) fatty acids. Due to a wide range of beneficial compounds and nutritive contents, the market for increased algae production for nutraceuticals is getting more attention and it is more profitable [15,17,18].

Algae serve as a viable source for combating numerous diseases and nutrient deficiencies experienced by people around the world. Very recently, the four most prevalent global diseases such as malnutrition, nutritional anemia, xerophthalmia (vitamin A deficiency), and endemic goiter (iodine deficiency) were reported [16]. These nutrient deficiencies can be remediated by just a single tablespoon of appropriate edible algae per day. Consumption of algae promotes regular healthy functioning of major body systems, including the immune system, humoral system, cardiovascular system, respiratory system, and nervous system [19,20]. In addition to these major functions, algae have been used as a sun protectant, a moisturizing agent, and as a treatment for wounds, burns, and bruises, due to its antiinflammatory properties. Algae offer a number of possibilities for enhancing health and wellbeing of human and animals. Recent research has even suggested algae as a therapeutic solution for a number of serious conditions, including diabetes, heart disease, autoimmune diseases, cancer, and cognitive decline, in the form of dementia and Alzheimer disease [21,22], considering the above said fact that alga offers attractive health benefits in a commercially viable manner. The present chapter discusses the nature of nutraceuticals, bioactive compounds, and its health benefits in a comprehensive manner with appropriate illustrations.

2. NUTRACEUTICALS AND FUNCTIONAL FOOD FROM ALGAE

2.1 Polyunsaturated Fatty Acids

Fatty acids play a vital role in metabolism, as an essential component of all the organelle membranes and as a regulatory molecule. Polyunsaturated fatty acids (PUFAs) are essential dietary lipids that are the major precursor molecules for active metabolism. Marine microalgae are potential sources of long-chain polyunsaturated fatty acids. PUFAs are fatty acids that contain more than one double bond in their backbone. PUFAs are mainly divided into two categories, i.e., omega-3 fatty acids and omega-6 fatty acids. These two are come under the group of methylene-interrupted polyenes in which two or more cis double bonds are present that are separated by a single methylene bridge.

2.1.1 Omega-3 Fatty Acids

In omega-3fatty acids, the first double bond may be found between the third and fourth carbon atom from the omega carbon. These fatty acids are important dietary fats. Although, humans and other mammals can synthesize saturated fatty acids and some monounsaturated fatty acids from carbon groups in carbohydrates and proteins, they lack the necessary enzymes to insert a cis double bond at the n-6 or the n-3 position of a fatty acid. Humans and animals lack the enzymes necessary for the synthesis of PUFAs more than 18 carbon atoms. The conventional source of such omega-3 fatty acids is fish oil; however, fish do not synthesize these fatty acids but, acquire them through their diet, which consists of marine microalgae. Fish consumption has several disadvantages like danger from contaminants like mercury and the rapid decline in fish stocks. Moreover, microalgae have several advantages over fish. They can be grown in controlled environments that eliminate the risk of contamination from chemical pollutants. Omega-3 fatty acids are the major components of cell membranes and important in antiinflammatory processes and viscosity of cell membranes. Mainly three types of omega-3 fatty acids involved in human health and physiology are α-linolenic acid (ALA) (found in vegetable oils), EPA,and DHA (both commonly found in marine oils) (Fig. 4.1). Among these, DHA and EPA are commercially important algal omega-3 fatty acids.

2.1.2 Chemistry of Omega-3 Fatty Acids

Alpha-linolenic acid (ALA) is a carboxylic acid with an 18-carbon chain and three *cis* double bonds. ALA is the most common omega-3 fatty acid in the western diet. It comes from plants, and is found in vegetable oils, primarily flaxseed, walnut, canola, and soybean oils. It is an essential fatty acid, but human body cannot synthesize it, thus needs to be supplemented in the diet. ALA is the precursor for two nutritionally important fatty acids, namely DHA and EPA. In chemical structure, EPA is a carboxylic acid with a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega end (Table 4.1). It also has the trivial name of timnodonic acid. It serves as precursor for prostaglandin-3 and thromboxane-3 families. The major source of EPA in humans is fish or algae. The richest reported sources of algae are *Phaeodactylum tricornutum*, *N. oculata*,

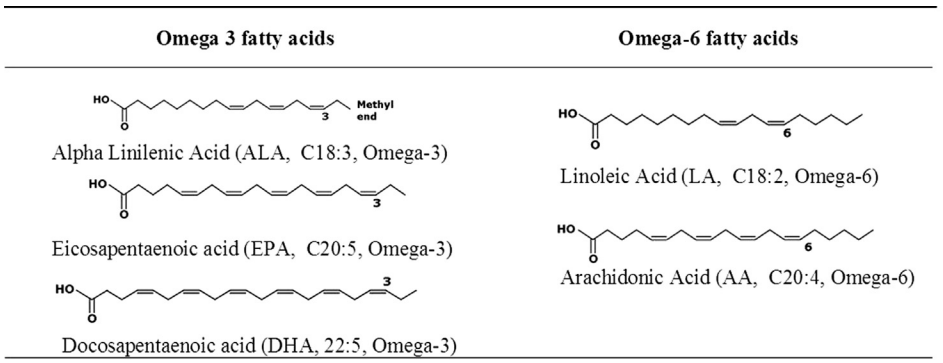


FIGURE 4.1 Chemical structure of omega 3 and omega 6 fatty acids.

TABLE 4.1 Biochemical Properties and Nutritional Applications of Omega 3 Fatty Acids

Common Name	Chemical Name	Shorthand Notation	Major Source	Potential Applications
α-Linolenic acid (ALA)	*all*-cis9,12,15-octadecatrienoic acid	C18:3, n-3	*Arthrospira*	Infant formulas for full-term infants, nutritional supplements
Eicosapentaenoic acid (EPA)	*all-cis*-5,8,11,14,17-eicosapentaenoic acid	C20:5, n-3	*Nannochloropsis, Phaeodactylum, Nitzschia*	Nutritional supplements, aquaculture
Docosahexaenoic acid (DHA)	*all-cis*-4,7,10,13,16,19-docosahexaenoic acid	C22:6, n-3	*Crypthecodinium, Schizochytrium*	Infant formulas for full-term/preterm infants. Nutritional supplements, aquaculture

M. subterraneus. Depending upon cultivation conditions the proportion of EPA varies in different species. Pure EPA supplementation in the ethyl ester form always has a greater effect than fish oil because of the easier absorption of the ethyl ester through the intestinal wall without requiring lipase. DHA (22: 6) is a 22-carbon chain polyunsaturated omega-3 fatty acid. DHA is essential for the growth and functional development of the brain in infants and adults. More specifically, it is a major structural lipid in the brain and the retina of the eye and is a key component of the heart. In humans DHA is biosynthesized by the conversion of EPA to docosapentaenoic acid, subsequently converted to DHA.

2.1.3 Health Benefits of Omega-3 Fatty Acids

Omega-3 fatty acids have a major role in the diet as well as prevention of major diseases like cardiovascular diseases. These omega-3 fatty acids protect the heart by decreasing arrhythmias, blood clot formation, blood triglycerides, growth rate of atherosclerotic build-up, as well as improve the function of arteries. Adequate supplementation of DHA and EPA in the diet is known to reduce blood pressure. ALA also appears to have a protective effect for the heart. In populations with low levels of fish consumption, higher ALA intake is associated with a reduced risk of heart disease [23]. DHA and EPA have antiinflammatory properties and are effectively used to treat the inflammatory bowel disease, eczema, psoriasis, and rheumatoid arthritis [24]. The antiinflammatory and cardio protective functions of n-3 omega PUFAs improve the immune system. Thus, immune system–compromised diseases like HIV/AIDS requires DHA- and EPA-enriched nutrients to strengthen the immune system for long-term survival [25].

In diabetic patients, plasma glucose and low-density lipoproteins (LDL) level may be high and high-density lipoproteins level may be very low. Omega-3 PUFAs significantly decreases LDL levels with plasma triglyceride [26]. DHA and EPA are also used in the treatment of gastrointestinal diseases and also help to decrease platelet aggregation [27]. Increased concentrations of long-chain fatty acids and EPA (major proportions of n-3 omega PUFAs) reported to have a protective role against colorectal cancer [28]. Dietary supplementation of n-3 PUFAs has been shown to produce beneficial effects in patients with pancreatic cancer, such as the suppression of tumor formation [29] and reducing weight loss [30].

Deficiency in EPA during childhood related to development of mental health issues like depression, heart problems, joint and bone conditions, and neurodegenerative diseases like Parkinson disease. The DHA level is significantly reduced after the development of brain and CNS (after the age of five). This is a good time to increase EPA in the diet, as studies show that EPA can help with childhood behavior and academic performance, like focus, attention, and reducing aggression. Dry skin conditions, asthma, and allergies are also common in children, and good levels of EPA at this time can help to reduce the inflammation. EPA also protects our genes and cell cycle as well as helps to keep our stress response regulated, thus, an adequate supply of EPA in day-to-day diet prevents a range of chronic illness. DHA is an essential component of the retina. Omega-3 fatty acids also prevent eye diseases like age-related macular degeneration, an illness associated with progressive degeneration of the macula (needed for sharp, central vision), a small spot near the center of the retina. Alzheimer disease is the most common cause of dementia in elderly adults. Research findings suggest that lower DHA levels are also associated with Alzheimer, DHA perhaps inhibit the progression of the disease. Moreover, DHA inhibits the formation of amyloid plaques and decreases the chances of development of Alzheimer diseases (Fig. 4.2).

2.1.4 *Algal Sources of Omega-3 Fatty Acids*

Algae are the primary producers of aquatic ecosystem and are rich in omega-3 fatty acids that are subsequently consumed by other marine life forms. DHA-rich algal oil is derived from the heterotrophic fermentation of the marine alga, *Schizochytrium* sp. The *Schizochytrium* sp. neither produces any toxic chemicals nor it is pathogenic, thus it is commercially exploited for the production of omega-3 fatty acids. Among the other marine microalgae, *Crypthecodinium cohnii* is the prolific producer of DHA. Since it produces only DHA, and no other PUFAs in its lipid content make easy separation of DHA. Successful cultivation of *C. cohnii* for commercial edible oil containing DHA has been achieved by Martek Corporation in Maryland, USA. Martek has developed a GMP process utilizing a wild-type *C. cohnii* growing on glucose as the main carbon source to produce single cell oil (DHASCO) with a DHA

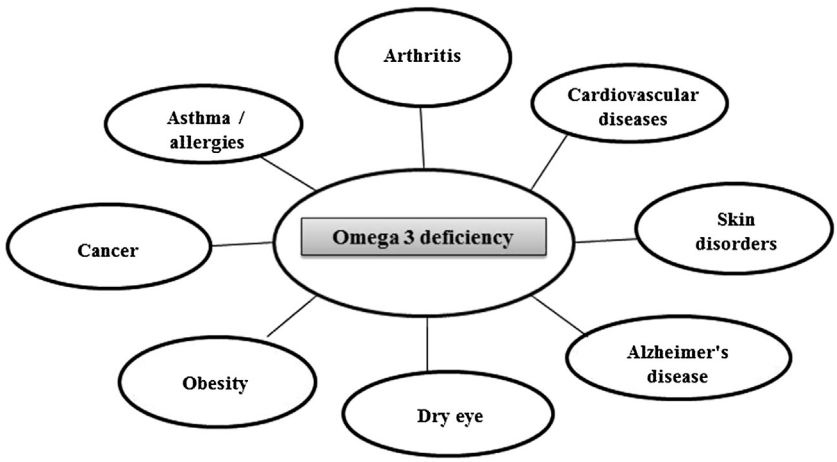


FIGURE 4.2 Omega 3 fatty acid deficiency.

enrichment of above 40% [31]. Single cell edible oil containing DHA can be used in infant formulas and baby foods, pharmaceutical products, and dietary supplements. As *C. cohnii* is a photosynthetic protists, but can be cultured in heterotophic mode as well, it has the potential as model organism (genetic and biochemical tool) for the characterization of the enzymes involved in the production of 22:6ω-3. *C. cohnii* possesses all the enzymes necessary for *de-novo* synthesis of 22:6ω-3, thus, becoming an ideal candidate for engineering of the fatty acid elongation and saturation metabolic pathways [32]. *Nannochloropsis* is considered as a promising alga for industrial applications because of its ability to accumulate high levels of PUFA. It is used as both food for humans and animals. The EPA content of *Nannochloropsis* is highly influenced by the culturing conditions. A diatom, *Phaeodactylum tricornutum*, naturally accumulates high levels of EPA (but trace amount of DHA) and is considered as a good source for large-scale commercial production.

2.2 Omega-6 Fatty Acids

Omega-6 fatty acids are PUFAs where the first double bond is present at the sixth carbon (Table 4.2 and Fig. 4.1). These are important fatty acids for human health. But, humans cannot synthesize omega-6 fatty acids in their body. Omega-6 fatty acids have an important role in human growth and development. These have important role in skin and hair growth, maintaining bone health, regulating metabolism, and maintaining reproductive system. But, some omega-6 fatty acids have an adverse effect on health, hence they promote inflammation, but not all promote inflammation. Most omega-6 fatty acids come from vegetable oils. Omega-6 fatty acids help to reduce diabetic neuropathy, allergy, rheumatoid arthritis, breast cancer, high blood pressure, multiple sclerosis, and osteoporosis.

2.3 Algal Pigments and Its Nutraceutical Values

Carotenoids are natural pigments widely distributed in nature and occur extensively in plants, animals, and microorganisms. Carotenoids are synthesized principally by plants and microalgae (Figs. 4.3 and 4.4). Animals, including humans are not able to synthesize carotenoids themselves and they obtain these pigments from plants and microalgae. Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. The word "carotene" was suggested by Wachenroder (1831) for the hydrocarbon pigment he had crystallized

TABLE 4.2 Different Types of Omega 6 Fatty Acids

Common Name	Lipid Name	Chemical Name	Major Health Benefits of Omega 6 Fatty Acids
Linoleic acid	18:2,n-6	9,12-Octadecadienoic acid	Antiinflammatory
Gamma linoleic acid	18:3,n-6	6,9,12-Octadecatrienoic acid	Cardiac defense Combats diabetes and obesity
Eicosadienoic acid	20:2,n-6	11,14-Eicosadienoic acid	Potent anticancer activity
Arachidonic acid	20:4,n-6	5,8,11,14-Eicosatetraenoic acid	Prevents skin diseases

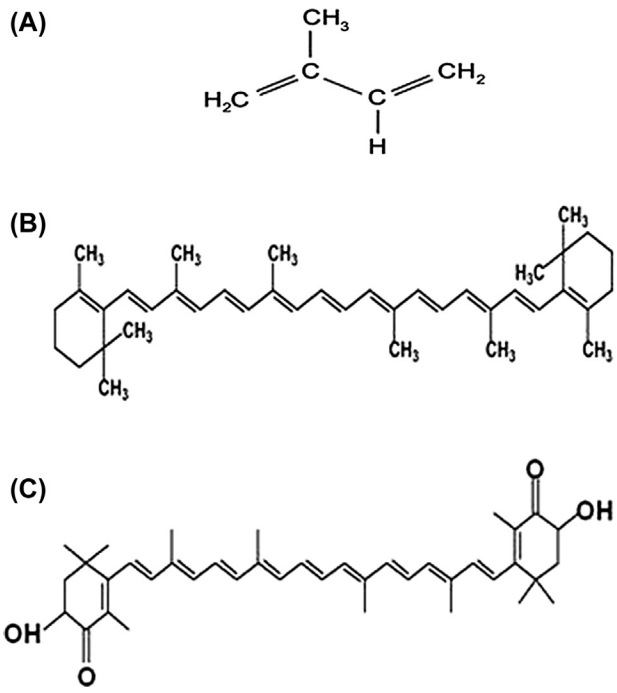


FIGURE 4.3 The structure of isoprene unit (A), β-carotene (B), and Asthaxanthin (C).

from carrot roots. The global carotenoid market is rising every year and will reach US$1.2 billion by 2015 because of the awareness of peoples about the health benefits of various carotenoids (Global Industry Analysts, Inc. http://www.strategy.com/Carotenoids Market Report.asp/). As per the latest predictions, it was projected that the carotenoid demand in the US will reach a target of US$1.3 billion in 2017 (http://www.prweb.com/pdfdownload/8849957.pdf). According to latest reports, US markets tend to reach US$1.3 billion by 2017. In many markets, microalgal carotenoids are in competition with a synthetic form of carotenoids because microalgal carotenoids have the advantage of supplying natural isomers in their natural ratio [33].

2.3.1 *Chemistry of Algal Carotenoids*

The basic structure of carotenoid is a symmetrical tetraterpene formed by tail to tail linkage of two C_{20} units. All carotenoids are derivatives of $C_{40}H_{56}$, a long acyclic hydrocarbon chain with conjugated double bonds. This structure can be considered as chemically identical to lycopene. This base structure (Fig. 4.3A) can be chemically altered by different chemical processes such as oxidation, cyclization, hydrogenation, and dehydrogenation or any combination of these to make a huge variety of carotenoids. This forty carbon polyene chain serves as a molecular backbone that provides different molecular structures to carotenoids, which are involved in the chemical properties like light absorption features and for the existence in the presence of oxygen [34]. Carotenoids can be classified into different groups on the basis of the criteria used. Based on the basic chemical structure and the oxygen presence, carotenoids are classified into two types: carotenes or carotenoid hydrocarbons, composed of carbon and

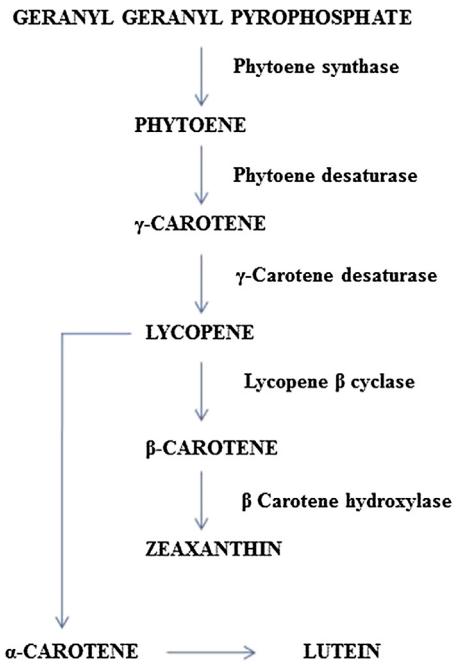


FIGURE 4.4 Biosynthetic pathway of carotenoids.

hydrogen only, and xanthophylls or oxygenated carotenoids, which are oxygenated and may contain epoxy, carbonyl, hydroxyl, methoxy, or carboxylic acid functional groups [35]. Lycopene and β-carotene are examples of carotene carotenoids and lutein, canthaxanthin, zeaxanthin, violaxanthin, capsorubin, and astaxanthin are xanthophyll carotenoids [36].

Basically, carotenoids are hydrophobic and soluble only in organic solvents because of their limited solubility in water. Carotenoids are very sensitive to high temperatures. The addition of hydroxyl groups to the end groups makes them more polar. One of the most characteristic features of carotenoids is their strong coloration, which is a consequence of light absorption due to the presence of an extensive system of conjugated double bonds. The presence of these conjugated double bonds is important for the proper functioning of carotenoids, especially in light absorption in photosynthetic organisms and photoreception in all living organisms [37].

In algae and higher plants carotenoids have important role in photosynthesis. They are important components in light harvesting complexes, scavenging the reactive oxygen species, and dissipating excess energy [38]. Oxygen is very important for humans, although prooxidants like ROS can damage biologically important properties of different molecules such as DNA, protein, carbohydrates, and lipids. Carotenoids are important in the scavenging of reactive oxygen species formed as a result of this damage. The efficacy of carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule, which determines their lowest triplet energy level. The powerful antioxidative properties make carotenoids a class of important nutrients in health promotion. In animals and humans, these compounds are precursors of vitamin A (pro-vitamin A activity) and

retinoid compounds required for morphogenesis and embryonic development [39]. Carotenoids have an important role in food and feed industry and they are mainly used as natural colorants in food products and cosmetics, as vitamin supplements and health food products, and as feed additives for poultry, livestock, fish, and crustaceans. In addition to these, carotenoids have intrinsic antiinflammatory properties due to their quenching action on reactive oxygen species and also have anticancer effects.

2.3.2 *Microalgae as Source of Carotenoids*

Microalgae serve as an outstanding natural source of carotenoids. Microalgae have several advantages over higher plants and other microorganism. Compared with higher plants, algae can be cultivated in a controlled system like bioreactors for obtaining continuous culturing in contamination free manner. Another advantage is that the growth of microalgae on photo bioreactors is not dependent on season and weather so that a homogenous biomass can be obtained throughout the year. Production of carotenoids has been one of the most successful activities in microalgal biotechnology achieving great commercial success. Carotenoids accumulated in microalgae can be classified into two types, primary and secondary ones. Primary carotenoids (Lutein and β-carotene) act as the major functional components of the cellular photosynthetic apparatus and therefore they are essential for survival. Secondary carotenoids accumulate only after exposure to specific environmental stimuli like caratenogen or UV light or any extreme stress [40]. If the amount of primary carotenoids is not sufficient, secondary carotenoids, e.g., astaxanthin, canthaxanthin, and adonixanthin will accumulate out of thylakoid membrane to protect cells against oxidative damage [41].

Several microalgal strains have been reported with carotenoid producing activities such as β-carotene from *Dunaliella salina* [41,42], zeaxanthin from *Synechocystis* sp. [42], lutein from *Chlorella protothecoides* [43], astaxanthin from *Haematococcus pluvialis* [44], and *Chlorella zofingiensis* [45]. Some of the most important carotenoids in terms of biotechnological and biomedical uses explored so far are: Astaxanthin, β-Carotene, Canthaxanthin, β-Cryptoxanthin, Fucoxanthin, Lycopene, Lutein, Zeaxanthin, and Violaxanthin. Among these β-Carotene, Astaxanthin, Lutein, and Canthaxanthin are the major carotenoids with commercial interest (Table 4.3).

2.3.2.1 β-CAROTENE

β-Carotene is an important fat soluble primary pigment in the carotenoid family. β-Carotene is a natural carotenoid that converts to vitamin A in the body. It is a pigment with increasing demand in markets. It occurs as orange-colored carbon-hydrogen carotenoid, abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables. It is also the most widely distributed carotenoid in foods. β-Carotene was first isolated by Wackenroder in 1831. The structure of β-carotene was elucidated by Karrer in 1931 and this was the first time that the structure of any vitamin or provitamin had been established. For this work Karrer got the Nobel Prize (Fig. 4.3B). The first total synthesis of β-carotene was achieved in 1950 and in 1954 Roche started its commercial production. It is widely used as a food coloring agent, provitamin A (retinol) in food and animal feed, additive to cosmetics and multivitamin preparations, and as a health food product under the antioxidant category.

Because of limited supply from natural sources, several companies are interested in synthetically produced carotenoids to meet the high demands in the global market. *Dunaliella*

TABLE 4.3 Commercially Important Microalga Carotenoids and Their Health Benefits

Carotenoid	Microalgae	Main Effect	References
β-Carotene	*Dunaliellasalina*	Reduced plasma cholesterol and atherogenesis, reduced fat accumulation and inflammation in liver.	[46]
	Dunaliellabardawil		[47]
Astaxanthin	*Haematococcuspluvialis*	Prevent obesity and fatty liver disease.	[48,49]
	Chlorella zofingiensis		
Zeaxanthin	*Dunaliellasalina*	Vision and antioxidant protection of body	[50,51]
	Microcystis aeruginosa		
	Nannochloropsis		
Lutein	*Chlorella zofingiensis*	Protective antioxidative effect	[31,52]
	Chlorella protothecoides		
	Muriellopsissp		

is the most potent producer of β-carotene. *Dunaliella* is a genus of unicellular and motile green alga commonly found in marine water. It stores secondary carotenoids (mostly β-carotene) in the chloroplast as lipid globules emitting red fluorescence originating from the chlorophyll in the thylakoid membrane. During stress conditions, the red fluorescence from chlorophyll partly disappears as the thylakoid membranes are broken down. During this time the cells start to produce carotenoid globules and green fluorescence appears simultaneously [52]. Natural β-carotene has several advantages over the synthetic entity in the presence of two isomers, the trans-isomer and the 9-cis isomer. Synthetic β-carotene contains only the fully trans-isomer, which has lower liposolubility and antioxidant activity than the 9-cis isomer, which is found exclusively in the natural environment.

Dunaliella sp. occurs in the oceans; as they are halophilic organisms they also occur in brine lakes, salt marshes, salt lagoon, and salt water ditches near sea, predominantly in water bodies containing more than 2 M salt and high level of magnesium. *Dunaliella* sp. is recognized as being the most halotolerant eukaryotic photosynthetic organism, showing a remarkable degree of adaptation to a variety of salt concentrations from as low as 0.1 M to salt saturation about 4 M [53]. The high-quantity production of β-carotene by *Dunaliella* is inversely proportional to high salinity and nutrient limited conditions. According to the reports of global markets for carotenoids, β-carotene has the largest share of the market. Valued at $247 million in 2007, this segment is expected to be worth $285 million by 2015, a CAGR of 1.8% (http://www.companiesandmarkets.com/Market-Report/the-global-market-for-carotenoids-696137.asp) (Table 4.4).

Use of *Dunaliella* as a nutraceutical positively influences the intracellular communication and immune response [54]. Several studies have reported the role of β-carotene in preventing different types of cancer like pancreas, lungs, stomach, cervix, colon, rectum, ovaries, prostrate, and breast by its antioxidant activity. Daily dose of β-carotene can reduce asthma induced as a result of exercise [55]. Age-related macular degeneration is one of the most

TABLE 4.4 Companies Involved in Algal Beta Carotene Production

Company	Location
Cognis nutrition and health	Australia
Cyanotech	Hawaii, USA
Aquacarotene	USA
Tianjin Lantai biotechnology	China
Parry Nutraceuticals	India
Nikken Sohonsha Corporation	Japan
Seambiotic	Israel
Muradel	Australia

common eye diseases causing severe or permanent loss of vision; an antioxidant therapy using combinations of high-dosage antioxidant vitamins C, E, β-carotene, and zinc is employed [56]. The price for natural β-carotene ranges from US$300 to US$3000 per kilogram. Naturally produced purified β-carotene is usually accompanied with other *Dunaliella* sp. carotenoids mix, which includes lutein, neoxanthin, zeaxanthin, violaxanthin, cryptoxanthin, and α-carotene. Variety of natural β-carotene can be found for sale as health food and supplement under vitamin section. In powder form, natural β-carotene is used for colorization and provitamin A for animal (cattle and poultry) and aquaculture (shrimp and fish) feed [53]. Even though natural β-carotene from *Dunaliella* has several advantages, more than 90% of commercialized β-carotene is produced synthetically. But natural β-carotene shows higher bioavailability compared to the synthetic analogue of β-carotene [57]. The production of antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase is significantly greater in β-carotene from *Dunaliella* and there are no risks associated with consumption of supplements containing this alga.

2.3.2.2 ASTAXANTHIN

Astaxanthin is a reddish pigment that belongs to a group of chemicals called carotenoids. It is a symmetric molecule consisting of a short ring of polyene terminated by rings. The keto (O) and hydroxyl group present helps to distinguish it from other carotenoids such as β-carotene (Fig. 4.3C). It is widely used in aquaculture industry as a pigmentation source. Astaxanthin is synthesized naturally by microalgae or phytoplankton. It is produced in the cytoplasm of these organisms. Zooplankton and other marine microorganisms consume these microalgae and as a result astaxanthin will accumulate in their body and elicit pinkish-red color in the flesh of these fishes. This pigment has been considered as super vitamin E due to its natural antioxidant activities.

Natural astaxanthin can be produced by *Haematococcus* microalgae, *Chlorella zofingiensis*, *Chlorococcum* sp., and the yeast *Xanthophyllomyces dendrorhous* [34,58–60]. Fatty acids are esterified to the 3 or 3′ hydroxyl groups resulting in mono and diesters of astaxanthin. This esterification increases its solubility and makes it more stable to oxidation. Synthetic

astaxanthin is in free form; algae-produced astaxanthin is a mixture of mono and diesters. Normally, astaxanthin is a red carotenoid pigment, but when it forms complexes with various proteins, its light absorbance property changes and causes crustaceans to range in color from green, yellow, and blue to brownish. When these crustaceans cooked red color is produced because these proteins will denature on cooking and will release astaxanthin from their protein group. Animals cannot synthesize astaxanthin and they get this pigment through diet. The ability to synthesize astaxanthin or converting dietary astaxanthin into vitamin A is absent in mammals. Unlike β-carotene, astaxanthin has no provitamin A activity in these animals [61]. Natural sources of astaxanthin are numerous, but astaxanthin is present in very low concentrations. The prominent source of astaxanthin is the green alga *Haematococcus pluvialis*, which is also referred as *Haematococcus lacustris* or *Sphaerella lacustris* belongs to Haematococcaceae family.

Haematococcus is a motile green alga that occurs in nature worldwide, which utilizes the available phosphate, nitrate, and other nutrients to grow and reproduce. This alga has different forms during its life cycle. Massive amounts of astaxanthin are produced by the algae when the cells undergo a dormant stage until the next influx of water and nutrients. When the algae are stressed due to low nutrients or other unfavorable environmental conditions, spores will form, which rapidly accumulate astaxanthin. It is thought that during unfavorable environmental conditions, *Haematococcus* is exposed to harsh ultraviolet rays and produces astaxanthin as a protective barrier. The astaxanthin level in *Haematococcus* is about 1.5−3% of its dry weight [62].

Astaxanthin has many important applications and it is widely used in neutraceuticals, cosmetics, food, and feed industries. Astaxanthin is a potent antioxidant and its antioxidant activity is more than 10 times stronger than β-carotene and close to 1000 times more effective than vitamin E. A report showed that *Haematococcus pluvialis* especially in dose of 3 g/kg feed administration may effectively enhance the antioxidant system and some biochemical parameters in rainbow trout [63]. The antioxidant properties of astaxanthin have a key role in several other properties such as protection against UV-radiation, photo oxidation, inflammation, cancer, ulcer, *Helicobacter pylori* infection, aging, and age-related ailments, or the promotion of the immune response, liver function and heart, eye, joints, and prostate health [63].

In Japan, the international research center for traditional medicine investigated the effects of astaxanthin in hypertensive (i.e., high blood pressure) rats. They found that after 14 days, astaxanthin provided significant reduction in the arterial blood pressure. After five weeks, astaxanthin significantly reduced blood pressure in stroke-prone rats [64]. The researchers suggested that astaxanthin can exert beneficial effects in protection against hypertension and stroke, and in improving memory in vascular dementia. Astaxanthin is essential for cellular health and they also mediate gene expression and cell to cell communication (Fig. 4.5). The price for astaxanthin varies in the market and depends on the percentage contents in algal source; however, the overall maintained price of 5% astaxanthin is about US$1900 kg^{-1} (http://www.herbridge.com/bencandy.php). Companies that involved in the production of astaxanthin from *Haematococcus* involve Cynotech Corporation, Parry Nutraceuticals, Fuji Health Science, Bioreal, Aqua Search Inc, Valensa International, and Alga Technologies. To date, there is no report on the negative consequences of *Haematococcus* as a source of astaxanthin as a direct dietary supplement.

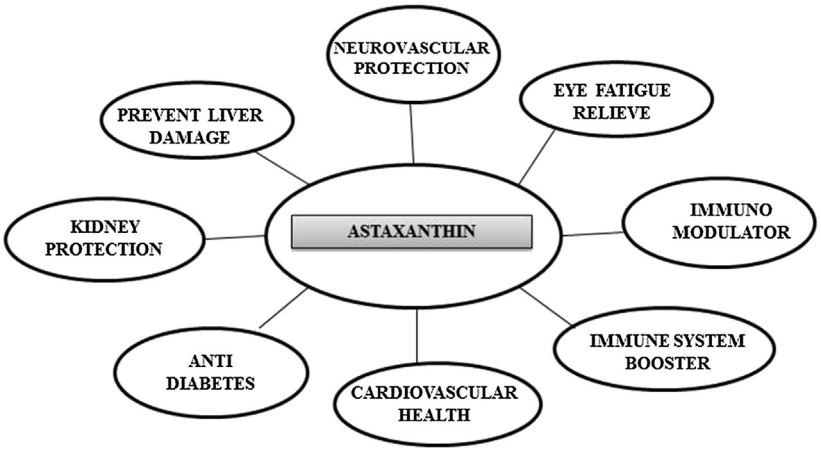


FIGURE 4.5 Health benefits of astaxanthin.

2.3.2.3 LUTEIN

Lutein belongs to the xanthophyll family of carotenoids and is one of the major components of the macular pigment of the retina [65]. These are synthesized within dark green leafy plants such as spinach and in algae. It is a yellow pigment and humans cannot synthesize this pigment and depends entirely on dietary sources such as leafy vegetables or algae. Lutein is mainly used for the natural coloration of foods, drugs, and cosmetics and it is also widely used for the pigmentation of animal tissues and products.

Lutein is an oxycarotenoid with one beta and one epsilon ionone ring end groups. It contains extended conjugated double bond system. The backbone polyene chain exists in two confirmations, either a cis or trans-confirmation (Fig. 4.6). Among these two confirmations trans form is more predominant. These two confirmations give rise to different mono-cis and poly-cis lutein isomers. During stress conditions the geometric isomerization converts all-trans isomers into a cis configuration. All-trans isomer of lutein is present in the lens and macular region of retina. The bioavailability of all-trans lutein is higher than that of cis-luteins in human body [66,67]; therefore, commercial production of lutein mainly focuses on all-trans lutein content.

The free hydroxyl groups at the two ends of lutein reacts with fatty acids and produces lutein mono-ester or lutein di-ester. Esterification helps to increase the bioavailability of lutein and it had no effects on antioxidant activity of lutein [68,69]. Global lutein market in 2004 accounted for US$139 million and was considered as the fastest projected growth in

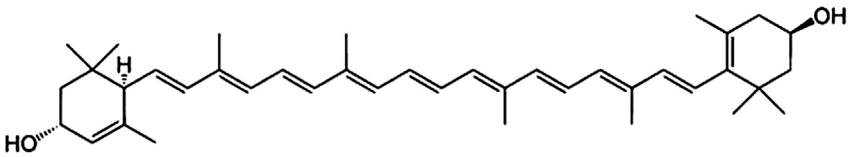


FIGURE 4.6 Structure of lutein.

individual carotenoids sales [70]. The market value of lutein in 2010 was approximately US$233 million and is expected to reach US$309 million by 2018 with a compound annual growth rate of 3.6% (http://www.companiesandmarkets.com/). French marigold (*Tagetes patula*) is currently the most widely applied source for lutein production in the world. In the USA, two lutein-containing products, Aztec Marigold and Tagetes have been successfully commercialized.

Marigold is the major producer of lutein. Three different varieties used for the production of lutein are Marigold orange, Marigold yellow, and Marigold red. Among these, Marigold orange variety has high content of lutein [71]. But, one disadvantage is the harvesting of this Marigold flower is a labor intensive process. Another disadvantage is that the mass cultivation of Marigold requires a large land area and is influenced by weather changes. To overcome these problems, microalgae can be used as a potential source of lutein. Several microalgae are a rich source of lutein. The microalgae biomass from Chlorophycean is also considered as the major source of lutein, especially *Chlorella* sp., *Scenedesmus* sp., and *Muriellopsis* sp. Nevertheless, there is no established commercial cultivation system for the production of lutein from microalgae. Studies showed that heterotrophically cultivated *Chlorella* accumulates high content of lutein [72] and makes it a suitable source for commercial production of lutein.

Lutein is the major component in the retina of eye and it has an important role in eye health. The macular region of the primate retina is yellow in color due to the presence of the macular pigment, composed of two dietary xanthophylls, lutein and zeaxanthin. Age-related macular degeneration (AMD) is an important cause of blindness. In AMD the pigmented epithelium and photoreceptors are mainly affected. It was found that individuals with the highest intake of lutein/zeaxanthin (6 mg daily) showed 57% decreased risk for AMD compared to those who consumed the lowest level of 0.5 mg daily [73]. Lutein and zeaxanthin are the only two carotenoids that have been identified in the human crystalline lens. Numerous studies found that increased consumption of foods rich in lutein/zeaxanthin decreases the chances of cataract in men and women. The antioxidant activity of lutein helps to scavenge super oxide radicals, free hydroxyl radicals, and inhibits lipid peroxidation. It has a role in cardiovascular diseases.

Lutein deposited in many body areas is prone to free radical damage such as eyes and skin. The only way to capitalize on lutein's antioxidant benefits is to consume it or, for skin care products, to apply it on the skin. Commercial lutein is sold as granules, powders, capsules, or oleoresin with 3–80% w/w lutein content. Products with 5–20% lutein is used as fodder additives while others are used in food industry as additives or further processed into health products.

2.4 Chlorophyll Pigments as Natural Colorant

Chlorophyll molecules are the important energy producers of photosynthesis. They are group of tetrapyrrolic compounds with common structural elements and function. Chemically, they are cyclic tetrapyrroles of the porphyrin, chlorin, or bacteriochlorin oxidation state. These are the most important components of chloroplast lamellae. The name chlorophyll was first coined by Pelletier and Caventou in 1818 but the chemical structure describing chlorophyll as magnesium complexes was obtained by Willstatter and Stoll in 1913.

Chlorophyll is an important natural coloring agent used in the food industry. But it has some disadvantages. Chlorophyll in its natural environment that is inside the chloroplast is conjugated with phospholipids, polypeptides, and tocopherols, and also protected by a hydrophobic membrane. When this chlorophyll is taken out from the chloroplast the magnesium ions will be replaced by a weak acid and it becomes unstable. So in the food industry this problem is resolved by the substitution of magnesium ion with a copper ion, which forms the highly stable blue/green complex [74]. This copper complex is not absorbed by the body and it will be excreted as waste product so it is used in many countries as a food additive. Although it is a natural food coloring agent, it loses its stability at different pH conditions of the food and is very expensive to produce.

Chlorophyll is widely used in pharmaceutical industries. It inhibits bacteria, stimulates tissue growth, and is involved in the wound healing process. Because the structure of chlorophyll is closely related to a hemoglobin molecule it facilitates easy carbon dioxide and oxygen interchange. So it is widely used in the treatment of ulcers, oral sepsis, and protocology. The application of ointments containing chlorophyll derivatives can eliminate the pain and improve the affected tissues. In addition to this, chlorophyll is a very good antioxidant capable of neutralizing free radicals in the body. Eating chlorophyll rich-foods can prevent degenerative diseases. One important property of chlorophyll is its ability to inhibit the growth of cancerous cells. It inhibits some carcinogenic compounds like nitrosamine, aflatoxin etc. According to studies on animals, chlorophyll can reduce the level of triglycerides and cholesterol in the body.

2.5 Pigment-Protein Complexes

2.5.1 *Phycobiliproteins*

Phycobiliproteins are a group of accessory light-harvesting pigment molecules and are important water soluble fluorescent pigment—protein complexes organized in supramolecular phycobilisome complexes. This phycobilisomes serves as photosynthetic apparatus in cyanobacteria and in some eukaryotic algae. The major classes of phycobiliproteins are phycocyanin, phycoerythrin, and allophycocyanin. In cyanobacteria, these phycobiliproteins comprise up to 40% of the total soluble protein content. These phycobiliproteins have wide spectrum applications in the field of biotechnology, nutraceuticals, pharmaceuticals, food industry, cosmetics, clinical diagnostics, and biomedical research.

2.5.2 *Phycocyanin*

Most of the blue-green algae contain c-phycocyanin as a major phycobiliprotein. *Spirulina* is an important source of c-phycocyanin. The dark blue color of blue-green algae is due to the presence of phycocyanin, which emits red color on excitation. c-Phycocyanin molecule is widely used as a natural dye because of its deep intense color. They can be used as a fluorescent reagent for immunological analysis without any toxic effect and also can be used as fluorescent probes for the analysis of cells and molecules [75]. The use of c-phycocyanin in fluorescent probes is dependent on chemical cross linking of peptides to form stable trimers [76].

Phycocyanin has a wide range of application in food industry as a coloring agent. They are used as a colorant in jelly gum and soft candy. Cyanobacteria rich in c-phycocyanin improve the immune system of humans. *Spirulina* rich in phycocyanin is a potent free radical

scavenger and it also inhibits lipid peroxidation in microsomes. Phycocyanin helps to protect renal failure caused by certain drugs. It also has anticancerous activity. C-phycocyanin also inhibits the growth of cancer cell lines.

2.5.3 *Phycobilins*

It is a group of photoreceptor pigments. These have structural similarity with mammalian bile pigments. There are two classes of phycobilins and they occur only in cyanobacteria and Rhodophyta. The phycobilin component is similar to the porphyrin without a metallic atom. These phycobilins have an important role in food, pharmaceutical, and textile industries.

2.5.4 *Phycoerythrins*

Phycoerythrin is a major component of light-harvesting complex in red algae and cyanobacteria. It has important fluorescent properties and it is widely used as a fluorescent probe. It is also used as an analytical reagent. Its value as a fluorescent probe exceeds 10,000 per kg. There are two major isomeric forms of phycoerythrin namely R-phycoerythrin and B- phycoerythrin. R-phycoerythrin is the predominant natural form.

2.6 Vitamins

A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. A compound is called vitamin, when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. The term vitamin does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids, or does not encompass the large number of other nutrients that promote health but are otherwise required less often. Vitamins are classified based on their activity, instead of their chemical structure. Vitamins are broadly classified as water-soluble or fat-soluble. In humans, there are 13 vitamins, which includes four fat-soluble (A, D, E and K) and nine water-soluble (8 B vitamins and vitamin C) (Table 4.5). Fat-soluble vitamins are absorbed through the intestinal tract with the help of lipids (fats), because they are more likely to accumulate in the body, which in turn leads to hypervitaminosis. Water-soluble vitamins dissolve easily in water, and in general, are readily excreted from the body, to the degree that urinary output is a strong predictor of vitamin consumption. Because they are not readily stored, consistent daily intake is very important.

Microalgae are considered as a rich, nonconventional source of vitamins for human nutrition. *Spirulina* and *Chlorella* are rich in vitamin B12. A number of vitamins are present in higher concentrations in the microalgae than in conventional food, traditionally considered rich in vitamins such as vitamin E, -carotene [provitamin β (tocopherols)], C (ascorbic acid), A, and B12. *Dunaliella* is rich in lipid soluble vitamins. *D. tertiolecta* is very rich in vitamin B2, vitamin B12, folic acid, vitamin C, nicotinic acid, and vitamin E, which accounted 31.2, 0.7, 4.8, 163.2, 79.3, and 116.3 (mg/kg of dry weight), respectively.

2.7 Polysaccharides

Polysaccharides are ubiquitous biopolymers that occur widely in nature. These are polymers of simple sugars that are monosaccharides linked together by glycosidic linkages. There

TABLE 4.5 Vitamins—Functions and Deficiencies

Vitamin	Function	Deficiency-Diseases
A	Night vision	Night blindness
	Healthy skin	Skin diseases
B	Healthy nervous system	Beriberi
	Healthy skin, formation of red blood cells	Anemia
C	Wound healing	Scurvy (bleeding gums)
	Disease resistance	
D	Bone health	Ricketts
	Strong teeth	
E	Disease resistance reproduction	Sterility
K	Blood clotting	Prolonged bleeding

are different types of polysaccharides. Polysaccharides exhibit a molecular structure that can be linear or highly branched, composed by the same (homopolysaccharide) or different (heteropolysaccharide) monosaccharide units. Structural differences confer distinct physical and chemical properties. Polysaccharides are nontoxic, natural biodegradable biopolymers. Algae are an important source of polysaccharides. Algae are cultivated in different parts of the world for the commercial production of phycocolloids such as alginates and carrageenan. Marine microalgae contain different types of polysaccharides namely cell wall polysaccharides, mucopolysaccharides, and storage polysaccharides. Sea weeds, especially the red macro algae are rich sources of these polysaccharides. Harvesting of seaweeds from natural habitat is very easy. These polysaccharides have wide range of commercial applications. They are extensively used gelling agents, thickeners, stabilizers, and as emulsifiers in food products. They also have application in pharmaceuticals, photographic films, and tertiary oil recovery. The highest content of polysaccharides is found in species such as *Porphyra* and *Palmaria* with a polysaccharide content of 65%.

Sulfated polysaccharides are another important group of polysaccharides from algae. They act as a protective barrier against pathogen. These sulfated polysaccharides act as promising ingredients in nutraceutical, pharmaceutical, and food industry. Important sulfated polysaccharides are agar and carrageenan [77]. Agar is one of the important polysaccharide from red algae. It is extracted from red seaweeds like Gracillariidae and Gelidiaceae. Agar is used as a gelling agent in food and pharmaceutical industry. Some of these polysaccharides impart functional value to food. Sulfated polysaccharides such as fucoidan, porphyrin, and furcellaran have been shown to demonstrate biological activities along with nonsulfated polysaccharide laminarin. β-1,3 Glucan is an important group of polysaccharide from green algae *Chlorella.* It is important for human health. It acts as an immune-stimulant and also used in prophylactic treatment.

2.8 Mycosporine and Mycosporine-like Amino acids

Mycosporines are chemical compounds that contain an aminocyclohexenimine ring linked to an amino acid, amino alcohol, or amino group. They have absorption maxima between 310 and 360 nm. Mycosporine-like amino acids (MAAs) are imine derivatives of mycosporine with a molecular weight of <400 Da. They are colorless water-soluble substances. MAA is transparent to visible light and has a high molar absorptivity for UV-A and UV-B. MAAs are more common in coral reef algae [78].

MAA-producing cyanobacteria are more abundant in hyper saline environment. *Microcystis aeruginosa* is a common cyanobacterium with bloom-forming ability to synthesize MAAs that can directly absorb UVR. The photoprotective effects of MAAs make it a good component in sunscreen. Some MAAs may protect the cell not only against UV radiation by absorbing the high-energy photons and dissipating the energy as heat, but also by scavenging reactive oxygen species such as singlet oxygen, superoxide anions, hydroperoxyl radicals, and hydroxyl radicals.

2.9 Bioactive Peptides and Proteins

Bioactive peptides are small peptide molecules that consist of 2–20 amino acids. These peptides have biological activities and health benefits. In order to cope-up the stress conditions microalgae synthesizes various bioactive chemicals. These chemicals are usually secondary metabolites, which include carbohydrates, organic acids, amino acids and peptides, vitamins, antibiotics, growth substances, enzymes, and toxic compounds. These bioactive peptides show anticancer, antiviral, antioxidant, and immunomodulatory effects (Table 4.6).

Marine microalgae produce a wide range of secondary metabolites, hence they considered as a rich source of antioxidants. The protease extracted from brown seaweeds *Scytosiphon lomentaria* and *Ishigeo kamurae* displayed high free radical scavenging activities [79]. *Chlorella vulgaris* contains a pepsin hydrolyzed peptide with strong antioxidant activity. This peptide is a protein waste and it is resistant against gastrointestinal enzymes [80]. *Chlorella vulgaris* also contains a bioactive peptide with antiproliferation effects [81].

TABLE 4.6 Microalgal Bioactive Peptides and Its Applications

Type	Peptide Name/Sequence	Source	References
Anticancer peptides	VECYGPNRPQF	*Chlorella vulgaris*	[82]
	Polypeptide CPAP	*Chlorella pyrenoidosa*	[84]
	Polypeptide Y2	*Spirulina platensis*	[85]
Antihypertension peptides	VECYGPNRPQF	*Chlorella vulgaris*	[83]
	VEGY	*Chlorella ellipsoidea*	[86]
	GMNNLTP, LEQ	*Nannochloropsis oculta*	[87]
Immunomodulatory peptides	Protein hydrolysates	*Chlorella vulgaris*	[88]
Antiartherosclerosis peptide	VECYGPNRPQF	*Chlorella sp*	[89]

3. NUTRITIONALLY IMPORTANT ALGAE

3.1 Haematococcus pluvialis

Haematococcus pluvialis is a green unicellular alga in Chlorophyceae species with worldwide distribution and most common in temperate regions. *Haematococcus* is a common component for nutraceuticals, cosmetics, pharmaceuticals, aquaculture, and numerous food products. It is a green free-swimming organism under optimal conditions of growth, and when conditions are unfavorable they form spores and rapidly accumulate astaxanthin. *Haematococcus* is the largest natural source of astaxanthin. Astaxanthin products from *Haematococcus* are available in the public domain from past 15 years. A recent survey of consumers of a commercial *Haematococcus* astaxanthin supplement indicates several benefits of astaxanthin supplementation. *Haematococcus* algae meal provides a minimum of 1.5% astaxanthin in an esterified form similar to that from krill and crawfish. *H. pluvialis* has been approved as a color additive in salmon feeds and a dietary-supplement ingredient for human consumption in USA by FDA, Japan, as well as several European countries [89].

3.2 Chlorella

Chlorella is a unicellular, green alga found in many aquatic systems. It is mass cultured for the commercial production of health foods in the form of pills and powder. First commercial production plant of *Chlorella* was established in Japan. The size of *Chlorella* is roughly similar to human blood corpuscle and it was first discovered by M.W Beijerinck. *Chlorella* is almost 60% protein and it is able to produce protein 50 times more efficiently than other protein crops. *Chlorella* contains all eight essential amino acids, the constituents of protein. Its amino acid content compares favorably with that of animal-derived protein, except that it has a slightly lower amount of methionine. It contains relatively high amounts of vitamin C, β-carotene (provitamin A), Bl, B2, B6, B12, niacin, pantothenic acid, folic acid, biotin, choline, inositol, PABA, vitamin E, and vitamin K. Its mineral content includes phosphorus, potassium, magnesium, sulfur, iron, calcium, manganese, copper, zinc, iodine, and cobalt. It is also a source of lipoic acid, an important microbial growth factor [90]. It is now widely available as a food supplement in tablet, granules, or in liquid form and is easily digested and hence, it is suitable for infants also.

3.3 Spirulina

Spirulina (*Arthrospira*) belongs to the group of oxygenic photosynthetic bacteria and are filamentous, nonheterocystous cyanobacteria commonly found in tropical and subtropical regions. This alga is known as "superfood" of nature because its nutrient profile is more potent than any other food, plant, grain or herb. *Spirulina* is an ideal food and dietary supplement for the twenty-first century by the Food and Agriculture Organization (FAO) of the United Nations. *Spirulina* contains almost all biochemical and it helps to build a healthy immune system that scavenges free radicals. Compounds extracted from *Spirulina* have inhibitory activity against a wide range of viruses [91]. *Spirulina* is claimed as a nontoxic, nutritious food, with some corrective properties against viral attacks, anemia, and tumoral growth, and as a source

of the yellow coloration of egg yolk when consumed by hens and growth. This contains proteins, carbohydrates, essential fatty acids, vitamins, minerals, carotenes, chlorophyll a, and phycocyanin. There has been a significant change in functional properties of *Spirulina* under stressed conditions.

3.4 Nannochloropsis

Nannochloropsis is a unicellular green alga that is rich in PUFA. Nowadays, it is widely used as an energy-rich source in aquaculture. It is also used for biodiesel production from photosynthetic organisms. *Nannochloropsis* is actually in use as a food additive for human nutrition and it is also served at the restaurant "A Poniente" of El-Puerto de Santa Maria (Cadiz, Spain) close to the natural environment where *Nannochloropsis gaditana* was first isolated and still grows. *Nannochloropsis* is an emerging microalgae with high omega-3 fatty acid content especially EPA. Many companies are using *Nannochloropsis* for the commercial production of omega-3 fatty acids.

4. CONCLUSION

Algae from marine and freshwater ecosystems are known for their potential to produce food ingredients and bioactive compounds since ancient times besides being a biofuel feedstock. Algal biomasses are now being widely cultivated to make the commercial formulation of functional foods and nutraceutical application. Algae contain many valuable compounds, which includes omega-3 fatty acids, vitamins and pigment–protein complexes etc. Even terrestrial plants could not be the producer of certain essential food ingredients and bioactive compounds, for which we majorly depend on algal sources. Considering the current shrinking arable land, persistent pesticides in food grains and leafy vegetables, and lifestyle-related diseases, there is no doubt that the algal biomass-derived food ingredients will serve as a "wonder molecule" for the mankind.

References

[1] E.K. Kalra, Nutraceutical-definition and introduction, AAPS Pharmsci. 5 (3) (2003) 27–28.
[2] E.E. Jarvis, Aquatic Species Program (ASP): Lessons Learned, National Renewable Energy Laboratory (NREL), Golden, CO, 2008.
[3] M. Arumugam, A. Agarwal, M. Arya, Z. Ahmed, Microalgae: a renewable source for second generation biofuels, Curr. Sci. 100 (8) (2011) 1141–1142.
[4] A. Pandey, D.J. Lee, Y. Chisti, C.R. Soccol, Biofuels from Microalgae, Elsevier Inc., San Diego, USA, 2013.
[5] L. Rodolfi, G. Chini Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, M.R. Tredici, Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotechnol. Bioeng. 102 (1) (2009) 100–112.
[6] G. Breuer, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains, Bioresour. Technol. 124 (2012) 217–226.
[7] M. Arumugam, A. Agarwal, M.C. Arya, Z. Ahmed, Influence of nitrogen sources on biomass productivity of microalgae Scenedesmus bijugatus, Bioresour. Technol. 131 (2013) 246–249.
[8] M. Arumugam, A. Agarwal, M. Arya, Z. Ahmed, Influence of organic waste and inorganic nitrogen source on biomass productivity of *Scenedesmus* and *Chlorococcum* sp. Int. J. Energy Environ. 2 (6) (2011) 1125–1132. www.IJEE.IEEFoundation.org.

[9] J. Anand, M. Arumugam, Enhanced lipid accumulation and biomass yield of *Scenedesmus quadricauda* under nitrogen starved condition, Bioresour. Technol. 188 (2015) 190–194.
[10] A. Muller-Feuga, Microalgues marines: les enjeux de la recherche, 1997.
[11] J.-P. Cadoret, M. Bardor, P. Lerouge, M. Cabigliera, V. Henriquez, A. Carlier, Producing recombinant commercial proteins, MS Médecine Sci. 24 (4) (2008) 375–382.
[12] S.M. Kim, Y.-J. Jung, O.-N. Kwon, K.H. Cha, B.-H. Um, D. Chung, C.-H. Pan, A potential commercial source of fucoxanthin extracted from the microalga Phaeodactylum tricornutum, Appl. Biochem. Biotechnol. 166 (7) (2012) 1843–1855.
[13] M.A. Borowitzka, Pharmaceuticals from algae, Biotechnology 7 (2011).
[14] V. Pasquet, J.-R. Chérouvrier, F. Farhat, V. Thiéry, J.-M. Piot, J.-B. Bérard, R. Kaas, B. Serive, T. Patrice, J.-P. Cadoret, Study on the microalgal pigments extraction process: performance of microwave assisted extraction, Process. Biochem. 46 (1) (2011) 59–67.
[15] T.C. Adarme-Vega, S.R. Thomas-Hall, P.M. Schenk, Towards sustainable sources for omega-3 fatty acids production, Curr. Opin. Biotechnol. 26 (2014) 14–18.
[16] I.M. Berquin, I.J. Edwards, S.J. Kridel, Y.Q. Chen, Polyunsaturated fatty acid metabolism in prostate cancer, Cancer Metast. Rev. 30 (3–4) (2011) 295–309.
[17] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sust. Energ. Rev. 14 (1) (2010) 217–232.
[18] P. Kumari, M. Kumar, V. Gupta, C. Reddy, B. Jha, Tropical marine macroalgae as potential sources of nutritionally important PUFAs, Food Chem. 120 (3) (2010) 749–757.
[19] A.L. Kau, P.P. Ahern, N.W. Griffin, A.L. Goodman, J.I. Gordon, Human nutrition, the gut microbiome and the immune system, Nature 474 (7351) (2011) 327–336.
[20] B.P. Chew, J.S. Park, Carotenoid action on the immune response, J. Nutr. 134 (1) (2004) 257S–261S.
[21] N.D. Riediger, R.A. Othman, M. Suh, M.H. Moghadasian, A systemic review of the roles of n-3 fatty acids in health and disease, J. Am. Diet. Assoc. 109 (4) (2009) 668–679.
[22] S. Lordan, R.P. Ross, C. Stanton, Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases, Mar. Drugs 9 (6) (2011) 1056–1100.
[23] L. Bellows, J. Clifford, K. Niebaum, M. Bunning, Omega 3 fatty Acids, Food and Nutrition Series, 2015. Fact sheet no. 9, 382.
[24] L.G. Cleland, M.J. James, S.M. Proudman, The role of fish oils in the treatment of rheumatoid arthritis, Drugs 63 (9) (2003) 845–853.
[25] J. Zimmerman, Optimal nutrition for HIV/AIDS wellness, J. Am. Diet. Assoc. 97 (9) (1997) A18.
[26] R. Levy, G. Herzberg, Effects of a meal of fish oil or corn oil on bile flow and composition in rats previously adapted to diets containing fish oil or corn oil, Nutr. Res. 16 (5) (1996) 805–816.
[27] M.E. Mohamed, E.S. Elswefy, M. Shawky, Diabetic renal injury induced in Exp rats. Role of curcuminoids as probable therapeutic agent, Biomed. Eng. Res. 2 (4) (2013) 175–181.
[28] A. Nkondjock, B. Shatenstein, P. Maisonneuve, P. Ghadirian, Specific fatty acids and human colorectal cancer: an overview, Cancer Detect. Prev. 27 (1) (2003) 55–66.
[29] C.A. Gogos, P. Ginopoulos, B. Salsa, E. Apostolidou, N.C. Zoumbos, F. Kalfarentzos, Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy, Cancer 82 (2) (1998) 395–402.
[30] M. Barber, J. Ross, A. Voss, M. Tisdale, K. Fearon, The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer, Br. J. Cancer 81 (1) (1999) 80.
[31] D.J. Kyle, Production and use of a single cell oil which is highly enriched in docosahexaenoic acid, Lipid Technol. 8 (5) (1996) 107–110.
[32] R.J. Henderson, E.E. Mackinlay, Polyunsaturated fatty acid metabolism in the marine dinoflagellate Crypthecodinium cohnii, Phytochemistry 30 (6) (1991) 1781–1787.
[33] W. Becker, 18 Microalgae in Human and Animal Nutrition. Handbook of Microalgal Culture: Biotechnology and Applied Phycology, 2004, p. 312.
[34] J. Del Campo, H. Rodriguez, J. Moreno, M. Vargas, J. Rivas, M. Guerrero, Accumulation of astaxanthin and lutein in Chlorella zofingiensis (Chlorophyta), Appl. Microbiol. Biotechnol. 64 (6) (2004) 848–854.
[35] S. Rivera, R. Canela-Garayoa, Analytical tools for the analysis of carotenoids in diverse materials, J. Chromatogr. 1224 (2012) 1–10.

[36] R.G. Fassett, J.S. Coombes, Astaxanthin in cardiovascular health and disease, Molecules 17 (2) (2012) 2030–2048.
[37] P. Palozza, S. Serini, M. Ameruso, S. Verdecchia, Modulation of intracellular signalling pathways by carotenoids, in: Carotenoids, Springer, 2009, pp. 211–234.
[38] B. Demmig-Adams, W.W. Adams, Antioxidants in photosynthesis and human nutrition, Science 298 (5601) (2002) 2149–2153.
[39] M.H. Zile, Vitamin A and embryonic development: an overview, J. Nutr. 128 (2) (1998) 455S–458S.
[40] C. Grewe, C. Griehl, Time and media dependent secondary carotenoid accumulation in Haematococcus pluvialis, Biotechnol. J. 3 (9–10) (2008) 1232–1244.
[41] D. Lagarde, L. Beuf, W. Vermaas, Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to *Synechocystis* sp. strain PCC 6803, Appl. Environ. Microbiol. 66 (1) (2000) 64–72.
[42] A.H. Tafreshi, M. Shariati, Pilot culture of three strains of *Dunaliella salina* for β-carotene production in open ponds in the central region of Iran, World J. Microbiol. Biotechnol. 22 (9) (2006) 1003–1006.
[43] X.M. Shi, F. Chen, High-yield production of Lutein by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture, Biotechnol. Prog. 18 (4) (2002) 723–727.
[44] B. Zhang, X. Zhang, Separation and nanoencapsulation of antitumor polypeptide from Spirulina platensis, Biotechnol. Prog. 29 (5) (2013) 1230–1238.
[45] J. Liu, Z. Sun, Y. Zhong, H. Gerken, J. Huang, F. Chen, Utilization of cane molasses towards cost-saving astaxanthin production by a *Chlorella zofingiensis* mutant, J. Appl. Phycol. 25 (5) (2013) 1447–1456.
[46] M. García-González, J. Moreno, J.C. Manzano, F.J. Florencio, M.G. Guerrero, Production of *Dunaliella salina* biomass rich in 9-cis-β-carotene and lutein in a closed tubular photobioreactor, J. Biotechnol. 115 (1) (2005) 81–90.
[47] A. Harari, D. Harats, D. Marko, H. Cohen, I. Barshack, Y. Kamari, A. Gonen, Y. Gerber, A. Ben-Amotz, A. Shaish, A 9-cis β-carotene–enriched diet inhibits atherogenesis and fatty liver formation in LDL receptor knockout mice, J. Nutr. 138 (10) (2008) 1923–1930.
[48] R.T. Lorenz, G.R. Cysewski, Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin, Trends Biotechnol. 18 (4) (2000) 160–167.
[49] M. Ikeuchi, T. Koyama, J. Takahashi, K. Yazawa, Effects of astaxanthin in obese mice fed a high-fat diet, Biosci. Biotechnol. Biochem. 71 (4) (2007) 893–899.
[50] E. Jin, B. Feth, A. Melis, A mutant of the green alga *Dunaliella salina* constitutively accumulates zeaxanthin under all growth conditions, Biotechnol. Bioeng. 81 (1) (2003) 115–124.
[51] F. Chen, H.-B. Li, R.N.-S. Wong, B. Ji, Y. Jiang, Isolation and purification of the bioactive carotenoid zeaxanthin from the microalga *Microcystis aeruginosa* by high-speed counter-current chromatography, J. Chromatogr. 1064 (2) (2005) 183–186.
[52] D.M. Kleinegris, M.A. van Es, M. Janssen, W.A. Brandenburg, R.H. Wijffels, Carotenoid fluorescence in Dunaliella salina, J. Appl. Phycol. 22 (5) (2010) 645–649.
[53] A. Ben-Amotz, 13 Industrial production of microalgal cell-mass and secondary products–major industrial species, in: Handbook of Microalgal Culture: Biotechnology and Applied Phycology, 2004, p. 273.
[54] R. Baker, C. Günther, The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption, Trends Food Sci. Technol. 15 (10) (2004) 484–488.
[55] I. Neuman, H. Nahum, A. Ben-Amotz, Prevention of exercise-induced asthma by a natural isomer mixture of β-carotene, Ann. Allergy Asthma Immunol. 82 (6) (1999) 549–553.
[56] H.R. Taylor, G. Tikellis, L.D. Robman, C.A. Mc Carty, J.J. Mc Neil, Vitamin E supplementation and macular degeneration: randomised controlled trial, The BMJ 325 (7354) (2002) 11.
[57] G.D. Lio-Po, E.M. Leaño, M.M.D. Peñaranda, A.U. Villa-Franco, C.D. Sombito, N.G. Guanzon, Anti-luminous Vibrio factors associated with the 'green water' grow-out culture of the tiger shrimp Penaeus monodon, Aquaculture 250 (1) (2005) 1–7.
[58] L. Zhao, G. Chen, G. Zhao, X. Hu, Optimization of microwave-assisted extraction of astaxanthin from Haematococcus pluvialis by response surface methodology and antioxidant activities of the extracts, Sep. Sci. Technol. 44 (1) (2009) 243–262.
[59] M. Rodríguez-Sáiz, J.L. de la Fuente, J.L. Barredo, *Xanthophyllomyces dendrorhous* for the industrial production of astaxanthin, Appl. Microbiol. Biotechnol. 88 (3) (2010) 645–658.
[60] J.P. Yuan, J. Peng, K. Yin, J.H. Wang, Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae, Mol. Nutr. Food Res. 55 (1) (2011) 150–165.

[61] H. Jyonouchi, S. Sun, M. Gross, Effect of Carotenoids on In Vitro Immunoglobulin Production by Human Peripheral Blood Mononuclear Cells: Astaxanthin, a Carotenoid without Vitamin A Activity, Enhances in Vitro Immunoglobulin Production in Response to At-dependent Stimulant and Antigen, 1995, pp. 173–183.
[62] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2) (2006) 87–96.
[63] N. Sheikhzadeh, H. Tayefi-Nasrabadi, A.K. Oushani, M.H.N. Enferadi, Effects of Haematococcus pluvialis supplementation on antioxidant system and metabolism in rainbow trout (*Oncorhynchus mykiss*), Fish Physiol. Biochem. 38 (2) (2012) 413–419.
[64] G. Hussein, H. Goto, S. Oda, T. Iguchi, U. Sankawa, K. Matsumoto, H. Watanabe, Antihypertensive potential and mechanism of action of astaxanthin: II. Vascular reactivity and hemorheology in spontaneously hypertensive rats, Biol. Pharm. Bull. 28 (6) (2005) 967–971.
[65] J.T. Landrum, R.A. Bone, Lutein, zeaxanthin, and the macular pigment, Arch. Biochem. Biophys. 385 (1) (2001) 28–40.
[66] F. Khachik, P.S. Bernstein, D.L. Garland, Identification of lutein and zeaxanthin oxidation products in human and monkey retinas, Invest. Ophthalmol. Visual Sci. 38 (9) (1997) 1802–1811.
[67] C. Chitchumroonchokchai, S.J. Schwartz, M.L. Failla, Assessment of lutein bioavailability from meals and a supplement using simulated digestion and Caco-2 human intestinal cells, J. Nutr. 134 (9) (2004) 2280–2286.
[68] P.E. Bowen, J.P. Clark, Lutein Esters Having High Bioavailability (Google Patents), 2001.
[69] A. Subagio, N. Morita, No effect of esterification with fatty acid on antioxidant activity of lutein, Food Res. Int. 34 (4) (2001) 315–320.
[70] J.A. Del Campo, M. García-González, M.G. Guerrero, Outdoor cultivation of microalgae for carotenoid production: current state and perspectives, Appl. Microbiol. Biotechnol. 74 (6) (2007) 1163–1174.
[71] S. Bhattacharyya, S. Datta, B. Mallick, P. Dhar, S. Ghosh, Lutein content and in vitro antioxidant activity of different cultivars of Indian marigold flower (*Tagetes patula* L.) extracts, J. Agric. Food Chem. 58 (14) (2010) 8259–8264.
[72] Z.-Y. Wu, C.-L. Shi, X.-M. Shi, Modeling of lutein production by heterotrophic *Chlorella* in batch and fed-batch cultures, World J. Microbiol. Biotechnol. 23 (9) (2007) 1233–1238.
[73] J.M. Seddon, U.A. Ajani, R.D. Sperduto, R. Hiller, N. Blair, T.C. Burton, M.D. Farber, E.S. Gragoudas, J. Haller, D.T. Miller, Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration, JAMA 272 (18) (1994) 1413–1420.
[74] A. Humphrey, Chlorophyll as a color and functional ingredient, J. Food Sci. 69 (5) (2004) C422–C425.
[75] S. Kulkarni, S. Badakere, J. Oswald, M. Kamat, Fluorescent phycocyanin from Spirulina platensis. Application for diagnosis, Biotech. Lab Int. (1996) 14–16.
[76] L. Sun, S. Wang, Z. Qiao, Chemical stabilization of the phycocyanin from cyanobacterium Spirulina platensis, J. Biotechnol. 121 (4) (2006) 563–569.
[77] I. Wijesekara, R. Pangestuti, S.-K. Kim, Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae, Carbohyd. Polym. 84 (1) (2011) 14–21.
[78] K.H. Cardozo, T. Guaratini, M.P. Barros, V.R. Falcão, A.P. Tonon, N.P. Lopes, S. Campos, M.A. Torres, A.O. Souza, P. Colepicolo, Metabolites from algae with economical impact, Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 146 (1) (2007) 60–78.
[79] C.-B. Ahn, Y.-J. Jeon, D.-S. Kang, T.-S. Shin, B.-M. Jung, Free radical scavenging activity of enzymatic extracts from a brown seaweed *Scytosiphon lomentaria* by electron spin resonance spectrometry, Food Res. Int. 37 (3) (2004) 253–258.
[80] I.-C. Sheih, T.J. Fang, T.-K. Wu, Isolation and characterisation of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste, Food Chem. 115 (1) (2009) 279–284.
[81] I.-C. Sheih, T.J. Fang, T.-K. Wu, P.-H. Lin, Anticancer and antioxidant activities of the peptide fraction from algae protein waste, J. Agric. Food Chem. 58 (2) (2009) 1202–1207.
[82] I.-C. Sheih, T.-K. Wu, T.J. Fang, Antioxidant properties of a new antioxidative peptide from algae protein waste hydrolysate in different oxidation systems, Bioresour. Technol. 100 (13) (2009) 3419–3425.
[83] X. Wang, X. Zhang, Separation, antitumor activities, and encapsulation of polypeptide from Chlorella pyrenoidosa, Biotechnol. Prog. 29 (3) (2013) 681–687.
[84] S.-C. Ko, N. Kang, E.-A. Kim, M.C. Kang, S.-H. Lee, S.-M. Kang, J.-B. Lee, B.-T. Jeon, S.-K. Kim, S.-J. Park, A novel angiotensin I-converting enzyme (ACE) inhibitory peptide from a marine *Chlorella ellipsoidea* and its antihypertensive effect in spontaneously hypertensive rats, Process. Biochem. 47 (12) (2012) 2005–2011.

[85] J.-Y. Ko, J.-H. Lee, K. Samarakoon, J.-S. Kim, Y.-J. Jeon, Purification and determination of two novel antioxidant peptides from flounder fish (*Paralichthys olivaceus*) using digestive proteases, Food Chem. Toxicol. 52 (2013) 113–120.

[86] H.J. Morris, O. Carrillo, A. Almarales, R.C. Bermúdez, Y. Lebeque, R. Fontaine, G. Llauradó, Y. Beltrán, Immunostimulant activity of an enzymatic protein hydrolysate from green microalga *Chlorella vulgaris* on undernourished mice, Enzym. Microb. Technol. 40 (3) (2007) 456–460.

[87] M.F. Shih, L.C. Chen, J.Y. Cherng, *Chlorella* 11-peptide inhibits the production of macrophage-induced adhesion molecules and reduces endothelin-1 expression and endothelial permeability, Mar. Drugs 11 (10) (2013) 3861–3874.

[88] M. Murakami, K. Ishida, T. Okino, Y. Okita, H. Matsuda, K. Yamaguchi, Aeruginosins 98-A and B, trypsin inhibitors from the blue-green alga *Microcystis aeruginosa* (NIES-98), Tetrahedron Lett. 36 (16) (1995) 2785–2788.

[89] J. Liu, Z. Sun, H. Gerken, Recent Advances in Microalgal Biotechnology, 2014.

[90] H. William, L. Michael, E. Rosenbaum, Chlorella, Mcgraw and Hill, New York, 2003.

[91] S. Ayehunie, A. Belay, T.W. Baba, R.M. Ruprecht, Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*), J. Acquir. Immune Defic. Syndr. 18 (1) (1998) 7–12.

CHAPTER

5

Natural Antioxidants From Algae: A Therapeutic Perspective

R.R. Sonani, R.P. Rastogi, D. Madamwar

Sardar Patel University, Anand, Gujarat, India

OUTLINE

1. INTRODUCTION

Free radicals and reactive oxygen species (ROS) are believed as slow poison due to their adverse effects on biologically significant molecules [1,2]. To cope up with harmful free radicles, every organism has its inherent antioxidant system comprising of several characteristics enzymatic and nonenzymatic antioxidants. The accumulation of excessive ROS, due to inefficiency of the antioxidant system in body will cause serious damage to various biomolecules, which in turn, results in occurrence of several life-threatening diseases including cancer, brain aging, diabetes, atherosclerosis, arthritis, immature aging, and neurodegenerative diseases

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00005-9

[3–5]. The fact that "the supplement of exogenous antioxidant can help the endogenous antioxidant system in combating against free radicals" has generated the thirst of the worthy antioxidant [1,2]. As result of which, many antioxidants have been synthesized chemically or purified from natural resources during last few decades. Among them, the synthetic ones are documented to have more side effects like liver damages and carcinogenesis [6]. This fact turned the consumers and researches toward the natural antioxidants with little or no side effects. Algae are considered as largest and oldest taxonomic group of photoautotrophs bearing an immense treasure of therapeutically important antioxidant molecules [7]. The present chapter summarizes the recent updates in the field of algal antioxidants with therapeutic perspective.

2. OXIDATIVE STRESS

The majority of ROS are the by-products of mitochondrial respiration [8]. Both the inner and outer mitochondrial membranes are involved in the production of free radicals. Monoamine oxidase, localized on outer mitochondrial membrane produces large amount of hydrogen peroxide (H_2O_2) as by-product during the oxidative deamination of biogenic amines [9]. This H_2O_2 contributes to the increase in the steady state concentration of ROS in both the cytosol and mitochondrial matrix [10]. In the case of inner membrane, several redox centers of mitochondrial electron transport chain (Complex I and III) are found to leak the electrons, serving as the primary site for ROS production in all mammalian tissues via different routes [11,12] (Fig. 5.1).

Leaked electrons reduce the minor portion of consumed oxygen in to relatively stable superoxide anions (O_2^-), which is the first precursor for the generation of most of the ROS [8] (Fig. 5.1). The O_2^- is further neutralized by the enzyme superoxide dismutase in to H_2O_2 (Fig. 5.1). Further, this H_2O_2 participates in the generation of highly reactive hydroxyl radicle [OH^-] through either Haber–Weiss reaction (reaction between O_2^- and H_2O_2) or Fenton reaction (Fe^{+2} driven cleavage of H_2O_2) (Fig. 5.1) [13]. Under oxidative stress, the production of ROS surpasses to that of its demolition despite the existence of mitochondrial

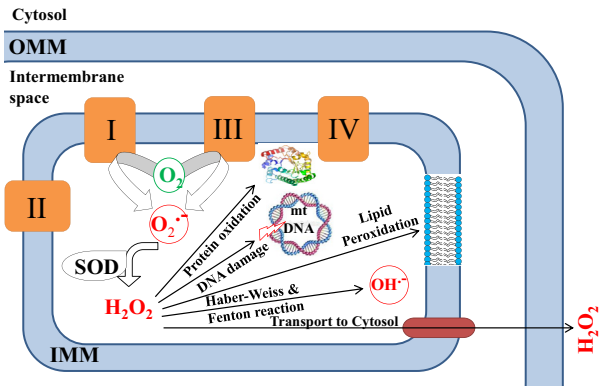


FIGURE 5.1 Schematic overview of reactive oxygen species (ROS) production and its consequences on mitochondrial DNA, proteins, and lipids. *I, II, III, IV*, electron transport chain complex I, II, III, IV; *IMM*, inner mitochondrial membrane; *mtDNA*, mitochondrial DNA; *OMM*, outer mitochondrial membrane; *SOD*, superoxide dismutase.

antioxidant defense system. Gradual accumulation of ROS inside mitochondria leads to the damages of mitochondrial biomolecules and impacts substantial negative effects on cell viability (Fig. 5.1).

2.1 Oxidative Stress–Associated Irregularities in Mitochondria

ROS damages the mitochondrial macromolecules, indispensible for life, specifically including DNA, proteins, and lipids. Mitochondrial-DNA (mtDNA) encodes for several proteins and RNAs having crucial role in electron transport chain and in energy (i.e., ATP) generation [14]. mtDNA is highly susceptible to ROS-induced damages due to the absence of protective histones [8]. ROS affects the mtDNA by triggering the formation of the most carcinogenic oxidative stress–induced lesion 8-hydroxydeoxyguanosine (8-OHdG) [8]. ROS-induced mtDNA damage is probably the major cause of mitochondrial genomic instability since oxidatively modified bases are found 10–20 folds higher in mtDNA as compared to nuclear DNA [8]. Such modified bases are believed as major causatives for aging–associated, neurodegenerative, and other carcinogenic deformities.

The major targets of O_2^- is the iron-sulphur (Fe-S)–containing proteins including aconitase, NADH dehydrogenase, and ATPase [10,15,16]. Aconitase is found to be involved in metabolic activity–regulated mitochondrial gene expression by interacting with the mtDNA-protein complex [15]. Impaired function of aconitase might adversely alter the mitochondrial gene expression. Moreover, aconitase performs two other major functions: first, it holds the reactive Fe^{+2} in the form of inactive $(3Fe^{+2}\text{-}4S)^+$ cluster [17]; second, it catalyzes the conversion of citrate to isocitrate, a crucial step of Krebs cycle. Therefore, the inactivation of mitochondrial aconitase results in the amplification of oxidative stress and impaired energy production by providing the reactants for Haber–Weiss/Fenton reactions and dysfunction of Krebs cycle, respectively. Other proteins like NADH dehydrogenase and ATPase having major role in Krebs cycle are also highly prone to the ROS-mediated oxidation and results in the hampered energy generation [10].

Inner mitochondrial membrane contains proteinaceous channel, named as a permeability transition pore (PTP). The thiol groups of PTP-proteins are highly susceptible to Ca^{+2}–induced oxidative stress (as describes in next paragraph). The cysteine and methionine residues are easily oxidized to disulfide and methionine sulfoxide by even the lesser amount of hydroxyl radicals [18]. The variation in protein structures due to cyc/met residues oxidation promotes the nonspecific permeability of inner mitochondrial membrane referred to as mitochondrial permeability transits (MPT) [19–21]. Gradually, MPT results in the complete loss of permeability control, which allows the uncontrolled bilateral flow of protons, osmolytes, and small proteins [19–21]. At developed stage, MPT drastically alters the mitochondrial structure and functional activity leading to the cell death.

Lipid peroxidation in outer (OMM) and inner mitochondrial membrane (IMM) is majorly identified as free radical–caused mitochondrial deformation [22,23]. Free radical oxidizes the unsaturated fatty acids of membrane ($L \rightarrow L^{\bullet}$) to lipid radicals. Lipid radical then combines with molecular oxygen to generate the highly active lipid peroxide ($L^{\bullet} \rightarrow LOO^{\bullet}$), which initiate the chain of lipid peroxidation [24]. Thus, mitochondrial lipid peroxidation causes complete loss of mitochondrial functions. OMM lipid peroxidation impaired the Ca^{+2} buffering capacity, which triggers the Ca^{+2}-induced oxidative stress and associated

malformations including MPT [16,25,26]; whereas, IMM lipid peroxidation affects the crucial functions of mitochondria including respiration, oxidative phosphorylation, inner membrane barrier properties, and maintenance of mitochondrial membrane potential ($\Delta\psi$). Specifically, cardiolipin peroxidation in IMM activates the prematured apoptotic pathway [24].

2.2 Mitochondrial Irregularities Result in Life-Threatening Diseases

During past several years, the pivotal role of oxidative stress and free radicals in occurrence of several life-challenging diseases like diabetes, cancer, aging, and other neurodegenerative diseases has been uncovered (Fig. 5.2).

There is considerable evidence that the oxidative stress plays major role in the development of muscular and cardiovascular complications in diabetes. The diabetic retinopathy, nephropathy, and cardiomyopathy could be prevented by trans-gene–mediated antioxidant enzymes expression and provision of antioxidant compounds in the diabetic mice [27–33]. Content of oxidative stress–modified bases (8-OHdG) was found significantly higher in the DNA of cancerous cells as compared to the adjacent normal cells [34]. Moreover, the elevated level of 8-OHdG in the urine of cancer patients has also suggested oxidative stress as the major causative of cancer [35]. Increased oxidative stress causes the gradual malfunctioning of almost all organs, results in aging [36]. Role of oxidative stress in aging is reasonably supported by some concrete evidences. First, long-lived animal mutant/transgenic (flies, worms, and mice) is linked with either reduced oxidative stress or increased antioxidant defense. Second, the provision of exogenous antioxidant compounds vitamin E, α-tocopherol [37], trolox [38], and α–lipoic acid [39,40] alleviates the onset of aging. The neurons astrocytes and microglia dysfunctionality is majorly supposed to happen due to premature apoptosis, notably triggered by altered ion transport and calcium homeostatic in mitochondria [41]. As discussed above, the respiratory chain of mitochondria is site of the ROS-generation. The oxidative stress, indirectly, by raising many adverse conditions like genetic mutation,

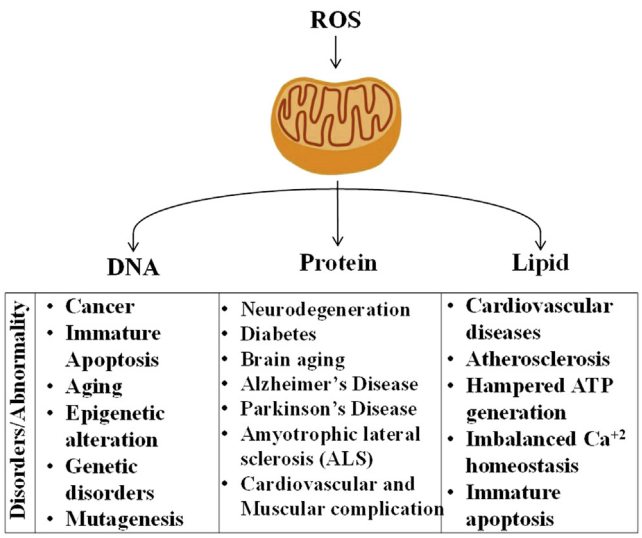


FIGURE 5.2 Reactive oxygen species (ROS)-associated disorders.

protein misfolding, and proteins aggregation, leads to the neurological disease including Alzheimer disease [42], Parkinson disease, amyotrophic lateral sclerosis, and many others.

3. WHY ALGAE?

Algae are oldest photoautotrophs, surviving and booming across wide range of habitats including bare rocks, deep oceans, ice shelves, deserts, barren soil, rivers, ponds, hot springs, and even in Arctic and Antarctic regions [43–47]. They are biggest contributors to the Earth's primary production. They have been exposed to several abiotic stresses, which include high light, high oxygen concentration, UV-radiation, high salinity, and desiccation [48,49]. The long-term exposure to such unique environmental conditions have positively pressurized the algae toward the metabolic evolution, which results in the production of various secondary metabolites with great biological, ecological, and economic importance. Based on the current literature, algae are perceived as emerging source of biomolecules like fatty acids, carotenoids, polysaccharides, and pigment proteins with diverse bioactivity [50]. Moreover, the biomolecules originated from algae are most likely to possess the biomedically and therapeutically important properties. The conjugated pi-double system of the algal molecule enables them to accept the electron and oxidize easily. This property is the primary requirement to be the antioxidants. Several algal-compounds have been found to show the antioxidant activity during last few decades [51]. Moreover, algal antioxidants are very diverse in terms of their size, solubility, and range of antioxidant activities.

4. ALGAL ANTIOXIDANTS

Numerous genera of algae including *Ahnfeltiopsis*, *Colpomenia*, *Gracilaria*, *Halymenia*, *Hydroclathrus*, *Laurencia*, *Padina*, *Polysiphonia*, and *Turbinaria* have been reported to produce the antioxidant compounds [52]. Algal compounds with antioxidant activity are also found to express some biologically significant activities viz. antiproliferative, anticancer, antiaging, neuroprotection etc. Algal antioxidants like phycobiliproteins, phlorotannins, carotenoids, sulfated polysaccharides, scytonemin and mycosporine-like amino acids (MAAs) are discussed in detail with respect to its occurrence, structure, mode of action, and putative roles in therapeutics.

4.1 Phycobiliproteins

Phycobiliprotein (PBPs), the water-soluble pigment proteins harvest the sunlight in broader region of solar spectrum for algal photosynthesis [53,54]. They are found in the cyanobacteria and certain algae like *Rhodophyta* and *Cryptomonas*. Generally, they are composed of two dissimilar peptide chains containing the covalently attached chromophore/s (Fig. 5.3) [55,56]. PBPs are classified in to three major divisions viz. phycoerythrin (PE, $\lambda^{A}_{max} = 540–570$ nm; $\lambda^{F}_{max} = 575–585$), phycocyanin (PC, $\lambda^{A}_{max} = 610–620$ nm; $\lambda^{F}_{max} = 645–653$), and allophycocyanin (APC, $\lambda^{A}_{max} = 651–655$ nm; $\lambda^{F}_{max} = 657–665$), based on the type of chromophore/s they contain (Fig. 5.3) [57–62]. APC and PC contain

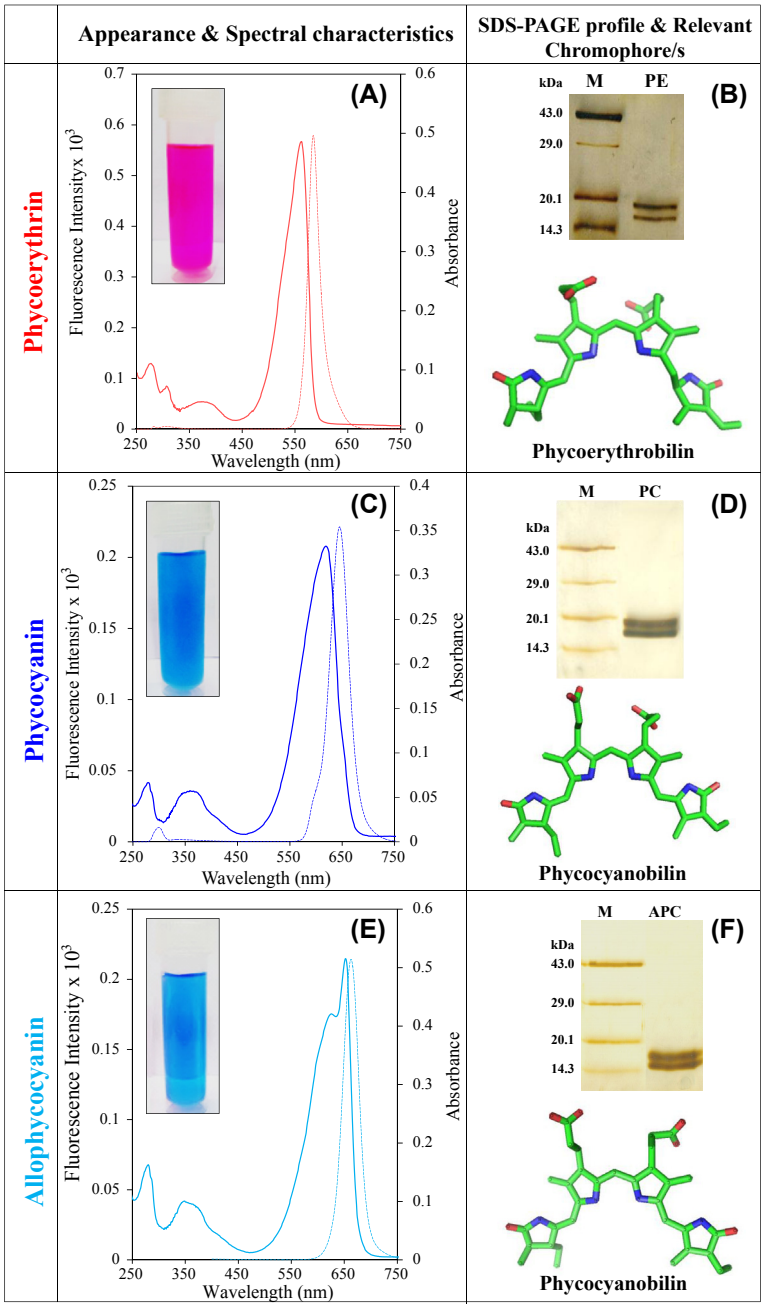


FIGURE 5.3 Spectral characteristics, apoproteins, and chromophores composition of phycobiliproteins. UV-visible (intact line) and fluorescence emission (fragmented line) spectra of phycoerythrin (A), phycocyanin (C), and allophycocyanin (E) upon excitation at 559, 589, and 645 nm. The phycoerythrin, phycocyanin, and allophycocyanin absorb the maximum visible light in the range of 540–570, 610–620, and 651–655 nm, respectively and give fluoresce emission between 575–585, 645–653, and 657–665 nm. The optical appearance of phycoerythrin, phycocyanin, and allophycocyanin has been shown in the inset of Figures A, C, and E, respectively. SDS-PAGE profiles of phycoerythrin (B, upper), phycocyanin (D, upper), and allophycocyanin (F, upper) showing the existence of two dissimilar type of α- and β-apoproteins in each phycobiliproteins. The chormophores (and its conformation) attached with the apoproteins of phycoerythrin (B, lower), phycocyanin (D, lower), and allophycocyanin (F, lower) [53,58,62,65].

phycocyanobilin and/or phycoviobilin, and PE contains phycoerythrobilin and/or phycourobilin as a chromophore/s with their peptide chains (Fig. 5.3) [54].

The substantial evidences during last two decades suggested that the PBPs encompass the antioxidant and free-radicals scavenging activity (Table 5.1). The amino acid composition of PBPs and conjugated double bond system of chromophore offer wide range of antioxidant

TABLE 5.1 In Vitro and In Vivo Antioxidant Activity Studies on Phycobiliproteins

Compound	Source	Experimental Details	References
Phycocyanin	*Arthospira maxima*	• In vivo • Colitis rat model • Myeloperoxidase (MPO) assay	González et al. [66]
Phycocyanin	Commercial preparation	• In vivo • Sprague—Dawley rat • Heat shock protein 27kD expression	Rimbau et al. [67]
Phycocyanin	*Spirulina* sp.	• In vitro • Human erythrocytes • Glutathione measurement and lipid peroxidation assay under Azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress	Romay and González [68]
Phycocyanin	*Arthospira maxima*	• In vitro • Hydroxyl and peroxide radicle scavenging activity • In vivo • Myeloperoxidase (MPO) and lipid peroxidation assay activity assay in CCl_4-treated rat	Romay et al. [69]
Phycocyanin	*Aphanizomenon flosaquae*	• In vitro • Human erythrocytes • Haemolysis assay under (AAPH) induced oxidative stress	Benedetti et al. [70]
Phycocyanin	*Spirulina platensis*	• In vivo • Wistar rat (Male) • Oxalate induced renal calculi formation monitoring by microscopic evaluation of urinary crystals • Histopathology and enzymes (alkaline phosphatase, acid phosphatase and γ-glutamyl transferase) activity of renal tissues	Farooq et al. [71]
Phycocyanin	*Spirulina* sp.	• In vitro • Rat ventricular cardiomyocytes • Cell viability assay, TUNEL assay, caspase-3 assay, DNA laddering assay • Intracellular reactive oxygen species (ROS) measurement	Khan et al. [72]

(*Continued*)

TABLE 5.1 In Vitro and In Vivo Antioxidant Activity Studies on Phycobiliproteins—cont'd

Compound	Source	Experimental Details	References
Phycocyanin	*Spirulina fusiformis*	• In vivo • Wistar rat (Male) • Lipid peroxidation assay • Glutathione peroxidase and catalase assay • Histopathology of brain tissue	Sathyasaikumar et al. [73]
Phycocyanin	*Spirulina platensis*	• In vivo • Golden Syrian hamster (Male) • Physiological parameters like body weight, food intake, and organs weight measurement • Plasma cholesterol (TC) and HDL cholesterol (HDL-C) measurement	Riss et al. [74]
Phycocyanin	*Phormidium fragile*	• In vitro • Ferrous ion reducing ability (FRAP) assay	Soni et al. [75]
Phycocyanin	*Spirulina platensis*	• In vitro • Superoxide, hydroxyl, hydrogen peroxide radicals scavenging activity, iron chelating activity	Bermejo et al. [76]
Phycocyanin	NA	• In vivo • Golden Syrian hamsters (Male) • Cu-induced low density lipoprotein (LDL) oxidation • Superoxide dismutase (SOD), catalase (CAT), and glutathion peroxidase (GSH-Px) assays	Sheu et al. [77]
Phycocyanin	*Oscillatoria tenuis*	• In vitro • DPPH, superoxide, hydroxyl, hydrogen peroxide radicals scavenging activity, ferric ion chelating ability, reducing power	Thangam et al. [78]
Phycocyanin and phycocyanin-β-subunit apoprotein	*Spirulina* sp. and genetically engineered *Escherichia coli*	• In vitro • Human erythrocytes • Azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress • Haemolysis assay under AAPH-induced oxidative stress	Pleonsil et al. [79]
Phycoerythrin, phycocyanin and allophycocyanin	*Phormidium* sp. A09DM	• In vitro • DPPH, ABTS, superoxide, hydroxyl radicals scavenging activity, FRAP assay, ferric ion chelating ability, reducing power • In vivo • *Caenorhabdit elegans (N2)* • Survival assay • Physiological marker (pharyngeal pumping, locomotion) assay • Paraquat sensitivity assay	Sonani et al. [65]

TABLE 5.1 In Vitro and In Vivo Antioxidant Activity Studies on Phycobiliproteins—cont'd

Compound	Source	Experimental Details	References
Phycoerythrin	*Phormidium* sp. A09DM	• In vivo • *C. elegans* (CL4176—Alzheimer disease model, mu86, e1370, hx546 mutants) • Survival assay • Physiological marker (pharyngeal pumping, locomotion) assay • Stress resistance assay • Paralysis assay	Sonani et al. [80]
Phycocyanin	*Leptolyngbya* sp. N62DM	• In vitro • DPPH, superoxide radicals scavenging activity, FRAP assay, reducing power • In vivo • *C. elegans* (CL4176—Alzheimer disease model, AM141—Huntington disease model) • Survival assay • Physiological marker (Pharyngeal pumping, locomotion) assay • polyQ::YFP aggregation assay • Stress resistance assay • Paralysis assay	Singh et al. [81]

potential. The antioxidants activity of PBPs is cumulative contribution of all accessible functional groups of their constituting amino acids [63]. PBPs express their antioxidant activity by both primary (by scavenging the already produced ROS through redox reaction) and secondary (by chelating the metal ion that produces ROS) routes, depending on their amino acid composition and distribution [64]. Moreover, the acidic, basic and aromatic amino acids of PBPs, having metal ion sequestration ability, are also the substantial contributors of PBP's total antioxidant activity [6,65]. Collectively, PBP's antioxidant activity is the combined outcome of its redox potential and metal ions chelating ability.

The biomedical applications of PBPs have been widely demonstrated. PBPs have been reported to display anticancerous, neuroprotective, antiinflammatory, hepatoprotective, and hypocholesterolemic activities during diverse studies. PE has been found beneficial in averting the physiological anomalies in various experimental animals [67,73,74,82,83]. The treatment of mouse tumor cells S180 and human liver carcinoma cells SMC 7721 with PE was noted to improve selectivity of photodynamic therapy [84]. PC was noticed to show neuroprotective effect in rat cerebellar granule cell cultures [69]. Furthermore, PC have been demonstrated to moderate the rise in tumor necrosis factor α level in the blood serum of mice upon endotoxin treatment [69]. The cell proliferation of human leukemia cell line was observed to inhibit by the provision of PC [76]. The enterovirus 71—mediated cytopathicity, plaque formation, and apoptosis in host cell were remarked to be vapid in the presence of APC [85]. PC, isolated from freshwater algae *Leptolyngbya* sp. N62DM was seen to act as potential drug in Alzheimer disease in both in silico by docking analysis and in vivo by using *Caenorhabditis elegans* CL4176, the *C. elegans* Alzheimer model [86]. Recently, an antioxidant-

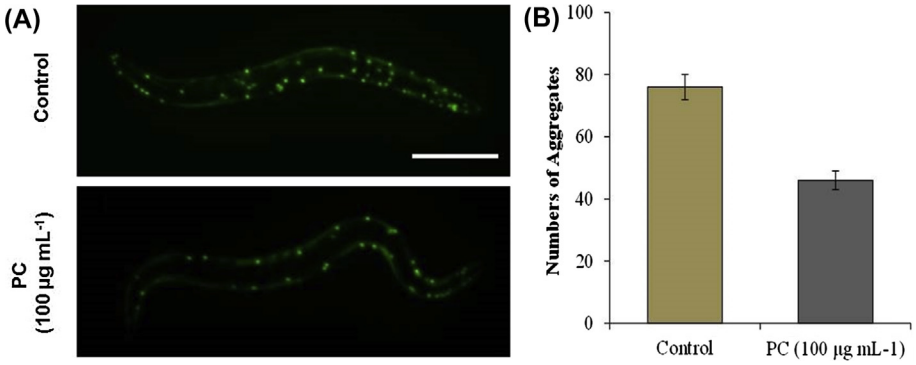


FIGURE 5.4 **Phycocyanin feeding to *Caenorhabditis elegans* AM141 results in the suppression of polyQ aggregation and Huntington disease.** (A) Representative images of phycocyanin treated and untreated *C. elegans* AM141 showing clear influence of phycocyanin treatment on Huntington disease. (B) Averaged number of polyQ::YFP aggregates in phycocyanin treated and untreated *C. elegans* AM141. Mean number of polyQ::YFP aggregates: Control 77.5 ± 3.5; PC treated 46.5 ± 3.6 [81].

based antiaging, anti-Alzheimeric and anti-Huntigton potential of PC have been recognized in wild-type and transgenic *C. elegans* [65,80,81]. PC was found to suppress the polyQ aggregation−mediated proteotoxicity in *C. elegans* AM141 (Huntington disease model) (Fig. 5.4) [81].

The potential antioxidant activity, easy availability, bright color, and nontoxic nature of PBPs enable them to being employed as active nutraceutical and pharmaceutical component in food, cosmetics, pharmaceutical, and biomedical application.

4.2 Phlorotannins

Phlorotannins, the polymer of phloroglucinol (1,3,5-trihydroxybenzene), is majorly found in marine brown algae. They are the products of acetate-malonate (polyketide) pathway [87]. Phlorotannins are classified in to four major categories based on the type of linkage between their monomers: fuhalols/phlorethols (ether linkage), fucols (phenyl linkage), fucophloroethols (ether and phenyl linkage), and eckols (dibenzodioxin linkage). Chemical structures of several algal-derived phlorotannins are shown in Fig. 5.5.

Ecklonia cava is a marine brown alga well known as a rich source of phlorotannins [88]. However, other brown algae like *Ecklonia stolonifera*, *Ecklonia kurome*, *Eisenia bicyclis*, *Ishige okamurae*, *Sargassum thunbergii*, *Hizikia fusiformis*, *Undaria pinnatifida*, and *Laminaria japonica* are also reported to produce the phlorotannins [89−92]. Phlorotannins have shown great potential in scavenging the 1,1-diphenyl 1,2-picrylhydrazyl (DPPH), hydroxyl, superoxide, peroxyl radicals, and moderates the lipid peroxidation during in vitro and in vivo studies (Table 5.2) [93−97]. The phlorotannins are thought to have antioxidant because of its radical scavenging potential, reducing power, and metal chelating properties (Table 5.2).

The antioxidant virtues of phlorotannins have been suggested to be effective for various biomedical and therapeutic applications. Phlorotannins like phlorofucofuroeckol A, dieckol

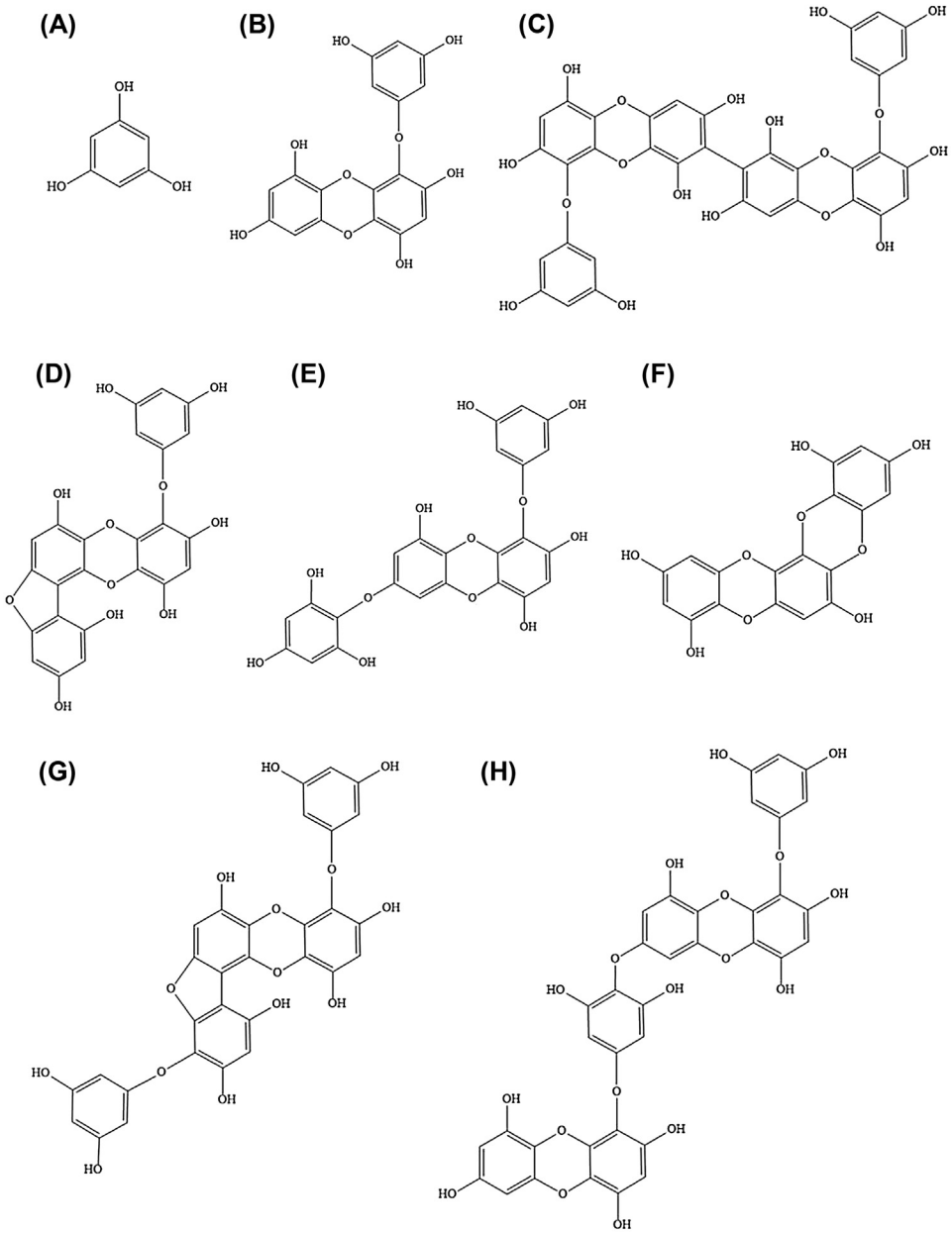


FIGURE 5.5 Chemical structure of phlorotannins derived from algae. (A) Phloroglucinol, (B) eckol, (C) 8,8′ bieckol, (D) fucofuroeckol-A, (E) 7-phloroeckol, (F) dioxinodehydroeckol, (G) phlorofucofuroeckol-A, (H) dieckol.

TABLE 5.2 Antioxidant Activity Studies on Algae-Derived Phlorotannins

Compound	Source	Experimental Details	References
Eckol and phloroglucinol	*Ecklonia cava*	• In vitro • Chinese hamster lung fibroblast (V79-4) cells • H_2O_2-induced oxidative stress • DPPH, hydrogen peroxide (H_2O_2), hydroxy radical scavenging activity, and lipid peroxidation assay • Intracellular reactive oxygen species (ROS) measurement • Catalase expression and activity • Cell viability assay	Kang et al. [96,98]
Phlorotannin-rich crude extract	*Sargassum ringgoldianum*	• In vitro • Superoxide and hydroxyl radicals scavenging activity	Nakai et al. [99]
Phloroglucinol, eckol and dieckol	*Ecklonia cava*	• In vitro • L5178 mouse T-cell lymphoma cell lines (L5178Y-R) • Electron spin resonance spectrometry (ESR) DPPH, alkyl, hydroxyl ($HO^{\bullet}$), and superoxide anion radical ($O_2^{\bullet -}$) scavenging activity • Comet assay	Ahn et al. [100]
Phloroglucinol, diphlorethohydroxycarmalol, and 6,6′-bieckol	*Ishige okamurae*	• In vitro • HL-60 cells and RAW 264.7 murine macrophage cells • Intracellular reactive oxygen species (ROS) measurement • Lipid peroxidation assay • Electron spin resonance spectrometry (ESR) DPPH, alkyl, hydroxyl ($HO^{\bullet}$), and superoxide anion radical ($O_2^{\bullet -}$) scavenging activity	Zou et al. [101]
Phloroglucinol, eckol, and dieckol	*Ecklonia cava*	• In vitro • Human fibroblast • UV-B–induced oxidative stress • Intracellular ROS measurement • Cell viability assay • Comet assay	Heo et al. [102]
Phlorofucofuroeckol A, dieckol, and dioxinodehydroeckol	*Ecklonia stolonifera*	• In vitro • RAW 264.7 murine macrophage cells • Intracellular reactive oxygen species (ROS) measurement • NO synthetase and cyclooxygenase 2 expression • Cellular NO and PGE2 production • DPPH radical scavenging activity	Kim et al. [103]

TABLE 5.2 Antioxidant Activity Studies on Algae-Derived Phlorotannins—cont'd

Compound	Source	Experimental Details	References
Phlorotannins-rich purified fraction	*Fucus vesiculosus*	• In vitro • Human mononuclear cells • Intracellular ROS detection by chemiluminescence techniques • DPPH radical scavenging activity, reducing power assay, ferrous ion chelating ability	Wang et al. [104]
Phloroglucinol, eckol, dieckol, 7-phloroeckol, phlorofucofuroeckol A, and dioxinodehydroeckol	*Eisenia bicyclis*	• In vitro • RAW 264.7 murine macrophages • Intracellular reactive oxygen species (ROS) measurement • Cellular NO production detection • Inhibition of iNOS and COX-2 protein • Cell viability assay	Jung et al. [105]
Phlorotannin-rich crude extract	*Lessonia nigrescens* and *Durvillaea antarctica*	• In vitro • Lipid peroxidation assay • DPPH radicle scavenging activity	Cruces et al. [106]

and, 8,8′-bieckol have been supposed to reduce the chronic diabetic complications like cataracts, neuropathy, and retinopathy by controlling the activity of some key enzymes including α-amylase, α-glucosidase, α-fucosidase, and aldose reductase [107]. The phlorotannins, 6,6′-bieckol and diphlorethohydroxycarmalol, from *I. okamurae* have been observed to inhibit the acetylcholinesterase, the possible drug targets to treat the Alzheimer disease [108]. Angiotensin-I-converting enzyme (ACE) is the major player in the regulation of blood pressure [109]. Phlorotannins and associated phenolic compounds present in *E. cava* extracts are anticipated to inhibit the in vitro ACE activity [110,111]. The proliferation of human breast cancer cells (MCF-7) is found to pause by dioxinodehydroeckol (phlorotannins derivative from *E. cava*) [112]. Similarly, the other phlorotannins derivatives like fucodiphloroethol G, dieckol, eckol, and phlorofucofurofuroeckol (from *E. cava*) have shown adverse effect on the growth of various cancer cell lines including HT-29, HT1080, HeLa and A549, and HT-29 as compared with human normal lung fibroblasts, MRC-5 [113]. Furthermore, phlorotannins are proposed for anti-HIV drugs since some eckol family phlorotannins (8,8′-bieckol, 8,4‴-dieckol, and 6,6′-bieckol) have demonstrated to inhibit the HIV-1 transcriptase and protease at reasonable IC_{50} values [114,115]. Phlorotannins are suggested to have potential application in the cosmeceuticals as they have been observed to inhibit tyrosinase activity and free radical generation under high-energy radiations [116,117]. Being the strong inhibitors of histamine release, phlorotannins are considered to have an antiallergic potential [118].

The series of recent studies on the algal phlorotannins pronounces its substantial biological activity. Algal phlorotannins could be exploited in designing of new functional foods and pharmaceutical forms to prevent and fight against several chronic human disorders.

TABLE 5.3 Occurrence of Various Carotenoids Among Algal Taxa

Types of Carotenoids	Source	References
α-Carotene	*Ulva fenestrata, Dunaliella salina*	Vershinin and Kamnev [119] and Hu et al. [120]
β-Carotene	*Laminaria* sp., *Porphyra* sp., *Dunaliella salina, Chlorella* sp., *Scenedesmus* sp., *Spirulina* sp.	Mohammed and Mohd [121], Okai et al. [122], Murthy et al. [123], Del Campo et al. [124], Inbaraj et al. [125], Haugan [126]
Astaxanthin	*Hematococcus pluvialis*	Kobayashi and Sakamoto [127]
Lutein	*Porphyra* sp., *Scenedesmus sp.*	Del Campo et al. [124], Okai et al. [122]
Zeaxanthin	*Porphyra* sp., *Dunaliella salina*, *Spirulina* sp.	Maoka [128], Esteban et al. [129], Yokthongwattana et al. [130]
Violaxanthin	*Laminaria* sp., *Ulva fenestrare*	Vershinin and Kamnev [119], Haugan [126]
Fucoxanthin	*Laminaria* sp., *Undaria pinnatifida*	Haugan [126], Sachindra et al. [132], Terasaki et al. [131]
Neoxanthin	*Ulva fenestrare*	Vershinin and Kamnev [119]
Siphonoxanthin	*Ulva fenestrare*	Vershinin and Kamnev [119]
Canthaxanthin	*Hematococcus pluvialis, Chlorella* sp.	Inbaraj et al. [125], Wu et al. [134], Grewe and Griehl [133]
β-Cryptoxantin	*Chlorella* sp.	Inbaraj et al. [126], Wu et al. [134]

4.3 Carotenoids

Carotenoids, the organic pigment compound, are generally found in the chloroplast and chromoplast of various algal taxa (Table 5.3). Structurally, it is hydrophobic tetraterpenoids that contain the C40 methyl-branched hydrocarbon backbone (Fig. 5.6).

Around 600 types of carotenoids are documents to date [124]. Carotenoids are classified in two major subcategories: carotenes, the linear hydrocarbons with cyclic end groups and xanthophylls, the oxygenated derivatives of carotenes.

Algal carotenoids have been found to express the wide range of antioxidant and radicals scavenging activity (Table 5.4). Moreover, carotenoid also deactivates the electronically exited molecules involved in the free ROS generation. The unsaturated nature of carotenoids enables them to be oxidized while reacting with the free radicals. Instead of chemical reaction, the interaction between carotenoids and ROS (O_2^-) occurs majorly via physical quenching, in which, the ROS transfer its energy to carotenoid to achieve ground state. Exited carotenoid returns to ground state by dissipation its energy in surrounding solvent [138–141]. The efficiency of carotenoid for physical quenching depends on the numbers of double bond, type of end-groups, and nature of substituent in cyclic end group present in its structure [142,143]. β-Carotene and other structurally related carotenoids (α-carotene, zeaxanthin, and cryptoxanthin) are the best scavengers of O_2^- radical. Due to their lipophilic nature, carotenoids can efficiently moderate the lipid peroxidation and thus, are believed as the best protectors of

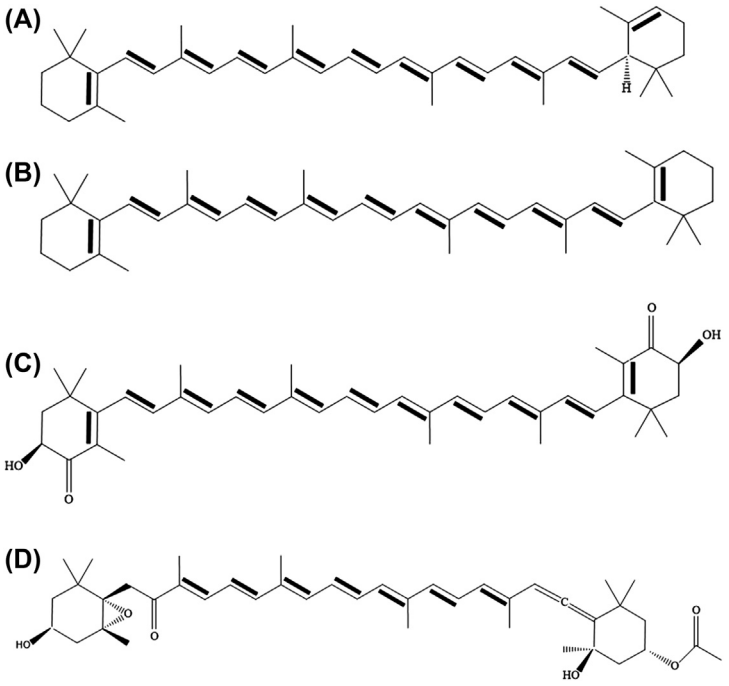


FIGURE 5.6 Chemical structure of various algal-derived carotenoids. (A) α-Carotene, (B) β-carotene, (C) astaxanthin, (D) fucoxanthin.

biological membranes during oxidative stress. The antioxidant capacity of carotenoids is highly dependent on the oxygen tension present in the system. Carotenoids express better antioxidant activity at low oxygen tension as compared to the higher oxidative stress [144,145].

β-Carotene was noticed to suppress the lipid peroxidation in mouse and rat tissues upon oxidative stress induced by UV-A exposure, CCl_4 injection, or oxidized oil consumption [146–148]. Moreover, β-carotene was noted to inhibit the ROS-induced lipid peroxidation in the thymocytes at 150 mm Hg PO_2 [149]. Low-density lipoprotein (LDL), the early target for atherosclerosis, was appeared to be protected against oxidative stress by the provision of α-carotene [150,151]. On the other hand, fucoxanthin (derived from algae *Sargassum siliquastrum*) has shown cytoprotective effect against H_2O_2-induced consequences including ROS formation, DNA damages, and apoptosis [152]. Moreover, fucoxanthin is also observed to protect the human dermal fibroblast (HDF cells) against UV-B radiation [153]. Astaxanthin, the hydroxyl- and keto-end containing carotenoid, was found to alleviate UV-A radiation–induced DNA alterations in human dermal fibroblasts and melanocytes [154,155]. Furthermore, astaxanthin is documented to efficiently moderate the free radical–induced and Fe^{+2}-catalyzed lipid peroxidation in rat mitochondria [156,157]. This bunch of considerable evidences clearly substantiates that the antioxidant virtues of algal carotenoids (beta-carotene, alpha-carotene, fucoxanthin, and astaxanthin) and their possible application in food, cosmetics, and therapeutics.

TABLE 5.4 In Vitro and In Vivo Antioxidant Activity Studies on Algal-Derived Carotenoids

Compound	Source	Experimental Details	References
Astaxanthin	*Haematococcus pluvialis*	• In vitro • $^1O_2$ quenching activity by linoleic acid photooxidation method	Kobayashi and Sakamoto [127]
α-Carotene and β-carotene, lutein, astaxanthin	Commercial preparation	• In vitro • Newely developed fluorometric assay (based on 4,4-difluoro-3,5-bis(4-phenyl-1,3- butadienyl)-4-bora-3a,4a-diaza-s-indacene (BODIPY 665/676) as an indicator; 2,2′-azobis-2,4-dimethylvaleronitrile (AMVN) as a peroxyl radical generator)	Naguib et al. [135]
β-Carotene-rich extract	*Dunaliella salina*	• In vivo • Albino Wistar rats • CCl_4-induced oxidative toxicity in	Murthy et al. [123]
Astaxanthin and β-carotene	Commercial preparation	• In vivo • *Hyphessobrycon callistus*, an ornamental fish • TAS (total antioxidant status), SOD (superoxide dismutase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and GPx (glutathione peroxidases) activity assays	Wang et al. [136]
Carotene-rich extract (α-carotene and β-carotene isomers)	*Dunaliella salina*	• In vitro • Trolox equivalent antioxidant capacity (TEAC) by ABTS radical scavenging activity	Jaime et al. [137]
α-Carotene and β-carotene isomers	*Dunaliella salina*	• In vitro • Trolox equivalent antioxidant capacity (TEAC) by ABTS radical scavenging activity • DPPH radical scavenging activity • Reducing power	Hu et al. [120]
Fucoxanthin and metabolites	*Undaria pinnatifida*	• In vitro • DPPH radical scavenging activity • ABTS radical scavenging activity • Hydroxyl radical scavenging activity • Analyses for hydroxyl radical scavenging	Sachindra et al. [132]

4.4 Sulfated Polysaccahrides

Sulfated polysaccharides (SPs) are the polysaccharide derivatives having covalently attached sulfate (SO_4^-) group/s at various places with main chain carbon atoms. Chemical structures of some well-known sulfated polysaccharides are shown in Fig. 5.7. Algae are believed as the largest source of nonanimal SPs. The type and amount of SPs varies greatly among various algal species [158]. *Chlorophyceae*, *Rhodophyceae*, and *Phaeophyceae* are commonly found to produce fucoidan/porphyran, carrageenan, and ulvan as major SPs, respectively.

Various SPs including fucoidan, porphyran, carrageenans, and ulvan have been found to scavenge the free radicles like DPPH, ABTS, NO, super oxide, and hydroxyl radicals (Table 5.5) [164,164,171]. The antioxidant activity of SPs depends on their chemical nature,

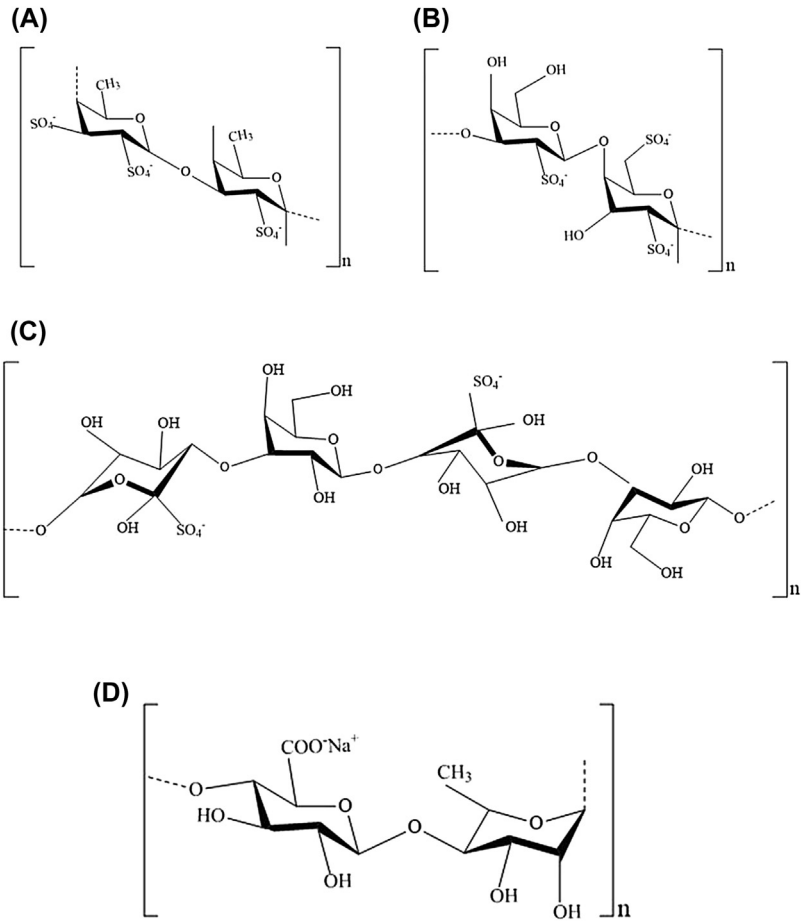


FIGURE 5.7 Chemical structure of sulfated polysaccharides. (A) Fucoidan, (B) carrageenan, (C) porphyran, (D) ulvan.

structure, extent of sulfating, size, monomer type, and glycosidic branching [159,172]. The correlation between the sulfate content and its free radicle scavenging activity has been documented for *Laminaria japonica*—derived fucoidan [165]. Moreover, it is also well established that the smaller SP are more effective in ROS-scavenging [173] as they might penetrate and donate proton more effectively than the larger SP [174].

Anticancer activity of SPs has been widely reported. The application of porphyran (*Rhodophyceae* sp.) can trigger the apoptosis in cancerous cells without affecting the normal cells [175]. The SP, isolated from *E. cava*, was observed to moderate the proliferation of human monocyte lymphoma cell line (U-937) [176]. In addition, the *Porphyra haitanensis*-derived SP was perceived to reduce the oxidative stress in aged mice [159]. Furthermore, fucoidan is noticed to prevent the UV-B—induced metalloproteinase-1 (MMP-1, the potential drug target for skin photoaging) expression in human skin fibroblast [177]. Moreover, several

TABLE 5.5 In Vitro and In Vivo Antioxidant Activity Studies on Algal-Derived Sulfated Polysaccharides

Compound	Source	Experimental Details	References
Sulfated polysaccharide fractions	*Porphyra haitanesis*	• In vitro • Ferric ion reducing ability (FRAP) assay	Zhang et al. [159]
Porphyran	*Porphyra haitanensis*	• In vivo • Total antioxidant capacity and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in aging mice	Zhang et al. [160]
Low molecular weight sulfated polysaccharide (LMWF)	*Laminaria japonica*	• In vitro • Superoxide radical, hydroxyl radical and hypochlorous acid scavenging activity • In vivo • Hepatoprotective effect in CCl_4-treated mice	Zhao et al. [161]
Gamma-carrageenan	*Commertial preparation*	• In vitro • Ion chelating ability, reducing power, linoleic acid peroxidation assay • Rat thymic lymphocyte • H_2O_2 induced stress and MTT assay	Yuan et al. [162]
Ulvans	*Ulva pertusa*	• In vitro • Hydroxyl, superoxide radicle scavenging activity, ferrous ion chelating ability	Qi et al. [163]
Carrageenans, fucoidan and fucans	*Fucus vesiculosus* and *Padina gymnospora*	• In vitro • Hydroxyl, superoxide radicle scavenging activity • Lipid peroxidation assay	de Souza et al. [164]
Fucoidan	*Laminaria japonica*	• In vitro • Hydroxyl, superoxide radicle scavenging activity, ferrous ion chelating ability, reducing power	Wang et al. [165]
SPs (Rhamnose polymer)	*Undaria pinnitafida*	• In vitro • DPPH, hydroxyl, superoxide radicle scavenging activity, ion chelating ability	Hu et al. [166]
Crude sulfate polysacchcaride crude (high sulfate:sugar ratio)	*Turbinaria ornata*	• In vitro • DPPh, ABTS, and NO scavenging activity • In vivo • Female Sprague–Dawley rats and Balb/c mice • Vascular permeability test	Ananthi et al. [167]

TABLE 5.5 In Vitro and In Vivo Antioxidant Activity Studies on Algal-Derived Sulfated Polysaccharides—cont'd

Compound	Source	Experimental Details	References
Crude sulfated polysaccharide (high sulfate:sugar ratio)	*C. sertularioide, Dictyota cervicornis, Sargassum filipendula* and *Dictyopteris delicatula*	• In vitro • Hydroxyl, superoxide radicle scavenging activity, reducing power, ferrous ion chelating ability • Cell proliferation study (HeLa cells)	Costa et al. [158]
Formulated mixtures of SPs	*Laminaria japonica, Porphyra haitanensis Ulva pertusa, Enteromorpha linza* and *Bryopsis plumose*	• In vitro • Superoxide and hydroxyl radicals scavenging activity, reducing power	Zhang et al. [168]
Heterofucans	*Sargassum filipendula*	• In vitro • Hydroxyl, superoxide radical scavenging activity, ferrous ion chelating ability	Costa et al. [169]
CCB-F0.3, CCB-F0.5, CCB-F1.0, and CCB-F2.0 (SPs with high sulfate and Galactose as major sugar)	*Caulerpa cupressoides* var. *flabellata*	• In vitro • Hydroxyl, superoxide radical scavenging activity, ferrous ion chelating ability	Costa et al. [170]

SPs like fucoidan, carrageenans, and sulfated rhamnogalactans showed antiviral activity against herpex simplex viruses (HSV-1, HSV-2), cytomegalovirus, and HIV [178].

Fucoidan, the SPs extracted from marine brown seaweeds, possess several biological activities. It showed the antiviral activity against infectious diseases, such as HIV, herpes simplex virus types (HSV-1 and HSV-2), and cytomegalovirus [178–182]. SPs like ulvan and porphyran have been revealed as promising putative drugs in cardiovascular therapeutics due to their antihyperlipidemic activity [183,184]. Ulvan, isolated from *Ulva pertusa* has successfully reduced the serum triglycerol (RG), the LDL-cholesterol in mice [185], and reduces the cardiovascular abnormalities. Similarly, porphyran, isolated from *Porphyra yezoensis* moderates the apolipoprotein B100 (apoB100, responsible for blood LDL) secretion by suppression of lipid synthesis in human liver cells [186]. The aforementioned reports greatly substantiate the promising utility of algal-SPs in therapeutics of various diseases. Due to its valuable biological functions and health beneficial effects, the algal-SPs could be suggested as an active ingredient in neutraceutical, cosmeceutical, and pharmaceutical formulations.

4.5 Scytonemin

Scytonemin is lipid soluble yellow to dark brown color pigment molecules of 544 Da, found in the extracellular sheath of the blue-green algae [187,188]. Structurally, scytonemin is tryptophan and tyrosine derivatives and found in dimeric form attached through olefinic

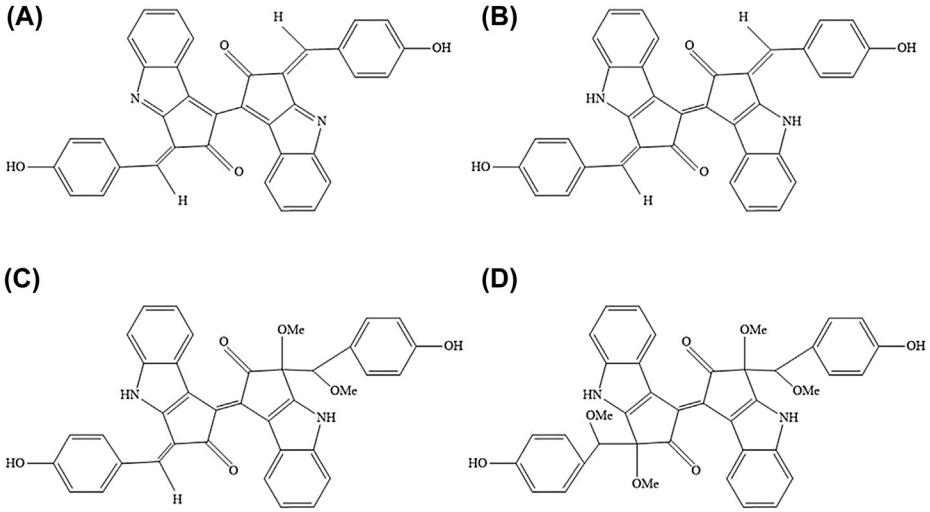


FIGURE 5.8 Chemical structure of scytonemin (A), reduced scytonemin (B), dimethoxyscytonemin (C), and tetramethoxyscytonemin (D) [203].

carbon atom. Scytonemin have characteristic absorbance in UV-A, B, and C region due its unique indole-alkaloid moiety [188,189]. Thus, it acts as potential sunscreen and precludes 90% of incident solar radiation from entering the cell [190,191]. Pure scytonemin displays three minor peaks at 252 ± 2, 278 ± 2, and 300 ± 2 nm, and one major peak at 386 ± 2 nm in its characteristic absorbance spectrum [192,193]. Scytonemin is generally found in two different forms: green/yellow (oxidized)- fuscochlorin and red (reduced)- fuscorhodin [194]. Based on the ^{1}H and ^{13}C NMR, and mass spectroscopy, some compounds namely dimethoxyscytonemin, tetramethoxyscytonemin, scytonin [195], and scytonemin-3a-imine [196] are perceived to be the derivatives of scytonemin skeleton and thus, clubbed with scytonemin family (Fig. 5.8).

Scytonemin occurs in more than 300 species of cyanobacteria and other algae. Especially, it is the stress response molecule that offers the organism to grow under high UV-radiation load, high osmotic stress, desiccated state, and nutrient deprivation [197–201].

Scytonemin is also recognized to scavenge the harmful free-radicals including DPPH and ABTS [202,203]. Moreover, scytonemin was shown to protect the cells from the UV-induced ROS [202,204].

Due to having UV-protecting and ROS-scavenging potential of scytonemin, it has been widely studied for its possible application in therapeutics [7,205]. Scytonemin has been reported to have great efficacy against UV-induced ROS, cyclobutane purine/pyrimidine dimer (CPD) formation and associated abnormalities [205]. Recently, Rastogi et al. has described that the provision of scytonemin reduces the ROS and CPD formation in *Scytonemin* sp. R77DM under UV-radiation [206].

It has been assigned that the first small–size inhibitor of human Polo like kinase 1 plays an important role in carcinogenesis by maintaining the genome integrity of cancerous cells [207,208]. Scytonemin exhibits a concentration-dependent inhibition of PKL1-mediated

cdc25c-phosphorylation and arrest the cancerous cell at the G2/M transition [209,210]. The anti(serine/threonine) kinase activity of scytonemin has been exploited to suppress the cell proliferation of HUVEC cell cancerous line and PMA-induced mouse ear edema [203,207,208]. Similarly, scytonemin has been shown to express anticancerous activity in multiple myeloma and renal cancer cells [211,212]. Moreover, human leukemia Jurkat (T) cell's proliferation has been observed to be arrested by the scytonemin-induced apoptosis. Such significant evidences suggest the conceivable use of scytonemin in cosmetics and therapeutics.

4.6 Mycosporine-Like Amino Acids

MAAs are water-soluble, colorless, small molecules found in cyanobacteria and micro-/macroalgae. MAAs are organic moiety having 5-hydroxy-5-hydroxymethylene ring backbone appended with substituting chemical groups at C1 and C3 carbons [213]. The conjugated nature of MAA backbone provides it with the UV (UV-A and -B) radiation-absorbing capacity. MAAs undergoes the electronic transition upon UV absorption and release the absorbed energy by the phenomenon called heat dissipation [214]. In this way, it protects the algae from harmful consequences of UV radiation like ROS generation and DNA damage. More than 20 types of MAAs have been identified in various algae, differing mainly in the type the functional groups attached to C1 and C3 carbon atom [215,216]. Each MAAs has unique absorbance in UV-region ranging from 309 to 362 nm [190]. Several studies have revealed the antioxidant and radical scavenging activity of MAAs. Readers are suggested to see Chapter 3 for details of MAAs.

5. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Algae are immense source of natural antioxidant biomolecules of diverse size, solubility, and radical-scavenging activity. Research during last few decades greatly enlightens the potency of algal-antioxidants to be exploited in food, cosmetics, pharmaceuticals, and therapeutics. Their usage in development of novel medicine, cosmetics, neutraceuticals, and drug to fight against various ROS-associated disorders is promising. Since these properties of biomolecules vary among diverse taxonomic group of algae, an extensive research is still needed to search the biomolecules with high bioactivity. Furthermore, the question "How do and at what extent these biomolecules work against oxidative stress?" still remains elusive. Still, there is considerable scope in the algal-antioxidant research including elucidation of exact mode of action, measurement of pharmacological parameters, and development of novel formulation from the algal-antioxidants.

Acknowledgments

Datta Madamwar acknowledges University Grant Commission (UGC), New Delhi for financial help in form of Centre of Advanced Study (CAS) program. Ravi R Sonani is highly thankful to the Department of Science and Technology (DST), New Delhi and Indo-French Center for the Promotion of Advance Research (IFCPAR) for financial support in form of INSPIRE (IF120712) and Raman-Charpak bi-national fellowships, respectively. Rajesh P Rastogi acknowledges University Grant Commission, New Delhi for Dr. DS Kothari post-doctoral grant.

References

[1] D. Harman, Free radical theory of aging, Mutat. Res. 275 (1992) 257–266.
[2] A.B. Salmon, A. Richardson, V.I. Pérez, Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? Free Rad. Biol. Med. 48 (2010) 642–655.
[3] R.S. Sohal, R.J. Mockett, W.C. Orr, Mechanisms of aging: an appraisal of the oxidative stress hypothesis, Free Rad. Biol. Med. 33 (2002) 575–586.
[4] M. Pan, T.S. Jiang, J.L. Pan, Antioxidant activities of rapeseed protein hydrolysates, Food Bioprocess Tech. 4 (2011) 1144–1152.
[5] W. Droge, Free radicals in the physiological control of cell function, Physiol. Rev. 82 (2002) 47–95.
[6] B.H. Sarmadi, A. Ismail, Antioxidative peptides from food proteins: a review, Peptides 31 (2010) 1949–1956.
[7] R.P. Rastogi, R.P. Sinha, Biotechnological and industrial significance of cyanobacterial secondary metabolites, Biotechnol. Adv. 27 (2009) 521–539.
[8] M. Ott, V. Gogvadze, S. Orrenius, B. Zhivotovsky, Mitochondria, oxidative stress and cell death, Apoptosis 12 (2007) 913–922.
[9] N. Hauptmann, J. Grimsby, J.C. Shih, E. Cadenas, The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA, Arch. Biochem. Biophys. 335 (1996) 295–304.
[10] E. Cadenas, K.J. Davies, Mitochondrial free radical generation, oxidative stress, and aging, Free Rad. Biol. Med. 29 (2000) 222–230.
[11] S. de Vries, The pathway of electron transfer in the dimeric QH_2: cytochrome c oxidoreductase, J. Bioenerg. Biomembr. 18 (1986) 195–224.
[12] F.L. Muller, Y. Liu, H. Van Remmen, Complex III releases superoxide to both sides of the mitochondrial inner membrane, J. Biol. Chem. 279 (2004) 49064–49073.
[13] A.Y. Andreyev, Y.E. Kushnareva, A.A. Starkov, Mitochondrial metabolism of reactive oxygen species, Biochemistry (Moscow) 70 (2005) 200–214.
[14] S. Anderson, A.T. Bankier, B.G. Barrell, M.H.L. De Bruijn, A.R. Coulson, J. Drouin, et al., Sequence and organization of the human mitochondrial genome, Nature 290 (1981) 457–465.
[15] X.J. Chen, X. Wang, B.A. Kaufman, R.A. Butow, Aconitase couples metabolic regulation to mitochondrial DNA maintenance, Science 307 (2005) 714–717.
[16] Y. Zhang, O. Marcillat, C. Giulivi, L. Ernster, K.J. Davies, The oxidative inactivation of mitochondrial electron transport chain components and ATPase, J. Biol. Chem. 265 (1990) 16330–16336.
[17] S.I. Liochev, I. Fridovich, The relative importance of HO^- and $ONOO^-$ in mediating the toxicity of O^-, Free Rad. Biol. Med. 26 (1999) 777–778.
[18] S.B. Berlett, E.R. Stadtman, Protein oxidation in aging, disease, and oxidative stress, J. Biol. Chem. 272 (1997) 20313–20316.
[19] T.E. Gunter, K.K. Gunter, S.S. Sheu, C.E. Gavin, Mitochondrial calcium transport: physiological and pathological relevance, Am. J. Physiol. 267 (1994) C313–C339.
[20] A. Vercesi, A. Kowaltowski, M. Grijalba, A. Meinicke, R. Castilho, The role of reactive oxygen species in mitochondrial permeability transition, Biosci. Rep. 17 (1997) 43–52.
[21] M. Zoratti, I. Szabò, The mitochondrial permeability transition, Biochim. Biophys. Acta 1241 (1995) 139–176.
[22] H. Kappus, Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance, Oxidative Stress (1985) 273–310.
[23] B. Halliwell, S. Chirico, Lipid peroxidation: its mechanism, measurement, and significance, Am. J. Clin. Nutr. 57 (1993) 715S–724S.
[24] A.J. Kowaltowski, A.E. Vercesi, Mitochondrial damage induced by conditions of oxidative stress, Free Rad. Biol. Med. 26 (1999) 463–471.
[25] E. Albano, G. Bellomo, M. Parola, R. Carini, M.U. Dianzani, Stimulation of lipid peroxidation increases the intracellular calcium content of isolated hepatocytes, Biochim. Biophys. Acta 1091 (1991) 310–316.
[26] B.R. Bacon, R. O'Neill, R.S. Britton, Hepatic mitochondrial energy production in rats with chronic iron overload, Gastroenterology 105 (1993) 1134–1140.
[27] X. Shen, S. Zheng, N.S. Metreveli, P.N. Epstein, Protection of cardiac mitochondria by overexpression of MnSOD reduces diabetic cardiomyopathy, Diabetes 55 (2006) 798–805.
[28] A.M. Vincent, J.W. Russell, K.A. Sullivan, C. Backus, J.M. Hayes, L.L. McLean, E.L. Feldman, SOD2 protects neurons from injury in cell culture and animal models of diabetic neuropathy, Exp. Neurol. 208 (2007) 216–227.

[29] P. Otero, B. Bonet, E. Herrera, A. Rabano, Development of atherosclerosis in the diabetic BALB/c mice: prevention with vitamin E administration, Atherosclerosis 182 (2005) 259–265.
[30] Y. Zhang, J. Wada, I. Hashimoto, J. Eguchi, A. Yasuhara, Y.S. Kanwar, et al., Therapeutic approach for diabetic nephropathy using gene delivery of translocase of inner mitochondrial membrane by reducing mitochondrial superoxide production, J. Am. Soc. Nephrol. 17 (2006) 1090–1101.
[31] R.A. Kowluru, V. Kowluru, Y. Xiong, Y.S. Ho, Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress, Free Rad. Biol. Med. 41 (2006) 1191–1196.
[32] F.R. DeRubertis, P.A. Craven, M.F. Melhem, Acceleration of diabetic renal injury in the superoxide dismutase knockout mouse: effects of tempol, Metabolism 56 (2007) 1256–1264.
[33] F. Giacco, M. Brownlee, Oxidative stress and diabetic complications, Circ. Res. 107 (2010) 1058–1070.
[34] S. Toyokuni, K. Okamoto, J. Yodoi, H. Hiai, Persistent oxidative stress in cancer, FEBS Lett. 358 (1995) 1–3.
[35] L.L. Wu, C.C. Chiou, P.Y. Chang, J.T. Wu, Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics, Clin. Chim. Acta 339 (2004) 1–9.
[36] H. Si, D. Liu, Dietary antiaging phytochemicals and mechanisms associated with prolonged survival, J. Nutr. Biochem. 25 (2014) 581–591.
[37] L.A. Harrington, C.B. Harley, Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*, Mech. Ageing Dev. 43 (1988) 71–78.
[38] N. Ishii, N. Senoo-Matsuda, K. Miyake, K. Yasuda, T. Ishii, P.S. Hartman, S. Furukawa, Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress, Mech. Ageing Dev. 125 (2004) 41–46.
[39] M.K. Brown, J.L. Evans, Y. Luo, Beneficial effects of natural antioxidants EGCG and α-lipoic acid on life span and age-dependent behavioral declines in *Caenorhabditis elegans*, Pharmacol. Biochem. Behav. 85 (2006) 620–628.
[40] M.G. Benedetti, A.L. Foster, M.C. Vantipalli, M.P. White, J.N. Sampayo, M.S. Gill, et al., Compounds that confer thermal stress resistance and extended lifespan, Exp. Gerontol. 43 (2008) 882–891.
[41] J. Emerit, M. Edeas, F. Bricaire, Neurodegenerative diseases and oxidative stress, Biomed. Pharmacother. 58 (2004) 39–46.
[42] M.F. Beal, Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann. Neurol. 31 (1992) 119–130.
[43] N.G. Carr, B.A. Whitton, The Biology of Blue–Green Algae, University of California Press, 1973.
[44] W.F. Vincent, A. Quesada, Ultraviolet radiation in Antarctica: measurements and biological effects, in: C.S. Weiler, P.A. Penhale (Eds.), Antarctic Research Series, vol. 63, American Geophysical Union, Washington, DC, 1994, p. 111.
[45] A. Quesada, W.F. Vincent, Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation, Eur. J. Phycol. 32 (1997) 335–342.
[46] P. Sanchez-Baracaldo, P.K. Hayes, C.E. Blank, Morphological and habitat evolution in the cyanobacteria using a compartmentalization approach, Geobiology 3 (2005) 145–165.
[47] N. Thajuddin, G. Subramanian, Cyanobacterial biodiversity and potential applications in biotechnology, Curr. Sci. 89 (2010) 47–57.
[48] A.Y. Mulkidjanian, E.V. Koonin, K.S. Makarova, S.L. Mekhedov, A. Sorokin, Y.I. Wolf, et al., The cyanobacterial genome core and the origin of photosynthesis, Proc. Natl. Acad. Sci. 103 (2006) 13126–13131.
[49] R.P. Rastogi, D. Madamwar, UV-induced oxidative stress in cyanobacteria: how life is able to survive? Biochem. Anal. Biochem. 4 (2015) 173, http://dx.doi.org/10.4172/2161-1009.1000173.
[50] S. Lordan, R.P. Ross, C. Stanton, Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases, Mar. Drugs 9 (2011) 1056–1100.
[51] N. Munir, N. Sharif, S. Naz, F. Manzoor, Algae: a potent antioxidant source, Sky J. Microbiol. Res. 1 (2013) 22–31.
[52] M.L. Cornish, D.J. Garbary, Antioxidants from macroalgae: potential applications in human health and nutrition, Algae 25 (2010) 155–171.
[53] R.R. Sonani, R.P. Rastogi, R. Patel, D. Madamwar, Recent advances in production, purification and applications of phycobiliproteins, World J. Biol. Chem. 7 (2016) 100–109.
[54] N.K. Singh, R.R. Sonani, R.P. Rastogi, D. Madamwar, The phycobilisomes: an early requisite for efficient photosynthesis in cyanobacteria, Exp. Clin. Sci. J. 14 (2015) 268–289.
[55] R.R. Sonani, R.P. Rastogi, M. Joshi, D. Madamwar, A stable and functional single peptide phycoerythrin (15.45 kDa) from *Lyngbya* sp. A09DM, Int. J. Biol. Macromol. 74 (2015) 29–35.

[56] K. Anwer, R. Sonani, D. Madamwar, P. Singh, F. Khan, K. Bisetty, et al., Role of N-terminal residues on folding and stability of C-phycoerythrin: simulation and urea-induced denaturation studies, J. Biomol. Struct. Dyn. 33 (2015) 121–133.
[57] N.K. Singh, A. Parmar, R.R. Sonani, D. Madamwar, Isolation, identification and characterization of novel thermotolerant *Oscillatoria* sp. N9DM: change in pigmentation profile in response to temperature, Process Biochem. 47 (2012) 2472–2479.
[58] R.R. Sonani, M. Sharma, G.D. Gupta, V. Kumar, D. Madamwar, *Phormidium* phycoerythrin forms hexamers in crystals: a crystallographic study, Acta Crystallogr. F: Struct. Biol. Commun. 71 (2015) 998–1004.
[59] R.P. Rastogi, R.R. Sonani, A.B. Patel, D. Madamwar, Occurrence of a functionally stable photoharvesting single peptide allophycocyanin α-subunit (16.4 kDa) in the cyanobacterium *Nostoc* sp. R76DM, RSC Adv. 5 (2015) 87598–87608.
[60] R.P. Rastogi, R.R. Sonani, D. Madamwar, Physico-chemical factors affecting the in vitro stability of phycobiliproteins from *Phormidium rubidum* A09DM, Bioresour. Technol. 190 (2015) 219–226.
[61] R.P. Rastogi, R.R. Sonani, D. Madamwar, Effects of PAR and UV radiation on the structural and functional integrity of phycocyanin, phycoerythrin and allophycocyanin isolated from the marine cyanobacterium *Lyngbya* sp. A09DM, Photochem. Photobiol. 91 (2015) 837–844.
[62] R.R. Sonani, G.D. Gupta, D. Madamwar, V. Kumar, Crystal structure of allophycocyanin from marine cyanobacterium *Phormidium* sp. A09DM, PLoS One 10 (2015) e0124580, http://dx.doi.org/10.1371/journal.pone.0124580.
[63] R.R. Sonani, R.P. Rastogi, D. Madamwar, Antioxidant potential of phycobiliproteins: role in anti-aging research, Biochem. Anal. Biochem. 4 (2015) 172, http://dx.doi.org/10.4172/2161-1009-1000172.
[64] S. Khantaphant, S. Benjakul, M.R. Ghomi, The effects of pretreatments on antioxidative activities of protein hydrolysate from the muscle of brownstripe red snapper (*Lutjanus vitta*), LWT-Food Sci. Technol. 44 (2011) 1139–1148.
[65] R.R. Sonani, N.K. Singh, J. Kumar, D. Thakar, D. Madamwar, Concurrent purification and antioxidant activity of phycobiliproteins from *Lyngbya* sp. A09DM: an antioxidant and anti-aging potential of phycoerythrin in *Caenorhabditis elegans*, Process. Biochem. 49 (2014) 1757–1766.
[66] R. Gonzalez, S. Rodriguez, C. Romay, A. González, J. Armesto, D. Remirez, N. Merino, Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats, Pharmacol. Res. 39 (1999) 55–59.
[67] V. Rimbau, A. Camins, C. Romay, R. González, M. Pallàs, Protective effects of C-phycocyanin against kainic acid-induced neuronal damage in rat hippocampus, Neurosci. Lett. 276 (1999) 75–78.
[68] C. Romay, R. Gonzalez, Phycocyanin is an antioxidant protector of human erythrocytes against lysis by peroxyl radicals, J. Pharm. Pharmacol. 52 (2000) 367–368.
[69] C.H. Romay, R. Gonzalez, N. Ledon, D. Remirez, V. Rimbau, C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects, Curr. Protein Pept. Sci. 4 (2003) 207–216.
[70] S. Benedetti, F. Benvenuti, S. Pagliarani, S. Francogli, S. Scoglio, F. Canestrari, Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*, Life Sci. 75 (2004) 2353–2362.
[71] S.M. Farooq, D. Asokan, P. Kalaiselvi, R. Sakthivel, P. Varalakshmi, Prophylactic role of phycocyanin: a study of oxalate mediated renal cell injury, Chem. Biol. Interact. 149 (2004) 1–7.
[72] M. Khan, S. Varadharaj, J.C. Shobha, M.U. Naidu, N.L. Parinandi, V.K. Kutala, P. Kuppusamy, C-phycocyanin ameliorates doxorubicin-induced oxidative stress and apoptosis in adult rat cardiomyocytes, J. Cardiovasc. Pharmacol. 47 (2006) 9–20.
[73] K.V. Sathyasaikumar, I. Swapna, P.V.B. Reddy, C.R. Murthy, K.R. Roy, A.D. Gupta, P. Reddanna, Co-administration of C-phycocyanin ameliorates thioacetamide-induced hepatic encephalopathy in Wistar rats, J. Neurol. Sci. 252 (2007) 67–75.
[74] J. Riss, K. Décordé, T. Sutra, M. Delage, J.C. Baccou, N. Jouy, et al., Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters, J. Agric. Food Chem. 55 (2007) 7962–7967.
[75] B. Soni, U. Trivedi, D. Madamwar, A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium fragile* and its characterization for antioxidant property, Bioresour. Technol. 99 (2008) 188–194.
[76] P. Bermejo, E. Piñero, Á.M. Villar, Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*, Food Chem. 110 (2008) 436–445.

[77] M.J. Sheu, Y.Y. Hsieh, C.H. Lai, C.C. Chang, C.H. Wu, Antihyperlipidemic and antioxidant effects of C-phycocyanin in golden Syrian Hamsters fed with a hypercholesterolemic diet, J. Tradit. Complement Med. 3 (2013) 41–47.
[78] R. Thangam, V. Suresh, W.A. Princy, M. Rajkumar, N. Senthilkumar, P. Gunasekaran, et al., C-Phycocyanin from *Oscillatoria tenuis* exhibited an antioxidant and in vitro antiproliferative activity through induction of apoptosis and G0/G1 cell cycle arrest, Food Chem. 140 (2013) 262–272.
[79] P. Pleonsil, S. Soogarun, Y. Suwanwong, Anti-oxidant activity of holo- and apo-c-phycocyanin and their protective effects on human erythrocytes, Int. J. Biol. Macromol. 60 (2013) 393–398.
[80] R.R. Sonani, N.K. Singh, A. Awasthi, B. Prasad, J. Kumar, D. Madamwar, Phycoerythrin extends life span and health span of *Caenorhabditis elegans*, Age 36 (2014) 1–14.
[81] N.K. Singh, R.R. Sonani, A. Awasthi, B. Prasad, A.R. Patel, J. Kumar, D. Madamwar, Phycocyanin moderates aging and proteotoxicity in *Caenorhabditis elegans*, J. Appl. Phycol. (2015) 1–11, http://dx.doi.org/10.1007/s10811-015-0772-5.
[82] C.H. Romay, J. Armesto, D. Remirez, R. González, N. Ledon, I. García, Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae, Inflamm. Res. 47 (1998) 36–41.
[83] Y. Liu, L. Xu, N. Cheng, L. Lin, C. Zhang, Inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells, J. Appl. Phycol. 12 (2000) 125–130.
[84] H. Bei, W. Guang-Ce, Z. Chen-Kui, L. Zhen-gang, The experimental research of R-phycoerythrin subunits on cancer treatment: a new photosensitizer in PDT, Cancer Biother. Radiopharm. 17 (2002) 35–42.
[85] S.R. Shih, K.N. Tsai, Y.S. Li, C.C. Chueh, E.C. Chan, Inhibition of enterovirus 71-induced apoptosis by allophycocyanin isolated from a blue-green alga *Spirulina platensis*, J. Med. Virol. 70 (2003) 119–125.
[86] N.K. Singh, S.S. Hasan, J. Kumar, I. Raj, A.A. Pathan, A. Parmar, et al., Crystal structure and interaction of phycocyanin with β-secretase: a putative therapy for Alzheimer's disease, CNS Neurol. Disord. Drug Targets 13 (2014) 691–698.
[87] M.A. Ragan, K.W. Glombitza, Handbook of Physiological Methods, Cambridge University Press, Cambridge, 1986.
[88] S.J. Heo, E.J. Park, K.W. Lee, Y.J. Jeon, Antioxidant activities of enzymatic extracts from brown seaweeds, Bioresour. Technol. 96 (2005) 1613–1623.
[89] N.Y. Yoon, H.Y. Chung, H.R. Kim, J.S. Choi, Acetyl-and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*, Fish. Sci. 74 (1) (2008) 200–207.
[90] K. Nagayama, T. Shibata, K. Fujimoto, T. Honjo, T. Nakamura, Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae, Aquaculture 218 (2003) 601–611.
[91] N.Y. Yoon, Cholinestarase and Lens Aldose Reductase Inhibitory Activities of Phlorotannins from *Ecklonia Stolonifera* and Their Protective Effects on Tacrine-Induced Hepatotoxicity and Hyperlipidemic Rat Models, Pukyong National University, Republic of Korea, 2008.
[92] Y. Li, Z.J. Qian, B. Ryu, S.H. Lee, M.M. Kim, S.K. Kim, Chemical components and its antioxidant properties in vitro: an edible marine brown alga, *Ecklonia cava*, Bioorg. Med. Chem. 17 (2009) 1963–1973.
[93] T. Shibata, K. Ishimaru, S. Kawaguchi, H. Yoshikawa, Y. Hama, Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae, J. Appl. Phycol. 20 (2008) 705–711.
[94] S.J. Heo, P.J. Park, E.J. Park, S.K. Kim, Y.J. Jeon, Antioxidant activity of enzymatic extracts from a brown seaweed *Ecklonia cava* by electron spin resonance spectrometry and comet assay, Eur. Food Res. Technol. 221 (2005) 41–47.
[95] H.S. Kang, H.Y. Chung, J.H. Jung, B.W. Son, J.S. Choi, A new phlorotannin from the brown alga *Ecklonia stolonifera*, Chem. Pharm. Bull. 51 (2003) 1012–1014.
[96] H.S. Kang, H.Y. Chung, J.Y. Kim, B.W. Son, H.A. Jung, J.S. Choi, Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation, Arch. Pharm. Res. 27 (2004) 194–198.
[97] S.H. Lee, F. Karadeniz, M.M. Kim, S.K. Kim, α-Glucosidase and α-amylase inhibitory activities of phloroglucinal derivatives from edible marine brown alga, *Ecklonia cava*, J. Sci. Food Agric. 89 (2009) 1552–1558.
[98] K.A. Kang, K.H. Lee, S. Chae, R. Zhang, M.S. Jung, Y.M. Ham, et al., Cytoprotective effect of phloroglucinol on oxidative stress induced cell damage via catalase activation, J. Cell. Biochem. 97 (2006) 609–620.
[99] M. Nakai, N. Kageyama, K. Nakahara, W. Miki, Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*, Mar. Biotechnol. 8 (2006) 409–414.

[100] G.N. Ahn, K.N. Kim, S.H. Cha, C.B. Song, J. Lee, M.S. Heo, et al., Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage, Eur. Food Res. Technol. 226 (2007) 71–79.
[101] Y. Zou, Z.J. Qian, Y. Li, M.M. Kim, S.H. Lee, S.K. Kim, Antioxidant effects of phlorotannins isolated from *Ishige okamurae* in free radical mediated oxidative systems, J. Agric. Food Chem. 56 (2008) 7001–7009.
[102] S.J. Heo, S.C. Ko, S.H. Cha, D.H. Kang, H.S. Park, Y.U. Choi, et al., Effect of phlorotannins isolated from *Ecklonia cava* on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation, Toxicol. In Vitro 23 (2009) 1123–1130.
[103] A.R. Kim, T.S. Shin, M.S. Lee, J.Y. Park, K.E. Park, N.Y. Yoon, et al., Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties, J. Agric. Food Chem. 57 (2009) 3483–3489.
[104] T. Wang, R. Jonsdottir, H. Liu, L. Gu, H.G. Kristinsson, S. Raghavan, G. Olafsdottir, Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*, J. Agric. Food Chem. 60 (2012) 5874–5883.
[105] H.A. Jung, S.E. Jin, B.R. Ahn, C.M. Lee, J.S. Choi, Anti-inflammatory activity of edible brown alga *Eisenia bicyclis* and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264. 7 macrophages, Food Chem. Toxicol. 59 (2013) 199–206.
[106] E. Cruces, P. Huovinen, I. Gómez, Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae, Mar. Biol. 160 (2013) 1–13.
[107] T. Shibata, K. Yamaguchi, K. Nagayama, S. Kawaguchi, T. Nakamura, Inhibitory activity of brown algal phlorotannins against glycosidases from the viscera of the turban shell *Turbo cornutus*, Eur. J. Phycol. 37 (2002) 493–500.
[108] N.Y. Yoon, S.H. Lee, S.K. Kim, Phlorotannins from *Ishige okamurae* and their acetyl- and butyrylcholinesterase inhibitory effects, J. Funct. Foods 1 (2009) 331–335.
[109] G.H. Li, G.W. Le, Y.H. Shi, S. Shrestha, Angiotensin I–converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects, Nutr. Res. 24 (2004) 469–486.
[110] H.A. Jung, S.K. Hyun, H.R. Kim, J.S. Choi, Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*, Fish. Sci. 72 (2006) 1292–1299.
[111] Y. Athukorala, Y.J. Jeon, Screening for angiotensin 1-converting enzyme inhibitory activity of *Ecklonia cava*, J. Food Sci. Nutr. 10 (2005) 134–139.
[112] C.S. Kong, J.A. Kim, N.Y. Yoon, S.K. Kim, Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells, Food Chem. Toxicol. 47 (2009) 1653–1658.
[113] Y. Li, Z.J. Qian, M.M. Kim, S.K. Kim, Cytotoxic activities of phlorethol and fucophlorethol derivatives isolated from Laminariaceae *Ecklonia cava*, J. Food Biochem. 35 (2011) 357–369.
[114] M.J. Ahn, K.D. Yoon, S.Y. Min, J.S. Lee, J.H. Kim, T.G. Kim, et al., Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*, Biol. Pharm. Bull. 27 (2004) 544–547.
[115] M. Artan, Y. Li, F. Karadeniz, S.H. Lee, M.M. Kim, S.K. Kim, Anti-HIV-1 activity of phloroglucinol derivative, 6, 6′-bieckol, from *Ecklonia cava*, Bioorg. Med. Chem. 16 (2008) 7921–7926.
[116] Y.J. Kim, H. Uyama, Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future, Cell. Mol. Life Sci. 62 (2005) 1707–1723.
[117] R. Zhang, K.A. Kang, M.J. Piao, D.O. Ko, Z.H. Wang, I.K. Lee, et al., Eckol protects V79-4 lung fibroblast cells against γ-ray radiation-induced apoptosis via the scavenging of reactive oxygen species and inhibiting of the c-Jun NH 2-terminal kinase pathway, Eur. J. Pharmacol. 591 (2008) 114–123.
[118] Q.T. Le, Y. Li, Z.J. Qian, M.M. Kim, S.K. Kim, Inhibitory effects of polyphenols isolated from marine alga *Ecklonia cava* on histamine release, Process. Biochem. 44 (2009) 168–176.
[119] A.O. Vershinin, A.N. Kamnev, Xanthophyll cycle in marine macroalgae, Bot. Mar. 39 (1996) 421–426.
[120] C.C. Hu, J.T. Lin, F.J. Lu, F.P. Chou, D.J. Yang, Determination of carotenoids in *Dunaliella salina* cultivated in Taiwan and antioxidant capacity of the algal carotenoid extract, Food Chem. 109 (2008) 439–446.
[121] M.K. Mohammed, M.K. Mohd, Production of carotenoids (antioxidants/colourant) in *Spirulina platensis* in response to indole acetic acid (IAA), Int. J. Eng. Sci. Technol. 3 (2011) 4973–4979.
[122] Y. Okai, K. Higashi-Okai, Y. Yano, S. Otani, Identification of antimutagenic substances in an extract of edible red alga, *Porphyra tenera* (Asadusa-nori), Cancer Lett. 100 (1996) 235–240.

[123] K.C. Murthy, A. Vanitha, J. Rajesha, M.M. Swamy, P.R. Sowmya, G.A. Ravishankar, In vivo antioxidant activity of carotenoids from *Dunaliella salina*—a green microalga, Life Sci. 76 (2005) 1381–1390.
[124] J.A. Del Campo, M. García-González, M.G. Guerrero, Outdoor cultivation of microalgae for carotenoid production: current state and perspectives, Appl. Microbiol. Biotechnol. 74 (2007) 1163–1174.
[125] B.S. Inbaraj, J.T. Chien, B.H. Chen, Improved high performance liquid chromatographic method for determination of carotenoids in the microalga *Chlorella pyrenoidosa*, J. Chromatogr. A 1102 (2006) 193–199.
[126] J.A. Haugan, Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae), Biochem. Syst. Ecol. 22 (1994) 31–41.
[127] M. Kobayashi, Y. Sakamoto, Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*, Biotechnol. Lett. 21 (1999) 265–269.
[128] T. Maoka, Carotenoids in marine animals, Mar. Drugs 9 (2011) 278–293.
[129] R. Esteban, B. Martínez, B. Fernández-Marín, J. Maria Becerril, J.I. García-Plazaola, Carotenoid composition in Rhodophyta: insights into xanthophyll regulation in *Corallina elongata*, Eur. J. Phycol. 44 (2009) 221–230.
[130] K. Yokthongwattana, T. Savchenko, J.E. Polle, A. Melis, Isolation and characterization of a xanthophyll-rich fraction from the thylakoid membrane of *Dunaliella salina* (green algae), Photochem. Photobiol. Sci. 4 (2005) 1028–1034.
[131] M. Terasaki, A. Hirose, B. Narayan, Y. Baba, C. Kawagoe, H. Yasui, et al., Evaluation of recoverable functional lipid components of several brown seaweeds (phaeophyta) from Japan with special reference to fucoxanthin and fucosterol contents, J. Phycol. 45 (2009) 974–980.
[132] N.M. Sachindra, E. Sato, H. Maeda, M. Hosokawa, Y. Niwano, M. Kohno, K. Miyashita, Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites, J. Agric. Food Chem. 55 (2007) 8516–8522.
[133] C. Grewe, C. Griehl, Time-and media-dependent secondary carotenoid accumulation in *Haematococcus pluvialis*, Biotechnol. J. 3 (2008) 1232–1244.
[134] Z. Wu, S. Wu, X. Shi, Supercritical fluid extraction and determination of lutein in heterotrophically cultivated *Chlorella pyrenoidosa*, J. Food Proc. Eng. 30 (2007) 174–185.
[135] Y.M. Naguib, Antioxidant activities of astaxanthin and related carotenoids, J. Agric. Food Chem. 48 (2000) 1150–1154.
[136] Y.J. Wang, Y.H. Chien, C.H. Pan, Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*, Aquaculture 261 (2006) 641–648.
[137] L. Jaime, J.A. Mendiola, E. Ibáñez, P.J. Martin-Álvarez, A. Cifuentes, G. Reglero, F.J. Señoráns, β-Carotene isomer composition of sub- and supercritical carbon dioxide extracts. Antioxidant activity measurement, J. Agric. Food Chem. 55 (2007) 10585–10590.
[138] C.S. Foote, R.W. Denny, Chemistry of singlet oxygen. VII. Quenching by beta-carotene, J. Am. Chem. Soc. 90 (1968) 6233–6235.
[139] D. Baltschun, S. Beutner, K. Briviba, H.D. Martin, J. Paust, M. Peters, et al., Singlet oxygen quenching abilities of carotenoids, Liebigs Ann. 9 (1997) 1887–1893.
[140] P.F. Conn, W. Schalch, T.G. Truscott, The singlet oxygen and carotenoid interaction, J. Photochem. Photobiol. B 11 (1991) 41–47.
[141] P. Di Mascio, S. Kaiser, H. Sies, Lycopene as the most efficient biological carotenoid singlet oxygen quencher, Arch. Biochem. Biophys. 274 (1989) 532–538.
[142] G. Britton, Structure and properties of carotenoids in relation to function, FASEB J. 9 (1995) 1551–1558.
[143] W. Stahl, H. Sies, Antioxidant defence: vitamins E and C and carotenoids, Diabetes 46 (Suppl. 2) (1997) S14–S18.
[144] G.W. Burton, K.U. Ingold, Beta-carotene: an unusual type of lipid antioxidant, Science 224 (1984) 569–573.
[145] P. Palozza, Prooxidant actions of carotenoids in biologic systems, Nutr. Rev. 56 (1998) 257–265.
[146] L. Lomnitski, S. Grossman, M. Bergman, Y. Sofer, D. Sklan, In vitro and in vivo effects of beta-carotene on rat epidermal lipoxygenases, Int. J. Vitam. Nutr. Res. 67 (1996) 407–414.
[147] T. Iyama, A. Takasuga, M. Azuma, beta-Carotene accumulation in mouse tissues and a protective role against lipid peroxidation, Int. J. Vitam. Nutr. Res. 66 (1995) 301–305.
[148] G. Levin, M. Yeshurun, S. Mokady, In vivo antiperoxidative effect of 9-cis β-carotene compared with that of the all-trans isomer, Nutr. Cancer 27 (1997) 293–297.

[149] P. Palozza, C. Luberto, G. Calviello, P. Ricci, G.M. Bartoli, Antioxidant and prooxidant role of β-carotene in murine normal and tumor thymocytes: effects of oxygen partial pressure, Free Rad. Biol. Med. 22 (1997) 1065–1073.
[150] R. Stocker, V.W. Bowry, B. Frei, Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol, Proc. Natl. Acad. Sci. 88 (1991) 1646–1650.
[151] H. Esterbauer, M. Dieber-Rotheneder, G. Striegl, G. Waeg, Role of vitamin E in preventing the oxidation of low-density lipoprotein, Am. J. Clin. Nutr. 53 (1991) 314S–321S.
[152] S.J. Heo, S.C. Ko, S.M. Kang, H.S. Kang, J.P. Kim, S.H. Kim, et al., Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H_2O_2-induced cell damage, Eur. Food Res. Technol. 228 (2008) 145–151.
[153] S.J. Heo, Y.J. Jeon, Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage, J. Photochem. Photobiol. B 95 (2009) 101–107.
[154] N.M. Lyons, N.M. O'Brien, Modulatory effects of an algal extract containing astaxanthin on UVA-irradiated cells in culture, J. Dermatol. Sci. 30 (2002) 73–84.
[155] S.A. Turujman, W.G. Wamer, R.R. Wei, R.H. Albert, Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin, J. AOAC Int. 80 (1997) 622–632.
[156] M. Kurashige, E. Okimasu, M. Inoue, K. Utsumi, Inhibition of oxidative injury of biological membranes by astaxanthin, Physiol. Chem. Phys. Med. NMR 22 (1990) 27–38.
[157] P. Palozza, N. Krinsky, Astaxanthin and canthaxanthin are potent antioxidant in a membrane model, Arch. Biochem. Biophys. 297 (1992) 291–295.
[158] L.S. Costa, G.P. Fidelis, S.L. Cordeiro, R.M. Oliveira, D.A. Sabry, R.B.G. Câmara, et al., Biological activities of sulfated polysaccharides from tropical seaweeds, Biomed. Pharmacother. 64 (2010) 21–28.
[159] Q. Zhang, N. Li, G. Zhou, X. Lu, Z. Xu, Z. Li, In vivo antioxidant activity of polysaccharide fraction from *Porphyra haitanensis* (Rhodephyta) in aging mice, Pharmacol. Res. 48 (2003) 151–155.
[160] Q. Zhang, N. Li, X. Liu, Z. Zhao, Z. Li, Z. Xu, The structure of a sulfated galactan from *Porphyra haitanensis* and its in vivo antioxidant activity, Carbohydr. Polym. 339 (2004) 105–111.
[161] X. Zhao, C.H. Xue, B.F. Li, Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*, J. Appl. Phycol. 20 (2008) 431–436.
[162] H. Yuan, W. Zhang, X. Li, X. Lü, N. Li, X. Gao, J. Song, Preparation and in vitro antioxidant activity of κ-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives, Carbohydr. Polym. 340 (2005) 685–692.
[163] H. Qi, T. Zhao, Q. Zhang, Z. Li, Z. Zhao, R. Xing, Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta), J. Appl. Phycol. 17 (2005) 527–534.
[164] M.C.R. De Souza, C.T. Marques, C.M.G. Dore, F.R.F. da Silva, H.A.O. Rocha, E.L. Leite, Antioxidant activities of sulfated polysaccharides from brown and red seaweeds, J. Appl. Phycol. 19 (2007) 153–160.
[165] J. Wang, Q. Zhang, Z. Zhang, Z. Li, Antioxidant activity of sulphated polysaccharide fractions extracted from *Laminaria japonica*, Int. J. Biol. Macromol. 42 (2008) 127–132.
[166] T. Hu, D. Liu, Y. Chen, J. Wu, S. Wang, Antioxidant activity of sulfated polysaccharide fractions extracted from *Undaria pinnitafida* in vitro, Int. J. Biol. Macromol. 46 (2010) 193–198.
[167] S. Ananthi, H.R.B. Raghavendran, A.G. Sunil, V. Gayathri, G. Ramakrishnan, H.R. Vasanthi, In vitro antioxidant and in vivo anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (Marine Brown Alga), Food Chem. Toxicol. 48 (2010) 187–192.
[168] Z. Zhang, F. Wang, X. Wang, X. Liu, Y. Hou, Q. Zhang, Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro, Carbohydr. Polym. 82 (2010) 118–121.
[169] L.S. Costa, G.P. Fidelis, C.B.S. Telles, N. Dantas-Santos, R.B.G. Camara, S.L. Cordeiro, et al., Antioxidant and antiproliferative activities of heterofucans from the seaweed *Sargassum filipendula*, Mar. Drugs 9 (2011) 952–966.
[170] M.S.S.P. Costa, L.S. Costa, S.L. Cordeiro, J. Almeida-Lima, N. Dantas-Santos, K.D. Magalhães, et al., Evaluating the possible anticoagulant and antioxidant effects of sulfated polysaccharides from the tropical green alga *Caulerpa cupressoides* var. flabellata, J. Appl. Phycol. 24 (2012) 1159–1167.
[171] P. Ruperez, O. Ahrazem, A. Leal, Potential antioxidant capacity of sulphated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*, J. Agric. Food Chem. 50 (2002) 840–845.

[172] H. Qi, Q. Zhang, T. Zhao, R. Chen, H. Zhang, X. Niu, Z. Li, Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro, Int. J. Biol. Macromol. 37 (2005) 195–199.
[173] L. Sun, C. Wang, Q. Shi, C. Ma, Preparation of different molecular weight from *Porphyridium cruentum* and their antioxidant activities, Int. J. Biol. Macromol. 45 (2009) 42–47.
[174] I. Wijesekara, N.Y. Yoon, S.K. Kim, Phlorotannins from *Ecklonia cava* (Phaeophyceae): biological activities and potential health benefits, Biofactors 36 (2010) 408–414.
[175] M.J. Kwon, T.J. Nam, Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines, Life Sci. 79 (2006) 1956–1962.
[176] Y. Athukorala, G.N. Ahn, Y.H. Jee, G.Y. Kim, S.H. Kim, J.H. Ha, et al., Antiproliferative activity of sulfated polysaccharide isolated from an enzymatic digest of *Ecklonia cava* on the U-937 cell line, J. Appl. Phycol. 21 (2009) 307–314.
[177] H.J. Moon, S.R. Lee, S.N. Shim, S.H. Jeong, V.A. Stonik, V.A. Rasskazov, et al., Fucoidan inhibits UVB-induced MMP-1 expression in human skin fibroblasts, Biol. Pharm. Bull. 31 (2008) 284–289.
[178] M. Witvrouw, E. De Clercq, Sulfated polysaccharides extracted from sea algae as potential antiviral drugs, Gen. Pharmacol. 29 (1997) 497–511.
[179] K.D. Thompson, C. Dragar, Antiviral activity of *Undaria pinnatifida* against herpes simplex virus, Phytother. Res. 18 (2004) 551–555.
[180] N.M. Ponce, C.A. Pujol, E.B. Damonte, M.L. Flores, C.A. Stortz, Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structural studies, Carbohydr. Polym. 338 (2003) 153–165.
[181] C.A. Pujol, J.M. Estevez, M.J. Carlucci, M. Ciancia, A.S. Cerezo, E.B. Damonte, Novel DL-galactan hybrids from the red seaweed *Gymnogongrus torulosus* are potent inhibitors of herpes simplex virus and dengue virus, Antiviral Chem. Chemother. 13 (2002) 83–89.
[182] D.J. Schaeffer, V.S. Krylov, Anti-HIV activity of extracts and compounds from algae and cyanobacteria, Ecotoxicol. Environ. Saf. 45 (2000) 208–227.
[183] D. Ren, H. Noda, H. Amano, T. Nishino, K. Nishizawa, Study on antihypertensive and antihyperlipidemic effects of marine algae, Fish. Sci. 60 (1994) 33–40.
[184] K. Tsuge, M. Okabe, T. Yoshimura, T. Sumi, H. Tachibana, K. Yamada, Dietary effect of porphyran from *Porphyra yezoensis* on growth and lipid metabolism of Sprague-Dawley rats, Food Sci. Technol. Res. 10 (2004) 147–151.
[185] P. Yu, L. Ning, L. Xiguang, Z. Gefei, Z. Quanbin, L. Pengchen, Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta), Pharmacol. Res. 48 (2003) 543–549.
[186] N. Inoue, N. Yamano, K. Sakata, K. Nagao, Y. Hama, T. Yanagita, The sulfated polysaccharide porphyran reduces apolipoprotein B100 secretion and lipid synthesis in HepG2 cells, Biosci. Biotechnol. Biochem. 73 (2009) 447–449.
[187] F. Garcia-Pichel, R.W. Castenholz, Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment, J. Phycol. 27 (1991) 395–409.
[188] P.J. Proteau, W.H. Gerwick, F. Garcia-Pichel, R. Castenholz, The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria, Experientia 49 (1993) 825–829.
[189] C.S. Jones, E. Esquenazi, P.C. Dorrestein, W.H. Gerwick, Probing the *in vivo* biosynthesis of scytonemin, a cyanobacterial ultraviolet radiation sunscreen, through small scale stable isotope incubation studies and MALDI-TOF mass spectrometry, Bioorg. Med. Chem. 19 (2011) 6620–6627.
[190] F. Garcia-Pichel, R.W. Castenholz, Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity, Appl. Environ. Microbiol. 59 (1993) 163–169.
[191] Q. Gao, F. Garcia-Pichel, Microbial ultraviolet sunscreens, Nat. Rev. Microbiol. 9 (2011) 791–802.
[192] R.P. Rastogi, R.P. Sinha, A. Incharoensakdi, Partial characterization, UV-induction and photoprotective function of sunscreen pigment, scytonemin from *Rivularia* sp. HKAR-4, Chemosphere 93 (2013) 1874–1878.
[193] R.P. Rastogi, A. Incharoensakdi, Characterization of UV-screening compounds, mycosporine like amino acids, and scytonemin in the cyanobacterium *Lyngbya* sp. CU2555, FEMS Microbiol. Ecol. 87 (2014) 244–256.
[194] A.H. Squier, D.A. Hodgson, B.J. Keely, A critical assessment of the analysis and distributions of scytonemin and related UV screening pigments in sediments, Org. Geochem. 35 (2004) 1221–1228.
[195] V. Bultel-Ponc, F. Felix-Theodose, C. Sarthou, J.-F. Ponge, B. Bodo, New pigments from the terrestrial cyanobacterium *Scytonema* sp. collected on the Mitaraka Inselberg, French Guyana, J. Nat. Prod. 67 (2004) 678–681.

[196] C.S. Grant, J.W. Louda, Scytonemin-imine, a mahogany-colored UV/Vis sunscreen of cyanobacteria exposed to intense solar radiation, Org. Geochem. 65 (2013) 29–36.
[197] F. Garcia-Pichel, J. Belnap, Microenvironments and microscale productivity of cyanobacterial desert crusts, J. Phycol. 32 (1996) 774–782.
[198] T.-L. Nadeau, H. Howard-Williams, R.W. Castenholz, Effects of solar UV and visible irradiance on photosynthesis and vertical migration of *Oscillatoria* sp. (cyanobacteria) in an Antarctic microbial mat, Aqu. Microb. Ecol. 20 (1999) 231–243.
[199] J.G. Dillon, R.W. Castenholz, The synthesis of the UV-screening pigment, scytonemin, and photosynthetic performance in isolates from closely related natural populations of cyanobacteria (*Calothrix* sp.), Environ. Microbiol. 5 (2003) 484–491.
[200] E.D. Fleming, R.W. Castenholz, Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria, Environ. Microbiol. 9 (2007) 1448–1455.
[201] J. Rath, S. Mandal, S.P. Adhikary, Salinity induced synthesis of UV-screening compound scytonemin in the cyanobacterium *Lyngbya aestuarii*, J. Photochem. Photobiol. B 115 (2012) 5–8.
[202] K. Matsui, E. Nazifi, Y. Hirai, N. Wada, S. Matsugo, T. Sakamoto, The cyanobacterial UV-absorbing pigment scytonemin displays radical-scavenging activity, J. Gen. Appl. Microbiol. 58 (2012) 137–144.
[203] R.P. Rastogi, R.R. Sonani, D. Madamwar, Cyanobacterial sunscreen scytonemin: role in photoprotection and biomedical research, Appl. Biochem. Biotechnol. 176 (2015) 1551–1563.
[204] S. Takamatsu, T.W. Hodges, I. Rajbhandari, W.H. Gerwick, M.T. Hamann, D.G. Nagle, Marine natural products as novel antioxidant prototypes, J. Nat. Prod. 66 (2003) 605–608.
[205] S.P. Singh, S. Kumari, R.P. Rastogi, R. Singh, R.P. Sinha, Photoprotective and biotechnological potentials of cyanobacterial sheath pigment, scytonemin, Afr. J. Biotechnol. 9 (2010) 580–588.
[206] R.P. Rastogi, R.R. Sonani, D. Madamwar, The high-energy radiation protectant extracellular sheath pigment scytonemin and its reduced counterpart in the cyanobacterium *Scytonema* sp. R77DM, Bioresour. Technol. 171 (2014) 396–400.
[207] C.S. Stevenson, E.A. Capper, A.K. Roshak, B. Marquez, C. Eichman, J.R. Jackson, M. Mattern, W.H. Gerwick, R.S. Jacobs, L.A. Marshall, The identification and characterization of the marine natural product scytonemin as a novel antiproliferative pharmacophore, J. Pharmacol. Exp. Ther. 303 (2002) 858–866.
[208] C.S. Stevenson, E.A. Capper, A.K. Roshak, B. Marquez, K. Grace, W.G. Gerwick, R.S. Jacobs, L.A. Marshall, Scytonemin, a marine natural product inhibitor of kinases key in hyperproliferative inflammatory diseases, Inflamm. Res. 51 (2002) 112–114.
[209] K. Strebhardt, A. Ullrich, Targeting polo-like kinase 1 for cancer therapy, Nat. Rev. Cancer 6 (2006) 321–330.
[210] F.A. Barr, H.H. Silljé, E.A. Nigg, Polo-like kinases and the orchestration of cell division, Nat. Rev. Mol. Cell Biol. 5 (2004) 429–440.
[211] G. Zhang, Z. Zhang, Z. Liu, Polo-like kinase 1 is overexpressed in renal cancer and participates in the proliferation and invasion of renal cancer cells, Tumor Biol. 34 (2013) 1887–1894.
[212] G. Zhang, Z. Zhang, Z. Liu, Scytonemin inhibits cell proliferation and arrests cell cycle through downregulating Plk1 activity in multiple myeloma cells, Tumour Biol. 34 (2013) 2241–2247.
[213] A. Portwich, F. Garcia-Pichel, Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium *Chlorogloeopsis* sp. strain PCC 6912, Phycologia 42 (2003) 384–392.
[214] F.R. Conde, M.S. Churio, C.M. Previtali, The deactivation pathways of the excited states of the mycosporine-like amino acids shinorine and porphyra334 in aqueous solution, Photochem. Photobiol. Sci. 3 (2004) 960–967.
[215] M.J. Shick, W.C. Dunlap, Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms, Annu. Rev. Physiol. 64 (2002) 223–262.
[216] R.P. Rastogi, R.R. Sonani, D. Madamwar, A. Incharoensakdi, Characterization and antioxidant functions of mycosporine-like amino acids in the cyanobacterium *Nostoc* sp. R76DM, Algal Res. 16 (2016) 110–118.

CHAPTER

6

Microalgae as a Source of Bioplastics

A. Rahman[1], *C.D. Miller*[2]

[1]NASA Ames Research Center, Moffett Field, CA, United States; [2]Utah State University, Logan, UT, United States

OUTLINE

1. INTRODUCTION

As we look to reduce our dependence on crude oil for fuels, there is also a need to reduce petroleum being used for petrochemically produced plastics. Worldwide demand for plastic-based materials continues to grow, adding stress on current infrastructure. In addition to reducing plastics being made from petroleum, there is significant interest in reducing plastic accumulation in landfills. In 2011, the United States Environmental Protection Agency (EPA) reported that plastic waste accounted for 13% of all municipal solid waste [1]. Furthermore, petroleum-based plastics pose a great risk to the environment especially in marine ecosystems [2]. Presently, there are worldwide initiatives to reduce the usage of petrochemically derived plastics, and municipalities have taken steps to limit their usage especially with regards to packaging [3].

http://dx.doi.org/10.1016/B978-0-444-63784-0.00006-0

Alternatives to petrochemically derived plastics are biological-based plastics that are biodegradable. Bioplastics can be classified into three categories: (1) renewable bioplastics (plastics naturally produced by plants or from renewable resources), (2) petroleum-based bioplastics (plastics originating from petroleum but are biodegradable), and (3) mixed bioplastics (from petroleum and biological sources) [4]. Production of bioplastics was approximately 2 million tons in 2014, where the most commonly manufactured bioplastics were starch and polylatic acid (PLA)-based polymers [5]. Currently, commodity bioplastics are derived from terrestrial crops and thus compete with food supplies. In addition, crop-based bioplastics require fertile land, water, fertilizers, and are reliant on weather conditions [6]. This is not sustainable for long-term bioplastic production and supply chains, however, there have been several commercial attempts to produce biological plastics on a large scale and efforts are still ongoing in this arena [7].

Microalgae have been identified as an ideal source for biofuels in part due to not competing with food sources, ability to grow on waste resources, and high lipid accumulation [8]. Using microalgae as a platform for bioplastic production could be a means of direct carbon capture by removing CO_2 from the atmosphere, since CO_2 is utilized by microalgae for photosynthesis and converted to various forms in central metabolism [9]. If microalgae biomass is converted to bioplastics, atmospheric carbon is then directly trapped as a polymer. The process of trapping the CO_2 as bioplastic differs from most microalgae platforms that have the primary purpose of producing biofuels, which eventually returns CO_2 to the atmosphere upon combustion. While the concept of CO_2 conversion to bioplastics is a reasonable justification for the existence of such technologies, there are issues upon scale-up to a fully fledged system. One such issue is that bioplastics are currently not cost competitive to their petroleum counterparts. An example of cost reduction is to use a biorefinery model for microalgae cultivation, and production of multiple product streams to make microalgal biomass an economically feasible resource [10,11].

The current state of the art with regards to microalgae conversion to bioplastics is illustrated in Fig. 6.1. Different studies have been conducted ranging from direct usage of microalgal biomass as a bioplastic, blending microalgae biomass with petroleum and biological plastics, intermediate processing in a biorefinery context, and genetic engineering to create bioplastic producing strains of microalgae. This chapter will discuss the state of the field with regards to conversion systems of microalgal biomass to bioplastics.

Eukaryotic microalgae-based bioplastics are the major focus of this chapter and macroalgae-based bioplastics such as those from seaweed will not be discussed in great

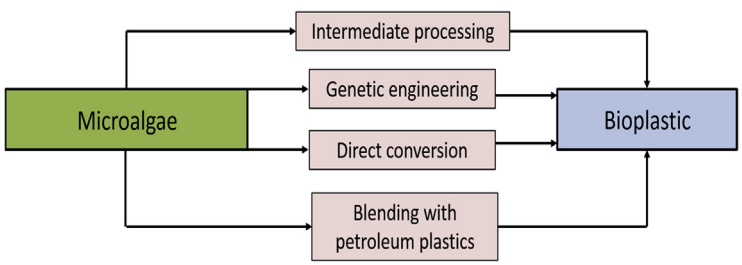


FIGURE 6.1 Conversion of microalgae to bioplastic.

detail. A recent review by Sawant and colleagues discuss bioplastics from marine biomass [12]. Blue–green algae or *Cyanobacteria* have also been studied extensively as a platform for production of bioplastics and there are several review papers that cover this aspect of *Cyanobacteria*, from genetic engineering to culturing [13–15].

2. DIRECT USE OF MICROALGAE BIOMASS FOR BIOPLASTIC PURPOSES

2.1 Blending Microalgal Biomass With Petroleum Plastics

Blending microalgal biomass with petroleum plastics could potentially extend the life of microalgae plastics and provide value-added mechanical properties. The opposite is also true, that conventional plastic blending with microalgae as the minor component could potentially free up the unused petroleum plastic for further applications. There are several studies that have investigated making hybrid materials with varying levels of microalgae biomass and petroleum plastics. While the overall mechanical properties of such hybrid materials is generally inferior than pure petrochemical counterparts, the combination of both the microalgae biomass and petrochemical plastics have serviceable properties and thus a wide range of potential applications.

A study by Zeller and colleagues focused on thermo-mechanical polymerization of microalgae protein biomass to produce bioplastics and blending of microalgae biomass with polyethylene [6]. Microalgae cells are small in size with a typical diameter of 50 μm; this is advantageous as it could potentially allow for better mixing when blended with conventional plastics. In this study, commercially acquired *Spirulina platensis* (57% protein) and *Chlorella vulgaris* (58% protein) were plasticized with glycerol in different ratios (0–30 wt%). It was determined that an algae:glycerol ratio of 80:20 produced the best material properties. All bioplastic samples were processed via thermomechanical molding for 20 min at 150°C to make ASTM standard "dog bones" for mechanical testing. The processing temperature was determined from thermogravimetric analysis and differential scanning calorimetry, where it was found that above 170°C the algae biomass was significantly degraded. When combined with polyethylene, the mechanical properties of *Spirulina*-derived plastics were better when compared to *Chlorella*-blended bioplastic. One hundred percent *Chlorella* did however have better loading and extension proprieties than 100% *Spirulina*, with tensile strengths of 5.7 and 3.0 MPa respectively (Table 6.1) [6].

Another study by Shi and co-workers [16] focused on thermoplastically processing *Nannochloropsis* and *Spirulina* with corn starch and subsequent blending with petroleum-based plastics. Dried algae biomass and relevant components were fed into a twin screw extruder to produce blends and films. Subsequent injection molding was used to make ASTM D638 standard test samples. For a *Nannochloropsis*/Starch/Polypropylene blend the final ratios produced were: 14/6/80%. The tensile strengths of a polypropylene blend with *Nannochloropsis*/Starch/Polypropylene and *Spirulina*/Starch/Polypropylene were 17.1 and 19.5 MPa, respectively (Table 6.1). The tensile strengths of the algae blends were lower than that of pure polypropylene that had a measured tensile strength of 24.2 MPa. From scanning electron microscope analysis it was determined that the algae blends with starch were completely

TABLE 6.1 Blending Microalgae Biomass With Different Plastics and Resulting Mechanical Properties

Microalgae Source	Plastic Blend	Ratio (Algae:Plastic)	Tensile Strength (MPa)	References
Chlorella	None	100/0	5.7	[6]
Spirulina	None	100/0	3.0	[6]
Nannochloropsis	Corn starch/PP	14/6/80	17.1	[16]
Chlorella	PE	10/90	22	[18]
Chlorella	PP	10/90	32	[20]
Chlorella	PVC	20/80	30	[23]
Botryococcus Braunii	PBS	20/80	21.6	[26]
Nannochloropsis gaditana	PBAT	20/80	10	[27]
"Green algae"	PLA	20/80	45	[32]

encapsulated within the petrochemical plastic and it was hypothesized that captured CO_2 (from algae photosynthesis) was fixed into the polymer matrix [16].

Chlorella biomass has also been combined with petrochemically derived polyethylene (PE), as it was suggested that natural cellulosic fibers could enhance thermoplastics. *Chlorella* grains have previously been demonstrated to not exhibit any change when subjected to 75 MPa of pressure, but significantly deform at pressures exceeding 150 MPa. This is significant as plastic processing occurs at pressures below 150 MPa making *Chlorella* an ideal candidate as a bioplastic or use in a blend with other petrochemical plastics [17]. It was found that chemically modifying polyethylene with maleic anhydride improved its compatibility with *Chlorella*. In this study, *Chlorella* was spray dried and fed into a roller mixer with modified PE at 160°C for 10 min. The resulting *Chlorella*-PE matrix was examined by Fourier transform infrared spectroscopy and it was suggested that the modified PE is connected to *Chlorella* at the hydroxy groups on the cellulose surface of *Chlorella* grains via esterification. From mechanical testing *Chlorella* with modified PE, the addition of *Chlorella* biomass fused to modified PE gave higher tensile strength and Young's modulus across a wide spectrum of *Chlorella* wt%. In the case of a polymer containing 10% *Chlorella*, the resulting mechanical properties compared to PE without *Chlorella* were, tensile strength: 22 MPa versus 20 MPa and Young's modulus: 0.47 GPa versus 0.36 GPa (Table 6.1) [18]. This group further elaborated on this study by developing a molding of a *Chlorella*-PE (30% *Chlorella*) dish 15 cm in diameter, demonstrating a practical application of the microalgae composite [19].

Another study by Zhang and colleagues focused on blending polypropylene (PP) with *Chlorella* [20]. This study was similar to the group's previous work, but in this case PP was used instead of PE for the maleic anhydride grafting process and a temperature of 180°C was used instead of 160°C. The results also demonstrated esterification reactions via the hydroxy groups present in cellulose and hemicellulose existing in *Chlorella*. The PP-graft-maleic anhydride with *Chlorella* (90/10 wt%) blend demonstrated tensile strength of 32 MPa and Young's modulus of approximately 0.65 GPa (Table 6.1). The strength and Young's modulus of 10 wt% *Chlorella* blended with PP was higher than that of similar concentration of *Chlorella*

blended with PE in the previous study [18,20]. PP has also been effectively used in combination with residual brown algae, *Laminaria japonica*, and green algae, *Enteromorpha crinite* [21].

Polyvinyl chloride (PVC) is the third most commonly used petroleum-based plastic after PE and PP and has a wide variety of applications [22]. A previous investigation studied the properties of a PVC-*Chlorella* composite material [23]. In these studies, PVC and *Chlorella* biomass were molded together at a ratio of 80% PVC to 20% *Chlorella*. At a molding temperature of approximately 180°C and pressure of 4.4 MPa, the resulting bioplastic had a tensile strength of 30 MPa (Table 6.1). This compares well to PVC without Chlorella, being 50 MPa. The authors also mentioned that increased water content in *Chlorella* had a negative effect on the tensile strength of the PVC-Chlorella composite with a water content of 25% yielding a tensile strength of approximately 10 MPa. However, depending on the application of the PVC-*Chlorella* composite, the lower tensile strength could have tangible applications [23]. Since dewatering microalgae biomass operations constitute a large fraction of costs, using a "wet" microalgae source could also be beneficial in the future [24].

The work carried out by Zhang and co-workers demonstrated a systematic approach to chemically fusing petroleum-based plastics (polyethylene, polypropylene, and polyvinyl chloride) to microalgal biomass. Future work could consist of using other strains of microalgae and other petroleum-based plastics. Other composite studies have been carried out with the sea algae *Ulva armoricana* and poly(vinyl alcohol) [25]. All the aforementioned studies used single strain microalgae to make composite bioplastics; attempting similar studies with mixed microalgal cultures such as those from wastewater would be of interest in the future. Using the previously discussed methods of creation of biocomposites, a technoeconomic analysis of the process could also be carried out to understand the potential scaling costs. Furthermore, removal of lipids from the microalgae biomass and then using the resultant cell walls for plastic production could be incorporated into future experimental designs, potentially reducing the overall cost as lipids could offset the costs from biodiesel production.

Recent studies by the Mohanty group focused on removal of lipids and processing the remaining biomass for use as a biomaterial. Lipids were removed from *Botryococcus Braunii* and the residual microalgae biomass (RBM) was processed with poly(butylene succinate) (PBS). Resulting biocomposites were 20%−30% RMB and 70%−80% PBS, where the authors calculated that PBS infused with 20% RMB could potentially reduce the cost of PBS by 17%. Results demonstrated a PBS/RMB (80/20%) had a tensile strength of approximately 21.6 MPa compared to native PBS that has a tensile strength of 33.3 MPa (Table 6.1). While the decrease in tensile strength was expected, the reduction in costs might make this acceptable depending on the final applications [26].

Another study focused on coextrusion of residual microalgae biomass (RBM, 10%, 20%, and 30%) with poly(butylene adipate-co-terephthalate) (PBAT) [27]. The microalgae used in this study were raceway grown *Nannochloropsis gaditana*. The microalgae were processed to produce biodiesel via transesterification and the residual algal biomass was dried for further processing. Upon mechanical testing, the PBAT/RBM (90/10) and PBAT/RBM (80/20) composition produced a tensile strength of approximately 12 and 10 MPa, respectively (Table 6.1). Native PBAT has a tensile strength of approximately 20 MPa, thus the substitution of PBAT with RBM reduces the overall tensile strength; but as mentioned previously by Toro et al. [26], this might be a justifiable sacrifice if the overall cost of the process is

reduced and there are suitable applications for the hybrid material. The findings of the study gave the recommendation that this type of bioplastic would be best suited for agricultural films that degraded over time. Furthermore, the authors demonstrated that a scaled-up raceway system was able to produce microalgae that had dual purpose of biodiesel and bioplastics, which represents a biorefinery approach to using microalgae for multiple purposes [27].

2.2 Blending Microalgal Biomass With Bioplastics

PLA is a type of bioplastic, derived from lactic acid and has origins from 100% biological resources such as corn and sugar beet [28]. PLAs are already produced on an industrial scale, which makes them ideal candidates for blending with microalgal biomass [29]. PLAs are biocompatible, are presently used in a wide variety of medical applications, and are approved by the United States Food and Drug Administration (FDA) [30]. PLAs are biodegradable and are broken down to lactic acid via hydrolysis, with the resulting lactic acid being incorporated into metabolic pathways [31].

A study by Bulota and co-workers [32] demonstrated that combining green algae (in 0–40 wt% fractions) with PLA had a decreasing effect on tensile strength when a higher ratio of algae was used. In this study, PLA and algae flakes were dried at 80°C and then mixed at various ratios (40 g final mass) at 180°C. Injection molding operating at 180°C was used to make ISO 527 standard "Dumbbell" bars; tensile strength and Young's modulus was then measured. At a 20 wt% of green algae with 80% PLA the report tensile strength was approximately 45 MPa (Table 6.1). When the weight fraction of algae combined with PLA was increased to 40 wt% the Young's modulus was higher than native PLA. These findings are attributed to green algae having higher cellulose content that contributed to the increase in Young's modulus. This is an interesting finding as increasing the Young's modulus of a material such as PLA could have implications for future applications [32].

With PLAs being an FDA-approved bioplastic, there could be potential applications for PLA-algae composites in the near future. Currently PLAs are primarily produced from biological sources with the monomer lactic acid and the use of an inorganic catalyst; however, research has been carried out to make this a completely biological process, including the catalyst and polymerization process [33]. Alternatively, lactic acid could also be produced economically using microbes growing on waste carbon substrates [34].

2.3 Microalgae Biomass as a Feedstock for Polyhydroxyalkanoate Production

While PLAs are widely used, their primary production competes with fertile land as they are derived from crops, such as corn and sugar beets [28]. Utilizing a biorefinery approach to using PLA producing microorganism that can grow on waste carbon substrates could be possible in the future with advances in genetic engineering and synthetic biology [33]. The most widely produced and studied bacterial polyesters are polyhydroxyalkanoates (PHAs) [35–38]. PHAs are naturally produced under environmental stress conditions by a number of different organisms such as *Ralstonia eutropha*, *Pseudomonas aeruginosa*, *Azotobacter vinelandii*, *Methylocystis parvus*, and *Cyanobacteria* [14,39–42]. PHAs are also produced in genetically engineered systems such as in bacteria, microalgae, and plants [43–46]. There are over 155

unique PHA monomers produced by a wide variety of microorganisms [47]. These differing monomers each have unique properties and thus different potential downstream applications. PHAs have a melting temperature range of 50–180°C, a crystallinity of 30–70% [48,49], a molecular weight range of 2×10^5 to 3×10^6 Da, and are biodegradable [50,51]. Due to their diverse range of properties there are many potential applications [52].

Currently there are several companies pursuing commercial implementation of PHAs. However, in order for PHA-based plastics to fully reach their potential, there are several barriers that need to be overcome: growth in low cost substrates, fast growing organisms, high density fermentation, and inexpensive downstream processing [53].

Of the 155 unique PHAs, one of the most studied are the polyhydroxybutyrates (PHBs). PHBs are classified as short chain length (scl) polymers having a methyl as the branched group [47]. PHBs have a melting temperature of approximately 179°C and a tensile strength of 40 MPa. Petrochemical plastics such as polypropylene and polystyrene have melting temperatures and tensile strengths of 170°C and 110°C and 35 MPa and 50 MPa, respectively (Table 6.2) [48]. Thus, PHBs have comparable properties to their petrochemical counterparts and merit investigation as suitable replacements.

PHB can be produced microbially from acetyl-CoA using three enzymes: β-ketothiolase (*pha*A), acetoacetyl-CoA reductase (*pha*B), and PHB polymerase (*pha*C). Two acetyl-CoAs are condensed with β-ketothiolase and the resulting product is reduced with acetoacetyl-CoA reductase. The final step is polymerization with PHB polymerase that forms the long-chain PHB polymer [44,47]. Typically, PHB polymers have a tendency to form amphipathic granules within the cell, keeping the PHB polymerase covalently attached [54].

PHAs are produced by a diverse group of microorganisms, providing opportunity for potential growth on an extensive range of substrates. This is important from a flexibility standpoint, where in a biorefinery system it would be desired to have production organisms with the ability to grow on different or variable carbon substrates. Systems for PHA production have been carried out with genetically engineered aerobic microorganisms such as *Escherichia coli* [55]. Using recombinant *E. coli* over native PHA producers has many advantages including high growth rate, high culture density, high product yield, and no degradation of product, since *E. coli* lacks the genetic ability to break down PHAs [56].

2.4 Biorefinery Approach to Producing PHAs

A biorefinery approach for microalgae bioproduct generation has the potential to reduce the overall cost of the system and thereby allowing microalgae-based bioproducts to be

TABLE 6.2 Comparison of Polyhydroxybutyrates With Petroleum-Based Plastics [48]

Polymer	Melting Point (°C)	Young's Modulus (GPa)	Tensile Strength (MPa)
Polyhydroxybutyrates	179	3.5	40
Polypropylene	170	1.7	34.5
Polystyrene	110	3.1	50

cost competitive [11,57,58]. Much like the petroleum industry uses refineries to produce multiple product streams, the same concept could be applied to microalgae systems. Utilizing lipids for biodiesel, hydrolyzing cell walls for solvent production or plastic production, carbohydrates for food, and using remaining biomass for anaerobic digestion could allow an integrated system to be cost competitive. Costs could be even further reduced if the primary source of microalgae are grown on domestic wastewater, which could be an essentially free source of nutrients [59]. Weak domestic wastewater is nutrient rich (mg/L N and P) and could theoretically produce up to 42.8 g biomass/L [60]. In addition, the harvesting technology used could have an effect on the product stream and the overall system costs [61–63]. Studies have also investigated the use of microalgae proteins as a source for recombinant microorganism growth and bioproduct production [64].

One such approach is using wastewater microalgae as a feedstock for a biorefinery model to produce multiple product streams [10]. In this system, naturally occurring domestic wastewater microalgae species (*Scenedesmus obliquus*) were grown in Solar Simulated Bioreactors and harvested using various cationic starches [65,66]. Central to this specific biorefinery model is the Wet Lipid Extraction Procedure (WLEP), where the harvested microalgae biomass was processed using the WLEP and generated three product streams. The first stage of the WLEP removed approximately 77–79% of all transesterifiable lipids from wet microalgae biomass (~84% moisture) via an acid/base hydrolysis and lipids were converted to fatty acid methyl esters (FAME or biodiesel). In addition, the first stage of the WLEP removed the majority of the chlorophyll in the FAME [67,68]. After lipid extraction, in the second stage, hydrolyzed microalgae biomass was used to make acetone, butanol, and ethanol via anaerobic fermentation by *Clostridium saccharoperbutylacetonicum* N1–4 [69]. In the third stage of the WLEP, the aqueous phase from stage 2 was used for recombinant *E. coli* cultivation and additional bioproduct generation (Fig. 6.2).

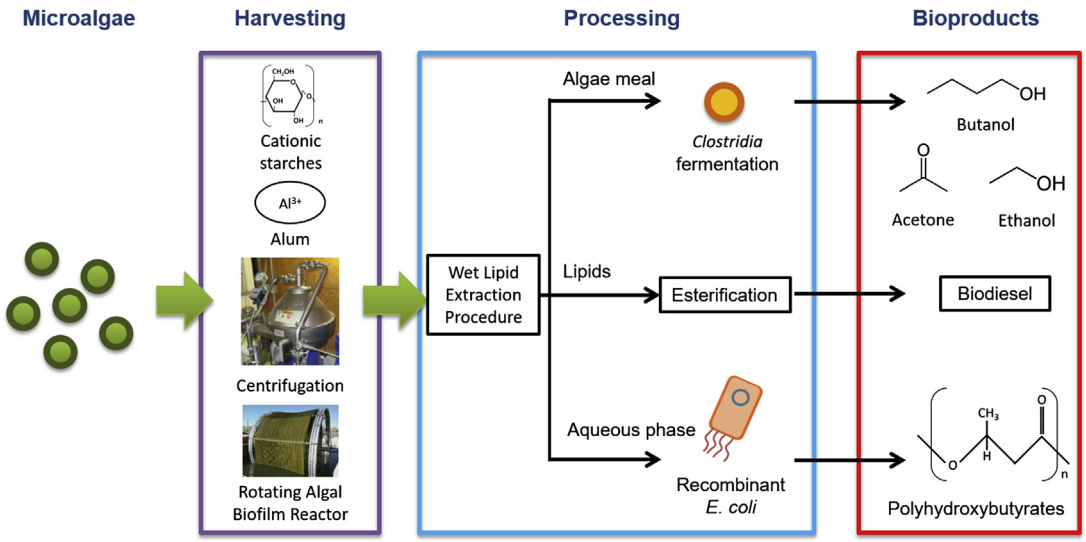


FIGURE 6.2 Wastewater microalgae harvesting and processing via wet lipid extraction procedure to produce multiple products in a biorefinery system, including the bioplastic polyhydroxybutyrate.

The results of the aforementioned study demonstrated that biodiesel, solvents, and *E. coli* biomass could be produced from a single microalgae source. Furthermore, it was found that the type of harvesting method had an effect on the product streams being generated by the WLEP. Using cationic corn and potato starches for microalgae harvesting produced 1.91 and 1.84 g/L butanol and 0.95 and 0.87 g/L acetone, respectively. Using centrifugation, the solvents produced were 1.44 g/L butanol and 0.71 g/L acetone, which was lower than that from harvested algae using cationic corn and potato starches. The highest yield of FAME achieved from the WLEP was in the centrifuged algae sample. In the case of recombinant *E. coli* growth, centrifuged microalgae media gave the highest levels of growth when compared to the other harvesting techniques [10].

From the previous biorefinery study by Anthony and co-workers where centrifugation was used and recombinant *E. coli* were grown on the aqueous phase media, it demonstrated high levels of *E. coli* growth that were comparable to standard *E. coli* growth media [10]. *E. coli* cultured on aqueous phase media was able to reach approximately 10^{13} CFU/mL, which corresponded to a dry cell weight of 0.1 g. The successful culturing of *E. coli* on a microalgae-based media is significant, as *E. coli* can be easily genetically engineered to produce a wide variety of bioproducts. *E. coli* is one of most widely used host organism for recombinant protein expression and demonstrating its productivity with an algae-based media could open up the possibilities for further product manufacturing [70]. The ability to genetically engineer *E. coli* to produce different products could be advantageous in a biorefinery context as it enables the overall system to be flexible and thus potentially economically feasible.

The aforementioned biorefinery study by Anthony and colleagues focused on using *S. obliquus* as the microalgae source grown in photobioreactors and was a demonstration of a biorefinery producing multiple products from a single source. The study used a photobioreactor for single strain microalgae growth and subsequent bioproduct production that might not be economically feasible from a biorefinery model standpoint, due to the cost of operation of a photobioreactor and economic value of the output. Additionally, using a single microalgae strain might be biased toward one or two product streams due to physiological biomass make up. Shifting toward a mixed culture microalgae source could prove to be advantageous for wastewater remediation and developing a bioproduct production platform [71].

Another biorefinery study focused on harvesting mixed wastewater microalgae from the Logan lagoon domestic wastewater treatment plant (Logan, UT, United States) using a variety of methods and processing via WLEP [72]. In this study, the harvesting techniques used were cationic potato starch, cationic corn starch, aluminum sulfate, and centrifugation. Harvested microalgae were processed via the WLEP to generate an aqueous phase. The aqueous phase was neutralized and sterilized to prepare for *E. coli* culturing, no other additional macro or micro nutrients were added. The only components that were added were 100 μg/mL ampicillin and 0.1 mM isopropyl β-D-1-thiogalactopyranoside to maintain the plasmid and induce gene expression, respectively. The recombinant *E. coli* strain XL1-blue harboring the pBHR68 plasmid (containing the phaABC operon under lac regulation) was used in this study [42]. The four different harvesting methods yielded four different media from the WLEP, which were used to grow *E. coli* for PHB production. The highest growth rate was observed in centrifuged microalgae aqueous phase media, with a CFU/mL of

10^{13} and PHB yield of 7.8% of dry cell weight after 48 h. Aqueous phase media from the potato starch, corn starch, and alum harvesting methods produced approximately 1%, 2%, and 0% PHB of dry cell weight. Interestingly, alum-harvested microalgae did not produce any PHB. While the PHB yields in this study were not significantly high when compared to *E. coli* grown on standard media (~50% dry cell weight) [73], the study demonstrated that a side stream from a microalgae biofuel process could produce a value-added compound from a mixed microalgae source.

In the two examples of a biorefinery that utilized the WLEP, centrifugation and coagulation/flocculation were the harvesting methods of choice as they produced the highest yields of bioproducts [10,72]. It would be interesting to see what affect other harvesting methods such as Rotating Algae Biofilm Reactor (RABR) or Dissolved Air Floatation (DAF) have on the WLEP and the bioproduct streams [60,74–76].

2.5 Hydrolysis of Microalgae Biomass for PHA Production

If desired, wastewater microalgae could also be hydrolyzed for production of bioplastics. It has been demonstrated that hydrolyzing microalgae biomass is suitable for media supplementation for *Clostridium saccharoperbutylacetonicum* N1–4 growth and production of acetone, butanol, and ethanol [69,77]. Another study showed that acid hydrolysis of *Chlorella* biomass produced fermentable sugars that were used as substrates for *Saccharomyces cerevisiae* bioethanol production [78]. In addition, it has been demonstrated that protein extracted from microalgae could be used for *E. coli* growth and production of biofuels [64]. Similar methods for microalgae pretreatment can be applied to microalgae biomass for the creation of a media for *E. coli* growth and PHB production [79]. It has been shown that the harvesting techniques used to concentrate microalgae had impacts on the usability of the treated biomass for recombinant PHB production by *E. coli* [10].

A recent study investigated the amount of PHB that could be produced by a recombinant *E. coli* strain expressing the *phaCAB* operon grown on hydrolyzed wastewater algal biomass. Weak domestic wastewater has approximately 20 mg/L nitrogen and 4 mg/L phosphorus, making it an ideal candidate for microalgae growth [80]. Wastewater microalgae from a wastewater treatment facility in Logan, UT, United States were harvested via continuous flow centrifugation (Fig. 6.3). The centrifuge unit had a theoretical capacity to process 9000 l/h, thus demonstrating the scalability for the harvesting process. Centrifugation was chosen as a previous study demonstrated that upstream centrifugation led to better overall recombinant *E. coli* growth and PHB production when compared to other harvesting methods [72]. Harvested microalgae was dried in a temperature-controlled oven, stored at −20°C and then hydrolyzed with 0.5 M sulfuric acid at 90°C. After neutralization and centrifugation the supernatant was used for supplementing recombinant *E. coli* M9 media, where the microalgae extract was the sole carbon substrate [81]. This study directly utilized the hydrolyzed microalgae supernatant fraction without any intermediate sterilization, thereby reducing the cost of the process. Supplementation of microalgae extract with M9 media generated approximately 31% PHB (dry cell weight). When comparing the microalgae-based media with standard *E. coli* media (e.g., M9 with 1.5% glucose), to make 1 kg of PHB would require 4.3 kg of dry microalgae biomass compared to 2.5 kg of glucose [79].

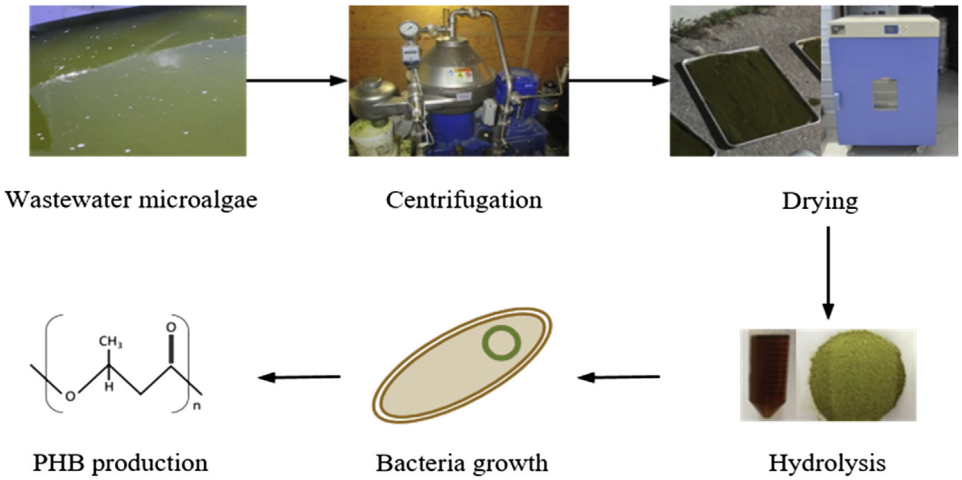


FIGURE 6.3 Schematic for harvesting wastewater microalgae from a wastewater treatment facility for production of polyhydroxybutyrates in *Escherichia coli*. *Adapted with permission from A. Rahman, R.J. Putman, K. Inan, F.A. Sal, A. Sathish, T. Smith, et al. Polyhydroxybutyrate production using a wastewater microalgae-based media. Algal Res. 8 (2015) 95–98.*

The yields of PHB accumulation in recombinant *E. coli* systems grown on microalgae-based media (maximum reported yield 31%) are not as high when compared to standard glucose media (>50%) [73]. However, considering that microalgae from a wastewater source are low in cost, the production of PHBs could be economically competitive. In addition, demonstrating that a recombinant system can produce bioplastics from microalgae-based media could also suggest that other bioproducts could be produced from the same recombinant systems simply by changing the genetic make-up of the microorganism. A plasmid-free PHB production system could also eliminate the need for expensive antibiotic addition. Further work could be carried out to optimize the upstream hydrolysis process of the system much like carried out in a previous study [77]. In addition, the insoluble fraction was not used for growing recombinant *E. coli*, following the steps outlined in another study, utilizing protein fractions for growing recombinant *E. coli* could potentially increase the yields of the bioproduct [64].

When combined in a biorefinery context to produce multiple product streams, PHB production could be economically viable; however, a complete technoeconomic analysis has not been performed. While the dual purpose of using wastewater microalgae to remediate wastewater and produce biological products is clear, there are still some obstacles that need to be overcome for such a platform to be successful.

3. GENETIC ENGINEERING OF ALGAL STRAINS FOR PHA PRODUCTION

Currently, widespread production of recombinant proteins in microalgae is low; however, there is significant research in developing techniques to improve efficiencies [82].

Genetically engineered microalgae systems are being used for biofuel production [83,84], biohydrogen production [85], produce therapeutic proteins [86–88], and to make PHAs [43].

Synthetic biology and metabolic engineering have already played a large role in industrial production of chemicals [89]. Moving forward, systems and synthetic biology approaches to improve microalgae strains to produce specialty chemicals will increase [90]. With the rate at which genetic engineering is progressing, there will most likely be easier and faster methods of moving various pathways into microalgae strains as researchers see the benefits of producing bioproducts in these organisms [91].

Currently, there are strains of microalgae that have been studied at the genetic level, with the most common strain being *Chlamydomonas reinhardtii*. The genome of *C. reinhardtii* was fully sequenced in 2007 and found to have similar phylogenetic properties as plants [92,93]. *Chlamydomonas reinhardtii* can grow photoautotrophically or with acetate and has a doubling time of 10 h [88]. In addition to *C. reinhardtii*, some other strains that are currently suitable for genetic manipulation are *Cyanidioschyzon merolae*, *Nannochloropsis* sp., *Ostreococcus tauri*, *Phaeodactylum tricornutum*, and *Thalassiosira pseudonana* [94]. In addition there are several other microalgae strains that are currently being sequenced that will add to the different possible products that could be produced [95].

PHB pathways have been incorporated into microalgae strains with great success. Chaogang and co-workers were able to successfully incorporate the *phb*B gene from *R. eutropha* into *C. reinhardtii* [96]. The major findings of this study demonstrated that part of a native bioplastic synthesis pathway could be incorporated into a nonnative host [97]. In 2010 the same group demonstrated successful incorporation of *phb*B and *phb*C genes from *R. eutropha* into *C. reinhardtii*, with the third enzyme *phb*A, already present in *C. reinhardtii*. The results of this study demonstrated detectable quantities of PHB (6 μg/g dry cell weight) produced by *C. reinhardtii*; however further studies need to be conducted to increase these yields [96]. It would be of further value to observe if overexpressing the *phb*A gene in *C. reinhardtii* increased its ability for PHB accumulation compared to the current system where *phb*A is expressed at native levels.

Another study by Hempel and colleagues [43] incorporated the full PHB pathway from *R. eutropha* H16 into *P. tricornutum.* This was the first instance of a complete PHA pathway being incorporated into a microalgal strain. The results of this study were impressive as it was reported that approximately 10.6% PHB (% dry cell weight) was achieved in this engineered strain after 7 days of culturing [43]. While 10.6% accumulation of PHA in this algal strain is low when compared to similar pathways in recombinant *E. coli* systems operating in defined media (~50% PHA accumulation in 24 h) [73], in this study, codon optimization and extensive screening were not carried out, thus further optimization of the system for increased PHB production could be conducted in the future. The demonstration of PHA production in a microalgae strain allows for the possible biological manufacturing of other PHAs, namely other scl PHAs and mcl PHAs. One advantage of using recombinant *P. tricornutum* over *E. coli* is evident from the cost of carbon substrate. In the case of *P. tricornutum* and *C. reinhardtii*, atmospheric CO_2 is essentially a free carbon source. For PHB production in *E. coli*, the cost of the carbon substrate is estimated to be around 38% of the total manufacturing cost, so eliminating this cost in a manufacturing context could make PHB production potentially economically viable in microalgae [98].

TABLE 6.3 Comparison of Polyhydroxybutyrate Production From Different Algae Sources

Organism	PHB Yield (% Dry Cell Weight)	Algae Source	References
Chlamydomonas reinhardtii	Detectable quantities	Self-produced	[96]
Phaeodactylum tricornutum	10.6	Self-produced	[43]
Escherichia coli	7.8	Wastewater	[72]
Escherichia coli	31	Wastewater	[79]

While the cost of the carbon substrate is significant, another issue that needs to be addressed is that of purification of PHB from microalgae biomass. Generally, cells are lysed and PHB is extracted; however, this is a major cost with biologically produced PHBs and alternative methods need to be developed to make PHB production economically viable [99].

Comparing the different production platforms for PHB (Table 6.3), currently engineered microalgae are not able to produce high yields of plastic when compared to genetically engineered *E. coli* grown with glucose as the carbon substrate. However, with development of genetic toolkits this could be increased in the near future. Furthermore, in a biorefinery context PHB-producing microalgae could have a dual purpose, thus providing biomaterials and biofuels from a single algae source.

As our understanding of genetic systems increases, there are advanced technologies that have been developed for genetic manipulation of microalgal system. A technique developed by Noor-Mohammadi and co-workers demonstrated pathway engineering in the baker's yeast *S. cerevisiae* and then successful transfer of the assembled pathway into the nucleus of *C. reinhardtii* [100]. Furthermore, similar techniques can be used to assemble genes in the chloroplast of *C. reinhardtii* [101]. With the recent increase in popularity of using clustered regularly interspaced short palindromic repeats (CRISPR) and Cas9 (an RNA guided cleaving enzyme) cloning systems [102], there could be potential to genetically engineer microalgae strains to produce PHAs in the near future. Studies using the CRISPR-Cas9 system have already been developed for use with the microalgae strain *C. reinhardtii* [103,104]. Transcription activator-like effectors and zinc-finger nucleases have also been successfully used in *C. reinhardtii* [105,106].

4. FUTURE OUTLOOK

Coupling modern genetic engineering with innovations in cultivation techniques has the potential to increase bioproduct production in microalgae [107]. As the demand for plastic products continues to increase, there will be a place for microalgae-derived bioplastics. Whether directly using microalgae biomass, using microalgae biomass derivatives, or genetic engineering of microalgae strains, there are multiple routes that could be taken to achieve an economically viable bioproduct. Future work could consist of a hybrid of both genetic engineering of microalgae strains to produce PHAs and blending with petrochemical plastics or other bio-based plastics. Combining macroalgae with PHBs have already been reported in

literature and using similar approaches with microalgae could also be considered [108]. A biorefinery model, where multiple bioproducts are produced from a single microalgal source, appears the mostly likely process by which microalgae-derived bioplastics will enter the market in the near future. Furthermore, microalgae feedstocks that are grown on waste streams will provide the lowest cost for a biorefinery system.

References

[1] EPA, Municipal Solid Waste in the United States, United States Environmental Protection Agency, 2012.
[2] C.J. Moore, Synthetic polymers in the marine environment: a rapidly increasing, long-term threat, Environ. Res. 108 (2008) 131–139.
[3] S. Chanprateep, Current trends in biodegradable polyhydroxyalkanoates, J. Biosci. Bioeng. 110 (2010) 621–632.
[4] M.M. Reddy, S. Vivekanandhan, M. Misra, S.K. Bhatia, A.K. Mohanty, Biobased plastics and bionanocomposites: current status and future opportunities, Prog. Polym. Sci. 38 (2013) 1653–1689.
[5] S. Sreedevi, K. Unni, S. Sajith, P. Priji, M. Josh, S. Benjamin, Bioplastics: Advances in Polyhydroxybutyrate Research, Springer, Berlin Heidelberg, 2015, pp. 1–30.
[6] M.A. Zeller, R. Hunt, A. Jones, S. Sharma, Bioplastics and their thermoplastic blends from *Spirulina* and *Chlorella* microalgae, J. Appl. Polym. Sci. 130 (2013) 3263–3275.
[7] B.E. DiGregorio, Biobased performance bioplastic: Mirel, Chem. Biol. 16 (2009) 1–2.
[8] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306.
[9] K.G. Satyanarayana, A.B. Mariano, J.V.C. Vargas, A review on microalgae, a versatile source for sustainable energy and materials, Int. J. Energy Res. 35 (2011) 291–311.
[10] R.J. Anthony, J.T. Ellis, A. Sathish, A. Rahman, C.D. Miller, R.C. Sims, Effect of coagulant/flocculants on bioproducts from microalgae, Bioresour. Technol 149 (2013) 65–70.
[11] J. Trivedi, M. Aila, D.P. Bangwal, S. Kaul, M.O. Garg, Algae based biorefinery-How to make sense? Renew. Sust. Energy Rev. 47 (2015) 295–307.
[12] S.S. Sawant, B.K. Salunke, T.K. Tran, B.S. Kim, Lignocellulosic and marine biomass as resource for production of polyhydroxyalkanoates, Korean J. Chem. Eng. (2016) 1–9.
[13] R.M.M. Abed, S. Dobretsov, K. Sudesh, Applications of *Cyanobacteria* in biotechnology, J. Appl. Microbiol. 106 (2009) 1–12.
[14] S. Balaji, K. Gopi, B. Muthuvelan, A review on production of poly β hydroxybutyrates from *Cyanobacteria* for the production of bio plastics, Algal Res. 2 (2013) 278–285.
[15] A.M. Ruffing, Engineered *Cyanobacteria*: Teaching an old bug new tricks, Bioengineered Bugs 2 (2011) 136–149.
[16] B. Shi, G. Wideman, J. Wang, A new approach of BioCO_2 fixation by thermoplastic processing of microalgae, J. Polym. Environ. 20 (2012) 124–131.
[17] F. Zhang, H. Kabeya, R. Kitagawa, T. Hirotsu, M. Yamashita, T. Otsuki, A novel polyethylene–chlorella composite. I. Characterization of *chlorella* biologically fixing CO_2, J. Appl. Polym. Sci. 77 (2000) 2278–2284.
[18] F. Zhang, H. Kabeya, R. Kitagawa, T. Hirotsu, M. Yamashita, T. Otsuki, Preparation and characterization of a novel polyethylene–*Chlorella* composite, Chem. Mater. 11 (1999) 1952–1956.
[19] J. Otsuki, F. Zhang, H. Kabeya, T. Hirotsu, Synthesis and tensile properties of a novel composite of *Chlorella* and polyethylene, J. Appl. Polym. Sci. 92 (2004) 812–816.
[20] F. Zhang, T. Endo, R. Kitagawa, H. Kabeya, T. Hirotsu, Synthesis and characterization of a novel blend of polypropylene with *Chlorella*, J. Mater. Chem. 10 (2000) 2666–2672.
[21] Y.H. Jang, S.O. Han, I.N. Sim, H.I. Kim, Pretreatment effects of seaweed on the thermal and mechanical properties of seaweed/polypropylene biocomposites, Composites Part A: Appl. Sci. Manuf. 47 (2013) 83–90.
[22] I. Fischer, W.F. Schmitt, H.-C. Porth, M.W. Allsopp, G. Vianello, Poly(Vinyl chloride), in: Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH Verlag GmbH & Co. KGaA., 2000.
[23] F. Zhang, H. Kabeya, R. Kitagawa, T. Hirotsu, M. Yamashita, T. Otsuki, An exploratory research of PVC-Chlorella composite material (PCCM) as effective utilization of Chlorella biologically fixing CO_2, J. Mater. Sci. 35 (2000) 2603–2609.

[24] N. Uduman, Y. Qi, M.K. Danquah, G.M. Forde, A. Hoadley, Dewatering of microalgal cultures: a major bottleneck to algae-based fuels, J. Renew. Sustain. Energy 2 (2010) 012701-01–012701-15.
[25] E. Chiellini, P. Cinelli, V.I. Ilieva, M. Martera, Biodegradable thermoplastic composites based on polyvinyl alcohol and algae, Biomacromolecules 9 (2008) 1007–1013.
[26] C. Toro, M. Reddy, R. Navia, M. Rivas, M. Misra, A. Mohanty, Characterization and application in biocomposites of residual microalgal biomass generated in third generation biodiesel, J. Polym. Environ. 21 (2013) 944–951.
[27] S. Torres, R. Navia, R. Campbell Murdy, P. Cooke, M. Misra, A.K. Mohanty, Green composites from residual microalgae biomass and poly(butylene adipate-co-terephthalate): processing and Plasticization, ACS Sustain. Chem. Eng. 3 (2015) 614–624.
[28] R.E. Drumright, P.R. Gruber, D.E. Henton, Polylactic acid technology, Adv. Mater. 12 (2000) 1841–1846.
[29] J. Lunt, Large-scale production, properties and commercial applications of polylactic acid polymers, Polym. Degrad. Stab 59 (1998) 145–152.
[30] R.E. Conn, J.J. Kolstad, J.F. Borzelleca, D.S. Dixler, L.J. Filer Jr., B.N. Ladu Jr., et al., Safety assessment of polylactide (PLA) for use as a food-contact polymer, Food Chem. Toxicol. 33 (1995) 273–283.
[31] K.A. Athanasiou, G.G. Niederauer, C.M. Agrawal, Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers, Biomaterials 17 (1996) 93–102.
[32] M. Bulota, T. Budtova, PLA/algae composites: Morphology and mechanical properties, Composites Part A: Appl. Sci. Manuf. 73 (2015) 109–115.
[33] S.J. Park, S.Y. Lee, T.W. Kim, Y.K. Jung, T.H. Yang, Biosynthesis of lactate-containing polyesters by metabolically engineered bacteria, Biotechnol. J. 7 (2012) 199–212.
[34] M. Eiteman, S. Ramalingam, Microbial production of lactic acid, Biotechnol. Lett. 37 (2015) 955–972.
[35] A.J. Anderson, E.A. Dawes, Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates, Microbiol. Rev. 54 (1990) 450–472.
[36] B. Laycock, P. Halley, S. Pratt, A. Werker, P. Lant, The chemomechanical properties of microbial polyhydroxyalkanoates, Prog. Polym. Sci. 39 (2014) 397–442.
[37] S.Y. Lee, Bacterial polyhydroxyalkanoates, Biotechnol. Bioeng. 49 (1996) 1–14.
[38] K. Sudesh, H. Abe, Y. Doi, Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters, Prog. Polym. Sci. 25 (2000) 1503–1555.
[39] M. Bassas, J. Diaz, E. Rodriguez, M.J. Espuny, M.J. Prieto, A. Manresa, Microscopic examination in vivo and in vitro of natural and cross-linked polyunsaturated mclPHA, Appl. Microbiol. Biotechnol. 78 (2008) 587–596.
[40] E. Linton, A. Rahman, S. Viamajala, R.C. Sims, C.D. Miller, Polyhydroxyalkanoate quantification in organic wastes and pure cultures using a single-step extraction and 1H NMR analysis, Water Sci. Technol. 66 (2012) 1000–1006.
[41] A.J. Pieja, E.R. Sundstrom, C.S. Criddle, Poly-3-Hydroxybutyrate metabolism in the type II Methanotroph *Methylocystis parvus* OBBP, Appl. Environ. Microbiol. 77 (2011) 6012–6019.
[42] P. Spiekermann, B.H.A. Rehm, R. Kalscheuer, D. Baumeister, A. Steinbüchel, A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds, Arch. Microbiol. 171 (1999) 73–80.
[43] F. Hempel, A.S. Bozarth, N. Lindenkamp, A. Klingl, S. Zauner, U. Linne, et al., Microalgae as bioreactors for bioplastic production, Microb. Cell Fact. (2011) 10.
[44] B.H.A. Rehm, Microbial Production of Biopolymers and Polymer Precursors: Applications and Perspectives, Caister Academic Press, Norfolk, UK, 2009.
[45] M.N. Somleva, O.P. Peoples, K.D. Snell, PHA bioplastics, biochemicals, and energy from crops, Plant Biotechnol. J. 11 (2013) 233–252.
[46] K.E.J. Tyo, P.K. Ajikumar, G. Stephanopoulos, Stabilized gene duplication enables long-term selection-free heterologous pathway expression, Nat. Biotech. 27 (2009) 760–765.
[47] D.E. Agnew, B.F. Pfleger, Synthetic biology strategies for synthesizing polyhydroxyalkanoates from unrelated carbon sources, Chem. Eng. Sci. 103 (2013) 58–67.
[48] S. Khanna, A.K. Srivastava, Recent advances in microbial polyhydroxyalkanoates, Process. Biochem. 40 (2005) 607–619.
[49] B.H.A. Rehm, Bacterial polymers: biosynthesis, modifications and applications, Nat. Rev. Microbiol. 8 (2010) 578–592.

[50] E. Akaraonye, T. Keshavarz, I. Roy, Production of polyhydroxyalkanoates: the future green materials of choice, J. Chem. Technol. Biotechnol. 85 (2010) 732–743.
[51] D. Jendrossek, R. Handrick, Microbial degradation of polyhydroxyalkanoates, Annu. Rev. Microbiol. 56 (2002) 403–432.
[52] A.M. Gumel, M.S.M. Annuar, Y. Chisti, Recent advances in the production, recovery and applications of polyhydroxyalkanoates, J. Polym. Environ. 21 (2013) 580–605.
[53] Y. Wang, J. Yin, G.-Q. Chen, Polyhydroxyalkanoates, challenges and opportunities, Curr. Opin. Biotechnol. 30 (2014) 59–65.
[54] B.H.A. Rehm, Polyester synthases: natural catalysts for plastics, Biochem. J. 376 (2003) 15–33.
[55] S.Y. Lee, J.-I. Choi, H.H. Wong, Recent advances in polyhydroxyalkanoate production by bacterial fermentation: mini-review, Int. J. Biol. Macromol. 25 (1999) 31–36.
[56] S.Y. Lee, *E. coli* moves into the plastic age, Nat. Biotech. 15 (1997) 17–18.
[57] M. Rizwan, J.H. Lee, R. Gani, Optimal design of microalgae-based biorefinery: economics, opportunities and challenges, Appl. Energy 150 (2015) 69–79.
[58] H.W. Yen, I.C. Hu, C.Y. Chen, S.H. Ho, D.J. Lee, J.S. Chang, Microalgae-based biorefinery—from biofuels to natural products, Bioresour. Technol. 135 (2013) 166–174.
[59] A. Rahman, J.T. Ellis, C.D. Miller, Bioremediation of domestic wastewater and production of bioproducts from microalgae using waste stabilization ponds, J. Biorem. Biodegrad. 3 (2012) 1000e113.
[60] M. Kesaano, R.C. Sims, Algal biofilm based technology for wastewater treatment, Algal Res. 5 (2014) 231–240.
[61] L. Brennan, P. Owende, Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sustain. Energy Rev. 14 (2010) 557–577.
[62] M.L. Gerardo, S. Van Den Hende, H. Vervaeren, T. Coward, S.C. Skill, Harvesting of microalgae within a biorefinery approach: a review of the developments and case studies from pilot-plants, Algal Res. 11 (2015) 248–262.
[63] D. Vandamme, I. Foubert, K. Muylaert, Flocculation as a low-cost method for harvesting microalgae for bulk biomass production, Trends Biotechnol. 31 (2013) 233–239.
[64] Y.-X. Huo, K.M. Cho, J.G.L. Rivera, E. Monte, C.R. Shen, Y. Yan, et al., Conversion of proteins into biofuels by engineering nitrogen flux, Nat. Biotech. 29 (2011) 346–351.
[65] R. Anthony, R. Sims, Cationic starch for microalgae and total phosphorus removal from wastewater, J. Appl. Polym. Sci. 130 (2013) 2572–2578.
[66] D. Dye, J. Muhs, B. Wood, R. Sims, Design and performance of a solar photobioreactor utilizing spatial light dilution, J. Sol. Energy-Trans. ASME 133 (2011) 1067–1076.
[67] A. Sathish, T. Marlar, R.C. Sims, Optimization of a wet microalgal lipid extraction procedure for improved lipid recovery for biofuel and bioproduct production, Bioresour. Technol. 193 (2015) 15–24.
[68] A. Sathish, R.C. Sims, Biodiesel from mixed culture algae via a wet lipid extraction procedure, Bioresour. Technol. 118 (2012) 643–647.
[69] J.T. Ellis, N.N. Hengge, R.C. Sims, C.D. Miller, Acetone, butanol, and ethanol production from wastewater algae, Bioresour. Technol. 111 (2012) 491–495.
[70] G.L. Rosano, E.A. Ceccarelli, Recombinant protein expression in *Escherichia coli*: advances and challenges, Front. Microbiol. 5 (2014) 172.
[71] L. Christenson, R. Sims, Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts, Biotechnol. Adv. 29 (2011) 686–702.
[72] A. Rahman, R.J. Anthony, A. Sathish, R.C. Sims, C.D. Miller, Effects of wastewater microalgae harvesting methods on polyhydroxybutyrate production, Bioresour. Technol. 156 (2014) 364–367.
[73] A. Rahman, E. Linton, A.D. Hatch, R.C. Sims, C.D. Miller, Secretion of polyhydroxybutyrate in *Escherichia coli* using a synthetic biological engineering approach, J. Biol. Eng. 7 (2013) 24.
[74] W.F.R. Bare, N.B. Jones, E.J. Middlebrooks, Algae removal using dissolved air flotation, J. (Water Pollut. Control Fed.) 47 (1975) 153–169.
[75] L.B. Christenson, R.C. Sims, Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products, Biotechnol. Bioeng. 109 (2012) 1674–1684.
[76] J.L. Wood, C.D. Miller, R.C. Sims, J.Y. Takemoto, Biomass and phycocyanin production from *Cyanobacteria* dominated biofilm reactors cultured using oilfield and natural gas extraction produced water, Algal Res. 11 (2015) 165–168.

[77] Y.A. Castro, J.T. Ellis, C.D. Miller, R.C. Sims, Optimization of wastewater microalgae saccharification using dilute acid hydrolysis for acetone, butanol, and ethanol fermentation, Appl. Energy 140 (2015) 14–19.
[78] N. Zhou, Y. Zhang, X. Wu, X. Gong, Q. Wang, Hydrolysis of *Chlorella* biomass for fermentable sugars in the presence of HCl and $MgCl_2$, Bioresour. Technol. 102 (2011) 10158–10161.
[79] A. Rahman, R.J. Putman, K. Inan, F.A. Sal, A. Sathish, T. Smith, et al., Polyhydroxybutyrate production using a wastewater microalgae based media, Algal Res. 8 (2015) 95–98.
[80] G. Tchobanoglous, F. Burton, Wastewater Engineering: Treatment, Disposal, and Reuse, McGraw-Hill, 1991.
[81] J. Sambrook, D.W. Russel, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.
[82] G. Potvin, Z. Zhang, Strategies for high-level recombinant protein expression in transgenic microalgae: a review, Biotechnol. Adv. 28 (2010) 910–918.
[83] J.L. Blatti, J. Michaud, M.D. Burkart, Engineering fatty acid biosynthesis in microalgae for sustainable biodiesel, Curr. Opin. Chem. Biol. 17 (2013) 496–505.
[84] J. Lu, C. Sheahan, P. Fu, Metabolic engineering of algae for fourth generation biofuels production, Energy Environ. Sci. 4 (2011) 2451–2466.
[85] O. Kruse, J. Rupprecht, K.-P. Bader, S. Thomas-Hall, P.M. Schenk, G. Finazzi, et al., Improved photobiological H_2 production in engineered green algal cells, J. Biol. Chem. 280 (2005) 34170–34177.
[86] S.P. Mayfield, S.E. Franklin, R.A. Lerner, Expression and assembly of a fully active antibody in algae, Proc. Natl. Acad. Sci. 100 (2003) 438–442.
[87] B.A. Rasala, M. Muto, P.A. Lee, M. Jager, R.M.F. Cardoso, C.A. Behnke, et al., Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of *Chlamydomonas reinhardtii*, Plant Biotechnol. J. 8 (2010) 719–733.
[88] E. Specht, S. Miyake-Stoner, S. Mayfield, Micro-algae come of age as a platform for recombinant protein production, Biotechnol. Lett. 32 (2010) 1373–1383.
[89] D. Jullesson, F. David, B. Pfleger, J. Nielsen, Impact of synthetic biology and metabolic engineering on industrial production of fine chemicals, Biotechnol. Adv. 33 (2015) 1395–1402.
[90] W. Fu, K. Wichuk, S. Brynjólfsson, Developing diatoms for value-added products: challenges and opportunities, New Biotechnol. 32 (2015) 547–551.
[91] J.-P. Cadoret, M. Garnier, B. Saint-Jean, Chapter eight—microalgae, functional genomics and biotechnology, in: P. Gwenaël (Ed.), Advances in Botanical Research, vol. 64, Academic Press, 2012, pp. 285–341.
[92] A.W.D. Larkum, I.L. Ross, O. Kruse, B. Hankamer, Selection, breeding and engineering of microalgae for bioenergy and biofuel production, Trends Biotechnol. 30 (2012) 198–204.
[93] S.S. Merchant, S.E. Prochnik, O. Vallon, E.H. Harris, S.J. Karpowicz, G.B. Witman, et al., The *Chlamydomonas* genome reveals the evolution of key animal and plant functions, Science 318 (2007) 245–250.
[94] M. Hlavova, Z. Turoczy, K. Bisova, Improving microalgae for biotechnology—from genetics to synthetic biology, Biotechnol. Adv. 33 (2015) 1194–1203.
[95] R. Radakovits, R.E. Jinkerson, A. Darzins, M.C. Posewitz, Genetic engineering of algae for enhanced biofuel production, Eukaryot. Cell 9 (2010) 486–501.
[96] W. Chaogang, H. Zhangli, L. Anping, J. Baohui, Biosynthesis of poly-3-hydroxybuytyrate (PHB) in then transgenic green alga *Chlamydomonas reinhardtii*, J. Phycol. 46 (2010) 396–402.
[97] C. Wang, Z. Hu, W. Hu, A. Lei, Expression and molecular analysis of *phbB* gene in *Chlamydomonas reinhardtii*, Chin. Sci. Bull. 49 (2004) 1713–1717.
[98] J. Choi, S.Y. Lee, Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation, Appl. Microbiol. Biotechnol. 51 (1999) 13–21.
[99] N. Jacquel, C.-W. Lo, Y.-H. Wei, H.-S. Wu, S.S. Wang, Isolation and purification of bacterial poly(3-hydroxyalkanoates), Biochem. Eng. J. 39 (2008) 15–27.
[100] S. Noor-Mohammadi, A. Pourmir, T.W. Johannes, Method for assembling and expressing multiple genes in the nucleus of microalgae, Biotechnol. Lett. 36 (2014) 561–566.
[101] S. Noor-Mohammadi, A. Pourmir, T.W. Johannes, Method to assemble and integrate biochemical pathways into the chloroplast genome of *Chlamydomonas reinhardtii*, Biotechnol. Bioeng. 109 (2012) 2896–2903.
[102] S.H. Sternberg, J.A. Doudna, Expanding the biologist's toolkit with CRISPR-Cas9, Mol. Cell 58 (2015) 568–574.
[103] W. Jiang, A.J. Brueggeman, K.M. Horken, T.M. Plucinak, D.P. Weeks, Successful transient expression of Cas9 and single guide RNA genes in *Chlamydomonas reinhardtii*, Eukaryot. Cell 13 (2014) 1465–1469.

[104] R.E. Jinkerson, M.C. Jonikas, Molecular techniques to interrogate and edit the *Chlamydomonas* nuclear genome, Plant J. 82 (2015) 393–412.
[105] H. Gao, D.A. Wright, T. Li, Y. Wang, K. Horken, D.P. Weeks, et al., TALE activation of endogenous genes in *Chlamydomonas reinhardtii*, Algal Res. 5 (2014) 52–60.
[106] I. Sizova, A. Greiner, M. Awasthi, S. Kateriya, P. Hegemann, Nuclear gene targeting in *Chlamydomonas* using engineered zinc-finger nucleases, Plant J. 73 (2013) 873–882.
[107] J.N. Rosenberg, G.A. Oyler, L. Wilkinson, M.J. Betenbaugh, A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution, Curr. Opin. Biotechnol. 19 (2008) 430–436.
[108] A. Barghini, V.I. Ivanova, S.H. Imam, E. Chiellini, Poly-(ε-caprolactone)(PCL) and poly (hydroxybutyrate)(PHB) blends containing seaweed fibers: Morphology and thermal-mechanical properties, J. Polym. Sci. Part A: Polym. Chem. 48 (2010) 5282–5288.

CHAPTER

7

Microalgae-Based Carotenoids Production

K. Rajesh[1,2], *M.V. Rohit*[1,2], *S. Venkata Mohan*[1,2]

[1]CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, Telangana, India;
[2]Academy for Scientific and Industrial Research (AcSIR), India

OUTLINE

1. INTRODUCTION

Intrinsic ability of microalgae to biocapture solar energy from sunlight and utilize CO_2 facilitates valorization of various biobased products viz., carotenoids, phycobilins, vitamins, triacylglycerols (TAGs), mono/poly-unsaturated fatty acids (MUFA,PUFA) etc., that have a wide spectrum of commercial applications in cosmetics, nutraceuticals and healthcare sectors. Photosynthetic microalgae-based solar energy biocapturing is gaining grounds in the field of photo-biotechnology employed for the production of value-added products [1–3]. One of the important metabolites from microalgae, gaining attention recently are carotenoids namely β-carotene and Astaxanthin.

Carotenoids are naturally occurring pigments and most widely distributed in the biosphere. There are more than 700 different carotenoid molecules collectively produced in

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00007-2

both photosynthetic and non-photosynthetic organisms [4–7]. They function as accessory light-harvesting pigments, structural components for photosystem assembly, moderators of non-photochemical quenching, and scavengers of reactive oxygen species (ROS) in photosynthetic organisms [8]. They are primarily divided into two classes: hydrocarbon-based carotenoids also called as carotenes (lycopene, α-carotene, β-carotene, etc.) and alcohol-based carotenoids (xanthophylls, lutein, canthaxanthin, astaxanthin). The color of these pigments range from yellow to red and some well-known examples are lycopene, zeaxanthin, β-caotene, astaxanthin. Microalgal species is particularly considered as a suitable cell factory due to high carotenoid accumulation capacity under certain stress conditions. Using microalgae as feedstock facilitates both production and accumulation of pigments simultaneously. Recent scientific advances in the fields of algal genomics, molecular genetics, metabolomics, and metabolic engineering, along with advances in enzyme and fermentation technology can guide for pigment enhancement, optimization and downstream processing of carotenoids [9,10]. Commercially carotenoids are important for industrial applications particularly colorants, food, pharmaceutical, and cosmetic markets when produced from a collection of microalgal strains [11,12]. This communication provides an overview of microalgae-based production of two important carotenoids namely β-carotene and Astaxanthin, with focus on the triggering factors influencing carotenogenesis along with their extraction, and delineates their practical applications, mainly in the food and health industries.

2. CAROTENOID SYNTHESIS PATHWAYS

2.1 β-Carotene

Beta carotene is a yellow/orange pigment that is primary precursor molecule for vitamin A and gives vegetables and fruits their rich colors. Microalgal species like *Dunaliella bardawil* have the property of producing β-carotene naturally under varied cultivation conditions. β-carotene synthesis takes place in the chloroplast through geranylgeranyl pyrophosphate (GPP) via phytoene and lycopene as precursor molecules (Fig. 7.1). β-Carotene generally accumulates in lipid globules associated with both triacylglycerol (TAG) and peripherally located carotene protein globule [14]. Biosynthesis of TAG and β-carotene are interdependent; the accumulation of β-carotene is functionally regulated by the enzyme acetyl CoA carboxylase, which catalyzes the TAG biosynthesis in the endoplasmic reticulum [13,15]. The C–C double bond in the polyene chain of carotenoids may exhibit two stereo configurations, i.e., geometric isomers cis or trans. Most carotenoids in nature are trans isomers and they are thermodynamically more stable than cis isomers. The amount of β-carotene and its stereogeometry depends on algal division time and light intensity, which is in turn are determined by the growth conditions [16]. β-Carotene globule is positioned according to their functional role in the inter-thylakoid space of the chloroplasts restricted in close vicinity to the plasma membrane, which facilitates light absorption to avoid damage to the photosynthetic machinery. Some microalgal species like *D. bardawil*, reported to produce β-carotene, are immune against photo-inhibition by high-intensity blue light or by UV-A radiation owing to the protection rendered by carotene [2]. β-carotene production is determined by the exposure to specific environmental stress conditions, such as hyper saline and high light intensities; high

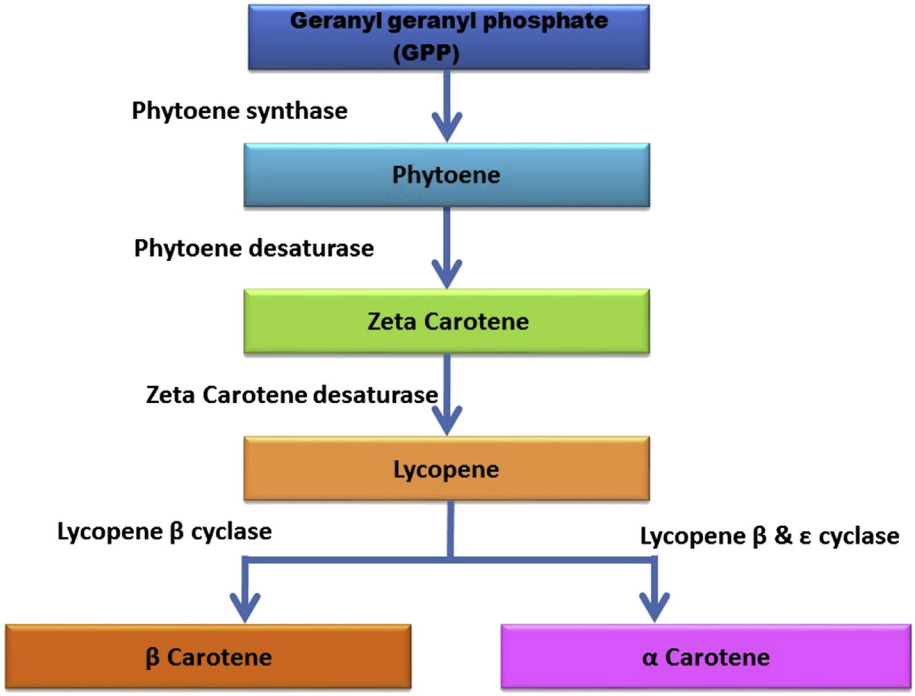


FIGURE 7.1 β carotene synthesis pathway.

temperatures and nutrient deprivation [17–21]. Unicellular green microalgae like *Dunaliella* sp. are wealthy sources of natural β-carotene. This imposes superior antioxidant properties, bioavailability, and physiological effects, validating commercial scale productivity interests for algal carotene [19,20,22].

2.2 Astaxanthin

Astaxanthin is a keto-carotenoid pigment with strong antioxidant property and is known for scavenging of ROS (reactive oxygen species) and neutralizing free radicals [23–25]. Microalgae produce astaxanthin in cytosolic lipid bodies (LBs) under environmental stress or adverse culture conditions, such as high light, high salinity, and nutrient deprivation [26,27]. Astaxanthin production was reported in different species like *Botryococcus braunii*, *Chlamydomonas nivalis*, *Chlorella zofingiensis*, *Chlorococcum* sp., *Chloromonas nivalis*, *Haematococcus pluvialis*, *Neochloris wimmeri*, *Protosiphon botryoides*, *Scenedesmus* sp., *Scotiellopsis oocystiformis*, and *Trachelomonas volvocina* [28–31]. Some green microalgae like *H. pluvialis* have exceptional pigment accumulation potential under stress conditions [32,33]. *Chlorella zofingiensis* has attracted interest as an alternative astaxanthin producer, due to its high capability to grow quickly (with a μmax of 1.03 d^{-1} and biomass concentration of 53 g/l when grown on glucose) and adopt a photoautotrophic, mixotrophic, or heterotrophic culture mode [34,35]. Carotenoid synthesis genes are expressed differentially in *H. pluvialis* and *C. zofingiensis* in response to cellular and molecular stress at the transcriptional level [36,37].

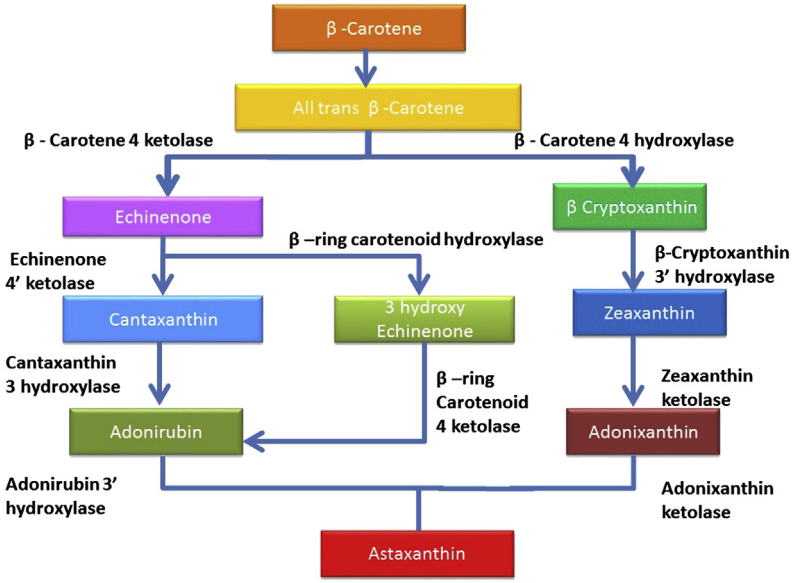


FIGURE 7.2 Astaxanthin synthesis pathway.

β-carotene forms a precursor for astaxanthin and is catalyzed by the enzymatic activity of β-carotene ketolase and hydroxylase (Fig. 7.2). The metabolic intermediates during the catalytic activity of ketolase and hydroxylase are canthaxanthin and zeaxanthin, respectively. The physiological change reported during the accumulation of astaxanthin is the decrease in photosynthetic activity or the limited oxygen evolution due to damaged PSII complex, which are the primary triggering factors for pigment synthesis [38–42]. The amount of astaxanthin relates inversely to photosynthetic activities, even though the amount of chlorophyll and PSII reaction center remains stable [41,43]. The photosynthetic imbalance between the input energy from the light adsorption by antennae and the output energy in the form of CO_2 fixation is quenched and it produces ROS. Carotenoids could prevent excessive damage by ROS by directly quenching triplet chlorophyll (3Chl) or singlet oxygen ($^1O_2$) produced from photodynamic reactions [44–46]. On the other hand, when CO_2 fixation is limited by stress environmental conditions such as nutrient starvation, high salinity, cold temperatures, or low CO_2 availability, the production of these ROS can occur even at moderate light intensity because of an energy surplus [47]. Under nutrient starvation, O_2 is probably the most effective ROS species that might involve in astaxanthin accumulation [48,49]. ROS may also activate the expression of genes coding for carotenogenesis enzymes [50].

Astaxanthin has wide nutraceutical value and pharmaceutical applications to fight against diseases related to liver, oral and colon cancers, cardiovascular, and degenerative eye diseases [2,51]. Astaxanthin from microalgae is preferred as a feed additive in aquaculture due to a higher extent of pigmentation [52]. The current astaxanthin market per kilo is around $2000 and is dominated by synthetic astaxanthins with a total market value above $240 million per year [53,54]. The growing concern about the safety of using synthetic astaxanthin for aquaculture/human consumption has lead to preference of natural astaxanthin [55].

3. FACTORS THAT INFLUENCE CAROTENOGENESIS

Microalgal carotenogenensis is primarily influenced by the factors like light intensity, temperature, salinity, and nutrient limitation [56]. Effect of incident light intensity has a remarkable influence on the production of carotenoids as their primary function is to overcome the irradiance stress developed [22]. During the division cycle of the microalgal species, exposure to visible light as well as UV light has an effect when cultivated in closed controlled conditions when compared to outdoor conditions where parametric controls is challenging [37,57,58]. Exposure to UV-A has specifically increased the production of total carotenoids, zeaxanthin, and lutein [59].

Lower temperatures have been reported to be favorable for the production of carotenoids. Drop in temperatures from 30 to 10°C showed an increase in the β-carotene production under high irradiances by four times [60]. Nutrient stress conditions affect the carotenoids production by affecting the photosynthetic process. The limitation in the supply of nitrogen, phosphorus, sulfates, and chloride leads to the accumulation of β-carotene in microalgae as they effect the growth of the cell, altering the ratio of the photoprotective pigments (α-carotene and β-carotene) to chlorophyll and influencing the photosynthetic pathway [61–65]. Nutrient stress and higher light intensity in combination with NaCl/sodium acetate enhanced total carotenoid and total astaxanthin content to 32 and 24.5 mg/g of dry biomass, respectively [66].

Genetic engineering and molecular approaches have been employed for over expression of carotenogenic genes, phytoene synthase, desaturase, lycopene cyclase, β-carotene ketolase (BKT), and β-carotene hydroxylase (CHY). Nutrient stress and high light intensity induced expression of astaxanthin biosynthetic genes, BKT, and CHY, rapidly. Enhanced expression of genes was observed with sodium acetate and NaCl/sodium acetate, while expression was delayed with NaCl [66]. The maximum content of astaxanthin recorded in cells grown in medium with sodium acetate and NaCl/sodium acetate correlated with the expression profile of the astaxanthin biosynthetic genes [67]. Studies using different inhibitors indicated that general carotenogenesis and secondary carotenoid induction were regulated at both the nuclear transcriptional and the cytoplasmic translational levels [67]. *Chlamydomonas* sp. is extensively used as a model organism for strategic genetic engineering studies with special emphasis on pigments [68,69]. The precursor molecules for carotenoid production are isopentenyl pyrophosphate [70], which are produced in two distinct pathways, i.e., cytosol-based mevalonate pathway (MVA) chloroplast-based non-mevalonate pathway (DOXP pathway or MEP pathway). Zhang and Lee [71,72] have studied the production of secondary carotenoids (mainly astaxanthin) in a mutant strain of *Chlorococcum* sp.; the mutant was stable and could build up two fold the amount of carotenoids than the wild strain.

4. EXTRACTION OF PIGMENTS

Microalgae cell wall disruption can be accomplished through various techniques viz., mortar-and-pestle, freezing, milling, microwave, ultrasound, thawing, chemical addition, etc. [68]. The mortar-and-pestle procedure provides good product recovery, but cannot be scaled up to industrial practice [69]. Solvent extraction is generally used for microalgal biomass for carotenoid extraction and can be used directly in the supplement formulation

or go through further multistep purification encompassing hydrolysis to release hydroxylated carotenoids from the complementary fatty acids and final recrystallization to polish the product. Obtaining a carotenoid-rich oleoresin from dried microalgae or in paste form is a more straightforward task and such extracts may then be subjected to obtain pure pigments [73–77]. Solvent extraction uses non-polar solvents, which converts to their free forms where carboxylic acids and chlorophylls are separated into aqueous phase [73–77]. Extraction with hexane or ethanol allows easy solvent removal afterward, as well as high-content extraction [76]. A green downstream process using vegetable oils was used for direct extraction of astaxanthin [76,77]. Supercritical fluid extraction permits more straightforward purification and shorter extraction times [78]. By controlling solvent density effective and selective extraction can be achieved. Supercritical fluid return to its gaseous form and leaving little or no residual solvent in the precipitate [79,80].

5. APPLICATIONS OF CAROTENOIDS

Of all the known carotenoids, very few are used commercially: β-carotene, astaxanthin and, of lesser importance zeaxanthin, lutein, and lycopene [77]. The most important applications are as antioxidants, food colorants, additive for animal feed, cosmetics, therapeutic, and nutraceutical applications. Certain carotenoids act as provitamin A, which can be converted into vitamin A and therefore, used in cosmetics [78]. Carotenoids have native anti-inflammatory properties owing to their quenching action on relative O_2 species and a therapeutic chemopreventive anti-cancer agent [10].

6. FUTURE PROSPECTS

The market demand for pigments from natural sources has promoted large-scale cultivation of microalgae for the synthesis of high-value compounds like carotenoids. The well-accepted therapeutic value of carotenoids (especially astaxanthin and β-carotene) in prevention and treatment of degenerative diseases has indeed opened new avenues. Advances in knowledge of the underlying physiology, biochemistry, and molecular genetics have helped the research fraternity in understanding the mechanism of carotenoid-producing microalgae which would have a major impact upon process optimization and development in a microalgal cell factory approach and enabling bio-based economy. However, to fully exploit the economic potential of microalgal cell factories, combined efforts are required for developing an understanding of the complex metabolic pathways and their regulation to attain desirable carotenoids production in microalgae.

References

[1] A. Solovchenko, I. Khozin-goldberg, Stress-induced changes in optical properties, pigment and fatty acid content of *Nannochloropsis* sp.: Implications for non-destructive assay of total fatty acids, Mar. Biotechnol. 13 (2015) 527–535.

[2] R.T. Lorenz, G.R. Cysewski, Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin, Trends Biotechnol. 18 (2000) 160–167.

[3] S. Venkata Mohan, M.P. Devi, G. Mohanakrishna, N. Amarnath, M.L. Babu, P.N. Sarma, Potential of mixed microalgae to harness biodiesel from ecological water-bodies with simultaneous treatment, Bioresour. Technol. 102 (2011) 1109–1117.
[4] P.P. Lamers, M. Janssen, R.C.H. De Vos, R.J. Bino, R.H. Wijffels, Exploring and exploiting carotenoid accumulation in *Dunaliella salina* for cell-factory applications, Trends Biotechnol. 26 (2008) 631–638.
[5] T.Y. Kim, H.U. Kim, J.M. Park, H. Song, J.S. Kim, S.Y. Lee, Genome- scale analysis of *Mannheimia succiniciproducen*s metabolism, Biotechnol. Bioeng. 97 (2007) 657–671.
[6] J.F. Sanchez, J.M. Fernandez-Sevilla, F.G. Acien, M.C. Ceron, J. Perez-Parra, E. Molina-Grima, Biomass and lutein productivity of *Scenedesmus almeriensis*: influence of irradiance, dilution rate and temperature, Appl. Microbiol. Biotechnol. 79 (2008) 719–729.
[7] G. Britton, S. Liaaen-Jensen, H. Pfander (Eds.), Carotenoids 4, Springer Science & Business Media, 2008.
[8] R.G. Hiller, The Photochemistry of carotenoids, in: H.A. Frank, A.J. Young, G. Britton, R.J. Cogdell (Eds.), Advances in Photosynthesis and Respiration 8, Kluwer Academic Publishers, 1999, pp. 81–98.
[9] D. Geng, Y. Wang, P. Wang, W. Li, Y. Sun, Stable expression of hepatitis B surface antigen gene in *Dunaliella salina* (Chlorophyta), J. Appl. Phycol. 15 (2003) 451–456.
[10] https://www.dsm.com/content/dam/dsm/cworld/en_US/documents/white-biotechmckinsey-feb-2009.pdf.
[11] O. Pulz, W. Gross, Valuable products from biotechnology of microalgae, Appl. Microbiol. Biotechnol. 65 (2004) 635–648.
[12] A. Richmond, Handbook of Microalgal Culture: Biotechnology and Applied Phycology, John Wiley & Sons, 2008.
[13] S. Venkata Mohan, J.A. Modestra, K. Amulya, B. Sai Kishore, G. Velvizhi, A circular bioeconomy with bio-based products from CO_2 sequestration, Trends Biotechnol. 34 (2016) 506–519.
[14] A. Katz, C. Jimenez, U. Pick, Isolation and characterization of a protein associated with carotene globules in the alga *Dunaliella bardawil*, Plant Physiol. 108 (1995) 1657–1664.
[15] S. Rabbani, P. Beyer, J.V. Lintig, P. Hugueney, H. Kleinig, Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular algae *Dunaliella bardawil*, Plant Physiol. 116 (1998) 1239–1248.
[16] A.L. White, L.S. Jahnke, Contrasting effects of UV-A and UV-B on photosynthesis and photoprotection of β-carotene in two *Dunaliella* sp, Plant Cell Physiol. 43 (2002) 877–884.
[17] M.A. Borowitzka, C.J. Siva, The taxonomy of the genus *Dunaliella* (Chlorophyta, Dunaliellales) with emphasis on the marine and halophilic species, J. Appl. Phycol. 19 (2007) 567–590.
[18] M.A. Gonzalez, A.W. Coleman, P.I. Gomez, R. Montoya, Phylogenetic relationship among various strains of *Dunaliella* (Chlorophyceae) based on nuclear its rDNA sequences, J. Phycol. 37 (2001) 604–611.
[19] A. Ben-Amotz, A. Katz, M. Avron, Accumulation of β-carotene in halotolerant algae: purification and characterization of b-carotene rich globules from *Dunaliella bardawil* (Chlorophyceae), J. Phycol. 18 (1982) 529–537.
[20] J.A. Del Campo, M. Garcaa-Gonzalez, M.G. Guerrero, Outdoor cultivation of microalgae for carotenoid production: current state and perspectives, Appl. Microbiol. Biotechnol. 74 (2007) 1163–1174.
[21] M.A. Hejazi, D. Kleinegris, R.H. Wijffels, Mechanism of extraction of β-carotene from microalga *Dunaliellea salina* in two-phase bioreactors, Biotechnol. Bioeng. 88 (2004) 593–600.
[22] A. Ben-Amotz, A. Shaish, M. Avron, Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation, Plant Physiol. 91 (1989) 1040–1043.
[23] W. Miki, Biological functions and activities of animal carotenoids, Pure Appl. Chem. 63 (1991) 141–146.
[24] A. Vershinin, Biological functions of carotenoids: diversity and evolution, Biofactors 10 (1999) 99–104.
[25] M.R. Droop, Conditions governing haematochrome formation and loss in the alga *Haematococcus pluvialis* Flotow, Arch. Microbiol. 20 (1954) 391–397.
[26] E.A. Johnson, G.H. An, Astaxanthin from microbial sources, Crit. Rev. Biotechnol. 11 (1991) 297–326.
[27] E.A. Johnson, W.A. Schroeder, Microbial carotenoids, Adv. Biochem. Eng. Biotechnol. 53 (1995) 119–178.
[28] M. Orosa, E. Torres, P. Fidalgo, J. Abalde, Production and analysis of secondary carotenoids in green algae, J. Appl. Phycol. 12 (2000) 553–556.
[29] Y.K. Lee, S.Y. Ding, Cell cycle and accumulation of astaxanthin in *Haematococcus lacustris* (Chlorophyta), J. Phycol. 30 (1994) 445–449.
[30] S. Qin, G.X. Liu, Z.Y. Hu, The accumulation and metabolism of astaxanthin in *Scenedesmus obliquus* (Chlorophyceae), Process. Biochem. 43 (2008) 795–802.
[31] J. Green, Occurrence of astaxanthin in euglenoid *Trachelomonas volvocina*, Comp. Biochem. Physiol. 9 (1963) 313–316.

[32] S. Boussiba, Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response, Physiol. Plant 108 (2000) 111–117.
[33] Y. Lemoine, B. Schoefs, Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress, Photosynth. Res. 106 (2010) 155–177.
[34] P.F. Ip, K.H. Wong, F. Chen, Enhanced production of astaxanthin by the green microalga *Chlorella zofingiensis* in mixotrophic culture, Process. Biochem. 39 (2004) 1761–1766.
[35] N. Sun, Y. Wang, Y.T. Li, J.C. Huang, F. Chen, Sugar-based growth, astaxanthin accumulation and carotenogenic transcription of heterotrophic *Chlorella zofingiensis* (Chlorophyta), Process Biochem. 43 (2008) 1288–1292.
[36] G. Hershkovits, Z. Dubinsky, D.J. Katcoff, A novel homologue of the prokaryotic htrA gene is differentially expressed in the alga *Haematococcus pluvialis* following stress, Mol. Gen. Genet. 254 (1997) 345–350.
[37] Y. Li, J. Huang, G. Sandmann, F. Chen, Glucose sensing and the mitochondrial alternative pathway are involved in the regulation of astaxanthin biosynthesis in the dark-grown *Chlorella zofingiensis* (Chlorophyceae), Planta 228 (2008) 735–743.
[38] Y.Y.R. Yong, Y.K. Lee, Do carotenoids play a photoprotective role in the cytoplasm of *Haematococcus lacustris* (Chlorophyta)? J. Phycol. 30 (1991) 257–261.
[39] C. Hagen, G. Bornman, W. Braune, Reversible lowering of modulated chlorophyll fluorescence after saturating flashes in *Haematococcus lacustris* (Volvocales) at room temperature, Physiol. Plant 86 (1992) 593–599.
[40] I.S. Zlotnik, A. Sukenik, Z. Dubinsky, Physiological and photosynthetic changes during the formation of red aplanospores in the chlorophyte *Haematococcus pluvialis*, J. Phycol. 29 (1993) 463–469.
[41] S. Boussiba, W. Bing, A. Zarka, J.P. Yuan, F. Chen, Changes in pigment profiles of *Haematococcus pluvialis* during exposure to environmental stresses, Biotechnol. Lett. 21 (1999) 601–604.
[42] F. Lu, A. Vonshak, S. Boussiba, Effect of temperature and irradiance on growth of *Haematococcus plulialis* (Chlorophyceae), J. Phycol 30 (1994) 829–833.
[43] S. Tan, F.X. Cunningham, M. Youmans, B. Grabowski, Z. Sun, E. Gantt, Cytochrome f loss in astaxanthin accumulating red cells of *Haematococcus pluvialis* (Chlorophyceae): Comparison of photosynthetic activity, photosynthetic enzymes and thylakoid membrane polypeptides in red and green cells, J. Phycol. 31 (1995) 897–905.
[44] A.H. Mehler, Studies on reaction of illuminated chloroplast. I. Mechanisms of the reduction of oxygen and other Hill reagents, Arch. Biochem. Biophys. 33 (1951) 65–77.
[45] E.F. Elstner, Oxygen activation and oxygen toxicity, Annu. Rev. Plant Physiol. 33 (1982) 73–96.
[46] N.I. Krinsky, Carotenoid protection against oxidation, Pure Appl. Chem. 51 (1979) 649–660.
[47] R.D. Allen, Dissection of oxidative stress tolerance using transgenic plants, Plant Physiol. 107 (1995) 1049–1054.
[48] M. Kobayashi, T. Kakizono, S. Nagai, Enhanced carotenoid biosynthesis by oxidative stress in acetate-induced cyst cells of a green unicellular alga, *Haematococcus pluvialis*, Appl. Environ. Microbiol. 5l9 (1993) 867–873.
[49] F. Lu, A. Vonshak, A. Zarka, S. Boussiba, Does astaxanthin protect *Haematococcus* against light damage? Z. Naturforsch 53 (1998) 93–100.
[50] F. Bouvier, R.A. Backhaus, B. Camara, Induction and control of chromoplast-specific carotenoid genes by oxidative stress, J. Biol. Chem. 273 (1998) 30651–30659.
[51] M. Guerin, M.E. Huntley, M. Olaizola, *Haematococcus* astaxanthin: applications for human health and nutrition, Trends Biotechnol. 21 (2003) 210–216.
[52] M.J. Barbosa, R. Morais, G. Choubert, Effect of carotenoid source and dietary lipid content on blood astaxanthin concentration in rainbow trout (*Oncorhynchus mykiss*), Aquaculture 176 (1999) 331–341.
[53] N. Misawa, Pathway engineering of plants toward astaxanthin production, Plant Biotechnol. 26 (2009) 93–99.
[54] J. Li, D. Zhu, J. Niu, S. Shen, G. Wang, An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*, Biotechnol. Adv. 29 (2011) 568–574.
[55] J.C. Huang, Y. Wang, G. Sandmann, F. Chen, Isolation and characterization of a carotenoid oxygenase gene from *Chlorella zofingiensis* (Chlorophyta), Appl. Microbiol. Biotechnol. 71 (2006) 473–479.
[56] Z.W. Ye, J.G. Jiang, G.H. Wu, Biosynthesis and regulation of carotenoids in *Dunaliella*: progresses and prospects, Biotechnol. Adv. 26 (2008) 352–360.
[57] H. Senger, C. Wagner, D. Hermsmeier, N. Hohl, T. Urbig, N.I. Bishop, The influence of light intensity and wavelength on the contents of α and β-carotene and their xanthophylls in green algae, J. Photochem. Photobiol. B. Biol. 18 (1993) 273–279.

[58] H. Herrmann, D.P. Hader, F. Ghetti, Inhibition of photosynthesis by solar radiation in *Dunaliella salina*: relative efficiencies of UV-B, UV-A and PAR, Plant Cell Environ. 20 (1997) 359–365.
[59] A. Salguero, R. Leon, A. Mariotti, B. de la Morena, J.M. Vega, C. Vílchez, UV-A mediated induction of carotenoid accumulation in *Dunaliella bardawil* with retention of cell viability, Appl. Microbiol. Biotech. 66 (2005) 506–511.
[60] A. Ben-Amotz, Effect of low temperature on the stereoisomer composition of β-carotene in the halotolerant alga, *Dunaliella bardawil* (Chlorophyta), J. Phycol. 32 (1996) 272–275.
[61] W. Becker, Microalgae for aquaculture. The nutritional value of microalgae for aquaculture, in: A. Richmond (Ed.), Handbook of Microalgal Culture, Blackwell, Oxford, 2004, pp. 380–391.
[62] S.N. Coesel, A.C. Baumgartner, L.M. Teles, A.A. Ramos, N.M. Henriques, L. Cancela, et al., Nutrient limitation is the main regulatory factor for carotenoid accumulation and for *Psy* and *Pds* steady state transcript levels in *Dunaliella salina* (Chlorophyta) exposed to high light and salt stress, Mar. Biotechnol. 10 (2008) 602–611.
[63] R. Geider, H.L. Macintyre, L. Graziano, R.M. McKay, Responses of the photosynthetic apparatus of *Dunaliella tertiolecta* (Chlorophyceae) to nitrogen and phosphorus limitation, Eur. J. Phycol. 33 (1998) 315–332.
[64] M.D. Krom, N. Kress, S. Brenner, L.I. Gordon, Phosphorus limitation of primary productivity in the eastern Mediterranean Sea, Limnol. Oceanogr 36 (1991) 424–432.
[65] A. Ben-Amotz, M. Avron, On the factors which determine massive β-carotene accumulation in the halotolerant alga *Dunaliella bardawil*, Plant Physiol. 72 (1983) 593–597.
[66] A.M.S. Albino, M.D.V.C. Penteado, F.M. Lanças, J.H.Y. Vilegas, Carotenoids extraction using CO_2 in supercritical state, from kale (*Brassica oleracea*, Lin. var. *acephala*), in: M.I.M. Mosquera, M.J. Galan, D.H. Mendez (Eds.), Proceedings of International Congress on Pigments in Food Technology, 1999, pp. 65–69.
[67] R. Vidhyavathi, L. Venkatachalam, R. Sarada, G. Aswanthanrayana, G.A. Ravishankar, Regulation of carotenoid biosynthetic genes expression and carotenoid accumulation in the green alga *Haematococcus pluvialis* under nutrient stress conditions, J. Exp. Bot. 59 (2008) 1409–1418.
[68] A.R. Grossman, M. Lohr, C.S. Im, *Chlamydomonas reinhardtii* in the landscape of pigments, Annu. Rev. Genet. 38 (2004) 119–173.
[69] G. Sandmann, Genetic manipulation of carotenoid biosynthesis: strategies, problems and achievements, Trends Plant Sci. 6 (2001) 14–17.
[70] H.K. Lichtenthaler, The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 47–65.
[71] J.A. Del Campo, J. Moreno, H. Rodríguez, M.A. Vargas, J. Rivas, M.G. Guerrero, Carotenoid content of chlorophycean microalgae. Factors determining lutein accumulation in *Muriellopsis* sp. (Chlorophyta), J. Biotechnol. 76 (2000) 51–59.
[72] D.H. Zhang, Y.K. Lee, Enhanced accumulation of secondary carotenoids in a mutant of the green alga, *Chlorococcum* sp, J. Appl. Phycol. 9 (1997) 459–463.
[73] J.M. Fernandez-Sevilla, F.G. Acien-Fernández, E. Molina-Grima, Biotechnological production of lutein and its applications, Appl. Microbiol. Biotechnol. 86 (2010) 27–40.
[74] M.I. Minguez-Mosquera, B. Gandul-Rojas, M.L. Gallardo-Guerrero, Rapid method of quantification of chlorophylls and carotenoids in virgin olive oil by high-performance liquid chromatography, J. Agric. Food Chem. 40 (1992) 60–63.
[75] M.C. Ceron, I. Campos, J.F. Sanchez, F.G. Acien, E. Molina, J.M. Fernandez-Sevilla, Recovery of lutein from microalgae biomass: development of a process for *Scenedesmus almeriensis*, J. Agric. Food Chem. 56 (2008) 11761–11766.
[76] A.M. Nonomura, Process for Producing a Naturally-Derived Carotene/oil Composition by Direct Extraction From Algae, 1987. US Patent 4,680,314.
[77] C.D. Kang, S.J. Sim, Direct extraction of astaxanthin from *Haematococcus* culture using vegetable oils, Biotechnol. Lett. 30 (2008) 441–444.
[78] E. Bravi, G. Perretti, L. Motanari, F. Favati, P. Fantozzi, Supercritical fluid extraction for quality control in beer industry, J. Supercrit. Fluids 42 (2007) 342–346.
[79] M.M. Bath, C. Zhou, K.M. Kute, G.A. Rosenthal, Determination of optimum conditions for supercritical fluid extraction of carotenoids from carrot (*Daucus carota* L.) tissue, J. Agric. Food Chem. 43 (1995) 2876–2878.
[80] D.M.M. Kleinegris, M. Janssen, W.A. Brandenburg, R.H. Wijffels, Continuous production of carotenoids from *Dunaliella salina*, Enzym. Microb. Technol. 48 (2011) 253–259.

CHAPTER

8

Low-Molecular-Weight Nitrogenous Compounds (GABA and Polyamines) in Blue–Green Algae

S. Jantaro, S. Kanwal

Chulalongkorn University, Bangkok, Thailand

OUTLINE

1. INTRODUCTION

Cyanobacteria are considered as earliest photosynthetic organisms forming an evolutionary link toward plants. They are morphologically diverse organisms ranging from unicellular to filamentous species and known to inhabit various kinds of environments such as deserts, freshwater bodies, and sea. Although cyanobacteria are considered as algae due to

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00008-4

their aquatic habitat and photoautotrophic nature, they are classified as prokaryotes owing to lack of membrane bound organelles. They carry out photosynthesis using blue–green pigment system known as phycobilins, hence called as blue–green algae.

Cyanobacteria tend to accumulate low-molecular-weight nitrogenous compounds to encounter physiological stresses imposed by environment, such as to attain osmotic equilibrium with respect of surroundings. GABA is a nonprotein amino acid with the general formula of $C_4H_9NO_2$, and polyamines are organic nitrogenous compounds involved in performing essential functions in all living organisms. These low–molecular-weight nitrogenous compounds are produced by both unicellular and filamentous cyanobacteria but detailed studies about their metabolism are scarce. Efficiency of GABA and polyamine productions is highly dependent on different cyanobacterial strains and growth conditions. The GABA production is higher after late-log phase of growth in unicellular and freshwater cyanobacterium, *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), but the marine cyanobacterium *Aphanothece halophytica* produces higher GABA at mid-log phase of cell growth [1,2]. The high accumulation of polyamines in cyanobacteria is apparently found in early-lag and log phases of cell growth [3]. In this chapter, we encompass various pathways possibly involved in synthesis and degradation of both GABA and polyamines, and also up-to-date knowledge available regarding their roles against physiological stresses in cyanobacteria.

2. GABA BIOSYNTHESIS

GABA metabolism in cyanobacteria occurs by circumventing two reactions in TCA cycle forming a shunt pathway commonly called as "GABA shunt" [4,5]. GABA metabolism is ubiquitous in all life forms ranging from prokaryotes to eukaryotes [5,6]. There are recent reports about the absence of typical α-ketoglutarate dehydrogenase (αKGDH) complex in cyanobacterial TCA cycle and also that the complex is replaced by a recently known α-ketoglutarate decarboxylase (Kgd) that sequentially converts α-ketoglutarate into succinic semialdehyde and succinate [4,7,8]. In connection to these TCA cycle reactions, GABA shunt becomes a very important pathway in cyanobacteria because it may act as a major contributor to convert α-ketoglutarate into succinate independent of the Kgd bypass. Like other organisms, GABA metabolism in cyanobacteria is associated with carbon-nitrogen balance by serving as a link between amino acid metabolism and TCA cycle [9]. Although GABA is produced by both unicellular and filamentous cyanobacteria, the detailed studies about its metabolism are scarce. In this part of chapter we discuss pathways of GABA formation and degradation in cyanobacteria particularly in model cyanobacterium *Synechocystis* and also the effect of physiological stresses on GABA metabolism.

2.1 GABA Formation via Glutamate Catabolism

In cyanobacteria, GABA is formed primarily by the irreversible action of glutamate decarboxylase enzyme (GAD, EC 4.1.1.15), a pyridoxal 5′-phosphate-dependent enzyme, using L-glutamate as a substrate [10]. The pathway is generally named as GAD pathway. GAD is well characterized in mammals, plants, and prokaryotes [11–13]. In case of cyanobacteria, GABA shunt is not appearing to be universal since many cyanobacterial strains are apparently

lacking the annotated gene encoding GAD (namely *gad*) [8]. The genome database of various cyanobacterial species reveals the ORF of *gad* gene, such as for unicellular *Synechocystis* and *Microcystis aeruginosa* NIES-843 with respective gene IDs of *sll1641* and *MAE41860* [http://genome.microbedb.jp/Cyanobase/]. *Synechocystis* GAD is distinct from plant GADs in many aspects, such as a monomeric protein unlike multimeric forms in plants and also other organisms [10,12,14]. Also, the plant GAD is a calmodulin-binding protein unlike *Synechocystis* GAD. Glutamate that is the key substrate for GAD pathway plays the pivotal role in carbon and nitrogen metabolism particularly in ammonia and nitrate/nitrite assimilation in bacteria, cyanobacteria, green algae, and plants. The connection of nitrogen metabolism with glutamate synthesis creates the perception as how GABA formation in cyanobacteria might be effected directly by nitrogen metabolism (Fig. 8.1).

Keeping in view on the potential ability of cyanobacteria to uptake and utilize variable nitrogenous compounds such as nitrite, nitrate, ammonia, urea, and glutamate [15], the relationship of exogenous nitrogen source provision with GABA synthesis was tried to establish previously in unicellular cyanobacterium *Synechocystis* [9]. GAD activity and intracellular GABA levels were affected in *Synechocystis* cells grown in media deprived of nitrogen source or supplemented with alternative nitrogen sources. The genetic engineering of *Synechocystis*

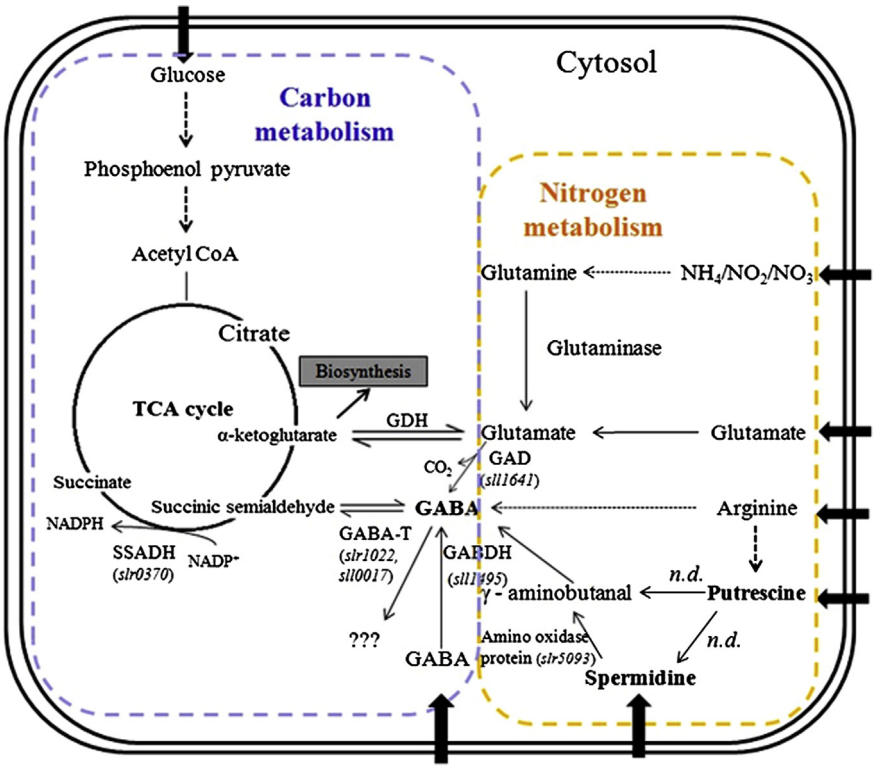


FIGURE 8.1 Metabolic map of cyanobacterial GABA metabolism in connection with TCA cycle and polyamine degradation pathway. Gene IDs from CyanoBase are indicated for the genes associated exclusively with GABA metabolism. Please refer to the text for abbreviations. *Dashed arrows* denote cascade of intermediate reactions. *n.d.*, Not detected.

to generate *Δgad* strain (a mutant strain lacking functional GAD) has led to gain better understanding about GABA metabolism in cyanobacteria. The Δ*gad* strain could not accumulate higher GABA levels in contrast to the wild-type (WT) strain irrespective of the nitrogen source supplementation. Exogenous glutamate supply also affected the intracellular GABA levels directly. Despite the fact that there are additional catabolic routes for glutamate in cyanobacteria, the Δ*gad* mutant could accumulate three times higher intracellular glutamate content as compared to WT strain.

Increase in GABA levels with concomitant increase in GAD activity in *Synechocystis* WT strain demonstrates a conspicuous response of GAD pathway toward nitrogen supplementation and deprivation in *Synechocystis* indicating the clear connection of nitrogen metabolism with GABA formation in cyanobacteria. In another study, the glutamate supplementation accompanied by high salt and acid stresses increased the intracellular GABA content in *A. halophytica* [2]. However, our experimental evidences suggest that there are various alternative routes and factors attributed to the metabolic link between nitrogen metabolism and GABA formation that shall be discussed hereafter. The normal growth and phenotype of Δ*gad* mutant indicates that the GAD pathway is optional under photoautotrophic conditions and also it is not indispensable for the survival of cyanobacteria. Other than nitrogen source, carbon source also affected GABA synthesis. Glucose, the chief raw material for glycolysis, which directly links to TCA cycle, plays important role in GABA formation. Apart from nitrogen assimilation, glutamate synthesis also occurs via glutamate dehydrogenase (GDH) in cyanobacteria using α-ketoglutarate from TCA cycle as a substrate. Glucose supplementation as a carbon source upregulated the GAD pathway in *Synechocystis* by elevating the *gad* transcript levels as well as enhancing the GAD activity and intracellular GABA levels [1]. Similarly in lactic acid bacteria, the increased GABA production was reported in response to glucose supplementation [16]. It means that in different life forms, GABA formation is contributed significantly by both nitrogen and carbon metabolism. Recently, we observed the slower growth rate of engineered *Synechocystis* strain overexpressing *gad* and having higher intracellular GABA levels. Glutamate is an important raw material not only for protein synthesis but also for the formation of other amino acids such as proline, glutamine, and arginine. The overexpression of *gad* gene might cause increased glutamate consumption by GAD pathway resulting in unavailability of this important amino acid to other metabolic pathways at the cost of higher GABA production.

2.2 GABA Formation via Spermidine Catabolism

Among the latest advancements made regarding study of GABA metabolism in cyanobacteria, the most interesting one is the availability of evidences for possible involvement of polyamine catabolism in GABA formation. Both the green algae and cyanobacteria are able to transport or synthesize polyamines because of the numerous cellular roles performed by these nitrogenous compounds. A large number of studies have investigated the role of polyamines in GABA formation in plants and mammals [17,18]. However, a bacterium *Escherichia coli* could also catabolize putrescine to GABA [19]. Bioinformatic analyses show putative genes encoding enzymes involved in spermidine degradation to GABA in cyanobacteria [20]. It is assumed that spermidine is converted to γ-aminobutanal that is subsequently converted to GABA. This hypothesis was bolstered by the finding that intracellular GABA

content were enhanced by exogenous spermidine supply in both *Synechocystis* WT and Δ*gad* mutant strains [9]. Although the activities of concerned enzymes are not examined yet but the transcription of *gabdh*, a gene encoding γ-aminobutanal dehydrogenase (GABDH) that is possibly involved in conversion of γ-aminobutanal to GABA, was shown to be remarkably upregulated in Δ*gad* mutant. It supports the notion that polyamine catabolism has a substantial contribution in GABA formation in cyanobacteria [10]. Information regarding the concerned enzymatic activities relating putrescine degradation to GABA in green algae and cyanobacteria is still lacking except one study by our group reporting that exogenous putrescine supply also elevated the GABA levels in *Synechocystis* [9]. From experimental data, we proposed that polyamine supplementation contributes about 45% of the GABA content in *Synechocystis* and polyamine degradation can be an additional pathway for GABA formation via GABDH catalysis. However, keeping in view the association of increased polyamine production with stress relieving roles in organisms, the pathway could be considered as an advantage for organism because conversion of polyamines into GABA provides additional substrate for TCA cycle to produce more carbon skeleton and energy for organism to counteract the stress-related damages to cell.

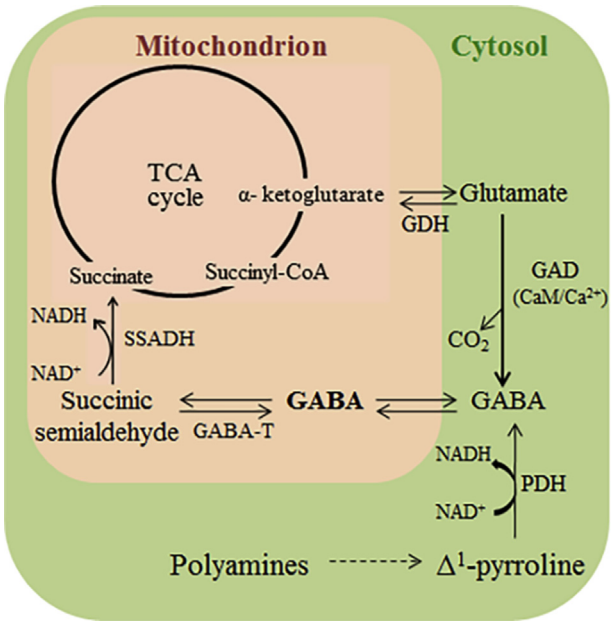
3. GABA CATABOLISM

GABA catabolism follows the subsequent action of two enzymes, first one is GABA transaminase (GABA-T, EC 2.6.1.19) and the second is succinic semialdehyde dehydrogenase (SSADH, EC 1.2.1.24). Both enzymes finally connect the GABA metabolism, with TCA cycle completing the GABA shunt pathway [6,7,21,22]. Like GABA synthesis, GABA degradation is also equally important for the organisms to carry out normal cellular functions. In plants, GABA degradation occurs inside the mitochondria (Fig. 8.2). Genes encoding GABA degradation enzymes are studied in both marine and freshwater cyanobacterial species.

GABA-T also known as γ-aminobutyrate aminotransferase does the reversible conversion of GABA and α-ketoglutarate into succinic semialdehyde by transferring the nitrogenous group. Succinic semialdehyde is further catalyzed by irreversible action of SSADH into succinate that is finally incorporated into TCA cycle. The *Δslr1022* and *Δslr0370* mutant strains of *Synechocystis* (knocked-out genes encoding GABA-T and SSADH, respectively) lacking functional GABA degradation pathways grew slower than the WT strain [8]. In plants also, the GABA-T mutants devoid of GABA degradation activity showed retarded pollen tube growth [23]. It suggests the importance of GABA-T in maintaining optimum GABA levels for normal cellular functions of organisms. The gene expressions of both GABA-T and SSADH were highly induced in the absence of functional GAD pathway in *Synechocystis*. It might be possible that the disruption of GAD pathway upregulated the polyamine degradation into GABA resulting in higher transcript levels of genes encoding GABA degrading enzymes. Due to the absence of experimental evidences, it would be interesting to investigate the role of polyamine degradation in GABA metabolism in cyanobacteria.

SSADH activity is dependent on $NADP^+$ as cofactor and ends up in closing the GABA shunt by forming succinate that adds up into TCA cycle. The SSADH reaction holds great significance in cyanobacterial cells that are apparently lacking αKGDH activity and provides an alternative energy source for organisms by generating NADPH to overcome αKGDH deficiency.

FIGURE 8.2 GABA metabolism in plants.



SSADH plays vital role in maintaining the toxic succinic semialdehyde levels inside the cells of cyanobacteria resulting from GABA degradation. *Synechocystis* mutant strain lacking SSADH is sensitive to SSA and GABA supplementation by depicting retarded cell growth as compared to WT strain [8]. The SSADH lacking *Synechococcus* mutant strain also revealed the retarded growth [4]. Interruption in enzyme activities involved in GABA degradation dropped the TCA succinate levels significantly. This supports the notion that GABA shunt is another active pathway bypassing the α-ketoglutarate in cyanobacteria to join the TCA cycle.

In other organisms also SSADH holds an important place in GABA catabolism by performing critically important roles. In humans, it is reported that an error in GABA catabolism owing to SSADH deficiency resulted in an autosomal recessive inherited disorder [24]. In *Agrobacterium tumefaciens*, SSADH affected the quorum sensing signal decay by the mechanism that is not clear yet [25]. *Arabidopsis* mutant lacking a functional SSADH tended to accumulate higher level of reactive oxygen intermediates than normal WT corresponding to growth retardation and hypersensitivity to UV radiation and heat stress [26]. This suggests that apart from contribution in energy production via GABA shunt pathway, SSADH has several stress relieving roles including scavenging of reactive oxygen species resulting from environmental stress factors.

4. ROLE OF GABA AGAINST PHYSIOLOGICAL STRESSES

Plants and animals undergo several crucial changes in their metabolism to counteract the adverse effects imposed by physiological stresses. In invertebrates and vertebrates, GABA

acts as an inhibitory neurotransmitter. Whilst in plants and microorganisms, GABA shunt pathway is known to play important role by responding to various stress conditions, such as pH variation, osmotic or ionic stress, and cold acclimation [27–29]. GAD enzyme converts glutamate into GABA utilizing cytosolic protons conferring acid resistance [30]. Stress induced by cytosolic acidosis increases the GAD activity with subsequent upregulation of GABA synthesis. In bacteria, GAD pathway is known to play role in acid resistance using the similar mechanism [31,32]. GABA accumulation in halotolerant cyanobacterium *A. halophytica* was investigated in response to variable concentrations of NaCl and pH values [2]. An external acidic pH increased the GABA accumulation and GAD activity in *A. halophytica* in addition to salt stress and glutamate supplementation. Higher GABA accumulation has been reported in freshwater cyanobacterium *Synechocystis* with concomitant upregulation of GAD activity and transcript levels under salt and sorbitol-induced osmotic stress [1]. In response to salt stress, GABA acts as a signaling molecule in plants by regulating the expression of various genes [33].

GABA shunt pathway links carbon and nitrogen metabolism inside the cell by connecting the amino acids and polyamines catabolism with TCA cycle. This important role of GABA shunt pathway to balance the intracellular carbon nitrogen metabolism is also observed in blue–green algae. *Synechocystis* tends to accumulate higher GABA content when supplemented with exogenous glutamate with parallel increase in GAD activity [9] and *gad* transcript levels (data not published). Growth of *Synechocystis* cells in buffered media is not affected by glutamate supplementation up to 40 mM, though a slight decline in growth occurred. But Δ*gad* strain displayed the complete inhibition of growth in response to glutamate supplementation higher than 20 mM with large accumulation of intracellular glutamate content. From these studies, it could be inferred that GABA metabolism serves as an ultimate pathway of glutamate utilization in cyanobacteria and plays an important role in maintaining intracellular glutamate homeostasis.

Involvement of GABA in mediating communication between plants and fungus or bacteria via multiple mechanisms was reported previously [27]. Coralline red algae (also known as *isoyake* in food industry) produced GABA to recruit specific habitats to larvae of sessile invertebrates like molluscs by stimulating their attachment and metamorphosis [28]. The increase in GABA accumulation under cold stress in charophycean green alga, *Klebsormidiumflaccidum*, suggests that GABA might act as a compatible solute in algae to offer freezing tolerance by the mechanism similar to that of terrestrial plants [34].

5. POLYAMINE BIOSYNTHESIS

Aliphatic polyamines are synthesized in living cells of both prokaryotic and eukaryotic organisms [35]. Three common polyamines found in the cells are diamine putrescine (Put, 1,4-diaminobutane, $NH_2(CH_2)_4NH_2$), triamine spermidine (Spd, 1,8-diamino-4-azaoctane, $NH_2(CH_2)_3NH(CH_2)_4NH_2$), and tetraminespermine (Spm, 1,12-diamino-4,9-diazadodecane, $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$). Generally, putrescine and spermidine are more abundant in the cells of living organisms, whereas spermine is found in trace amounts. Other aliphatic polyamines such as 1,3-diaminopropane and cadaverine (Cad, 1,5-diaminopentane) are also present in cyanobacteria [3,36]. Moreover, environmental stress such as osmotic

stress effectively induced high norspermidine accumulation via the alternative pathway of spermidine biosynthesis in *Synechocystis* (unpublished). After discovery of the functional role of polyamines in DNA stabilization in 1975 [37], much research regarding the roles of polyamines in living organisms has been carried out. These research works are done in various fields addressing the regulation of polyamine homeostasis in cellular metabolism during cancer or other hyperproliferative diseases [38], involvement in plant proliferation, and offering adaptation to physiological stresses in both plants and prokaryotes [3,39,40].

The main polyamine biosynthetic pathway or alternative ones in each organism varied depending on homeostasis response under normal and physiological stress conditions. However, the key substrate amino acids of de novo polyamine biosynthesis are basic amino acids of arginine and ornithine. In animal, the classical polyamine biosynthetic pathway revealed that ornithine decarboxylase (ODC, EC 4.1.1.17) is the directly controlled enzyme on rate-limiting step of polyamine biosynthesis from ornithine [39]. ODC rapidly turns over with a half-life shortest among mammalian enzymes [41]. ODC is under negative feedback regulation by polyamines for the protection of cells from the adverse effects of excess polyamine accumulation. Self-ODC degradation is accelerated by the increase in levels of intracellular polyamine and is regulated at four different levels of gene expression, namely gene transcription, mRNA degradation, mRNA translation, and enzyme degradation. In addition, ODC is specifically inhibited by antizyme, a unique regulatory protein of 26.5 kDa induced by polyamines. Antizyme reversibly binds to ODC with a high affinity inhibiting enzyme activity and rendering the enzyme susceptible to proteolysis [42].

At present, the arginine metabolism via agmatine to polyamines is an alternative pathway found in mammalian organisms, especially in their neurons and liver cells [43–45] (shown in Fig. 8.3A). The indirect decarboxylation of arginine by the enzyme arginine decarboxylase (ADC, EC 4.1.1.19) generates an intermediate agmatine, which is sequentially catalyzed by agmatinase (AGM, 3.4.3.11) or agmatine amidinohydrolase to putrescine producing urea as a by-product.

The conversion from arginine to ornithine is performed by the enzyme arginase (ARG, EC 3.5.3.1), the final enzyme of the urea/ornithine cycle. In higher plants (Fig. 8.3B), although both pathways of de novo polyamine biosynthesis exist, the arginine decarboxylation is the main pathway of putrescine production. In order to produce putrescine from arginine, two conversion reactions via agmatine and *N*-carbamoylputrescine intermediates are catalyzed by agmatineiminohydrolase (AIH, EC 3.5.3.12) and *N*-carbamoylputrescine amidohydrolase (NCPAH, EC 3.5.1.53), respectively. The alternative pathway to *N*-carbamoylputrescine is also found in some plants from citrulline, an intermediate in urea cycle, by citrulline decarboxylase (CDC, EC 4.1.1.-) [46]. For polyamine biosynthesis in cyanobacteria shown in Fig. 8.3C, only one indirect pathway from arginine is dominant although there are some predicted genes encoding ornithine decarboxylase reported in CyanoBase database (Table 8.1).

Interestingly, there are two routes for putrescine synthesis from an agmatine intermediate including the main conversion via AGM catalytic reaction and the other reaction via an *N*-carbamoylputrescine intermediate catalyzed by NCPAH enzyme [21]. Recently, the knockout mutant of putative AGM gene in *Synechocystis* contained small amount of putrescine via the latter reaction, which has been confirmed by the increased *NCPAH* transcripts (unpublished). Consequently, the biosynthesis of spermidine and spermine is performed by the sequential

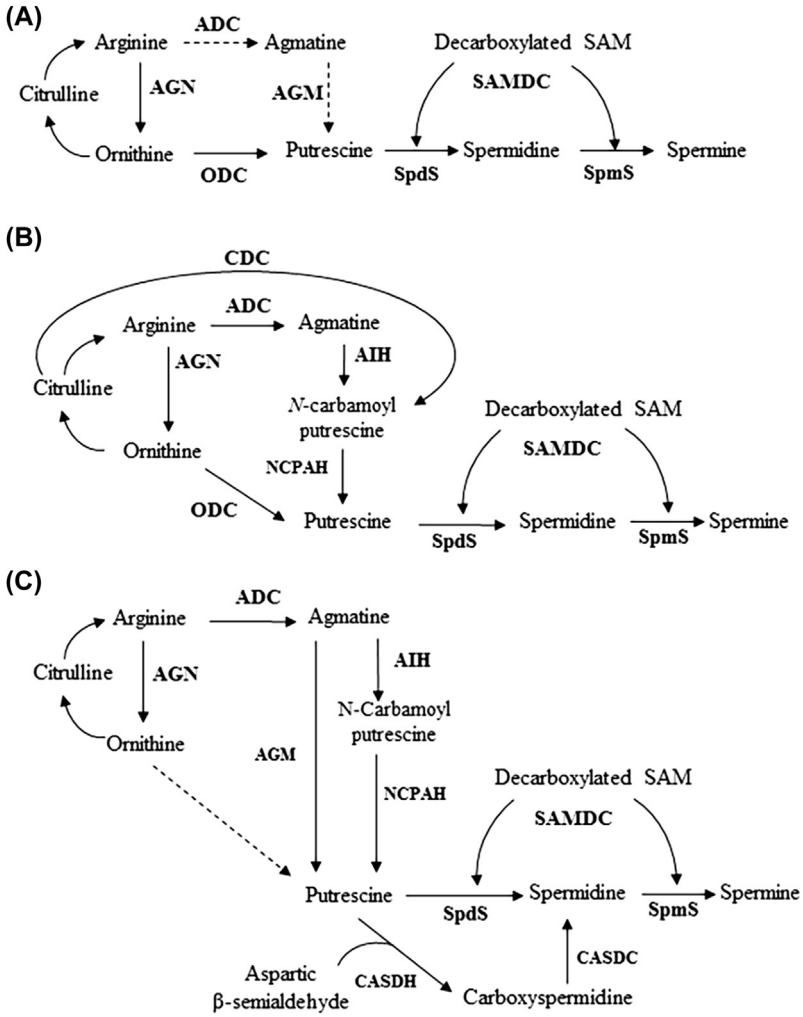


FIGURE 8.3 The pathways of polyamine synthesis in animal (A), higher plants (B), and blue–green algae (C). *ADC*, Arginine decarboxylase; *AGM*, agmatinase; *AGN*, arginase; *AIH*, agmatineiminohydrolase; *CASDC*, carboxyspermidine decarboxylase; *CASDH*, carboxyspermidine dehydrogenase; *NCPAH*, *N*-carbamoylputrescine amidohydrolase; *ODC*, ornithine decarboxylase; *SAMDC*, *S*-adenosyl-methionine decarboxylase; *SpdS*, spermidine synthase; *SpmS*, spermine synthase.

donation of an aminopropyl group from decarboxylated *S*-adenosyl-methionine (SAM) to putrescine and spermidine molecules, respectively. Those two enzymes that catalyzed each aminopropylation step are spermidine synthase (SpdS, EC 2.5.1.16) and spermine synthase (SpmS, EC 2.5.1.22), respectively. The decarboxylated SAM is catalyzed by SAM decarboxylase enzyme (SAMDC, EC 4.1.1.50). In general, both SAM and decarboxylated SAM are found in mammalian liver, whereas in higher plants and prokaryote, they are involved in methionine or cysteine biosynthesis [3,39]. On the other hand, the alternative pathway of

TABLE 8.1 Genes Related to Polyamine Biosynthesis

Enzymes	Blue–Green Algae	Genes: ID (symbol)	Sources
Arginine decarboxylase (ADC)	*Synechocystis* sp. PCC 6803	Slr1312 (*adc1*), slr0662 (*adc2*)	Cyanobase
	Synechococcus sp. PCC 7002	SYNPCC7002_A1808 (*adc*)	Cyanobase
	Synechococcus sp. WH8102	SYNW2359 (*speA*)	Cyanobase
	Microcystis aeruginosa NIES-843	MAE46810	Cyanobase
	Cyanothece sp. PCC 8801	PCC8801_1650	Cyanobase
	Trichodesmium erythraeum IMS101	Tery_1142 (*adc1*), Tery_1276 (*adc2*)	Cyanobase
	Anabaena sp. PCC 7120	All3401 (*adc*)	Cyanobase
	Anabaena variabilis ATCC 29413	Ava_2157 (*adc1*), Ava_3423 (*adc2*)	Cyanobase
	Nostoc punctiforme ATCC 29133	Npun_F6279 (*adc*)	Cyanobase
Ornithine decarboxylase (ODC)	*Synechocystis* sp. PCC 6803	Not detected	Cyanobase
	Synechococcus sp. PCC 7002	Not detected	Cyanobase
	Anabaena sp. PCC 7120	Not detected	Cyanobase
	Trichodesmium erythraeum IMS101	Not detected	Cyanobase
	Cyanothece sp. PCC 8801	Not detected	Cyanobase
	Synechococcus sp. RCC 307	SynRCC307_0782 (putative)	Cyanobase
	Synechococcus sp. WH 7803	SynWH7803_1540 (putative)	Cyanobase
Agmatinase (AGM)	*Synechocystis* sp. PCC 6803	Sll1077 (*speB2*), Sll0228 (*speB1*)	[20]
	Synechococcus sp. PCC 7002	SYNPCC7002_A1109 (putative), SYNPCC7002_A1751	Cyanobase
	Anabaena sp. PCC 7120	Alr2310 (putative)	Cyanobase
	Microcystis aeruginosa NIES-843	MAE18840	Cyanobase
	Cyanothece sp. PCC 7424	PCC7424_2696, PCC7424_5245	Cyanobase
	Trichodesmium erythraeum IMS101	Tery_3780 (putative)	Cyanobase
	Nostoc punctiforme ATCC 29133	Npun_R6035	Cyanobase
Agmatineiminohydrolase (AIH) or agmatine deiminase	*Synechocystis* sp. PCC 6803	Not detected	Cyanobase
	Synechococcus sp. PCC 7002	Not detected	Cyanobase
	Anabaena sp. PCC 7120	Not detected	Cyanobase
	Cyanothece sp. PCC 7425	Cyan7425_2586	Cyanobase
	Cyanothece sp. PCC 8801	PCC8801_0984	Cyanobase
	Nostoc punctiforme ATCC 29133	Not detected	Cyanobase
	Synechocystis sp. PCC 6803	Sll0601, Sll1640	[20]

TABLE 8.1 Genes Related to Polyamine Biosynthesis—cont'd

Enzymes	Blue–Green Algae	Genes: ID (symbol)	Sources
N-carbamoylputrescine amidohydrolase (NCPAH) or *N*-carbamoylputrescine hydrolase	*Synechococcus* sp. WH8102	SYNW 1008	[20]
	Synechococcus elongatus PCC 6301	Syc1946_d, Syc1745_c	[20]
	Synechococcus elongatus PCC 7942	Synpcc79422145, Synpcc79422358	[20]
	Anabaena variabilis ATCC 29413	Ava_5061	[20]
	Nostoc punctiforme PCC 73102	Npun02002053	[20]
Spermidine synthase (SpdS)	*Synechocystis* sp. PCC 6803	Not detected	Cyanobase
	Anabaena sp. PCC 7120	Not detected	Cyanobase
	Prochlorococcus marinus SS120	Pro1848 (*speE*)	Cyanobase
	Synechococcus sp. WH8102	SYNW2421 (*speE*, putative)	Cyanobase
	Synechococcus sp. PCC 7002	SYNPCC7002_A2283 (*speE*)	Cyanobase
	Synechococcus elongatus PCC 7942	Synpcc7942_0628	Cyanobase
	Cyanothece sp. ATCC 51142	Not detected	Cyanobase
	Nostoc punctiforme ATCC 29133	Not detected	Cyanobase
	Trichodesmium erythraeum IMS101	Tery_0722, Tery_0886	Cyanobase
Spermine synthase (SpmS)	*Synechocystis* sp. PCC 6803	Not detected	Cyanobase
	Anabaena sp. PCC 7120	Not detected	Cyanobase
	Microcystis aeruginosa NIES-843	MAE08080	Cyanobase
	Synechococcus sp. PCC 7002	Not detected	Cyanobase
	Cyanothece sp. ATCC 51142	Not detected	Cyanobase
	Cyanothece sp. PCC 7424	PCC7424_4961	Cyanobase
	Cyanothece sp. PCC 8801	PCC8801_2020	Cyanobase
	Trichodesmium erythraeum IMS101	Tery_0248	Cyanobase
S-adenosyl-methionine decarboxylase (SAMDC)	*Synechocystis* sp. PCC 6803	Not detected	Cyanobase
	Anabaena sp. PCC 7120	Not detected	Cyanobase
	Microcystis aeruginosa NIES-843	MAE40560	Cyanobase
	Prochlorococcus marinus SS120	Pro1727 (*speD*)	Cyanobase
	Synechococcus sp. WH8102	SYNW2050 (putative)	Cyanobase
	Synechococcus sp. PCC 7002	SYNPCC7002_A0430 (*speH*)	Cyanobase
	Cyanothece sp. PCC 7424	PCC7424_3322	Cyanobase
	Arthrospira platensis NIES-39	NIES39_K04910, NIES39_O04830	Cyanobase
Carboxyspermidine dehydrogenase (CASDH)	*Synechocystis* sp. PCC 6803	Slr0049	[47]
	Anabaena sp. PCC 7120	Not detected	[47]

(*Continued*)

TABLE 8.1 Genes Related to Polyamine Biosynthesis—cont'd

Enzymes	Blue–Green Algae	Genes: ID (symbol)	Sources
	Cyanothece sp. PCC 7424	Not detected	[47]
	Trichodesmium erythraeum IMS101	Not detected	[47]
Carboxyspermidine decarboxylase (CASDC)	*Synechocystis* sp. PCC 6803	Sll0873 (*napC*)	[47]
	Anabaena sp. PCC 7120	Not detected	[47]
	Cyanothece sp. PCC 7424	Not detected	[47]
	Trichodesmium erythraeum IMS101	Not detected	[47]
Lysine decarboxylase (LDC)	*Synechocystis* sp. PCC 6803	Sll1683 (*cad*)	Cyanobase
	Anabaena sp. PCC 7120	All4887	Cyanobase
	Cyanothece sp. ATCC 51142	Cce_4417	Cyanobase
	Synechococcus sp. PCC 7002	SYNPCC7002_A1154 (putative)	Cyanobase
	Microcystis aeruginosa NIE-843	MAE14460	Cyanobase
	Arthrospira platensis NIES-39	NIES39_A04310	Cyanobase

spermidine biosynthesis is demonstrated via a transformation of both putrescine and aspartic β-semialdehyde (ASA) into carboxyspermidine by carboxyspermidine dehydrogenase (CASDH, EC 1.5.1.43) and subsequent conversion of carboxyspermidine to spermidine by carboxyspermidine decarboxylase enzyme (CASDC, EC 4.1.1.96) [47]. Nevertheless, the uncommon polyamines are also found in higher plants and cyanobacteria, such as norspermidine (norSpd or caldine), norspermine (norSpm or thermine), and cadaverine, which are mainly induced by harsh environmental stresses. Consequently, there is another pathway for norspermidine biosynthesis, accomplished from the transformation of two intermediates, 1,3-diaminopropane and ASA, catalyzed by the same two enzymes CASDH and CASDC [39,48]. For cadaverine biosynthesis, the amino acid lysine is its substrate converted by lysine decarboxylase enzyme (LDC, EC 4.1.1.18). This enzyme is present in some bacteria, cyanobacteria, and several higher plants belonging particularly to Leguminose and Solanaceae families [35]. So far, there is somehow no clear metabolic correlation found between uncommon and common polyamines.

Until now, the bioinformatics technology has potentially helped us gaining more information about large number of genes related to polyamine metabolism in cyanobacteria (Table 8.1), publically available in CyanoBase (http://genome.microbedb.jp). Previously, the dominant polyamine-biosynthetic pathway in *Synechocystis* was apparently found via arginine–agmatine conversion route [3,49]. However, the predicted genes encoding ODC related to ornithine-putrescine conversion (Fig. 8.3) is currently reported in some cyanobacterial stains, such as unicellular *Synechococcus* sp. RCC 307 and *Synechococcus* sp. WH 7803. On the other hand, the two isoforms of *adc* genes (*adc1* and *adc2*) found in *Synechocystis* were reported to be expressed in different patterns depending on the surrounding environment [49]. Under normal condition, the *adc2* gene transcript showed higher expression

than *adc1* transcript level in *Synechocystis* cells, whereas under abiotic stress conditions, such as UV irradiation and osmotic stress, *adc1* transcript levels were highly induced as compared to *adc2* transcript [49,50]. Although, the employed WU-BLAST 2.0 program from TAIR and BLASTP 2.2.19 from CyanoBase revealed the nucleotide and amino acid identities of *Synechocystis* ADC with the higher plant *Arabidopsis* ADC of about 57–59% and 38–40%, respectively, their phylogenetic tree was clustered plausibly into the near clade [50]. Moreover, previously predicted two isoforms of AGM genes, *sll1077* (*speB2*) and *sll0228* (*speB1*) [20] demonstrated a tight correlation with polyamine contents, especially *speB1* transcript (unpublished). The in vivo biosynthesized polyamines might exist in free form within the cells or conjugated to large and small molecules like proteins and phenolic acids, or mostly to hydroxycinnamic acid [51]. In cyanobacterium *Synechocystis* cells, free-formed spermidine was the dominant polyamine under both normal and stress conditions [52].

6. POLYAMINE CATABOLISM

As in the case of any plant growth regulator, the intracellular free polyamine pool does not only depend on its synthesis, but also on several other processes including polyamine degradation (oxidative deamination), polyamine conjugation, and polyamine transport [39]. Polyamines are oxidatively deaminated by the action of amine oxidases (Fig. 8.4).

These enzymes include the copper-containing diamine oxidases (DAO; EC 1.4.3.6), defined on the basis of their higher substrate specificity toward diamines, and the flavin-containing polyamine oxidases (PAO; EC 1.5.3.3), which oxidize spermidine and spermine at their secondary amino groups. DAO, firstly found in Leguminose apoplast [53] and present practically in all the monocots and dicots families tested, have a broad specificity in oxidizing putrescine and other diamines [39,54]. In plants, peroxisomes and apoplast are the sites of polyamine catabolism by either back-conversion or terminal oxidation, respectively [54]. The DAO reaction products from putrescine are pyrroline, hydrogen peroxide, and ammonia,

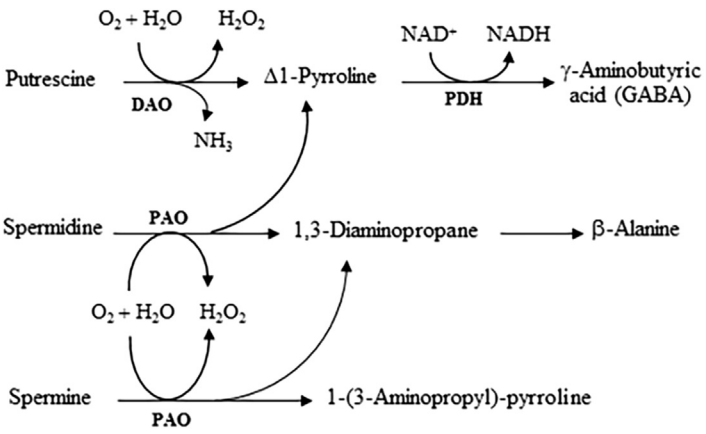


FIGURE 8.4 The pathway of polyamine catabolism in higher plants. DAO, diamine oxidase; PDH, pyrroline dehydrogenase; PAO, polyamine oxidase. *Modified from A. Bouchereau, F. Aziz, J., Larher, J. Martin-Tanguy. Polyamines and environmental challenges: recent development, Plant Sci. 140 (1999) 103–125.*

while PAO yields pyrroline and 1-(3-aminopropy)-pyrroline, respectively, from spermidine and spermine, along with diaminopropane (Dap) and hydrogen peroxide. Peroxisomal PAOs in higher plants are involved in a sequential back-conversion of diacetyl-Spm to acetyl-Spd and acetyl-Spd to acetyl-Put [55–57]. These oxidases are implicated in the production of free radicals and toxic aldehydes and in lignification processes of the cell wall [58]. Dap can be converted into β-alanine, whereas pyrroline can be further catabolized to GABA in a reaction catalyzed by pyrroline dehydrogenase as described above. Polyamines degradation enzymes vary in bacteria and fungi [35]. Far from being only a means of eliminating cellular polyamines, the enzymes involved in polyamine catabolism and the products derived from their action have been demonstrated to be involved in important physiological processes [39]. Moreover, the Spm oxidase (SMO, EC 1.5.3.3), a FAD-dependent amine oxidase recently identified in animal cells, potentially catalyzes the back-conversion of Spm to Spd, 3-aminopropanal, and H_2O_2 [59]. Later, the PAO1 and PAO3 in *Arabidopsis* were sequentially identified as the enzymes that completes the back-conversion pathway, converting Spm to Spd and Spd to Put [57,60]; however they are not yet reported in cyanobacteria. Up to date, genes related to polyamine catabolism of cyanobacteria reported in CyanoBase are shown in Table 8.2.

There are a number of putative genes encoding amine oxidase, copper- and flavin-containing amine oxidases in both freshwater and marine cyanobacterial species. It was observed that either freshwater or marine species could contain several types of amine oxidases that are not yet classified as either DAO or PAO owing to diverse polyamine precursors.

7. ROLE OF POLYAMINES AGAINST PHYSIOLOGICAL STRESSES

In nature, different polyamine's forms have influenced cellular function and living. The positively charged polyamines under physiological ionic condition not only occur as free molecular bases but they can also be conjugated or bound with negatively charged molecules inside the cell [39], such as phosphate groups of DNA in cell nucleus [61,62]. The polyamine-DNA interaction has direct influence on DNA stabilization and replication, which further regulates cell division, differentiation, and proliferation. Moreover, the polyamine biosynthesis, conjugation, transport, and catabolism are highly influenced by environmental stresses [39,40]. The intracellular polyamine homeostasis is mainly attributed to several functions during environmental changes. Endogenous polyamines frequently accumulate in living organisms in response to abiotic and biotic stresses [3,39,63], which supports a tight correlation between their titers and physiological perturbations on the protective effect of polyamines against those stresses. Recent data have shown the significant increase of polyamine contents and induction of transcript levels of genes related to polyamine biosynthetic pathway under osmotic pressure compared to those under normal condition in a model organism, cyanobacterium *Synechocystis* (typical scenario shown in Fig. 8.5). The cyanobacterial accumulation of common polyamines under normal growth condition normally directs to the dominant polyamine Spd via an intermediate Agm, whereas trace amount of only uncommon polyamine Cad is detectable. However, the significant responses to osmotic stress

TABLE 8.2 Genes Related to Polyamine Catabolism Available in CyanoBase

	CyanoBase Reported as		
Blue–Green Algae	**Amine Oxidase**	**Copper-Containing Amine Oxidase**	**Flavin-Containing Amine Oxidase**
FRESHWATER SPECIES			
Synechocystis sp. PCC 6803	—	—	Slr0782 (putative), Slr5093 (probable)
Anabaena sp. PCC 7120	—	Alr3431	—
Microcystis aeruginosa NIES-843	—	—	MAE40250 (putative)
Synechococcus elongatus PCC 6301	—	—	Syn1144_c (putative)
Synechococcus elongatus PCC 7942			Synpcc7942_0369 (putative)
Anabaena variabilis ATCC 29413	Ava_0368, Ava_0871, Ava_2342, Ava_2672		
Nostoc punctiforme ATCC 29133	Npun_R0498, Npun_R2646, Npun_R2772, Npun_R3302, Npun_R3653, Npun_F5429, Npun_F6425	Npun_R4934	—
Trichodesmium erythraeum IMS101	Tery_1366, Tery_4343	—	—
MARINE SPECIES			
Acaryochloris marina MBIC 11017	AM1_2003	—	—
Synechococcus sp. CC9605	—	—	Syncc9605_0745, Syncc9605_1906
Cyanothece sp. ATCC 51142	Cce_2233 (putative)	—	—
Cyanothece sp. PCC 7424	PCC7424_4196, PCC7424_4330, PCC7424_4868	—	—
Cyanothece sp. PCC 7425	Cyan7425_3736, Cyan7425_3896, Cyan7425_4465	Cyan7425_1871	—
Cyanothece sp. PCC 8801	PCC8801_0517, PCC8801_3473	—	PCC8801_0817

include higher accumulation of common polyamines, as well as their enhanced gene expression level [49–52].

The recent data also show the significant enhancement of uncommon polyamines including Cad and especially inducible norspermidine (norSpd), which is not detectable under normal condition (unpublished). Although norSpd as well as norSpm are present inside the cells of some eukaryotic algae, belonging to phyla Glaucophyta, Rhodophyta,

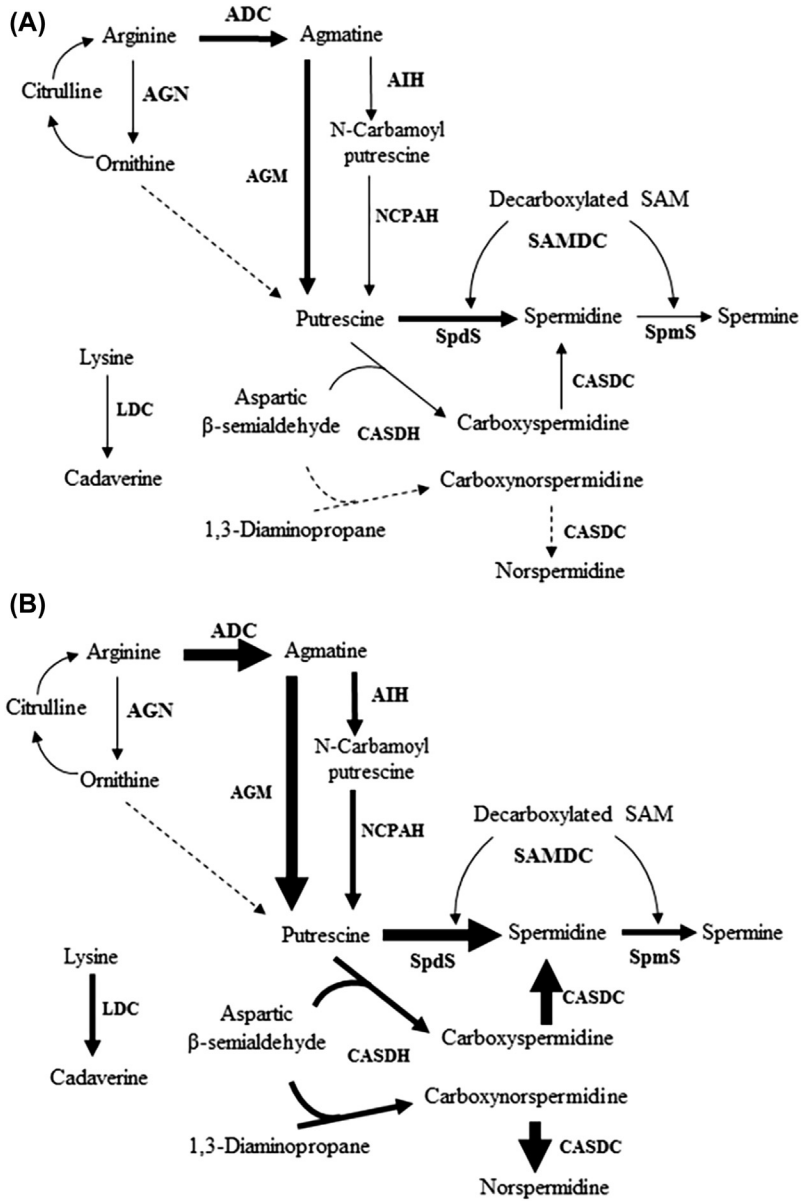


FIGURE 8.5 Changes in the pathway polyamine of synthesis in cyanobacterium *Synechocystis* sp. PCC 6803 under normal condition (A) and osmotic stress (B). The width of the arrows represents the intensity of metabolic fluxes through the pathway. *ADC*, Arginine decarboxylase; *AGM*, agmatinase; *AGN*, arginase; *AIH*, agmatineiminohydrolase; *CASDC*, carboxyspermidine decarboxylase; *CASDH*, carboxyspermidine dehydrogenase; *LDC*, lysine decarboxylase; *NCPAH*, *N*-carbamoyl-putrescine amidohydrolase; *SAMDC*, *S*-adenosyl-methionine decarboxylase; *SpdS*, spermidine synthase; *SpmS*, spermine synthase.

Cryptophyta, and Haptophyta, their possible role is still unclear [64,65]. The rationale design of preinduction of polyamine by osmotic stress has also proved that it can protect *Synechocystis* cells and *adc* transcripts from UV-B damage [50]. There is extensive literature describing the correlation between the content ratios of either endogenous Spd/Put or Spm/Put relatively implicated to the stress tolerance capability of organism [39,66–69]. The transgenic plants with overexpressing polyamine biosynthetic genes, such as SpdS and ADC, apparently increased their stress tolerance [70,71]. Moreover, it is hypothesized that the polycationic nature of polyamines could affect physiological systems by binding to anionic sites and play important cellular functions, such as free radical scavenging and antioxidant activity [40]. The in vitro enzyme systems demonstrated that severe hydroxyl radical generated by the Fenton reaction was scavenged by diamines and polyamines, as well as with superoxide radical by senescing microsomal membranes from pea epicotyls [72]. This propensity of polyamine-scavenging ability subjected to inhibit lipid peroxidation and retard senescence by decreasing RNase and protease activities that normally associate with senescence of leaf discs [73]. Localization of polyamines is also found on different organelles, especially thylakoid membranes in photosynthetic organisms including plants, algae, and blue–green algae, which are sensitively affected by light or UV stress [74]. An evidence of Put role is its involvement in modulation of higher plant photosynthetic proton circuit by partitioning of proton motive force [75]. The supplementation of exogenous polyamines strikingly protected organisms against abiotic stresses [58,69,76]. Recent data indicate that the external Spd supplementation into BG_{11} medium for *Synechocystis* cells significantly alleviates UV-induced growth inhibition via the decrease of H_2O_2 and malonaldehyde, a product from lipid peroxidation process [69]. Similarly, the additional Cad supplementation allows *E. coli* cells to survive in 30 min exposure to acidic pH at 3.6 via its inhibition mechanism upon porin-mediated outer membrane permeability [77]. On the other hand, the activation of polyamine catabolism attributively responded to environmental stress via an increase of cellular oxidative stress through the generation of H_2O_2 [54]. The final product of polyamines, especially Dap, is involved in stress tolerance since it is precursor of β-alanine and uncommon norSpd production. The recent data demonstrate that high levels of PAO and H_2O_2 accumulation relatively facilitate abiotic stress responses by resulting in stress signaling and expression of defense genes [40].

8. FUTURE PERSPECTIVES

Latest studies regarding GABA and polyamines metabolism with their roles in cyanobacteria form basis to reveal the possible novel mechanisms of low-molecular-weight nitrogenous compounds to counteract the physiological stresses in these photosynthetic organisms. However, more work needs to be done to fully understand the contribution of GABA and polyamines in stress resistance. This can be done in future by elucidating the following points: (1) study of GABA metabolism and regulation in different cyanobacterial species, (2) metabolic engineering of GABA shunt pathway in cyanobacterial species with available genomes, (3) molecular and bioinformatics study of the mechanisms controlling polyamine metabolism and conjugation as well as investigation of molecular mechanisms involved in functioning of polyamines in cyanobacteria, and (4) study and gene manipulation

of related enzyme activities to elucidate the involvement of polyamine catabolism in GABA formation. Investigation of GABA overproducing strain of *Synechocystis* could form the basis for further development of biological synthesis of GABA by employing cyanobacteria that are safe and eco-friendly microorganisms. Up to our knowledge there is unavailability of information regarding GABA metabolism and function in algae. This missing gap of novel biochemical information in phycology needs to be full filled in future.

References

[1] S. Kanwal, R.P. Rastogi, A. Incharoensakdi, Glutamate decarboxylase activity and gamma-aminobutyric acid content in *Synechocystis* sp. PCC6803 under osmotic stress and different carbon sources, J. Appl. Phycol. 26 (2014) 2327–2333.
[2] B. Boonburapong, S. Laloknam, A. Incharoensakdi, Accumulation of gammaaminobutyric acid in the halotolerant cyanobacterium *Aphanothece halophytica* under salt and acid stress, J. Appl. Phycol. (2015), http://dx.doi.org/10.1007/s10811-015-0523-7.
[3] A. Incharoensakdi, S. Jantaro, W. Raksajit, P. Mäenpää, Polyamines in cyanobacteria: biosynthesis, transport and abiotic stress response, in: A. Méndez-Vilas (Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Formatex, Spain, 2010.
[4] S. Zhang, D.A. Bryant, The tricarboxylic acid cycle in cyanobacteria, Science 334 (2011) 155–552.
[5] N. Bouché, B. Lacomb, H. Fromm, GABA signaling: a conserved and ubiquitous mechanism, Trends Cell Biol. 13 (2003) 607–610.
[6] N. Bouché, H. Fromm, GABA in plants: just a metabolite? Trends Plant Sci. 9 (2004) 110–115.
[7] D. Steinhauser, A.R. Fernie, W.L. Araújo, Unusual cyanobacterial TCA cycles: not broken just different, Trends Plant Sci. 17 (2012) 503–509.
[8] W. Xiong, D. Brune, F.J. Vermaas, The γ-aminobutyric acid shunt contributes to closing the tricarboxylic acid cycle in *Synechocystis* sp. PCC 6803, Mol. Microbiol. 93 (2014) 786–796.
[9] S. Kanwal, W. Khetkorn, A. Incharoensakdi, GABA accumulation in response to different nitrogenous compounds in unicellular cyanobacterium *Synechocystis* sp. PCC6803, Curr. Microbiol. 70 (2015) 96–102.
[10] S. Kanwal, A. Incharoensakdi, Characterization of glutamate decarboxylase from *Synechocystis* sp. PCC6803 and its role in nitrogen metabolism, Plant Physiol. Biochem. 99 (2016) 59–65.
[11] M.L. Fonda, L-Glutamate decarboxylase from bacteria, Methods Enzymol. 113 (1985) 11–16.
[12] H. Zhang, H. Yao, F. Chen, X. Wang, Purification and characterization of glutamate decarboxylase from rice germ, Food Chem. 101 (2007) 1670–1676.
[13] D.C. Spink, T.G. Porter, S.J. Wu, D.L. Martin, Characterization of three kinetically distinct forms of glutamate decarboxylase from pig brain, Biochem. J. 231 (1985) 695–703.
[14] G. Capitani, D.D. Biase, C. Aurizi, H. Gut, F. Bossa, M.G. Grütter, Crystal structure and functional analysis of *Escherichia coli* glutamate decarboxylase, EMBO J. 22 (2003) 4027–4037.
[15] J. Labarre, P. Thuriaux, F. Chauvat, Genetic analysis of amino acid transport in the facultatively heterotrophic cyanobacterium *Synechocystis* sp. Strain 6803, J. Bacteriol. 169 (1987) 4668–4673.
[16] H. Li, Y. Cao, Lactic acid bacterial cell factories for gamma aminobutyric acid, Amino Acids 39 (2010) 1107–1116.
[17] R. Yang, y. Yin, Z. Gu, Polyamine degradation pathway regulating growth and GABA accumulation in germinating Fava Bean under hypoxia-NaCl stress, J. Agric. Sci. Technol. 17 (2015) 311–320.
[18] N. Seiler, G. Bink, J. Grove, Regulatory interrelations between GABA and polyamines. I. brain GABA levels and polyamine metabolism, Neurochem. Res. 4 (1979) 425–435.
[19] E. Shaibe, E. Metzer, Y.S. Halpern, Metabolic pathway for the utilization of L -arginine, L -ornithine, agmatine, and putrescine as nitrogen sources in *Escherichia coli* K-12, J. Bacteriol. 163 (1985) 933–937.
[20] S. Schriek, C. Rückert, D. Staiger, E.K. Pistorius, K.P. Michel, Bioinformatic evaluation of L-arginine catabolic pathways in 24 cyanobacteria and transcriptional analysis of genes encoding enzymes of L-arginine catabolism in the cyanobacterium *Synechocystis* sp. PCC 6803, BMC Genomics 8 (2007) 437.
[21] H. Knoop, M. Gründel, Y. Zilliges, R. Lehmann, S. Hoffmann, W. Lockau, et al., Flux balance analysis of cyanobacterial metabolism: the metabolic network of *Synechocystis* sp. PCC 6803, PLoS Comput. Biol. 9 (2013) e1003081.

[22] B.J. Shelp, A.W. Bown, M.D. McLean, Metabolism and functions of gamma-aminobutyric acid, Trends Plant Sci. 4 (1999) 446–452.
[23] R. Palanivelu, L. Brass, A.F. Edlund, D. Preuss, Pollen tube growth and guidance is regulated by POP2, an *Arabidopsis* gene that controls GABA levels, Cell 114 (2003) 47–59.
[24] M. Gupta, B.M. Hogema, M. Grompe, T.G. Bottiglieri, A. Concas, G. Biggio, et al., Murine succinate semialdehyde dehydrogenase deficiency, Ann. Neurol. 54 (2003) S81–S90.
[25] C. Wang, H.B. Zhang, L.H. Wang, L.H. Zhang, Succinic semialdehyde couples stress response to quorum-sensing signal decay in *Agrobacterium tumefaciens*, Mol. Microbiol. 62 (2006) 45–56.
[26] A. Fait, A. Yellin, H. Fromm, GABA shunt deficiencies and accumulation of reactive oxygen intermediates: insight from *Arabidopsis* mutants, FEBS Lett. 579 (2005) 415–420.
[27] B.J. Shelp, A.W. Bown, D. Faure, Extracellular γ-aminobutyrate mediates communication between plants and other organisms, Plant Physiol. 142 (2006) 1350–1352.
[28] A.N.C. Morse, D.E. Morse, Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surface of crustose red algae, J. Exp. Mar. Biol. Ecol. 75 (1984) 191–215.
[29] M.K. Alan, J.T. Frank, Gamma aminobutyric acid (GABA) and plant responses to stress, Crit. Rev. Plant Sci. 19 (2000) 479–509.
[30] C. Feehily, K.A. Karatzas, Role of glutamate metabolism in bacterial responses towards acid and other stresses, J. Appl. Microbiol. 114 (2013) 11–24.
[31] P.D. Cotter, C.G.M. Gahan, C. Hill, A glutamate decarboxylase system protects *L. monocytogenes* in gastric fluid, Mol. Microbiol. 40 (2001) 465–475.
[32] M.P. Castanie-Cornet, T.A. Penfound, D. Smith, J.F. Elliott, J.W. Foster, Control of acid resistance in *E. coli*, J. Bacteriol. 181 (1999) 3525–3535.
[33] S.Q. Shi, Z. Shi, Z.P. Jiang, L.W. Qi, X.M. Sun, C.X. Li, et al., Effects of exogenous GABA on gene expression of *Caragana intermedia* roots under NaCl stress: regulatory roles for H_2O_2 and ethylene production, Plant Cell Environ. 33 (2010) 149–162.
[34] M. Nagao, K. Matsui, M. Uemura, *Klebsormidium flaccidum*, a charophycean green alga, exhibits cold acclimation that is closely associated with compatible solute accumulation and ultrastructural changes, Plant Cell Environ. 31 (2008) 872–885.
[35] N. Bagni, A. Tassoni, Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants, Amino Acids 20 (2001) 301–317.
[36] Y. Ito, A. Butler, Structure of synechobactins, new siderophores of the marine cyanobacterium *Synechococcus* sp. PCC 7002, Limnol. Oceanogr. 50 (2005) 1918–1923.
[37] I. Flink, D.E. Pettijohn, Polyamines stabilise DNA folds, Nature 253 (1975) 62–63.
[38] R.A. Casero Jr., L.J. Marton, Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases, Nat. Rev. 6 (2007) 373–390.
[39] A. Bouchereau, F. Aziz, J. Larher, J. Martin-Tanguy, Polyamines and environmental challenges: recent development, Plant Sci. 140 (1999) 103–125.
[40] M.D. Groppa, M.P. Benavides, Polyamines and abiotic stress: recent advances, Amino Acids 34 (2008) 35–45.
[41] D.H. Russell, S.H. Snyder, Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase, Mol. Pharmacol. 5 (1969) 253–262.
[42] S. Hayashi, Y. Murakami, Rapid and regulated degradation of ornithine decarboxylase, Biochem. J. 306 (1995) 1–10.
[43] K. Otake, D.A. Ruggiero, S. Regunathan, H. Wang, T.A. Milner, D.J. Reis, Regional localization of agmatine in the rat brain: an immunocytochemical study, Brain Res. 787 (1998) 1–14.
[44] W.-P. Wang, A.H. Iyo, J. Miguel-Hidalgo, S. Regunathan, M.-Y. Zhu, Agmatine protects against cell damage induced by NMDA and glutamate in cultured hippocampal neurons, Brain Res. 1084 (2006) 210–216.
[45] X. Wang, W. Ying, K.A. Dunlap, G. Lin, M.C. Satterfield, R.C. Burghardt, et al., Arginine decarboxylase and agmatinase: an alternative pathway for de novo biosynthesis of polyamines for development of mammalian concept uses, Biol. Reprod. 90 (2014) 1–15.
[46] O.J. Crocomo, L.C. Basso, Accumulation of putrescine and related amino acids in potassium deficient *Sesamum*, Phytochemistry 13 (1974) 2659–2665.
[47] X. Zhu, Q. Li, C. Yin, X. Fang, X. Xu, Role of Spermidine in overwintering of cyanobacteria, J. Bacteriol. 197 (2015) 2325–2334.

[48] S. Yamamoto, S. Shinoda, M. Makita, Occurrence of norspermidine in some species of genera Vibrio and Beneckea, Biochem. Biophys. Res. Commun. 87 (1979) 1102–1108.
[49] S. Jantaro, A. Pothipongsa, S. Khanthasuwan, A. Incharoensakdi, Short-term UV-B and UV-C radiations preferentially decrease spermidine contents and arginine decarboxylase transcript levels of *Synechocystis* sp. PCC 6803, Curr. Microbiol. 62 (2011) 420–426.
[50] A. Pothipongsa, S. Jantaro, A. Incharoensakdi, Polyamines induced by osmotic stress protect *Synechocystis* sp. PCC 6803 cells and arginine decarboxylase transcripts against UV-B radiation, Appl. Biochem. Biotechnol. 168 (2012) 1476–1488.
[51] J. Martin-Tanguy, Metabolism and function of polyamines in plants: recent development (new approaches), Plant Growth Regul. 34 (2001) 135–148.
[52] S. Jantaro, P. Mäenpää, P. Mulo, A. Incharoensakdi, Content and biosynthesis of polyamines in salt and osmotically stressed cells of *Synechocystis* sp. PCC 6803, FEMS Microbiol. Lett. 228 (2003) 129–135.
[53] T.A. Smith, J.H.A. Barker, The di- and polyamine oxidases of plants, in: V. Zappia, A.E. Pegg (Eds.), Progress in Polyamine Research, Plenum Press, New York, 1988, pp. 573–587.
[54] P.N. Moschou, K.A. Paschalidis, K.A. Roubelakis-Angelakis, Plant polyamine catabolism: the state of the art, Plant Signal. Behav. 3 (2008) 1061–1066.
[55] M.E. Beard, R. Baker, P. Conomos, D. Pugatch, E. Holtzman, Oxidation of oxalate and polyamines by rat peroxisomes, J. Histochem. Cytochem. 33 (1985) 460–464.
[56] T. Wu, V. Yankovskaya, W.S. McIntire, Cloning, sequencing and heterologous expression of the murine peroxisomal flavoprotein N^1-acetylated polyamine oxidase, J. Biol. Chem. 278 (2003) 20514–20525.
[57] P. Tavladoraki, M.N. Rossi, G. Saccuti, M.A. Perez-Amador, F. Polticelli, R. Angelini, et al., Heterologous expression and biochemical characterization of a polyamine oxidase from *Arabidopsis* involved in polyamine back conversion, Plant Physiol. 141 (2006) 1519–1532.
[58] R. Angelini, R. Federico, P. Bonfante, Maize polyamine oxidase: antibody production and ultrastructural localization, J. Plant Physiol. 145 (1995) 686–692.
[59] T. Murray-Stewart, Y. Wang, W. Devereux, R.A. Casero, Cloning and characterization of multiple human polyamine oxidase splice variants that code for isoenzymes with different biochemical characteristics, Biochem. J. 368 (2003) 673–677.
[60] P.N. Moschou, M. Sanmartin, A.H. Andriopoulou, E. Rojo, J.J. Sanchez-Serrano, K.A. Roubelakis-Angelakis, Bridging the gap between plant and mammalian polyamine catabolism: a novel peroxisomal polyamine oxidase responsible for a full back-conversion pathway in *Arabidopsis*, Plant Physiol. 147 (2008) 1845–1857.
[61] J.E. Morgan, J.W. Blankenship, H.R. Matthews, Polyamines and acetylpolyamines increase the stability and alter the conformation of nucleosome core particles, Biochemistry 26 (1987) 3643–3649.
[62] T.J. Thomas, U.B. Gunnia, T. Thomas, Polyamine-induced B-DNA to Z-DNA conformational transition of a plasmid DNA with $(dG\text{-}dC)_n$ insert, J. Biol. Chem. 266 (1991) 6137–6141.
[63] D.R. Walters, Resistance to plant pathogens: possible roles for free polyamines and polyamine catabolism, New Phytol. 159 (2003) 109–115.
[64] K. Hamana, S. Matsuzaki, Widespread occurrence of norspermidine and norspermine in eukaryotic algae, J. Biol. Chem. 91 (1982) 1321–1328.
[65] K. Hamana, M. Niitsu, Cellular polyamines of lower eukaryotes belonging to the phyla Glaucophyta, Rhodophyta, Cryptophyta, Haptophyta and Percolozoa, J. Gen. Appl. Microbiol. 52 (2006) 235–240.
[66] A. Santa-Cruz, M. Acosta Pérez-Alfocea, C. Bolarin, Changes in free polyamine levels induced by salt stress in leaves of cultivated and wild tomato species, Physiol. Plant 101 (1997) 341–346.
[67] C. Lütz, E. Navakoudis, H.K. Seidlitz, K. Kotzabasis, Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UVB protection, Biochim. Biophys. Acta 1710 (2005) 24–35.
[68] S. Hamdani, H. Yaakkoubi, R. Carpentier, Polyamines interaction with thylakoid proteins during stress, J. Photochem. Photobiol. B: Biol. 104 (2011) 314–319.
[69] S. Jantaro, W. Baebprasert, C. Piyamawadee, O. Sodsuay, A. Incharoensakedi, Exogenous spermidine alleviated UV-induced growth inhibition of *Synechocystis* sp. PCC 6803 via reduction of hydrogen peroxide and malonaldehyde levels, Appl. Biochem. Biotechnol. 173 (2014) 1145–1156.
[70] Y. Kasukabe, L. He, K. Nada, S. Misawa, I. Ihara, S. Tachibana, Overexpression of spermidine synthase enhances tolerance to multiple environmental stress and upregulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*, Plant Cell Physiol. 45 (2004) 712–722.

[71] T. Capell, L. Bassie, P. Christou, Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress, Proc. Natl. Acad. Sci. USA 101 (2004) 9909–9914.
[72] G. Drolet, E.B. Dumbroff, R. Legg, J.E. Thompson, Radical scavenging properties of polyamines, Phytochemistry 25 (1986) 367–371.
[73] R.B. Popovic, D.J. Kyle, A.S. Cohen, S. Zalik, Stabilization of thylakoid membranes by spermine during stress-induced senescence of barley leaf discs, Plant Physiol. 64 (1979) 721–726.
[74] L. Sfichi-Duke, N.E. Ioannidis, K. Kotzabasis, Fast and reversible response of thylakoid-associated polyamines during and after UV-B stress: a comparative study of the wild type and a mutant lacking chlorophyll b of unicellular green alga Scenedesmus obliquus, Planta 228 (2008) 341–353.
[75] N.E. Ioannidis, J.A. Cruz, K. Kotzabasis, D.M. Kramer, Evidence that putrescine modulates the higher plant photosynthetic proton circuit, Plos ONE 7 (e29864) (2012) 1–6.
[76] D. Unal, I. Tuney, A. Sukatar, The role of external polyamines on photosynthetic responses, lipid peroxidation, protein and chlorophyll a content under the UV-A (352 nm) stress in *Physcia semipinnata*, J. Photochem. Photobiol. B: Biol. 90 (2008) 64–68.
[77] H. Samartzidou, M. Mehrazin, Z. Xu, M.J. Benedik, A.H. Delcour, Cadaverine inhibition of porin plays a role in cell survival at acidic pH, J. Bacteriol. 185 (2003) 13–19.

CHAPTER

9

Algal Pigments for Human Health and Cosmeceuticals

H. Chakdar[1], *S. Pabbi*[2]

[1]ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, India;
[2]ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

OUTLINE

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00009-6

1. INTRODUCTION

Algae are an extremely diverse group of organisms accounting for approximately 50% of the photosynthesis on this planet [1]. Due to their metabolic diversity they are distributed over a diverse habitat. Algae are considered as close relative of higher plants due to similarity in their photosynthesis. Like higher plants, algae harbor pigments like chlorophylls and carotenoids for absorption of light energy and protection from high irradiance. Apart from these pigments, algae also harbor xanthophylls and phycobiliproteins (PBPs) as major accessory photosynthetic pigments. Distribution of the pigments within the algal division is different and classical botanists classify the algae based on their color, more specifically based on the distribution of the pigments. Broadly, the algae can be divided into following classes: (1) green algae (contains chlorophyll *a* and *b*), (2) red algae (contains chlorophyll *a*, *d*, and PBP), (3) brown algae (contains chlorophyll *a*, *c1*, and *c2*), (4) yellow-green algae (contains chlorophyll *a*, *c*, and xanthophylls), (5) euglenoids (contains chlorophyll *a* and *b*), (6) dinoflagellates (contains chlorophyll *a* and *c2*), (7) cryptophytes (chlorophyll *a*, *c2*, and PBPs), (8) golden algae (contains chlorophyll *a*, *c1*, and *c2*), (9) haptophytes (contains chlorophyll *a*, *c1*, and *c2*), (10) diatoms (contains chlorophyll *a*, *c1*, and *c2*), (11) chloromonads (contains chlorophyll *a* and *c*), and (12) blue-green algae (chlorophyll *a* and PBPs). However, blue-green algae, despite its many similarities to eukaryotic algae, are classified in the bacterial domain due to its prokaryotic cellular organization. These algae due to their long evolutionary history are endowed with many other novel metabolites with distinct physiological functions and a number of beneficial properties.

Due to their brilliant colors, these pigments are considered as an eco-friendly alternative to the synthetic food colorants. Besides their distinct physiological roles, they can also impart several health-promoting properties upon crucial application to human and other animals. Many algal metabolites and pigments have antiaging, antioxidant, and neuroprotective properties making them suitable for use in cosmetics. Such compounds with pharmaceutical properties and application in cosmetic products are now being termed as cosmeceuticals, which more precisely can be defined as "cosmetic products having drug-like benefits that enhance or protect the appearance or order of the human body." Since the last decade, the cosmeceutical industry has grown significantly with a compound annual growth rate of 7.7% from 2012 to 2016. This is faster than the already strong growth witnessed in the pharmaceuticals and medical technology sector, which grew at a rate of 4.7% in the last growth period (1998–2008). The top market for cosmeceuticals is the USA. UK, Germany, France, Italy, and Spain are the leading European markets followed by Japan. The combined global market share of the five European countries is 65% ($20 bn). With the growing focus in algal research, the algal compounds including the pigments will be a major contender in the cosmeceutical industry.

In this chapter we will discuss the algal pigments and other metabolites, their properties related to human health, and prospects as cosmeceuticals.

2. ALGAL PIGMENTS

Algae harbor a number of strongly visible light-absorbing compounds known as pigments. They share a number of fundamental similarities in their chemical structure where molecular

backbones of such pigments are built with long-chain or closed rings of conjugated double bonds. Complex conjugated ring systems enable the pigments to strongly absorb in the visible range of light. Moreover, the conjugated double bonds make the structure amenable to resonance resulting in shifts of energy levels, which is key to the energy transduction during photosynthesis. Algal pigments can broadly be grouped into two categories *viz*. tetrapyrrole pigments and isoprene based on their chemical backbones. Chlorophyll and PBPs are made up of tetrapyrrole rings while carotenoids consist of isoprene units. These pigments are elaborately discussed in this section.

2.1 Chlorophylls

Chlorophyll is the principal photoreceptor in algal photosynthesis. The basic structure of chlorophyll is a ring made up of four pyrroles, a tetrapyrrole, which is also known as porphyrin. This cyclic tetrapyrrole, like the heme group of globins and cytochromes is biosynthetically derived from protoporphyrin IX. The Mg^{++} present in the center of the porphyrin is bound with N atoms by covalent and coordinate bondings. The pyrrole rings are designated as "A," "B," "C," and "D." To the ring "C," a cyclopentanone is attached while to the ring "D," a phytol side chain is attached through esterification with a propionic acid. Phytol is a long-branched hydrocarbon with a C═C. It is an isoprenoid derivative and formed from four isoprene units.

Two major types of chlorophylls viz. chlorophyll a (*Chl a*) and b (*Chl b*) are predominant in algae. The differentiation of *Chl a* and *Chl b* occurs at ring "B" with the presence of 7-methyl group for *Chl a* and 7-formyl group for *Chl b*. Intense absorption in the visible region is an important factor in light harvesting by *Chl a*, and it is the major pigment in all chlorophyllous antenna complexes of oxygenic organisms. Apart from these two major types, chlorophyll c (*Chl c*) and chlorophyll d (*Chl d*) have also been reported from many rhodophytes and chromophytes. *Chl d* differs from *Chl a* by the presence of a 3-formyl group. It has been found together with isochlorophyll *d* (of unknown structure) in extracts from rhodophytes [2,3]. *Chl c* has fully unsaturated porphyrin macrocycle. Three subtypes of *Chl c* viz. *Chl c*1, *Chl c*2, and *Chl c*3 have been reported. All of them has an acrylic side chain at C-17. *Chl c*1 and *Chl c*2 differ by the presence of an 8-ethyl- and 8-vinyl-substituent, respectively, while *Chl c*3 carry a $COOCH_3-$ substituent at ring "B." It has been observed that majority of the algae contain either of the *Chl b* or *Chl c*. Variations in the substituent of chlorophyll molecule results in absorption of light energy at different wavelength, i.e., the side chain substituents fine-tune the absorption of light. Table 9.1 presents a brief comparison of the different types of the chlorophylls.

Due to presence of alternate single and double bonds, chlorophyll molecules efficiently absorb light energy that excites electron from ground energy level to an excited state. In the presence of suitable electron acceptor, the electron moves further and creates a suitable condition for energy transduction.

2.2 Carotenoids

Carotenoids are orange or yellow colored lipophilic polyene compounds ending with "ionone" rings. Chemically they may be hydrocarbons lacking oxygen called carotenes like α-carotene, β-carotene, and lycopene, or oxygen-containing compounds called

TABLE 9.1 Types of Algal Chlorophylls and Their Molecular Structure

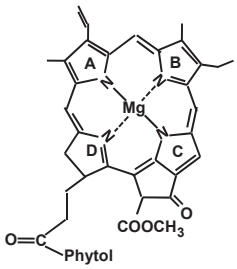
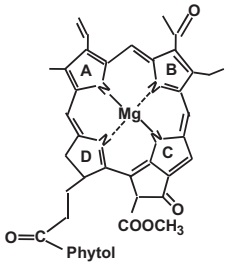
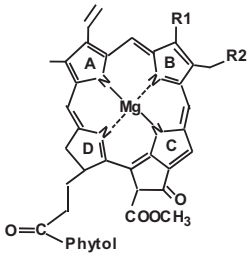
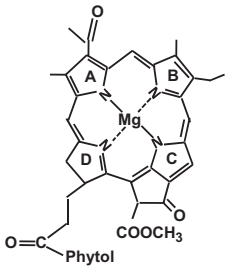
Chlorophyll	Formula	Molecular Structure	Molecular Weight (g/mol)	Esterifying Alcohol	Absorption Maxima (nm)
Chl a	$C_{55}H_{72}N_4O_5Mg$		892	Δ2	662, 430
Chl b	$C_{55}H_{70}N_4O_6Mg$		906	Δ2	644, 430
Chl c	$C_{35}H_{30}N_4O_5Mg$		610	H	626, 576, 444
Chl d	$C_{54}H_{70}N_4O_6Mg$		894	Δ2	688, 447

TABLE 9.2 Major carotenoids of algae

Carotenoid Type		Molecular Weight (g/mol)	Absorption Maxima (nm)	Distribution
Carotenes	α-Carotene	536.87	420, 440, 470	Cryptophyta and some Chlorophycean members
	β-Carotene	536.87	425, 450, 480	Cyanophyta, Rhodophyta, Chlorophyta, Glaucophyta, Haptophyta, Chlorarachinophyta, Euglenophyta,
Xanthophylls	Astaxanthin ($C_{40}H_{52}O_4$)	596.84	477	Some chlorophytes
	Diatoxanthin ($C_{40}H_{54}O_2$)	566.86	430, 453, 480	Xanthophycean members
	Diadinoxanthin ($C_{40}H_{54}O_3$)	582.85	428, 449, 479	Haptophytes, Euglenophytes, Dinophytes and Xanthophycean members
	Fucoxanthin ($C_{42}H_{58}O_6$)	658.91	420, 444, 467	Chrysophycean, Bacillarophycean and Phaeophycean members and Haptophytes
	Lutein ($C_{40}H_{56}O_2$)	568.87	425, 448, 476	Macrophytic rhopdophytes and some chlorophytes
	Neoxanthin ($C_{40}H_{56}O_4$)	600.87	416, 440, 469	Chlorophytes
	Siphonaxanthin ($C_{40}H_{56}O_4$)	600.87	441, 461	Some chlorophytes
	Violaxanthin ($C_{40}H_{56}O_4$)	600.87	415, 438, 467	Chlorophyta and some phaeophycean members
	Zeaxanthin ($C_{40}H_{56}O_2$)	568.87	428, 454, 481	Cyanophyta, Rhodophyta, Glaucophyta and some Phaeophycean members

xanthophylls with oxygen being present as hydroxyl groups (e.g., lutein), as oxy-groups (e.g., canthaxanthin), or in combination of both of them (e.g., astaxanthin). Many different kinds of carotenoids are found in algae, and Table 9.2 presents a brief account of major carotenoids present in algae. In algae, the carotenoids primarily function as accessory light-harvesting pigments and photoprotect the photosynthetic machinery from photobleaching by scavenging reactive oxygen species. β-Carotene performs the protective functions while other carotenoids are involved mainly in light harvesting. Carotenoids are also reported to play roles in phototropism and phototaxis [4]. Presence of unique bonding patterns (e.g., allenic bond C═C═C, acetylene C≡C), hydroxyl, or keto endings at ionone ring endow carotenoids with unusual antioxidant activities. Due to brilliant colors and health-promoting properties, carotenoids have found many commercial applications in food, feed, and pharmaceutical industries. β-carotene, fucoxanthin, astaxanthin, lutein, and zeaxanthin are some of the remarkable algal carotenoids with versatile commercial application.

2.3 Phycobiliproteins

Tetrapyrrole biliproteins known as PBPs absorb radiations in regions of the visible spectrum where *Chl a* has low absorptivity. PBPs are categorized into three types: phycoerythrins ($\lambda_{max}\sim565$ nm), phycocyanins ($\lambda_{max}\sim620$ nm), and allophycocyanins (APCs) ($\lambda_{max}\sim650$ nm). PBPs are commonly found to consist of heteromonomers composed of two different subunits, α and β [5,6]. These subunits are present in equimolar stoichiometry (αβ) and differ in molecular mass, amino acid sequence, and chromophore content. The brilliant colors of PBPs are mainly due to covalently bound prosthetic groups that are open-chain tetrapyrrole chromophores bearing A, B, C, and D rings named phycobilins. They are either blue-colored phycocyanobilin (PCB), red-colored phycoerythrobilin (PEB), the yellow-colored phycourobilin (PUB), or the purple-colored phycoviolobilin (PVB), also named cryptoviolin. These chromophores are generally bound to the polypeptide chain at conserved positions either by one cysteinylthioester linkage through the vinyl substituent on the pyrrole ring A of the tetrapyrrole or occasionally by two cysteinylthioester linkages through the vinyl substituent on both A and D pyrrole rings [7]. Their unique structure, brilliant color, high photostability, thermal and pH stability, and numerous health benefits have made PBPs suitable for many industrial applications.

2.3.1 *Phycoerythrin*

Phycoerythrin (PE) is the most abundant PBP in many red algae and in some cyanobacteria. These are characterized by strong absorption bands in the green region of the visible spectrum from 480 nm to 570 nm and by intensive fluorescence emissions at about 575–580 nm. Based on their absorption spectra, these red-colored proteins fall into three distinct species: (1) B-phycoerythrin (B-PE) ($\lambda_{max}\sim540$–560 nm, shoulder ~495 nm); (2) R-phycoerythrin (R-PE) ($\lambda_{max}\sim565$, 545 and 495 nm), and (3) C-phycoerythrin (C-PE) ($\lambda_{max}\sim563$, 543 and ~492 nm). The prefixes B-, R-, and C- were used historically for indicating the type of organisms from which (for example **B**angiales, Floridian **R**hodophyceae, and **C**yanophyceae) the pigment proteins were originally extracted.

2.3.2 *Phycocyanin*

Phycocyanins are one of the most widespread PBPs observed in almost all PBP-containing organisms, including cyanobacteria, red algae, glaucophytes, and some cryptophytes. They are most abundantly found in most cyanobacterial species that grow in natural environment. The phycocyanin (PC) from the PBsome-containing algae is subdivided into three types: (1) C-phycocyanin (C-PC, $\lambda_{max}\sim615$–620 nm) exclusively existing in cyanobacteria, (2) phycoerythrocyanin (PEC, $\lambda_{max}\sim575$ nm) inducible only in some cyanobacteria, and (3) R-phycocyanin (R-PC, $\lambda_{max}\sim615$ nm) mainly in red algae [8–14]. These blue- or blue-purple–colored PBPs have strong light absorption ability mainly in range from 580 to 630 nm and emit intensive red fluorescence at 635–645 nm.

2.3.3 *Allophycocyanin*

APCs are a type of core-constructing PBPs. These are a less-contained PBP species with respect to PCs and PEs, but they exist in all PBsome-containing organisms, cyanobacteria, glaucophytes, and red algae, which grow in natural environment. Compared with red algae, cyanobacteria may generally contain higher amounts of APCs with respect to total PBP content of the organisms.

3. HEALTH BENEFITS OF ALGAL PIGMENTS

Use of algae including cyanobacteria as a nutritious food is an age-old practice in many of the human communities. Due to the huge diversity of bioactive compounds present in algae, they have numerous health benefits apart from its rich nutritional factors. Algal pigments, due to their chemical structure, can be very useful to improve health because of a number of properties like antioxidant, anticancer, antiobesity, neuroprotective, antiaging, and so on. Table 9.3 lists various health-promoting properties of different algal pigments and detail about these properties is deliberated in the following sections.

TABLE 9.3 An Overview of Health-Promoting Properties of Algal Pigments

Pigment	Health-Promoting Property	References
Chlorophyll	Antioxidant activity	[17–19]
	Anticancer activity	[35]
β Carotene	Antioxidant activity	[20]
	Anticancer activity	[36]
Astaxanthin	Antioxidant activity	[20]
	Immune boosting activity	[31–34]
	Antiinflammatory activity	[50, 51]
	Neuroprotective activity	[62, 63]
	Dermatoprotective activity	[75]
	Photoprotective activity	[62, 76, 77]
Fucoxanthin	Antioxidant activity	[21, 22]
	Anticancer activity	[37–41]
	Antiinflammatory activity	[53, 54]
	Neuroprotective activity	[61]
	Antiobesity activity	[67–71]
	Dermatoprotective activity	[74]
Phycocyanin	Antioxidant activity	[23, 26–30]
	Anticancer activity	[45–49]
	Antiinflammatory activity	[56–59]
	Neuroprotective activity	[65, 66]
Phycoerythrin	Antioxidant activity	[25]
	Dermatoprotective activity	[25]

3.1 Algal Pigments as Antioxidants and Immune Boosters

Algal pigments are reported to be potent antioxidants. Antioxidant activity of these pigments mainly depends on their structural features like porphyrin ring, phytol chain, and extended system of conjugated double bonds. For example, chlorophyll, apart from its role as major light-absorbing molecule, can also show antioxidant activities, and the porphyrin ring is an essential structure for the antioxidant activity [15,16]. *Chl a* and some related compounds from brown algae have antioxidant activities in methyl linolenate systems [17]. Chlorophyll derivatives lacking the central $Mg^{2+}$and phytol chain are reported to be more potent antioxidant than chlorophyll [18]. *Chl b* derivatives have been reported to show stronger antioxidant activity than *Chl a* derivatives, suggesting that the presence of formyl group in place of methyl group provides a better antioxidant activity [19].

Carotenoids are also potent biological antioxidants that can absorb the excited energy of singlet oxygen onto the carotenoid chain and preventing other molecules or tissues from damage. β-carotene from *Dunaliella* have been reported to significantly elicit antioxidant enzymes like catalase, superoxide dismutase (SOD), peroxidise, and antilipid peroxidise. Rao et al. (2010) reported significant antioxidant activities of algal carotenoids (β-carotene from *Spirulina platensis*, Astaxanthin from *Haematococcus pluvialis*, and Lutein from *Botryococcus braunii*), which substantially increased the activities of SOD, peroxidise, and catalase [20]. Astaxanthin, which is a major carotenoid of its commercial algal source *H. pluvialis*, has been reported to have many folds stronger antioxidant activity than vitamin E and β-carotene. Another carotenoid, fucoxanthin has a strong radical scavenging activity [21]. The potential involvement of fucoxanthin in radical scavenging activity may correlate to the presence of unusual double allenic bonds. Sachindra et al. (2007) isolated fucoxanthin from *Undaria pinnatifida* and reported high antioxidant activity [22]. Fucoxanthin purified from marine diatom *Odontella aurita* showed strong antioxidant activity. Several studies have indicated that the number of hydroxyl groups on the ring structure of fucoxanthin is correlated with the effects of ROS suppression.

PBP-like PC is able to scavange alkoxyl, peroxyl, and hydroxyl radicals in vitro and could inhibit microsomal lipid peroxidation [23]. Yabuta et al. (2010) demonstrated antioxidant activity of phycoerythrobilin derived from *Porphyra* sp [24]. Sonani et al. (2014) reported antioxidant activity of PBPs from *Lyngbya* sp. A09DM and found phycoerythrin to have highest hydroxyl radical scavenging activity [25]. These workers established that the antioxidant activity of phycoerythrin was mainly targeted on the free radicals produced while PC and APC expressed their antioxidant action by chelating the free radical producing metal ions. Similar reports were also made by Bermejo et al. (2008) who observed excellent antioxidant activity of *Spirulina* extract containing PC and the same was attributed to both free radical scavenging and metal chelating activity [26]. Antioxidant properties in the PBPs in *Spirulina* have made it excellent for dietary applications. Benedetii et al. (2007) also reported lowering of AAPH induced hemolysis and lipid peroxidation in red blood cells treated with PC enriched *Aphanizomenon flos-aquae* extract [27].

The effect of PC is seen to enhance the biological defense activity by reducing allergic inflammation by the suppression of antigen-specific IgE antibody and through maintaining the mucosal immune system function against infectious diseases in C3H/HeN and BALB/cA mice [28]. An influence of selenium-enriched phycocyanin (Se-PC) on anaphylactic

reaction severity and circulating antibody response against model allergen (hen's egg white ovalbumin) was studied in rats. Rats receiving Se-PC demonstrated significantly increased specific IgG response [29]. Phycocyanin has also been shown to promote the expression of CD59 protein and reduce the reproduction of HeLa cells. With an ascendance of PC concentration, the expression quantities of CD59 protein and apoptosis inducing Fas protein increased and the multiplication activity of HeLa cells declined [30]. Immune response cells are particularly sensitive to oxidative stress and membrane damage by free radicals because they rely heavily on cell-to-cell communications via cell membrane receptors. Furthermore, the phagocytic action of some of these cells releases free radicals that can rapidly damage these cells if they are not neutralized by antioxidants. Astaxanthin significantly influences immune function in several in vitro and in vivo assays using animal models. Astaxanthin enhances in vitro antibody production by mouse spleen cells [31] and can also partially restore decreased humoral immune responses in old mice [32]. Other evidence also points to the immune-modulating activity of astaxanthin on the proliferation and functions of murine immune-competent cells [33]. Finally, studies on human blood cells in vitro have demonstrated enhancement by astaxanthin of immunoglobulin production in response to T-dependent stimuli [34].

3.2 Algal Pigments as Anticarcinogens

Algal pigments especially carotenoids and PBPs are well studied for their potential role as anticarcinogens. However, cancer preventive effects of chlorophyll and its derivatives have also been studied, with particular emphasis on their in vitro antimutagenic effect against numerous dietary and environmental mutagens [35]. Lutein, β-carotene, and *Chl a* isolated from *Porphyra tenera* have demonstrated antimutagenic activity in bacteria, *Salmonella typhimurium* [36]. Carotenoids like fucoxanthin are reported to exert antitumor activity through apoptosis induction [37–39]. Kim et al. (2010) reported that formation of reactive oxygen species was a critical target in fucoxanthin-induced apoptosis in HL-60 cells [40]. Ishikawa et al. (2008) assayed the antiproliferative effects of fucoxanthin, fucoxanthinol, β-carotene, and astaxanthin, and found that both fucoxanthin and fucoxanthinol had remarkable antiproliferative effects on human T-cell leukemia virus type1-infected T-cell lines and adult T-cell leukemia cells in vitro [41]. Fucoxanthin has been reported to reduce the viability of human colon cancer cell lines and induce apoptosis in a dose- and time-dependent manner [37]. Kotake-Nara et al. (2005) reported that fucoxanthin and neoxanthin were effective in reducing the viability of HCT116 cancer [39]. Fucoxanthin can inhibit the proliferation of human colon cancer cell lines WiDr and HCT116 cells by induction of cell cycle arrest at the G0/G1 phase [42,43]. Zhang et al. (2008) showed that fucoxanthin had remarkable antiproliferative effects on human urinary bladder cancer EJ-1 cells and reduced the viability of EJ-1 cells by inducing apoptosis, which was characterized by morphological changes [44].

Among PBPs, PC has been reported to have significant anticarcinogenic property. Selenium-containing PC has been identified as a potent antiproliferative agent against human melanoma and human breast adenocarcinoma. PC can promote the expression of CD59 protein in HeLa cells, hold back the reproductions of HeLa cells, and moreover, a dosage effect was found between them. C-PC was also effective on hepatocellular carcinoma [45] and has antineoplastic effects on colon carcinogenesis [46,47]. A significant downregulation of multi

drug resistance was observed in C-PC–treated human hepatocarcinoma cells [48]. C-PC could significantly reduce the expressions of N-methyl D-aspartate receptor subunit 2B, tumor necrosis factor- α (TNF-α), interleukin-1β (IL-1 β), and cyclooxygenase type 2 genes in the cochlea and inferior colliculus of mice [49].

3.3 Antiinflammatory Activity of Algal Pigments

Antiinflammatory activities of algal pigment are mainly due to modulation of macrophages function, which is the chief source of proinflammatory mediators like nitric oxide (NO), prostaglandin E2 (PGE2), TNF-α, IL-6 and IL-1b, and reactive oxygen species [50]. Carotenoid-like astaxanthin has been found to reduce induced swelling of rat paw [51] and reduced symptoms of gastric inflammation [52]. Fucoxanthin is known to be potent antiinflammatory agent and comparable with prednisolone, a commercially available steroidal antiinflammatory drug [53,54]. Fucoxanthin has been reported to attenuate the levels of TNF-α, IL-6, and IL-1b [54]. Biliprotein pigments like PC are known to reduce allergic inflammation by suppressing antigen-specific IgE antibody [55]. PCB chromophores found in blue-green algae are found to have potential antiinflammatory effects. Inhibition of NO and PGE2 overproduction, attenuation of TNF-α formation, and neutrophil infiltration into inflammatory sites by C-PC might contribute pain relieving activity [56]. Cyanobacterial PC has been found to be effective for treatment of LPS-related acute lung injury by inhibiting inflammatory responses and apoptosis in lung tissues [57]. Gonzalez et al. (1999) evaluated PC for treatment of acetic acid–induced colitis in rats, an experimental model of inflammatory bowel disease and demonstrated protective and antiinflammatory effects of PC, which was comparable to 200 mg/kg of 5-aminosalicylic acid, an antiinflammatory drug [58]. Phycocyanin has also been reported to exert antiinflammatory effect through the scavenging of hydroxyl radicals [59].

3.4 Neuroprotective Activity of Algal Pigments

A number of studies have reported neuroprotective properties of algal pigments. Okuzumi et al. (1990) found that fucoxanthin isolated from *Hijikia fusiformis* inhibited N-myc expression and cell cycle progression of GOT0 cells, a human neuroblastoma cell line [60]. Fucoxanthin from Wakame could attenuate cell damage in cortical neurons during hypoxia and oxygen reperfusion [61]. Studies have demonstrated that astaxanthin can cross the blood–brain barrier in mammals and can extend its antioxidant benefits beyond that barrier [62]. Astaxanthin is therefore an excellent candidate for testing in Alzheimer disease and other neurological diseases. Astaxanthin-mediated neuroprotection in subarachnoid hemorrhage is attributed to downregulation of increased nuclear factor kappa B activity and the expression of inflammatory cytokines and intercellular adhesion molecule-1 [63]. Rimbau et al. (1999) showed that administration of PC lowers the neurobehavioral alterations in rats receiving kainic acid, which causes neural damage [64]. Phycocyanin is reported to show protective role against hippocampus neuronal cell death and improved locomotive behavior in male Mongolian Gerbil [65]. The neuroprotective role of PC has been attributed toward its antioxidant, antiinflammatory and immunomodulatory properties [66].

3.5 Antiobesity Property of Algal Pigments

The excessive growth of adipose tissue in obese individuals has been suggested to be a result of adipocyte hypertrophy and engagement of new adipocytes from precursor cells [67]. Fucoxanthin isolated from *U. pinnatifida* has been reported to inhibit the differentiation of 3T3-L1 preadipocytes into adipocytes [68]. Fucoxanthin and neoxanthin are known to have significant suppressive effect on adipocyte differentiation, which may be due to the presence of allenic bond [69]. Fucoxanthin could significantly reduce plasma and hepatic triglyceride concentrations and the activities of adipocytic and hepatic fatty acid synthesis and cholesterol-regulating enzymes while resulting in increased concentrations of plasma high-density lipoprotein-cholesterol [70,71]. Fucoxanthins are reported to significantly lower mRNA expressions of proliferators-activated receptor γ and the activity of hepatic phosphatidate phosphohydrolase and significantly increase the mRNA expressions of β-oxidation-related acyl-coA oxidase 1 and proliferators-activated receptor α [70]. Green algal pigment siphonaxanthin could significantly lower the lipid accumulation in KK-Ay mice. This carotenoid pigment could lower the expression of key adipogenesis genes, such as *Cebpa*, *Pparg*, *Fabp4*, and *Scd1* [72].

Phycocyanin is also known to exert antiobesity effect, which might be due to hypocholesterolemic action of PC. Han et al. (2006) reported that PC might inhibit the pancreatic lipase resulting in the inhibition of intestinal absorption of dietary fat [73]. Phycocyanin can lower serum cholesterol, total cholesterol, triglyceride, and low-density lipids.

3.6 Algal Pigments as Antiaging Compounds, Skin, and Photo Protective Agents

Excessive exposure of unprotected skin to sunlight results in sunburn and can also lead to photo-induced oxidation, inflammation, immunosuppression, aging, and even carcinogenesis of skin cells. Preclinical studies show that typical dietary antioxidants like carotenoids can reduce such deleterious effects. Fucoxanthin from *Laminaria japonica* has been reported to suppress tyrosinase activity in UV-irradiated guinea pig and melanogenesis in UV-irradiated mice. Oral treatment of fucoxanthin significantly suppressed skin mRNA expression related to melanogenesis [74]. Astaxanthin derived from *H. pluvialis* showed improvements in skin wrinkle, skin texture, moisture content of corneocyte layer, and corneocyte condition [75]. Camera et al. (2009) reported astaxanthin as an effective agent imparting protection against photooxidative damage in cell culture [76]. Astaxanthin supplementation helped to protect the retinal photoreceptors in rats exposed to acute UV-light injury [62]. In vitro protective effect of astaxanthin against UV-induced photooxidation was stronger when compared with β-carotene and lutein [77]. As the algal pigments show strong antioxidative effect, they hold considerable potential to restrict aging due to generation of free radicals in the cell. Sonani et al. (2014) reported increased survival of *Caenerhabditis elegans* under oxidative stress when treated with phycoerythrin from *Lyngbya* sp. A09DM [25]. Huangfu et al. (2013) reported that astaxanthin from *H. pluvialis* can complement defective antioxidant defense system and thereby extending the life span of fruit flies [78].

4. APPLICATION IN COSMETICS AND SKIN CARE

4.1 Commercial Application as Cosmeceuticals

Colorants prepared from red and blue-green algae are also suitable for use in cosmetics. In 2009, Fujifilm Corporation launched an Astaxanthin based product, "Astalift Whitening Essence" to combat aging-related skin spots. Japanese cosmetic giant Kose has marketed Astaxanthin-based product AstaBlanc for combating wrinkles and spots. AstaReal AB (Sweden) has marketed AstaReal natural astaxanthin, which is claimed to revitalize photodamaged skin, removes wrinkle, and enhances the elasticity of the skin. A heat-and pH-stable low-molecular-weight (18,000 Da) PC produced from thermophilic blue-green algae has already been formulated as an eye shadow. Arad and Yaron (1996) prepared pink and purple cosmetics eye shadow, face make-up, and lipstick, in the form of powders or creams from pigments isolated from various red microalgae [79].Two mycosporine-like amino acids (MAA) viz. shinorine and porphyra-334 from the red macroalga *Porphyra umbilicalis* are already in market for use in cosmetics under the trade name Helioguard 365.

Protein-rich extract from *S. platensis* for repairing the signs of early skin aging (Protulines, Exsymol S.A.M., Monaco) is also commercially available. Spirulina Facial Moisturizer by Ferenes Cosmeticscontains natural proteins, essential fatty acids, and β-carotene from *Spirulina* and other herbal extract Spirulina Facial Scrub by Ferenes Cosmetics contain quality ingredients from *Spirulina* and other herbs that remove the dead skin cells and act as a cleanser to energize the face. Codif Recherche and Nature (Paris, France) has marketed a *Phormidium persicinum* product Phormiskin Bioprotech G, which has unique photoprotective property [23]. Biotherm (France) has marketed a product Blue Therapy (Lift and Blur), which contains extracts from *A. flos-aquae* and *Laminaria ochroleuca* is claimed to confer antiaging effects. Lucas Meyer Cosmetics (France) has marketed *A. flos-aquae* extract—based LanaBlue, which is also claimed as antiaging and antiwrinkling product.

A recently marketed new cosmeceuticals product from microalgae is a mixture of polysaccharides extracted from heterotrophic green algae [80], with the registered trade name "Alguronic Acid." Protanal alginates (FMC Health & Nutrition, USA) are ideal forms for stabilizing acidic skin-care preparations. Marinova Pty. Ltd. has marketed two fucoidan-rich skin-care products viz. Maritech Bright and Maritech Reverse. Maritech Bright is a 90% polyphenol-fucoidan (from *Fucus vesiculosus*) extract and is claimed to be effective in restricting skin darkening and patchiness. Maritech Reverse is low-molecular-weight extract of 80% fucoidan from *U. pinnatifida* and is claimed to have antiaging, soothing, and protective skin-care applications. Phycosaccharides marketed by Codif Recherche and Nature (France) is made from extracts of *Laminaria digitata* and is a known skin penetrant. Pentapharm (Basel, Switzerland) has marketed new skin tightening products from the extracts of *Nannochloropsis oculata*. Marestil prepared from marine algae extracts is a strong moisturizing, elasticizing, and toning complex [81]. Looking at the demand and benefits of algal cosmeceuticals many of the cosmeticians have developed their own microalgal production system (e.g., LVMH, Paris, France and Danial Jouvance, Carnac, France).

4.2 Prospects of Algal Pigments in Skin-Care Products

Urbanization and globalization have imposed the threat of pollution and stress on human life. With the growth of science, the human problems are also becoming complex. Pollution and stress have made the problem of early skin aging very prominent. To look more beautiful and healthy, people always demand an effective remedy. Antioxidants and antiaging creams have been the most common way outs for this and have an ever increasing demand. With the growing concern of biological safety and toxicity issues, people have been attracted toward herbal/botanical products, which are comparatively safer to use. It is reported that the demand of cosmeceutical products and chemicals are expected to rise from 8240 million dollar in 2012 to 11,520 million dollar in 2017 [82] and of these skin-care products contribute more than 50% of the share. Algal pigments especially, carotenoids and PBPs have excellent antioxidant and antiaging properties that make them highly suitable for use in cosmetics. Besides these, their brilliant bright colors can be an added advantage for the cosmetic products. At present, a few cosmetic products based on these pigments have been commercialized but many of them did not make sufficient market due to safety regulations. Algal pigments are highly exploited in food, beverages, and feeds as natural colorants, and due to many health benefits, there may be little hitch for their use in skin care. Properties of these pigments like free radical scavenging, inhibiting melanogenesis, and photoprotection make them ideal for skin care. Use of carotenoids may lead to glowing skin due to accumulation of carotenoids in body fat. Furthermore, other health benefits particularly anticancer and neuroprotective properties make these excellent candidates. Numerous health benefits, cheap and less energy intensive production of algal pigments and huge biodiversity of terrestrial and marine algae make algal pigments the ideal candidate for next-generation cosmetics with health benefits.

5. OTHER ALGAL COMPOUNDS AS COSMECEUTICALS

As stated earlier, algae are considered to be very rich repertoire of different bioactive compounds. Based on numerous scientific studies and clinical trials, these compounds have been attributed a number of biologically relevant functions. Besides the photosynthetic pigments, algae produce a number of other bioactive pigments, polysaccharides, vitamins, and polyphenols, which can be potent cosmeceuticals due to their versatile health benefits.

Apart from tetrapyrrole and isoprene pigments, another specific class of pigment molecules known as MAA are found in algae and predominantly involved in photoprotection especially against UV radiations. These are hydrophilic molecules of $<$400 Da size and contains basic structure of aminocyclohexenone or amino cyclohexenimine to which various amino acids are substituted. MAA have also shown antioxidant properties apart from its sunscreen activity against harmful radiations.

Fucoidan or "fucan" is a type of highly branched polysaccharide with substantial percentages of L-fucose and is generally sulfated and acetylated and obtained mainly from brown algae. This can be incorporated into creams and lotions, providing cosmetic antiaging and antiwrinkle benefits, such as inhibition of matrix enzymes against hyaluronidase, heparanase, phospholipase A2, tyrosine kinase and collagenase expression, and antiinflammatory activity. It was also found to increase the number of dermal fibroblasts and deposition of collagen,

collagen tightness, and facial elasticity. Fucoidan also minimizes elastase activity, resulting in the protection of human skin elastic fiber network against the enzymatic proteolysis.

Brown algae (Phaeophyta) accumulate a variety of phloroglucinol-based polyphenols known as phlorotannins. These phlorotannins consist of phloroglucinol units linked to each other in various ways, and are of wide occurrence among marine brown algae. Phlorotannins have multiple properties that make these highly potential for skin care. Its tyrosinase inhibition activity contributes to inhibition of melanogenesis thus making it suitable as skin whitener. Inhibition of matrix metalloprotease makes them to stop wrinkle formation in skin. Furthermore, its antioxidant activity can protect cells from free-radical−related aging. Sargachromanol E, which is extracted from *Sargassum horneri*, a marine brown algae, has demonstrated anabolic effects with regard to bone calcification both in vitro and in vivo, indicating that sargachromanol E can be further evaluated for its potential antiaging effects [83]. Unsaturated fatty acids are known to be produced by a number of micro and macro algae. Application of fatty acids can improve the skin condition and protect from dermatitis and dehydration. Algal polysachharides like carrageenan and alginates are also known to be used as stabilizers and thickening agents in skin-care products.

Apart from specific purified compounds from algae, algal extracts have been reported to contain health-promoting benefits thus making them suitable for cosmeceutical applications. Extract from *Chlorella vulgaris* has been reported as promising substance for use in skin-care products, as it boosts collagen synthesis, supporting skin tissue and thus reducing wrinkles [83]. Polysaccharides and mineral rich extract of *Chondrus crispus* has hydrating, soothing, healing, moisturizing, and conditioning effects on skin. The extract from *Codium tomentosum*, a green alga, is a good source of glucuronic acid, which can help in skin hydration. Extract from *C. vulgaris* is known to stimulate collagen synthesis in the skin and can be used for the products supporting tissue regeneration and wrinkle reduction [81]. Silica muds and algal extracts from Blue Lagoon of Iceland have been reported to have significant effect on preventing premature skin aging. Grether-Beck et al. (2008) showed that bioactive molecules in the silica mud and algal extracts have inhibitory effect on matrix metalloproteinase while upregulating the expression of collagen genes that are often downregulated due to UV damage [84].

6. CONCLUSION AND PERSPECTIVES

Versatile health promoting activity, attractive color, and easy mass production are the main keys for commercial exploitation of algal pigments. The industry is still in infancy and requires a good number of high yielding strains and in many cases commercially viable technologies. In depth information on the biology of the pigment-producing algae, biosynthetic pathway, and genetic organization can make the future of the algal pigments more bright and colorful. Engineering pigment-producing algae for higher production of pigments (e.g., metabolic engineering through biolistic transformation of *H. pluvial*) could show accelerated accumulation of astaxanthin under stress condition [85]; heterologous expression of pigments (e.g., Lemuth et al. reported engineered *E. coli* strains for sustainable production of astaxanthin) [86] has been used to increase the pigment production sustainably. However, with the growing field of DNA sequencing more information about the intricate metabolic

network of the pigment-producing algae will help to easily engineer the algae for higher production of pigment molecules. Exploitation of new and unexplored niches and more research initiatives for such explorations can be fruitful to uncover newer sources of high value pigments. Unique habitats like Klamath Lake and Blue Lagoons should be exploited for new high yielding pigment producers as well as novel bioactive pigment and other metabolites. Cheap, eco-friendly, and economically viable technologies coupled with coordinated clinical trials carried out globally would further enhance and establish the role of algal pigments as cosmeceuticals.

References

[1] D.M. John, Biodiversityandconservation, an algal perspective, Phycologist 38 (1994) 3–15.
[2] W.M. Manning, H.H. Strain, Chlorophyll *d*, a green pigment of red algae, J. Biol. Chem. 151 (1943) 1–19.
[3] H. Sagromsky, Pigments of red algae, Ber. Dtsch. Bot.Ges 71 (3–7) (1960) 358–362.
[4] M.A. Borowitzka, Vitamins and fine chemicals from microalgae, in: M.A. Borowitzka, L.J. Borowitzka (Eds.), Micro-algal Biotechnology, Cambridge University Press, Cambridge, 1988, pp. 153–196.
[5] K.S. Rowan, Photosynthetic Pigments of Algae, Cambridge University Press, New York, 1989, pp. 166–211.
[6] B.A. Zilinskas, L.S. Greenwald, Phycobilisome structure and function, Photosynth. Res. 10 (1986) 7–36.
[7] A.N. Glazer, Light harvesting by phycobilisomes, Annu. Rev. Biophys. Biophys. Chem. 14 (1985) 47–77.
[8] N. Adir, Elucidation of the molecular structures of components of the phycobilisome, reconstructing a giant, Photosynth. Res. 85 (2005) 15–32.
[9] E. Gantt, Structure and function of phycobilisomes, light harvesting pigment complexes in red and blue-green algae, Int. Rev. Cytol 66 (1980) 45–80.
[10] A.N. Glazer, L. Stryer, Phycofluor probes, Trends Biochem. Sci. 9 (1984) 423–427.
[11] R. MacColl, Cyanobacterial phycobilisomes, J. Struct. Biol. 124 (1998) 311–334.
[12] W. Reuter, C. Muller, Adaptation of the photosynthetic apparatus of cyanobacteria to light and CO_2, J. Photochem. Photobiol. B 21 (1993) 3–27.
[13] W.A. Sidler, Phycobilisomes and phycobiliprotein structures, in: D.A. Bryant (Ed.), The Molecular Biology of Cyanobacteria, Kluwer Academic Press, Dordrecht, The Netherlands, 1994, pp. 139–216.
[14] N. Tandeau de Marsac, Phycobiliproteins and phycobilisomes, the early observations, Photosynth. Res. 76 (2003) 197–205.
[15] Y. Endo, R. Usuki, T. Kaneda, Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects, J. Am. Oil Chem. Soc. 62 (1985) 1375–1378.
[16] Y. Endo, R. Usuki, T. Kaneda, Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll, J. Am. Oil Chem. Soc. 62 (1985) 1387–1390.
[17] B. Le Tutour, F. Benslimane, M. Gouleau, J. Gouygou, B. Saadan, F. Quemeneur, Antioxidant and pro-oxidant activitiesof the brown algae, *Laminaria digitata*, *Himanthalia elongata*, *Fucus vesiculosus*, *Fucus serratus* and *Ascophyllum nodosum*, J. Appl. Phycol 10 (1998) 121–129.
[18] H. Cahyana, Antioxidative activity of porphyrin derivatives, Biosci. Biotechnol. Biochem 57 (1993) 680–681.
[19] U.M. Lanfer-Marquez, R. Barros, P. Sinnecker, Antioxidant activity of chlorophylls and their derivatives, Food Res. Int. 38 (2005) 885–891.
[20] A. RangaRao, R.L. Raghunath Reddy, V. Baskaran, R. Sarada, G.A. Ravishankar, Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model, J. Agric. Food Chem. 58 (15) (2010) 8553–8559.
[21] X. Yan, Y. Chuda, M. Suzuki, T. Nagata, Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed, Biosci. Biotechnol. Biochem 63 (1999) 605–607.
[22] N. Sachindra, E. Sato, H. Maeda, M. Hosokawa, Y. Niwano, M. Kohno, et al., Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites, J. Agric. Food Chem. 55 (2007) 8516–8522.
[23] H. Chakdar, S.D. Jadhav, D.W. Dhar, S. Pabbi, Potential applications of blue green algae, J. Sci. Ind. Res. 71 (1) (2012) 13–20.

[24] Y. Yabuta, H. Fujimura, C.S. Kwak, T. Enomoto, F. Watanabe, Antioxidant activity of the phycoerythrobilin compound formed from a dried Korean purple laver (*Porphyra* sp.) during in vitro digestion, Food Sci. Technol. Res. 16 (2010) 347–352.
[25] R. Sonani, N.K. Singh, J. Kumar, D. Thakar, D. Madamwar, Concurrent purification and antioxidant activity of phycobiliproteins from *Lyngbya* sp. A09DM, an antioxidant and anti-aging potential ofphycoerythrin in *Caenorhabditis elegans*, Process. Biochem. 49 (2014) 1757–1766.
[26] P. Bermejo, E. Pinero, A.M. Villar, Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*, Food Chem. 110 (2008) 436–445.
[27] S. Benedetti, F. Benvenuti, S. Pagliarani, S. Francogli, S. Scoglio, F. Canestrari, Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*, Life Sci. 75 (2004) 2353–2362.
[28] T. Hayashi, K. Hayashi, M. Maeda, I.A. Kojima, Natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*, in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities, AIDS Res. Hum. Retroviruses 12 (15) (1996) 1463–1471.
[29] E.A. Egorova, I.V. Gmoshinskiĭ, S.N. Zorin, V.K. Mazo, Studies of immunomodulation caused by selenium-enriched phycocyanin, Voprosypitaniia 75 (2) (2005) 19–21.
[30] B. Li, X. Zhang, M. Gao, X. Chu, Effects of CD59 on antitumoral activities of phycocyanin from *Spirulina platensis*, Biomed. Pharmacotherapy 59 (10) (2005) 551–560.
[31] H. Jyonouchi, L. Zhang, Y. Tomita, Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation, Nutr. Cancer 19 (1993) 269–280.
[32] H. Jyonouchi, L. Zhang, M. Gross, Y. Tomita, Immunomodulating actions of carotenoids, enhancement of in vivo and in vitro antibody production to T-dependent antigens, Nutr. Cancer 21 (1994) 47–58.
[33] Y. Okai, K. Higashi-Okai, Possible immunomodulating activities of carotenoids in in vitro cell culture experiments, Int. J. Immunopharmacol 18 (1996) 753–758.
[34] H. Jyonouchi, S. Sun, Y. Tomita, M.D. Gross, Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen, J. Nutr. 124 (1995) 2483–2492.
[35] M.G. Ferruzzi, J. Blakeslee, Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives, Nutr. Res. 27 (2007) 1–12.
[36] Y. Okai, K. HiqashiOkai, Y. Yano, S. Otani, Identification of antimutagenic substances in an extract of edible red alga, *Porphyra tenera* (Asadusa-nori), Cancer Lett. 100 (1996) 235–240.
[37] M. Hosokawa, M. Kudo, H. Maeda, H. Kohno, T. Tanaka, K. Miyashita, .Fucoxanthin induces apoptosis and enhances the anti-proliferative effect of the PPARγ ligand, troglitazone, on colon cancer cells, Biochim. Biophys. Acta 1675 (2004) 113–119.
[38] Y. Nakazawa, T. Sashima, M. Hosokawa, K. Miyashita, Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthinin human cancer cell lines, J. Funct. Foods 1 (2009) 88–97.
[39] E. Kotake Nara, A. Asai, A. Nagao, Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells, Cancer Lett. 220 (2005) 75–84.
[40] K.N. Kim, S.J. Heo, S.M. Kang, G. Ahn, Y.J. Jeon, Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a RO S-mediated Bcl-xL pathway, Toxicol. Vitro 24 (2010) 1648–1654.
[41] C. Ishikawa, S. Tafuku, T. Kadekaru, S. Sawada, M. Tomita, T. Okudaira, et al., Antiadult T-cell leukemiaeffects of brown algae fucoxanthin and its deacetylated product fucoxanthinol, Int. J. Cancer 123 (2008) 2702–2712.
[42] S.K. Das, T. Hashimoto, K. Shimizu, T. Yoshida, T. Sakai, Y. Sowa, et al., Fucoxanthin induces cell cycle ar rest at G0/G1 phase in human colon carcinoma cells through up-regulation of p21WAF1/Cip1, Biochim. Biophys. Acta 1726 (2005) 328–335.
[43] S.K. Das, T. Hashimoto, K. Kanazawa, Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D, Biochim. Biophys. Acta 1780 (2008) 743–749.
[44] Z.Y. Zhang, P.J. Zhang, M. Hamada, S. Takahashi, G.Q. Xing, J.Q. Liu, et al., Potential chemoprevention effect of dietary fucoxanthin on urinary bladder cancer EJ-1 cell line, Oncol. Rep. 20 (2008) 1099–1103.
[45] K.R. Roy, K.M. Arunasree, A. Dhoot, R. Aparna, G.V. Reddy, S. Vali, et al., C-Phycocyanin inhibits 2-acetylaminofluorene-induced expression of MDR1 in mouse macrophage cells, ROS mediated pathway determined via combination of experimental and *in silico* analysis, Arch. Biochem. Biophys 459 (2) (2007) 169–177.

[46] M.K. Saini, S.N. Sanyal, Piroxicam and c-phycocyanin prevent colon carcinogenesis by inhibition of membrane fluidity and canonical Wnt/β-catenin signaling while up-regulating ligand dependent transcription factor PPARγ, Biomed. Pharmacother. 68 (5) (2014) 537–550.
[47] M.K. Saini, K. Vaiphei, S.N. Sanyal, Chemoprevention of DMH-induced rat colon carcinoma initiation by combination administration of piroxicam and C-phycocyanin, Mol. Cell. Biochem. 361 (1–2) (2012) 217–228.
[48] R.P. Nishanth, B.S. Ramakrishna, R.G. Jyotsna, K.R. Roy, G.V. Reddy, P.K. Reddy, et al., C-Phycocyanin inhibits MDR1 through reactive oxygen species and cyclooxygenase-2 mediated pathways in human hepatocellular carcinoma cell line, Eur. J. Pharmacol 649 (1) (2010) 74–83.
[49] J.H. Hwang, J.C. Chen, Y.C. Chan, Effects of C-phycocyanin and *Spirulina*on salicylate- induced tinnitus, expression of NMDA receptor and inflammatory genes, PloS One 8 (3) (2013) e58215.
[50] M. Block, L. Zecca, J. Hong, Microglia-mediated neurotoxicity, uncovering the molecular mechanisms, Nat. Rev.Neurosci 8 (2007) 57–69.
[51] M. Kurashige, E. Okimasu, M. Inoue, K. Utsumi, Inhibition of oxidative injury of biological membranes by astaxanthin, Physiol. Chem. Phys. Med. NMR 22 (1990) 27–38.
[52] M. Bennedsen, X. Wang, R. Willén, T. Wadström, L.P. Andersen, Treatment of *H. Pylori*infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes, Immunol. Lett 70 (1999) 185–189.
[53] S.J. Heo, W.J. Yoon, K.N. Kim, G.N. Ahn, S.M. Kang, D.H. Kang, et al., Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide stimulated RAW 264.7 macrophages, Food Chem. Toxicol 48 (2010) 2045–2051.
[54] K. Shiratori, K. Ohgami, I. Ilieva, X.H. Jin, Y. Koyama, K. Miyashita, et al., Effects of fucoxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo, Exp. Eye Res. 81 (2005) 422–428.
[55] C. Nemoto-Kawamura, T. Hirahashi, T. Nagai, H. Yamada, T. Katoh, O. Hayashi, Phycocyanin enhances secretary IgA antibody response and suppresses allergic IgE antibody response in mice immunized with antigen-entrapped biodegradable microparticles, J. Nutr. Sci.Vitaminol 50 (2) (2004) 129–136.
[56] C.M. Shih, S.N. Cheng, C.S. Wong, Y.L. Kuo, T.C. Chou, Anti-inflammatory and anti-hyperalgesic activity of C-phycocyanin, Anesthes. Analges 108 (4) (2009) 1303–1310.
[57] P.O. Leung, H.H. Lee, Y.C. Kung, M.F. Tsai, T.C. Chou, Therapeutic effect of C-phycocyanin extracted from blue green algae in a rat model of acute lung injury induced by lipopolysaccharide, Evidence-Based Complementary Altern. Med. (2013) 1–11. http://dx.doi.org/10.1155/2013/916590.
[58] R. González, S. Rodríguez, C. Romay, O. Ancheta, A. González, J. Armesto, et al., Pharmacol. Res. 39 (1999) 55–59.
[59] C. Romay, J. Armesto, D. Remirez, R. González, N. Ledón, I. García, Inflamm. Res. 47 (1998) 36–41.
[60] J. Okuzumi, H. Nishino, M. Murakoshi, A. Iwashima, Y. Tanaka, T. Yamane, et al., Inhibitory effects of fucoxanthin, a natural carotenoid, on N-myc expression and cell cycle progression in human malignant tumor cells, Cancer Lett. 55 (1990) 75–81.
[61] K. Khodosevich, H. Monyer, Signaling involved in neurite outgrowth of postnatally born subventricular zone neurons in vitro, BMC Neurosci. 11 (2010) 18.
[62] M.O.M. Tso, T.T. Lam, Method of Retarding and Ameliorating Central Nervous System and Eye Damage, U.S. Patent#5527533, 1996.
[63] X.S. Zhang, X. Zhang, Q. Wu, W. Li, C.X. Wang, G.B. Xie, et al., Astaxanthin offers neuroprotection and reduces neuroinflammation in experimental subarachnoid hemorrhage, J. Surg. Res. 192 (1) (2014) 206–213.
[64] V. Rimabau, A. Camnis, R. Romay, M. Pallas, Protective effectsof C-phycocyanin against kainic acid-induced neuronal damage in rat hippocampus, Neurosci. Lett 276 (1999) 75–78.
[65] G. Penton-Rol, J. Marin-Prida, G. Pardo-Andreua, G. Martínez-Sáncheza, E.F. Acosta-Medinaa, A. Valdivia-Acostaa, et al., C-Phycocyanin is neuroprotective against global cerebral ischemia/reperfusion injury in gerbils, Brain Res. Bull. 86 (2011) 42–52.
[66] S.K. Min, J.S. Park, L. Luo, Y.S. Kwon, H.C. Lee, H.J. Shim, et al., Assessment of C-phycocyanin effect on astrocytes-mediated neuroprotection against oxidative brain injury using 2D and 3D astrocyte tissue model, Sci. Rep. 5 (2015) 14418.
[67] T. Wang, Y. Wang, Y. Kontani, Y. Kobayashi, Y. Sato, N. Mori, et al., Evodiamine improves diet-induced obesity in auncoupling protein-1-independent manner, Involvement ofantiadipogenic mechanism and extracellularly regulatedkinase/mitogen-activated protein kinase signaling, Endocrinology 149 (2008) 358–366.

[68] M. Hayato, H. Masashi, S. Tokutake, T. Nobuyuki, K. Teruo, M. Kazuo, Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3L1 cells, Int. J. Mol. Med. 18 (2006) 147–152.
[69] T. Okada, M. Nakai, H. Maeda, M. Hosokawa, T. Sashima, K. Miyashita, Suppressive effect of neoxanthin on the differentiation of 3T3-L1 adipose cells, J. Oleo Sci. 57 (2008) 345–351.
[70] M.N. Woo, S.M. Jeon, H.J. Kim, M.K. Lee, S.K. Shin, Y.C. Shin, et al., .Fucoxanthin supplementation improves plasma and hepatic lipid metabolism and blood glucose concentration in high-fat fed C57BL/6N mice, Chem. Biol. Interact. 186 (2010) 316–322.
[71] S.M. Jeon, H.J. Kim, M.N. Woo, M.K. Lee, Y.C. Shin, Y.B. Park, et al., .Fucoxanthin-rich seaweed extract suppresses body weight gain and improves lipid metabolism in high-fat-fed C57BL/6J mice, Biotechnol. J. 5 (2010) 961–969.
[72] Z.S. Li, K. Noda, E. Fujita, Y. Manabe, T. Hirata, T. Sugawara, The green algal carotenoid Siphonaxanthininhibits adipogenesis in 3T3-L1 preadipocytes and the accumulation of lipids in white adipose tissue of KK-Ay Mice, J. Nutr. 145 (3) (2015) 490–498.
[73] L.K. Han, D.X. Li, L. Xiang, X.J. Gong, Y. Kondo, I. Suzuki, et al., Isolation of pancreatic lipase activity-inhibitory component of *Spirulina platensis* and it reduce postprandial triacylglycerolemia, J. Pharmaceut. Soc. Jpn. 126 (1) (2006) 43–49.
[74] H. Shimoda, J. Tanaka, S.J. Shan, T. Maoka, Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules, J. Pharm. Pharmacol. Res. 62 (2010) 1137–1145.
[75] K. Tominaga, N. Hongo, M. Karato, E. Yamashita, Cosmetic benefits of astaxanthin on humans subjects, Acta Biochim. Pol. 59 (1) (2012) 43.
[76] E. Camera, A. Mastrofrancesco, C. Fabbri, F. Daubrawa, M. Picardo, H. Sies, et al., .Astaxanthin, canthaxanthin and beta-carotene differently affect UV-A-induced oxidative damage and expression of oxidative stress-responsive enzymes, Exp. Dermatol. 18 (2009) 222–231.
[77] I. O'Connor, N. O'Brien, Modulation of UV-A light-induced oxidative stress by beta-carotene, lutein and astaxanthin in cultured fibroblasts, J. Dermatol. Sci. 16 (1998) 226–230.
[78] J. Huangfu, J. Liu, Z. Sun, M. Wang, Y. Jiang, Z.Y. Chen, et al., Antiagingeffects of astaxanthin-rich alga *Haematococcus pluvialis* on fruit flies under oxidative stress, J. Agric. Food Chem. 61 (32) (2013) 7800–7804.
[79] S. Arad, A. Yaron, Natural pigments from red microalgae for use in foods and cosmetics, Trends Food Sci. Technol. 3 (1992) 92–97.
[80] A. Coragliotti, S. Franklin, A.G. Day, S.M. Decker, Microalgalpolysaccharide Compositions, US Patent Application 2012/0202768, 2012.
[81] S. Kim, Y.D. Ravichandran, S.B. Khan, Y.T. Kim, Prospective of the cosmeceuticals derived from marine organisms, Biotechnol. Bioprocess Eng. 13 (2008) 511–523.
[82] F.S. Brandt, A. Cazzaniga, M. Hann, Cosmeceuticals, current trends and market analysis, Semin.Cutan. Med. Surg. 30 (3) (2011) 141–143.
[83] H.M.D. Wang, C.C. Chen, P. Huynh, J.S. Chang, Exploring the potential of using algae in cosmetics, Biores. Technol 184 (2015) 355–362.
[84] S. Grether-Beck, K. Muhlberg, H. Brenden, I. Felsner, A. Brynjolfsdottir, S. Einarsson, et al., Bioactive molecules from the Blue Lagoon, in vitro and in vivo assessment of silica mud and microalgae extracts for their effects on skin barrier function and prevention of skin ageing, Exp. Dermatol. 17 (2008) 771–779.
[85] J. Steinbrenner, G. Sandmann, Transformation of the green alga *Haematococcus pluvialis* with a phytoenedesaturase for accelerated astaxanthin biosynthesis, Appl. Env. Microbiol 72 (12) (2006) 7477–7484.
[86] K. Lemuth, S. Kristin, A. Christoph, Engineering of a plasmid-free *Escherichia coli* strain for improved in vivo biosynthesis of astaxanthin, Microb. Cell Fact. 10 (1) (2011) 29.

CHAPTER

10

Role of Algae as a Biofertilizer

A. Chatterjee, S. Singh, C. Agrawal, S. Yadav, R. Rai, L.C. Rai

Banaras Hindu University, Varanasi, India

OUTLINE

1. INTRODUCTION

Today with an increase in food demand one of the major issues of global concern is food security with rising populations and restricted lands under cultivation as a result of increasing land use for urbanization and industrialization. With the advent of green revolution in 1960, intensive agricultural practices that come into existence include use of high-yielding, disease-resistant crop varieties, and constant input of agrochemicals such as chemical fertilizers, pesticides etc. Application of such chemicals adversely affects the dynamic equilibrium of soil [1] and affects agro-biodiversity by destroying nontarget useful soil flora and fauna [2].

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00010-2

Rice, one of the world's most important food as more than half of the world's population depends on rice as a staple food, registered 741.3 million tons of production in 2014 as per rice market monitoring conducted by FAO (www.fao.org/economic/RMM). Fageria [3] estimated that by the year 2025, 60% more rice production than current production will be required. Several coordinated efforts for increasing rice production have come into existence to meet the demand of growing population with the help of research and development units worldwide. Such efforts also help in raising employment opportunities, increasing the income of farmers, and uplifting access of rice to the poor populations across the world. One such approach is sustainable agricultural practice including application of biofertilizer to enhance the soil nutrient content, which further results in better productivity. It has been reported that nitrogen deficiency affects rice productivity in several ways such as retarded growth and development, production of dwarf plants, yellowing of leaves, and lower yield [4–6]. In most of the cropping system recovery efficiency is less than 50% [3,7]. Moreover, a large amount of applied nitrogen is lost due to volatilization, leaching, denitrification, and soil erosion [8]. Algae with ubiquitous occurrence in almost all terrestrial environments are one of the most characteristic organism on the Earth having potential application in nutrition as food supplements, in agriculture as biofertilizers and amelioration of sodic soils, in waste water treatment, and as source of biofuel. Filamentous, heterocystous, nitrogen fixing, photosynthetic cyanobacteria (BGA) are part of tropical paddy field ecosystem assumed as an excellent source of global nitrogen economy of rice fields and embraced as better alternative to agrochemicals with significant economic and environmental benefits [9,10]. Such cyanobacterial strains differentiate heterocyst under nitrogen-deprived condition and hence employ both photoautotrophy and diazotrophy, thus requiring only water, mineral nutrients, carbon dioxide, and light for survival [11]. Apart from BGA, red algae and brown algae have also been utilized as a potential biofertilizer.

2. EMERGENCE OF ALGAE AS BIOFERTILIZER

Until the 20th century, plant nutrients, mainly nitrogen and phosphorus, were the limiting factors for agricultural yield. The Haber-Bosch process, an artificial nitrogen fixation process, which consumes more than 1–2% of the annual energy supply on the Earth is the major industrial phenomenon for the production of available form of nitrogen for plants. It is an irony that nitrogen is one of the most essential elements required in high amount for crops. Its availability is relatively very low to crop plants despite its plentiful abundance in the atmosphere. This is due to the fact that most crop plants lack the desired trait to assimilate atmospheric nitrogen. Thus it necessitates production of synthetic nitrogen fertilizers. Production of such chemical fertilizers primarily depends on dwindling nonrenewable resources hence unable to satisfy the nitrogen requirement of the crop plants. Moreover, the production cost prohibits poor farmers to use desired quantity of synthetic fertilizer. The synthetic nitrogen fertilizers so produced and utilized in agricultural practices are able to feed roughly one-third of the world's population today. Aggressive use of these synthetic fertilizers leads to soil erosion, degradation of local ecosystem hence opening gates for pests and diseases, enhanced water demand, and inhibiting crop productivity. Not only are these fertilizers also known to contribute toward greenhouse gas emissions as their production depends on fossil fuels, use of these chemicals

is inevitable to meet the growing demand for food in the world. Emergence of organic farming is an important area of priority in respect of the growing demand for healthy food and long-term sustainability. Biofertilizers have emerged as best alternative to synthetic fertilizers. A biofertilizer comprises living microorganisms, which on application colonizes the rhizosphere or the interior of the plant, plantlet or seed surfaces or soil, thus promoting growth by accelerating the availability of primary nutrients to the host plant. Biofertilizers comprise of microorganisms, including bacteria, fungi, cyanobacteria, and algae as well as their metabolites that are capable of enhancing soil, crop growth, and yield. Biofertilizers include symbiotic nitrogen fixers like *Rhizobium* spp. associated with leguminous crops, nonsymbiotic free-living nitrogen fixers like *Azotobacter*, which can be used for crops like maize, wheat, cotton, mustard, potato, and other vegetable crops, while *Azospirillium* is mainly used for sorghum, millets, maize, sugarcane, and wheat. Algal biofertilizers like the BGA such as *Nostoc* sp., *Anabaena* sp., *Tolypothrix* sp., *Aulosira* sp. etc., have the potential to fix atmospheric nitrogen and are used in paddy fields. Some other types include mycorrhizae, organic fertilizers, and phosphate-solubilizing bacteria. *Pantoea agglomerans*, one of the phosphate-solubilizing bacteria such as strain P5, and *Pseudomonas putida* strain P13 are capable of solubilizing the insoluble phosphate from organic and inorganic sources. The *Azolla-Anabaena* and *Rhizobium* form the most important group of biofertilizers. Biofertilizers have numerous benefits to soil quality and crop yield as they enhance nutrient transfer, increase population of beneficial microorganisms, stabilize soil aggregates, and decrease reliance on fossil fuels.

History of biofertilizers began with the launch of "Nitragin" by Nobble and Hiltner, a laboratory culture of *Rhizobium* in 1895, followed by the discovery of *Azotobacter* and BGA and some other microorganisms. Cyanobacteria have a great potential as source of fine chemicals, as a biofertilizer, as a source of renewable fuel accumulators and as degrader of different kinds of environmental pollutants including metal ions, salinity, and pesticides. The history of cyanobacteria extends about 3.5 billion years back into the Precambrian era [12]. Cyanobacteria can both photosynthesize and fix nitrogen and at the same time they can easily adapt to different soil types. The effect of cyanobacteria on rice yield was first studied in Japan in 1950s [13]. Better nitrogen fixing ability of BGA under flooded conditions has been determined when compared under dry land conditions, which might be correlated with relatively stable yield of rice under flooded condition. Key role played by cyanobacteria is maintenance and build-up of soil fertility, which further results in increasing rice growth and yield [14]. The contributions of these algae include (1) enhancement in soil porosity by a group of cyanobacteria having filamentous structure and production of adhesive substances [15]; (2) excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, and amino acids [16,17]; (3) increase in water holding capacity through their jelly structure [16]; (4) increase in soil biomass following their death and decomposition [18]; (5) decrease in soil salinity [19]; (6) prevention of weed growth [19]; and (7) increase in soil phosphate by excretion of organic acids [20]. Beneficial effects of cyanobacterial inoculation were also reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli, and lettuce [21]. Malliga [22] has reported that *Anabaena azollae* utilized as biofertilizer displayed lignolysis and released phenolic compounds, which induced profuse sporulation of the organism. This report discusses the usefulness of coir waste as carrier for cyanobacterial biofertilizer. Cyanobacteria play a spectrum of remarkable roles in the field of biofertilizer, energy production, human food, animal feed, polysaccharides, biochemical,

pharmaceutical, and changing up of the environment. The cyanobacteria provide inexpensive nitrogen to plants besides increasing crop yield by making the soil fertile and productive. BGA biofertilizer in rice popularly known as "algalization" helps in creating an environment-friendly agro-ecosystem that ensures economic viability in paddy cultivation while saving energy intensive inputs. At present data differentiating the utilization of microalgae as a biofertilizer is not enough although reports suggest that microalgae could represent a potential substitute to commercial or organic fertilizers. The University of Texas Algae Processing Program believes that microalgae may hold an important place in the future of agriculture.

3. ALGAL DIVERSITY IN PADDY FIELDS

Investigation of paddy fields in several countries like Japan, Thailand, China, Philippines, Bangladesh, and India revealed that cyanobacteria registered its dominance over other organisms [23]. Rice paddy field is differentiated into three compartments based on different physical and chemical soil condition, oxic surface, anoxic bulk, and the rhizosphere along with rhizoplane [24]. Due to high habitat diversity, a clear spatial and temporal heterogeneity can be seen, which is responsible for development of distinct ecological niches. The anoxic bulk is typically (but not exclusively) methanogenic. The oxic compartment is largely inhabited by diazotrophic cyanobacteria and dominated by *Nostoc*, *Anabaena*, and *Phormidium* [25]. Moreover, *Aulosira*, *Cylindrospermum*, *Fischerella*, *Lyngbya*, *Plectonema*, and *Stigonema* are also some frequent genera [26]. Salinity is also responsible for shifts in diversity in paddy fields; low salinity favors growth of heterocystous cyanobacteria, whereas high salinity supports growth of nonheterocystous genera [27]. Table 10.1 comprises algal species with potency in sustainable agriculture utilized as biofertilizer all over the world.

4. EFFECT ON SOIL PHYSICO-CHEMICAL AND BIOCHEMICAL PROPERTIES

Various kinds of soil indicators are used for assessing the effects of fertilizers. Some of them utilize soil physical and chemical properties whereas others focus on biochemical properties that directly reflect the size and activity of soil microbial biomass [28]. This section encompasses effects of algal biofertilizers on soil physico-chemical, biochemical properties, and indigenous soil microbial community as depicted in Fig. 10.1.

4.1 Algae as Biofertilizer Influences Soil Aggregation and Soil Porosity

Effect of surface growth of inoculated BGA on subsurface properties of a brown earth, silt loam soil was studied by Rao and Burns [29]. Significant increase in soil polysaccharides, dehydrogenase, urease, and phosphatase activities was recorded. Improvement in soil aggregation was also seen; stable soil aggregates are essential to soil fertility. Studies of Burns and Davies [30] suggested soil polysaccharides as major component responsible for soil stabilization. However, these effects were confined to surface layer of 0−0.7 cm depth. The results of Roychoudhary et al. [31] also demonstrated improvement in soil aggregation following

TABLE 10.1 Algae Used as Biofertilizer in Different Parts of the World

Major Class of Algal Biofertilizer	Species Name	Contribution	References
Brown macroalgae	*Laminaria digitata* (Oarweed), *Saccharina latissima* (Sugar Kelp), *Fucus vesiculosus* (Bladder wrack), *Ascophyllum nodosum* (Knotted wrack), *Ecklonia maxima*, *Stoechospermum marginatum*	1. Rich in nitrogen, potassium, and phosphorus 2. Carbohydrates (improve aeration and soil structure, especially in clay soils and have good moisture retention properties) 3. Used as source of naturally occurring plant growth regulators 4. Enhance plant growth, freezing, drought and salt tolerance; photosynthetic activity; and resistance to fungi, bacteria, and virus	[71–79]
Red macroalgae	*Phymatolithon calcareum* (Maerl[a]), *Lithothamnion corallioides* (Maerl[a])	Trace elements	[80,81]
Blue-green algae	*Nostoc*, *Anabaena*, *Aulosira*, *Tolypothrix*, *Nodularia*, *Cylindrospermum*, *Scytonema*, *Aphanothece*, *Calothrix*, *Anabaenopsis*, *Mastigocladus*, *Fischerella*, *Stigonema*, *Haplosiphon*, *Chlorogloeopsis*, *Camptylonema*, *Gloeotrichia*, *Nostochopsis*, *Rivularia*, *Schytonematopsis*, *Westiella*, *Westiellopsis*, *Wollea*, *Plectonema* and *Chlorogloea*	1. Fix 18–45 kg N/ha in submerged rice field 2. Produce growth-promoting substances	[32,82–84]
Anabaena Azolla association	*Anabaena azollae*	1. Fixes 40–80 kg N/ha 2. Used as green manure because of large biomass	[42,85]

[a]*UK Soil Association standards (2005) prohibit the use of Maerl from* Lithothamnium corallioides, Lithothamnium glaciale, *or* Phymatolithon calcareum, *since they are endangered species.*

incubation with BGA, which in turn provides a better water holding capacity to the soil. These findings were also supported by several other studies [32–34]. Algal proteoglycans possess adhesive properties, which can easily fasten cells to solid surfaces and thus aggregate soil particles [35]. Soil aggregation and arrangement of the soil aggregates seems very important as it directly affects temperature, aeration, and infiltration rates of the soil, which ultimately improves the physical environment of the crop [36].

Furthermore, not only soil aggregation but also soil porosity is fortified by inoculating *Nostoc* strains on clay soils due to the reduction of the damaging effects of water addition [36]. There are reports suggesting solubilization of insoluble forms of inorganic phosphate by cyanobacterial inoculation [32,37]. It was further evidenced by studies of Bose et al. [38], Cameron and Julian [39], and Roychoudhary and Kaushik [40], which advocated cyanobacterial phosphorous solubilizing activity on hydroxyapatite, tricalcium phosphate, and Mussorie rock phosphate. Apart from phosphorous, there are several evidence that witnessed an increase in N content and organic matter of soils inoculated with BGA [41–43].

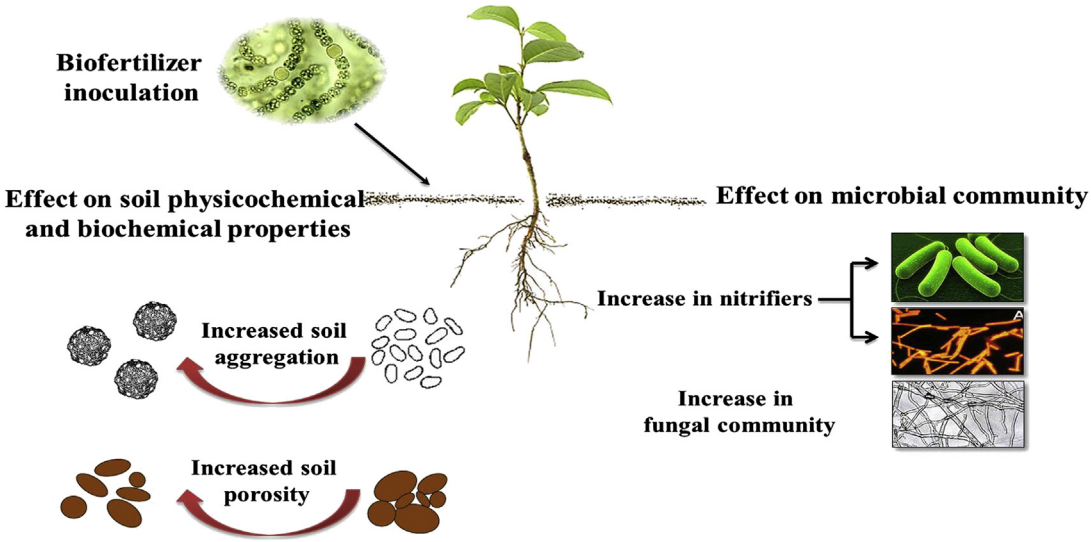


FIGURE 10.1 Effects of biofertilizers on physiological and biochemical properties of soil.

4.2 Effect on Soil pH and Chelation of Soil Elements

Soil pH is also known to be affected by algal biofertilizers. Saha and Mandal [44] reported an initial increase in soil pH, whereas contradictory to it Subhashini and Kaushik [45] reported a significant reduction not only in pH but also in hydraulic conductivity, electrical conductivity, and soil aggregation.

Cyanobacteria are also known for their ability to release trace elements from insoluble materials. Fe, Mn, and Zn are known to be influenced in rice fields by cyanobacterial growth [46]. Lange [47] reported chelation of Fe, Cu, Mo, Zn, Co, and Mn through gelatinous sheath of many cyanobacterial species. This sheath is also known to reduce particle erosion and may adsorb charged nutrient cations [26]. Thivy [48] reported that brown alga (*Sargassum*) serves as soil conditioner due to the presence of soluble alginates, which accelerates decomposition of organic matter by bacteria.

In summary, algal biofertilizers influence soil properties through soil particle aggregation, phosphate and trace element release from insoluble minerals, and N storage and its slow release.

4.3 Influence on Microbial Community

Although some workers have studied the effect of algal biofertilizer on soil microflora very little is known about the associated changes in soil microbial community following inoculation of blue-green or other algae. Rao and Burns [29] reported an eightfold increase in bacterial members in the BGA inoculated columns, whereas increase in fungal population was not significant. Ibrahim et al. [49] reported an increase in total microbial community in a pot

experiment specifically nitrifiers (genera of *Azotobacter* and *Clostridium*) after inoculation of *Tolypothrix tenuis*.

Acea et al. [50] reported greater than four logarithmic unit increases in heterotrophic bacteria, actinomycetes, algal, and fungal propagules and three logarithmic unit increases in fungal mycelia after inoculating burnt soils with cyanobacteria. Similarly, Rogers and Burns [51] reported a significant difference in the heterotrophic microbial population after inoculation of soil with *Nostoc muscorum*. These results suggest additional carbon and energy source due to cyanobacterial polysaccharides as one of the reason behind increase in heterotrophic microbial populations. Increment in total nitrogen content of inoculated soil also stimulates indigenous soil microorganisms. Nutrient status of soil specifically nitrogen and phosphorous determines the mineralization of available carbon and thus affects the microbial community [52].

5. ROLE OF ALGAE IN AMELIORATION OF SODIC SOIL

Soil salinization and sodification are the major problems associated with nearly one billion hectares of soil around the world [53] and severely affect the agricultural productivity and sustainability [54]. Therefore, concern arises to rehabilitate saline and/or sodic lands. To develop a successful strategy for amelioration of sodic soil, one should understand the term "sodicity" and "salinity." Soil has a definite capacity to adsorb cations (such as Ca^{2+}, Mg^{2+}, K^{+}, or Na^{+}) from the dissolved salts and this capacity is referred as cation exchange capacity. William Albrecht [55] formulated the recommended range of cation in soil as 60–75% Ca, 10–20% Mg, 2–5% K, 0.5–5.0% Na, and 5% other cations. When sodium makes up more than 5% of all cations adhered to the clay particles, the soil is said to be sodic. Sodic soils may be either nonsaline or saline. Sodicity can raise soil alkalinity to pH 10 and causes extensive clay dispersion, leading to poor hydraulic conductivity and reduced aeration of soil [56]. As a consequence, crop production in these soils is poor. Whereas, saline soils contain high concentrations of salts (predominantly NaCl, Na_2SO_4, and seldom $NaNO_3$) in liquid and solid phases, which results in high osmotic pressure and poor agricultural yield and productivity.

In the pursuit of new methods, which can ameliorate salt affected soils, algalization of paddy crops emerged as an effective biological remedy [57]. BGA or cyanobacteria, in general, are considerably tolerant to salinity and found to grow luxuriantly in alkaline or "Usar" soil in India [9] and saline soil of USSR [58]. The predominant forms recorded in salt-affected soil were species of *Plectonema*, *Nostoc*, *Calothrix*, *Scytonema*, *Hapalosiphon*, *Microchaete*, and *Westiellopsis* [59]. Considering that, recent years have witnessed the extensive research on cyanobacterial management of salt stress [60–63]. The studies revealed the existence of at least four major strategies that help cyanobacteria to thrive under saline environments: (1) production of extracellular polysaccharides; (2) synthesis and accumulation of osmoregulatory compounds; (3) maintenance of low internal Na^{+} (either by restricted uptake or efflux); and (4) expression of a set of salt-stress responsive proteins. It is believed that the ability of cyanobacteria to tolerate extreme of salinity also confers on them a reclamation potential in saline and alkali soils [64].

The possibility of ameliorating alkaline soils with BGA was first proposed by Singh [9,32]. Later, Kaushik and his group [65] strengthened this assumption through a series of

experiments. Reclamation of sodic soil requires displacement of sodium from the exchangeable site of clay particles by more useful cation such as Ca. The most effective and conventionally used method for this is addition of gypsum ($CaSO_4.2H_2O$) followed by leaching of replaced Na^+ from root zone through excess irrigation. Kaushik and Subhashini [57] reported that application of a mixture of BGA viz. *Calothrix braunii*, *Hapalosiphon intricatus*, *Scytonema tolypothricoides*, and *Tolypothrix ceylonica* for a period of about 105 days is equally efficient in reclamation of sodic soil as gypsum. Kaushik [64] also noted that for reclamation of highly sodic soils, single treatment of cyanobacteria may not be sufficient and require at least three subsequent treatments for a period of three years. However, combined application of BGA and 50% gypsum can give even better results. Apte and Thomas [65] reported the amelioration of moderately saline "Kharland soil" (soil conductivity 5−8.5 dSm^{-1}) by application of halotolerant cyanobacterium *Anabaena torulosa*. In laboratory as well as field experiments, algalization has proven to bring substantial reduction in pH, electrical conductivity, and exchangeable Na [32,45,57,65]. These changes improve chemical properties of salt-affected soil toward neutrality and resume crop vigor and yield. In addition, algalization is a low-cost technology that simultaneously enhances nutrient availability (C and N status of the soil), improves soil aggregation status, and hydraulic conductivity [32,45,66]. The precise mechanism by which BGA alleviate excess of sodium from soil is not known, but there are different theories claiming the possible mechanism: (1) BGA produce organic acids through microbial decomposition of organic matter, which react with calcium carbonate (invariably present in alkali soil) and release calcium. This allows the exchange of Ca with Na in the exchange complex [32,66]. (2) The extracellular polysaccharides production is enhanced several fold under salt stress, which chelate or temporarily immobilize excess Na^+ present in sodic soil [64,65,67].

In contrast, studies of Bhardwaj and Gupta [68] and Rao and Burns [29] do not support the above contention. Rao and Burns [29] reported that BGA are ineffective in amelioration of sodic soil primarily because of their inability to mobilize Ca and therefore, are not comparable with the chemical amendments such as gypsum. Therefore, in the light of available literature BGA could be the potential targets for future research to explore their potential in bioreclamation of sodic soil.

6. GENETICALLY MODIFIED ALGAE WITH POTENTIAL IN SUSTAINABLE AGRICULTURE

BGA are the most successful organism surviving under several abiotic stresses, which are the major constrain in crop productivity. Researchers have modified the genome of algae found in paddy fields to enhance their nitrogen fixing ability as well as stress tolerance potential, which further adds to the sustainable agriculture vision. Machete and Basalin are the common herbicides applied in paddy fields. These herbicide tolerant genes from an aerobic diazotrophic *Gloeocapsa* strain introduced into the genome of *Nostoc muscorum* provide herbicide resistance and hence possess considerable implications in biofertilizer technology [69]. Chaurasia et al. [70] reported the overexpression of *hetR* gene responsible for heterocyst differentiation in *Anabaena* sp. strain PCC7120, which resulted in the formation of multiple heterocysts and enhanced nitrogenase activity, hence better nitrogen fixation ability.

7. CONCLUSION AND FUTURE PERSPECTIVES

The present chapter concludes that algal species have great potential in biofertilizer technology in terms of cost effectiveness, eco-friendly soil binders, amelioration of sodic soil, as well as their natural occurrence in the paddy fields. Furthermore, the secretion of exopolysaccharides and bioactive substances by algae and cyanobacteria has proven role in recovering soil nutrients and mobilization of insoluble forms of inorganic phosphates. Development of genetically modified stress-tolerant cyanobacteria has better prospect as biofertilizer and transfer of stress coping genes into crop plants has a great future worth investing by the researchers.

Acknowledgment

L. C. Rai is thankful to ICAR, CSIR, DST for the projects and J. C. Bose National Fellowship. A. Chatterjee and S. Yadav thank the University Grants Commission for Junior Research Fellowship, S. Singh is thankful to Department of Science & Technology for Women Scientist (WOS-A) and C. Agrawal thanks the Department of Biotechnology for Senior Research Fellowship.

References

[1] C.N.B. Bambaradeniya, F.P. Amerasinghe, Biodiversity Associated with the Rice Field Agroecosystem in Asian Countries: A Brief Review, International Water Management Institute, Colombo, Sri Lanka, 2003. Working Paper 63.
[2] V. Galhano, G.J. Laranjo, E.F. Valiente, R. Videira, F. Peixoto, Impact of herbicides on non-target organisms in sustainable irrigated rice production systems: state of knowledge and future prospects, in: A. Kortekamp (Ed.), Herbicides and Environment, Intech, Croatia, 2011, pp. 45–72.
[3] N.K. Fageria, The Role of Plant Roots in Crop Production, CRC Press, Boca Raton, 2013.
[4] N. Borrell, M.J. Figueras, J. Guarro, Phenotypic identification of *Aeromonas* genomospecies from clinical and environmental sources, Can. J. Microbiol. 44 (1998) 7–12.
[5] A. Borrell, G. Hammer, E. Van Oosterom, Stay-green: a consequence of the balance between supply and demand for nitrogen during grain filling, Ann. Appl. Biol. 138 (2001) 91–95.
[6] X.M. Lian, Y.Z. Xing, H. Yan, C.G. Xu, X.H. Li, Q.F. Zhang, QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid, Theor. Appl. Genet. 112 (2005) 85–96.
[7] N.K. Fageria, V.C. Baligar, C.A. Jones, Growth and Mineral Nutrition Field Crops, CRC Press, Boca Raton, 2011.
[8] N.K. Fageria, V.C. Baligar, A.B. Heinemann, M.C.S. Carvalho, Nitrogen uptake and use efficiency in rice, in: A. Rakshit, H. Bahadur Singh, A. Sen (Eds.), Nutrient Use Efficiency: From Basics to Advances, Springer, India, 2015, pp. 285–296.
[9] R.N. Singh, Reclamation of "usar" lands in India through blue-green algae, Nature 165 (1950) 325–326.
[10] G.S. Venkataraman, Algal inoculation in rice field, in: Nitrogen and Rice Symposium Proceedings, International Rice Research Institute, Los Banos, Philippines, 1979.
[11] C.P. Wolk, Heterocyst formation, Annu. Rev. Genet. 30 (1996) 59–78.
[12] J.W. Schopf, Fossil evidence of Archaean life, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 361 (2006) 869–885.
[13] U. Mishra, S. Pabbi, Cyanobacteria: a potential biofertilizer for rice, Resonance 9 (2004) 6–10.
[14] T. Song, L. Martensson, T. Eriksson, W. Zheng, U. Rasmussen, Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China, FEMS Microbiol. Ecol. 54 (2005) 131–140.
[15] D. Sahu, I. Priyadarshani, B. Rath, Cyanobacteria—as potential biofertilizer, CIBTech J. Microbiol 1 (2012) 20–26.
[16] P.A. Roger, P.A. Reynaud, Free-living blue-green algae in tropical soils, in: Y. Dommergues, H. Diem (Eds.), Microbiology of Tropical Soil and Plant Productivity, MartinusNijhoff Publisher, La Hague, 1982.
[17] A.A. Rodriguez, A.M. Stella, M.M. Storni, G. Zulpa, M.C. Zaccaro, Effects of cyanobacterial extracelular products and gibberellic acid on salinity tolerance in *Oryza sativa* L, Saline Syst. 2 (2006) 7.

[18] H. Saadatnia, H. Riahi, Cyanobacteria from paddy-fields in Iran as a biofertilizer in rice plants, Plant Soil Environ. 55 (2009) 207–212.
[19] E.A. Al-Sherif, M.S. Abd El-Hameed, M.A. Mahmoud, H.S. Ahmed, American-Eurasian J. Agric. Environ. Sci. 15 (2015) 794–799.
[20] L.T. Wilson, Cyanobacteria: a potential nitrogen source in rice fields, Tex. Rice 6 (2006) 9–10.
[21] N. Thajuddin, G. Subramanian, Cyanobacterial biodiversity and potential applications in biotechnology, Curr. Sci. 89 (2005) 47–57.
[22] P. Malliga, L. Uma, G. Subramanian, Lignolytic activity of the cyanobacterium *Anabaena azollae* ML2 and the value of coir waste as a carrier for biofertilizer, Microbios 86 (1996) 175–183.
[23] P.A. Roger, S.A. Kulasooriya, Blue-green Algae and Rice, The International Rice Research Institute, Los Banos, Philippines, 1980.
[24] W. Liesack, S. Schnell, N.P. Revsbech, Microbiology of flooded rice paddies, FEMS Microbiol. Rev. 24 (2000) 625–645.
[25] S. Nayak, R. Prasanna, T.K. Dominic, P.K. Singh, Floristic abundance and relative distribution of different cyanobacterial genera in rice field soil at different crop growth stages, Phykos 40 (2001) 15–22.
[26] B.A. Whitton, Soils and rice-fields, in: B.A. Whitton, M. Potts (Eds.), The Ecology of Cyanobacteria: Their Diversity in Time and Space, Springer, Netherlands, 2000, pp. 233–255.
[27] A.K. Srivastava, P. Bhargava, A. Kumar, L.C. Rai, B.A. Neilan, Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India, Saline Syst. 5 (2009) 4.
[28] G.P. Sparling, Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter, Aust. J. Soil Res. 30 (1992) 195–207.
[29] D.L.N. Rao, R.G. Burns, The effect of surface growth on blue-green algae and bryophytes on some microbiological, biochemical and physical soil properties, Biol. Fert. Soils 9 (1991) 239–244.
[30] R.G. Burns, J.A. Davics, The microbiology of soil structure, Biol. Agric. Hortic. 3 (1986) 95–113.
[31] P. Roychoudhury, B.D. Kaushik, G.S.R. Krishnamurthy, G.S. Venkataraman, Effect of blue-green algae and *Azolla* application on the aggregation status of the soil, Curr. Sci. 48 (1979) 454–455.
[32] R.N. Singh, Reclamation of Usar Lands. "Role of Blue-green Algae in Nitrogen Economy of Indian agriculture", Indian Council of Agricultural Research, New Delhi, 1961.
[33] D.A. Bailey, A.P. Mazurak, J.R. Rosowski, Aggregation of soil particles by algae, J. Phycol. 9 (1973) 99–101.
[34] B. de Winder, J. Pluis, L. de Reus, L.R. Mur, Characterization of a cyanobacterial, algal dune crust in the coastal dunes of The Netherlands, in: Y. Cohen, E. Rosenberg (Eds.), Microbial Mats, Physiological Ecology of Benthic Microbial Communities, American Society for Microbiology, Washington, DC, 1989, pp. 77–83.
[35] A. Flaibani, Y. Olsen, T.J. Painter, Polysaccharides in desert reclamation: composition of exocellular proteoglycan complexes produced by filamentous blue-green and unicellular green edaphic algae, Carbohydr. Res. 190 (1989) 235–248.
[36] L. Falchini, E. Sparvoli, L. Tomaselli, Effect of *Nostoc* (cyanobacteria) inoculation on the structure and stability of clay soils, Biol. Fert. Soils 23 (1996) 246–252.
[37] K.T. Kleiner, K.T. Harper, Soil properties in relation to cryptogamic ground cover in Canyonlands National Park, J. Range Manage. 30 (1977) 202–205.
[38] P. Bose, U.S. Nagpal, G.S. Venkataraman, S.K. Goyal, Solubilization of tricalcium phosphate by blue-green algae, Curr. Sci. 7 (1971) 165–166.
[39] H.J. Cameron, G.R. Julian, Utilization of hydroxyapatite by cyanobacteria as their sole source of phosphate and calcium, Plant Soil 109 (1988) 123–124.
[40] P. Roychoudhury, B.D. Kaushik, Solubilization of Mussorie rock phosphate by cyanobacteria, Curr. Sci. 58 (1989) 569–570.
[41] A.L. Singh, P.L. Singh, Nitrogen Fixation in Indian Rice Fields (*Azolla* and Blue-green Algae), Agro-Botanical Publishers, Bikaner, India, 1989.
[42] A. Vaishampayan, R.P. Sinha, D.P. Hader, T. Dey, A.K. Gupta, et al., Cyanobacterial biofertilizers in rice agriculture, Bot. Rev. 67 (2001) 453–516.
[43] G.S. Venkataraman, Blue-green algae (cyanobacteria), in: S.N. Tata, A.M. Wadhwani, M.S. Mehdi (Eds.), Biological Nitrogen Fixation, Indian Council of Agricultural Research, New Delhi, 1993, pp. 45–76.
[44] K.C. Saha, L.N. Mandal, Effect of algal growth on the availability of phosphorus, iron and manganese in rice soil, Plant Soil 52 (1979) 139–146.

[45] D. Subhashini, B.D. Kaushik, Amelioration of sodic soils with blue-green algae, Aust. J. Soil Res. 19 (1981) 361–366.
[46] S.C. Das, M. Mandal, L.N. Mandal, Effect of growth and subsequent decomposition of blue-green algae on the transformation of iron and manganese in submerged soils, Plant Soil 138 (1991) 75–84.
[47] W. Lange, Speculations on a possible essential function of the gelatinous sheath of blue-green algae, Can. J. Microbiol. 22 (1976) 1181–1185.
[48] F. Thivy, Seaweed manure for perfect soil and smiling fields, Salt Res. Ind. 1 (1964) 1–4.
[49] A.N. Ibrahim, M. Kamel, M. El-sherbeny, Effect of inoculation of alga *Tolypothrix tenuis* on the yield of rice and soil nitrogen balance, Agrokem.Talajtan 20 (1971) 389–400.
[50] M.J. Acea, N. Diz, A. Prieto-Fernández, Microbial populations in heated soils inoculated with cyanobacteria, Biol. Fert. Soils 33 (2001) 118–125.
[51] S.L. Rogers, R.G. Burns, Changes in aggregate stability, nutrient status, indigenous microbial populations, and seedling emergence, following inoculation of soil with *Nostoc muscorum*, Biol. Fert. Soils 18 (1994) 209–215.
[52] T.H. Anderson, T.R.G. Gray, The influence of soil organic carbon on microbial growth and survival, in: W.S. Wilson (Ed.), Advances in Soil Organic Matter Research: The Impact on Agriculture and the Environment, Royal Society of Chemistry, Cambridge, 1991, pp. 253–266.
[53] J.D. Rhoades, A. Kandiah, A.M. Mashali, The Use of Saline Water for Crop Production-FAO Irrigation and Drainage Paper 48, FAO, Rome, 1992.
[54] L. Bernstein, Effects of salinity and sodicity on plant growth, Annu. Rev. Phytopathol. 13 (1975) 295–312.
[55] W.A. Albrecht, The Albrecht Papers Volume I, Foundation Concepts.Acres, Kansas City USA, 1975.
[56] R. Gupta, I.P. Abrol, Salt affected soils: their reclamation and management for crop production, Adv. Soil Sci. 9 (1990) 223–286.
[57] B.D. Kaushik, D. Subhashini, Amelioration of salt affected soils with blue green algae: II, Improvement Soil Properties Proc. Indian Nat. Sci. Acad. B 51 (1985) 386–389.
[58] M.M. Gollerbach, L.N. Novichkova, N.V. Sdubrikova, The Algae of takyrs.-Takyrs of Western Turkmenia and Routes of Their Agricultural Conquest, Nauk, Moscow, 1956.
[59] B.D. Kaushik, Cyanobacteria—some aspects related to their role as ameliorating agent of salt effected soils, in: V.N. Raja Rao (Ed.), Prospective of Phycology, Today and Tomorrow publication, New Delhi, 1990, p. 404.
[60] S. Ozturk, B. Aslim, Modification of exopolysaccharide composition and production by three cyanobacterial isolates under salt stress, Environ. Sci. Pollut. Res. Int. 17 (2010) 595–602.
[61] M. Hagemann, Molecular biology of cyanobacterial salt acclimation, FEMS Microbiol. Rev. 35 (2011) 87–123.
[62] S. Klähn, M. Hagemann, Compatible solute biosynthesis in cyanobacteria, Environ. Microbiol. 13 (2011) 551–562.
[63] S. Rai, C. Agrawal, A.K. Shrivastava, P.K. Singh, L.C. Rai, Comparative proteomics unveils cross species variations in *Anabaena* under salt stress, J. Proteomics 98 (2014) 254–270.
[64] B.D. Kaushik, Reclamation of Salt Affected Soil through Cyanobacteria. "Advances in Microbiology, IARI, New Delhi, 2005.
[65] S.K. Apte, J. Thomas, Possible amelioration of coastal soil salinity using halotolerant nitrogen-fixing cyanobacteria, Plant Soil 189 (1997) 205–211.
[66] P.K. Singh, S. Kishore, J. Prakash, S.K. Singh, M. Shukla, Cyanophycean algae inhabiting sodic soil exhibit diverse morphology: an adaptation to high exchangeable sodium, Ecoprint 15 (2008) 15–21.
[67] M.N. Jha, G.S. Venkataraman, B.D. Kaushik, Response of *Westiellopsis prolifica* and *Anabaena* sp. to salt stress, World J. Microbiol. Biotechnol. 3 (1987) 307–317.
[68] K.K.R. Bhardwaj, I.C. Gupta, Effect of Algae on the Reclamation of Salt Affected Soils. Annual Report, CSSRI, Karnal, 1971.
[69] D.T. Singh, K. Nirmala, D.R. Modi, S. Katiyar, H.N. Singh, Genetic transfer of herbicide resistance gene(s) from *Gloeocapsa* spp. to *Nostoc muscorum*, Mol. Gen. Genet. 208 (1987) 436–438.
[70] A.K. Chaurasia, A. Parasnis, S.K. Apte, An integrative expression vector for strain improvement and environmental applications of the nitrogen fixing cyanobacterium, *Anabaena* sp. strain PCC7120, J. Microbiol. Methods 73 (2008) 133–141.
[71] S.S. Ramya, N. Vijayanand, S. Rathinavel, Foliar application of liquid biofertilizer of brown alga *Stoechospermum marginatum* on growth, biochemical and *yield of Solanum melongena*, Int. J. Recycl. Org. Waste Agric. 4 (2015) 167–173.

[72] A. Eris, H.Ö. Sivritepe, N. Sivritepe, The effect of seaweed (*Ascophyllum nodosum*) extract on yield and quality criteria in peppers, in: R. Fernández-Muñoz, J. Cuartero, M.L. Gómez-Guillamón (Eds.), International Symposium on Solanacea for Fresh Market 412, vol. I, ISHS ActaHorticulturae 412, Malaga, Spain, 1995, pp. 185–192.
[73] J. Norrie, J.P. Keathley, Benefits of *Ascophyllum nodosum* marine-plant extract applications to 'Thompson seedless' grape production, ActaHortic 727 (2006) 243–247.
[74] H.S. Sharma, C. Fleming, C. Selby, J.R. Rao, T. Martin, Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses, J. Appl. Phycol. 26 (2014) 465–490.
[75] M.J. Stadnik, M.B. De Freitas, Algal polysaccharides as source of plant resistance inducers, Trop. Plant Pathol. 39 (2014) 111–118.
[76] J.S. Craigie, Seaweed extract stimuli in plant science and agriculture, J. Appl. Phycol. 23 (2011) 371–393.
[77] S.T. Zodape, Seaweeds as a biofertilizer, J. Sci. Ind. Res. 60 (2001) 378–382.
[78] I.J. Crouch, J. van Staden, Evidence for the presence of plant growth regulators in commercial seaweed products, J. Plant Growth Regul. 13 (1993) 21–29.
[79] G. Blunden, Agricultural uses of seaweeds and seaweed extracts, in: M.D. Guiry, G. Blunden (Eds.), Seaweed Resources in Europe: Uses and Potential, vol. II, John Wiley & Sons, Chichester, 1991, pp. 65–81.
[80] D.J. McHugh, A Guide to the Seaweed Industry, FAO Fisheries Technical Paper, Rome, 2003.
[81] A.W. Smit, What Is the Potential of Marine Algae, in Combination with Sewage Sludge, as a Composite of Biofertilizer?, NIVA-rapport, 5895, Norwegian Institute for water research, Norway, 2009.
[82] I. Watanabe, W. Cholikul, Field studies on nitrogen fixation in paddy soil, in: Nitrogen and Rice Symposium Proceedings, IRRI, Philippines, 1979, pp. 223–239.
[83] A. Vaishampayan, Physiological responses of genetically improved nitrogen-fixing cyanobacteria to agrochemicalization in relation to paddy culture: prospects as a source material for engineering herbicide sensitivity and resistance in plants, in: A. Hemantaranjan (Ed.), Advances in Plant Physiology, vol. I, Scientific Publishers, Jodhpur, India, 1998, pp. 191–220.
[84] P.K. De, The role of blue-green algae in nitrogen fixation in rice fields, Proc. R. Soc. B. 127 (1939) 121–139.
[85] A.W. Moore, *Azolla*: biology and agronomic significance, Bot. Rev. 35 (1969) 17–34.

CHAPTER

11

Modeling and Technoeconomic Analysis of Algae for Bioenergy and Coproducts

A.D. Kroumov, F.B. Scheufele, D.E.G. Trigueros, A.N. Modenes, M. Zaharieva, H. Najdenski

The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria

OUTLINE

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00011-4

1. INTRODUCTION

Development of any technology requires many research and scientific efforts during the scale-up and scale-down process development. Green technologies based on microalgae biomass as an alternative source of third-generation renewable energy (biofuels) is very promising and challenging. Production of algae biomass for biofuels—biodiesel, bioethanol, and biomethane—requires large-scale culturing systems, where many unit operations are involved. Unfortunately, nowadays bioenergy based on algae biomass cannot compete with fossil fuels. Hence, innovative approaches need to be applied in this field during all the steps of technology development and transfer to the industrial production. Mathematical modeling of algae growth kinetics and photobioreactors (PBRs) optimization and design are essential steps in development of cost-effective biofuels from microalgae biomass. There is great potential if algae lipids, proteins, and carbohydrates biomass are fully utilized. Technical-economical evaluation of mass culturing algae system is evitable and must be included in the loop procedure of system optimization, design, and realization in the industrial scale. This chapter presents an overview of modeling and technical-economical evaluation in the state of art of algae culturing system and the integrated use of the microalgae biomass and coproducts.

2. MODELING OF ALGAE PROCESSES FOR BIOENERGY AND COPRODUCTS

Bioindustry is presently engaged in developing new products and testing a new generation of algal-derived natural products of interests for human health, food, and others for energy production such as polyunsaturated fatty acids and polysaccharides. An especially challenging issue is to develop a cost-effective algae technology for CO_2 fixation and production of algae biomass for biofuels. Such technology is a complex chain formed by many unit

operations. Most important is the understanding of algae physiology and how this physiology can be optimized and used being covered with engineering specifications and approaches in order to meet the final goal of technology realization. A brilliant example of how to utilize theoretical achievements through engineering solutions are works of Wu et al. and Vunjak-Novakovic et al. [1,2] (see Fig. 11.1).

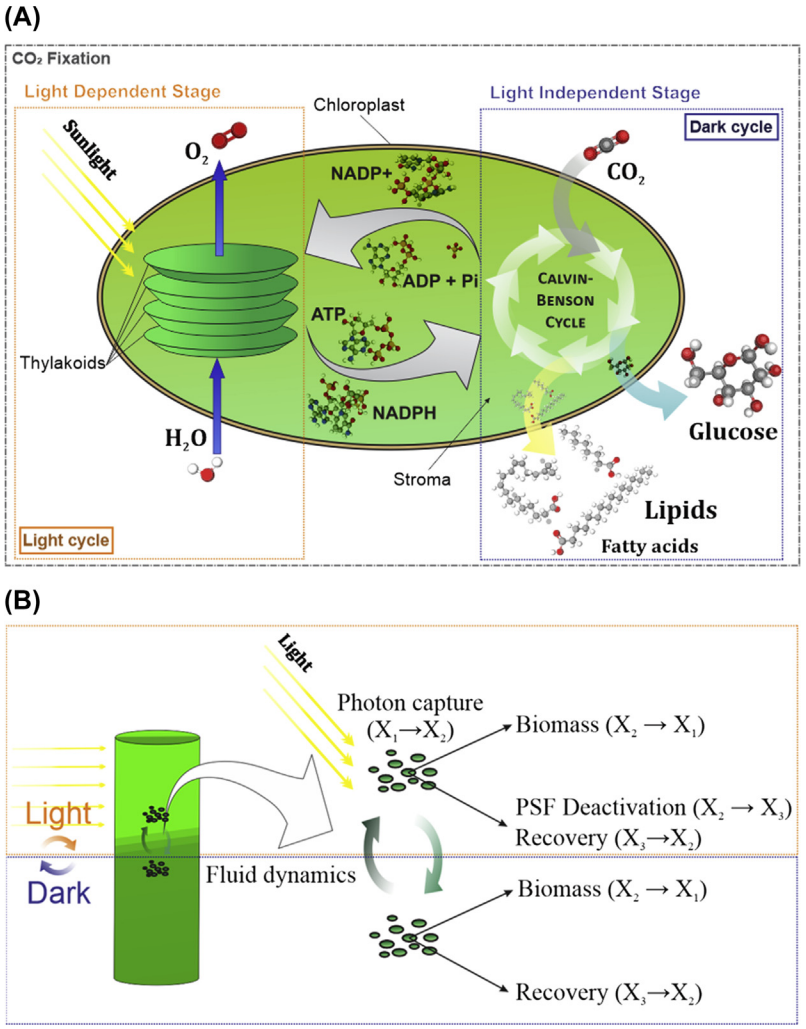


FIGURE 11.1 (A) Scheme of the photosynthesis process of microalgae. The environmental conditions and PBR design can be of any kind only to ensure unlimited microalgal growth. (B) Simple schematic representation of the interaction of light illumination and the fluid dynamics in a photosynthetic cell culture. *(B) Adapted from G. Vunjak-Novakovic, Y. Kim, X. Wu, I. Berzin, J.C. Merchuk. Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies, Ind. Eng. Chem. Res. 44 (2005) 6154–6163.*

By analyzing Fig. 11.1A one may recognize photosynthetic process of CO_2 fixation (e.g., from flue gas) as a two-stage/-phase process. The detailed description of the kinetic model was firstly published elsewhere [3]. Their dynamic three-state model describes the photosynthetic processes with photoinhibition and recovery from photoinhibition and is widely used for modeling microalgal kinetics in PBRs. In Fig. 11.1B, the energy for this process is provided by light, which is absorbed by pigments (primarily chlorophylls and carotenoids). Light energy is absorbed in light reactions of phase I (Light Dependent Stage), stored in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) and utilized for biomass production in dark reactions of phase II (Light Independent Stage). In light reactions, water is hydrolyzed and oxygen evolves. In dark reactions, CO_2 is incorporated into the cell components (biomass) via a Calvin–Benson cycle. The phenomenon of light/dark (L/D) cycles is successfully used to build high-efficient closed PBRs where hydrodynamic conditions are precisely controlled [1,2]. Further, the authors showed how to model microalgal kinetics in an actual tubular PBR [1,2]. Fig. 11.1B represents a block scheme view of Fig. 11.1A, where the PBR volume was divided into two regions, one dark and one illuminated. Photons are captured by the cells in the illuminated region by open photosynthetic factory (PSF) (state X_1), wherein both photosynthesis (transfer to state X_2) and PSF deactivation (transfer to state X_3) can take place. The cells are cyclically transported to the dark zone, where PSF recovery occurs. The kinetic model of the photosynthesis by algae cells [Eqs. (11.1)–(11.4)] is applied and experimentally verified by Wu and Merchuk [4]:

$$\frac{dx_1}{dt} = -\alpha I x_1 + \gamma x_2 + \delta x_3 \tag{11.1}$$

$$\frac{dx_2}{dt} = \alpha I x_1 - \gamma x_2 - \beta x_2 \tag{11.2}$$

$$x_1 + x_2 + x_3 = 1 \tag{11.3}$$

$$\mu = k\gamma x_2 - Me \tag{11.4}$$

where I is the incident light illumination (constant in this case) [$\mu E/m^2h$]; x_1, x_2, and x_3 the fractions of PSF in the open state, closed state, and inhibited state, respectively [dimensionless]; X the cell concentration [10^6 cells/cm^3]; X_f the final biomass concentration [10^6 cells/cm^3]; α the kinetic constant for $x_1 \rightarrow x_2$ [$m^2/\mu E$]; β the kinetic constant for $x_2 \rightarrow x_3$ [$m^2/\mu E$]; γ the kinetic constant for $x_2 \rightarrow x_1$ [1/h]; k the kinetic constant for growth [$\mu E/m^2h$]; μ the specific growth rate [1/h]; and Me the maintenance term (coefficient) [1/h].

2.1 Photosynthetic Factory

The authors assumed that the algae growth was a function of incident light (I_0), because nutrient saturation was achieved, and light was the only variable where four occurring simultaneous stages had been distinguished [1,2]:

1. Photon capture starting the chain of successive fast and slow biochemical reactions resulting in biomass synthesis (in terms of PSF, it would be indicated by $X_1 \rightarrow X_2$);
2. Initiation of the chain of dark reactions ($X_2 \rightarrow X_1$);

3. Reversible loss of photon trap activity due to high light intensity (photoinhibition) ($X_2 \rightarrow X_3$);
4. Photon trap recovery ($X_3 \rightarrow X_1$).

While the processes described in stages (1) and (3) (see Fig. 11.1B) occur only during the light period, steps (2) and (4) do not need light to proceed. The observed growth rate is the result of the integration of all of the processes described above over the history of the culture. Wu and Merchuk [1] slightly modified the original model of Eilers and Peeters [3] where a negative production rate at any photon flux density (PFD) is not accounted for. They took this fact into consideration by adding a term of the maintenance. Because of the difficulty in describing the maintenance process mathematically, Lee and Pirt [5] used *Me* as a constant.

Note: This assumption is usually acceptable if the biological material is in the growth phase and no secondary metabolite is being produced. Nevertheless, if the system produces a growth-associate secondary metabolite, the model can still be valid [4]. Further, the authors solved the dynamic model analytically and fitted the experimental data under different scenarios of light illumination conditions. The usefulness of their findings was systematically proven experimentally by other authors for different PBRs with L/D cycle conditions. The main conclusion from their work is that the proposed approach and the kinetics model can be applied for wide range of photosynthetic microalgal species with similar pathways of energy transfer. Their simulation and experimental results have shown that

- the specific growth rate (SGR) always increased with an elongation of illumination time at low photon flux intensity;
- the SGR eventually reached a constant value at moderate (PFD); and
- the SGR reached a maximum and then decreased at high illumination.

The incident light was used in their study, but the authors predicted how to represent the light illumination conditions when the PBR diameter increased. In the real industrial PBRs, position of the microalgae cells in the PBR is time dependent. Consequently, the light availability is also time dependent, thus $I_0(t)$ should be used, where $I_0(t)$ stands for the illumination history of the cells. In addition, it is noticeable that the hydrodynamics influences the $I_0(t)$ and cells trajectories, as well. On this base, by using appropriate mathematical modeling of the PBR and taking into account such characteristic one is able to calculate the PBR productivity more accurately. Hence, optimization and design of PBR can be robustly performed.

The influence of light availability as a main parameter of growth was studied very intensively. Moreover, the utilization of light by algae has to be analyzed at the macro- (population) and micro- (molecular) levels, and their interactions should be analyzed as well. This energy source needs special attention. At the micro level, light utilization is related to algal pigments located in chloroplasts [6]. The identification of pigments and their light absorption spectra are key factors for the selection of a light source and material used for PBR columns. Classification of algae pigments and their distribution in chloroplast can be found elsewhere [6–8]. In addition, energy transfer pathway is described by Lee [7]. Absorption spectrum and wavelength range associated with the different algae pigments can be found in the works of authors [6,7,9]. Physiological stress of algae under high light intensity should be done experimentally for the high biomass concentrations. Theoretical consideration of this adaptation can be found in [7,9], and the corresponding changes of cell composition are studied by Lee and Palsson

[10]. Light (photons) intake efficiency of photosynthetic algal cells can be determined theoretically. Light penetration into PBR algal suspension depends on the biomass concentration, which can be explained by Lambert–Beer law. Based on this, at low algae concentration, light intensity requirements must be low. On the other hand, under the higher biomass concentrations the requirements for light intensity should be high due to mutual shading. In this context, the PBR design and definition of working conditions, such as mixing properties for determination of L/D cycles [11–13] are function of high biomass concentration requirements of light. Design of flat plate PBRs is well documented in the literature [14].

For example, in bubble columns, effect of mixing conditions varies due to bubble size. When bubble size is small, more light is reflected or scattered (does not penetrate deeper) than when bubble size is large [15]. When small bubbles are deeply located in the system, the reflection helps in maximizing the light utilization. However, if they are located close to the walls of the system, light would not be able to penetrate deeper, but rather would be reflected back out of the PBR as it is reflected by the bubbles. These observations on the macro level show the importance of understanding the micro- and macro-level interactions. Photoinhibition is another phenomenon that has to be modeled during the photosynthetic process. When algae receive more photons than needed, the antenna structure may get damaged. In real applications, this phenomenon is well studied [4,13,16]. The chlorophyll a, accessory pigments (chlorophyll b; chlorophyll c; chlorophyll d; carotenoids; phycobilisomes—phycoerythrin, phycocyanin, and allophycocyanin), and xanthophylls are the photosynthetic machineries responsible for capturing and transferring the light energy in the form of ATP and NADPH. The second phase is the incorporation of CO_2 into the cells via Calvin–Benson cycle (see Fig. 11.1A).

Furthermore, glucose utilization and metabolic rates of intermediates must be interpreted in connection with the cell components synthesis (protein, lipids, and carbohydrates). All this knowledge has to be used as a tool to interpret algal growth and response to the changes of environmental conditions [17] and to build the kinetics model on the population level [1,2].

2.2 Algae "Pure" Kinetics on CO_2 and/or Real Flue Gas

This is a key point to the overall understanding, description, development, and control of the system behavior. In this step, two fundamental strategies and considerations must be distinguished: (1) development of "pure" kinetics on CO_2 from air and (2) development of real algal growth kinetics on CO_2 from flue gas.

1. The development of "pure" kinetics depends on CO_2 from air (0.04%) as well as from the concentration of flue gas (15% CO_2). Note: Under "pure" kinetics, the authors regard to a system in which limitation and other effects of hydrodynamics and mass transfer on microalgal physiology can be neglected [18].

   The determination of "pure" kinetics of algal growth and the algal tolerance to high CO_2 contents in the gas phase (up to 20%) in lab scale is an essential step for the process development and PBR scale-up procedures, because it includes an understanding of the water chemistry when there is bubbling flue gas or air is enriched with CO_2 in a cultural medium. These conditions allow studying of microalgal species' tolerance to high CO_2 concentrations and CO_2 aqueous species' uptake mechanisms in algae.

2. The development of real algal growth kinetics on CO_2 from flue gas (15% CO_2 and 6% O_2 and SO_2 up to 2000 ppm); algae tolerance to flue gas components (especially to SO_2, NO, HCl, SO_3 high content). Note: Under real algal growth kinetics, the authors consider experiments performed in a particular PBR vessel where limitation and other effects of hydrodynamics and mass transfer cannot be neglected.

   Here, important issues are CO_2 uptake from flue gas and how CO_2 is dissolved in the liquid phase as a function of pH. Photoautotrophic metabolism of SO_2, SO_3, and NO ionic forms in the liquid phase should also be taken into account. For most photoautotrophic fresh and sea water algae, growth on inorganic carbon (CO_2) occurs in the pH range of 5.5–11 [19,20].

2.3 $CO_{2(aq)}$ Concentrating Mechanism in Algae

The optimal photosynthesis of most microalgae occurs in the range of pH between 6 and 10, where the bicarbonate form dominates. Hence, it is crucial to understand the CO_2 concentrating mechanism (CCM) in algae by engineers in its general form and by plant physiologists in its detailed form. Recent advances about HCO_3^- uptake from fresh microalgae can be found elsewhere [21].

A simple schematic representation of HCO_3^- and CO_2 uptake in the algal cell without localization of carbonic anhydrase can be found in the work of Giordano et al. [22]. In addition, an excellent detailed analysis of CCM in cyanobacteria can also be found [23]. There are many excellent review articles discussing in detail CCM and carbonic anhydrase (CA) forms (α–CA, β–CA, and γ–CA, localization in plant cells and algae) [24].

The conclusion on CA involvement in CCM is that for practical applications, such as medium optimization for algal high biomass concentration, it is important to understand and interpret CA action in active transport of HCO_3^- and CO_2 uptake, photosystem I and photosystem II (PSI and PSII systems), and ATP involvement in this process. The strain's ability to grow fast at a high pH is expected to ensure a huge advantage when applied to flue gases.

Modeling of algal growth kinetics on CO_2 from flue gas (15% CO_2 and 6% O_2) includes understanding on dissociation of main components in the liquid phase and algae tolerance to high content of SO_3, SO_2, and NO_x as gases of interest. Water chemistry in solution of the main flue gas constituents are components of crucial importance when culturing algae on flue gas. The speciation of CO_2 and SO_2 in water are given by Eqs. (11.i)–(11.iv) and Eqs. (11.v)–(11.ix), respectively.

$$CO_{2(aq)} + H_2O \overset{K_{c,1}}{\leftrightarrow} H_2CO_3 \tag{11.i}$$

$$CO_{2(aq)} + OH^- \overset{K_{c,2}}{\leftrightarrow} HCO_3^- \tag{11.ii}$$

$$H_2CO_3 \overset{K_{f,c1}}{\leftrightarrow} HCO_3^- + H^+ \tag{11.iii}$$

$$HCO_3^- \overset{K_{f,c2}}{\leftrightarrow} CO_3^{2-} + H^+ \tag{11.iv}$$

$$SO_{2(aq)} + H_2O \overset{K_{s,1}}{\leftrightarrow} H_2SO_3 \tag{11.v}$$

$$H_2SO_3 + SO_2 \overset{K_{s,2}}{\leftrightarrow} H_2SO_4 \tag{11.vi}$$

$$SO_{2(aq)} + OH^- \overset{K_{s,2}}{\leftrightarrow} HSO_3^- \tag{11.vii}$$

$$H_2SO_4 \overset{K_{f,s1}}{\leftrightarrow} HSO_3^- + H^+ \tag{11.viii}$$

$$HSO_3^- \overset{K_{f,s2}}{\leftrightarrow} SO_3^{2-} + H^+ \tag{11.ix}$$

The chemical equilibrium in solution for such species is shown and described elsewhere [25–27]. It must be noted that the knowledge about the water chemistry of flue gas is crucially important, because SO_2 speciation in water can be toxic for some algae strains. The same is valid for NO_2 dissociation in water, but its concentration in flue gas is, generally, low. Nevertheless, these effects can easily be checked by simulations in the liquid phase by adding acids, which ensure different scenarios of toxic concentration of SO_3^{2-} and NO_3^- are already proven for algae strains with industrial interest. Thus, the understanding and simulation of flue gas composition with 15% CO_2 content will help to find the difference in the growth of algae when impurities and pure CO_2 are applied.

For instance, the dependence of the pH and, consequently, its influence on the growth rate as a function of the gas composition can be also modeled. The model in the liquid phase describing the water chemistry of CO_2 and SO_2 is presented in Eqs. (11.5)–(11.12).

1. Mass balances of CO_2 species in water

$$\frac{dCO_{2(aq)}}{dt} = K_L a_{CO_2}\left(CO_{2(eq)} - CO_{2(aq)}\right) + (k_{b,c1}\mathrm{pH} + k_{b,c3})\left[HCO_3^-\right] - \left(k_{f,c1} + \frac{k_{f,c3}K_w}{\mathrm{pH}}\right)CO_{2(aq)} \tag{11.5}$$

$$\frac{d\left[HCO_3^-\right]}{dt} = \left(k_{f,c1} + \frac{k_{f,c3}K_w}{\mathrm{pH}}\right)CO_{2(aq)} + k_{b,c2}\left[CO_3^{2-}\right]\mathrm{pH} - (k_{b,c3} + k_{f,c2} + k_{b,c1}\mathrm{pH})\left[HCO_3^-\right] \tag{11.6}$$

$$\frac{d\left[CO_3^{2-}\right]}{dt} = k_{f,c2}\left[HCO_3^{2-}\right] - k_{f,c2}\left[CO_3^{2-}\right]\mathrm{pH} \tag{11.7}$$

$$\frac{dC_T}{dt} = \frac{dCO_{2(aq)}}{dt} + \frac{d\left[HCO_3^-\right]}{dt} + \frac{d\left[CO_3^{2-}\right]}{dt} - \mu_{total}X(t)Y_{\frac{CO_2}{X}} \tag{11.8}$$

2. Mass balances of SO_2 species in water

$$\frac{dSO_{2(aq)}}{dt} = K_L a_{SO_2}\left(SO_{2(eq)} - SO_{2(aq)}\right) + (k_{b,s1}\mathrm{pH} + k_{b,s3})\left[HSO_3^-\right] - \left(k_{f,s1} + \frac{k_{f,s3}K_w}{\mathrm{pH}}\right)SO_{2(aq)} \tag{11.9}$$

$$\frac{d\left[HSO_3^-\right]}{dt} = \left(k_{f,s1} + \frac{k_{f,s3}K_w}{pH}\right)SO_{2(aq)} + k_{b,s2}\left[SO_3^{2-}\right]pH - \left(k_{b,s3} + k_{f,s2} + k_{b,s1}pH\right)\left[HSO_3^-\right] \tag{11.10}$$

$$\frac{d\left[SO_3^{2-}\right]}{dt} = k_{f,s2}\left[HSO_3^{2-}\right] - k_{b,s2}\left[SO_3^{2-}\right]pH \tag{11.11}$$

$$\frac{dS_T}{dt} = \frac{dSO_{2(aq)}}{dt} + \frac{d\left[HSO_3^-\right]}{dt} + \frac{d\left[SO_3^{2-}\right]}{dt} \tag{11.12}$$

3. Modeling of pH

A dynamic model of pH can be built by taking into account all the information from dynamics, equilibrium of CO_2 and SO_2, and electroneutrality balance, according to Eq. (11.13).

$$\frac{dpH}{dt} = \frac{\left(\frac{K_{c1}}{pH} + \frac{2\,K_{c1}K_{c2}}{pH^2}\right)\frac{dCO_{2(aq)}}{dt}}{1 + \frac{K_w}{pH^2} + \frac{K_{c1}CO_{2(aq)}}{pH^2} + \frac{8K_{c1}K_{c2}CO_{2(aq)}}{pH^3}} + \frac{\left(\frac{K_{s1}}{pH} + \frac{2K_{s1}K_{s2}}{pH^2}\right)\frac{dSO_{2(aq)}}{dt}}{1 + \frac{K_w}{pH^2} + \frac{K_{s1}SO_{2(aq)}}{pH^2} + \frac{8K_{s1}K_{s2}SO_{2(aq)}}{pH^3}} \tag{11.13}$$

where, $K_La_{CO_2}$ and $K_La_{SO_2}$ are the mass transfer coefficients (s^{-1}); K_{ci} and K_{si} are the equilibrium constants for CO_2 and SO_2, respectively; and $k_{f,ci}$, $k_{b,ci}$, $k_{f,si}$, and $k_{b,si}$ are the speciation rate constants for CO_2 and SO_2 species, respectively. The equilibrium and rate constants can be found elsewhere [26].

The influence of C_T and pH on the SGR can be represented by Eq. (11.14); moreover, by considering only these two state parameters, the microalgal biomass balance yields to Eq. (11.15), as follows (Box 11.1):

$$\mu_{total} = \mu_{max} \cdot \left(\mu_{C_T}\mu_{pH}\right) \tag{11.14}$$

BOX 11.1

CASE STUDY: SIMULATION OF PH AND WATER CHEMISTRY BASED ON THE ABOVE MODEL [EQS. (11.5)–(11.15)]

By using this model, we were able to prove that the pH value decreased very fast (for about 60 s) and reached value of pH = 2 (data not shown). Hence, culturing algae on flue gas requires an understanding of its composition as well as of the used medium. Therefore, buffering of the medium is obligatory. Developed models on this subject, but only for CO_2 dissociation, can be found elsewhere [28,29].

$$\frac{dX(t)}{dt} = \mu_{total} X(t) \tag{11.15}$$

2.4 Medium Optimization for Culturing of Microalgae

Surprisingly, the specific growth rate on flue gas can be higher than on pure 15% CO_2 for all other conditions being equal (PBR type, light illumination, mixing properties, and L/D cycles frequencies). This phenomenon can be attributed to a low O_2 content (6%) and to the presence of metals such as Co, Mo, V, and Ni, which are important microelements of the nutrients' medium. Calculations of the macro- and micronutrients' concentrations in the medium are based on elemental composition of the algal biomass [30]. On the other hand, macroelements (C, O, H, N, Na, K, Ca, P, S, Mg, Cl) and microelements (Fe, Zn, Mn, Br, Si, B, Mo, V, Sr, Al, Rb, Li, Cu, Co, I, Se, Ni) [31,32] of the medium are supplied as pure or hydrated salts. Using comprehensive kinetics models and taking into consideration the speciation of ions, the authors Concas et al. [27] were able to quantitatively describe the dynamics of pH evolution and its effect on microalgae growth, which is novel in the field. Further, in aqueous solutions, a dissociation, formation of complexes, or precipitation can be predicted by using MINEQL+4.5 software (Chemical speciation modeling software), which calculates dissociation constants, on the basis of thermodynamic properties of species. Active experiments on medium optimization for maximizing microalgal growth and achieving high biomass concentration can be found in the work of Mandalam and Palsson [33]. Linear programming was used by Kroumov et al. [34] to find minimum medium elemental composition giving unlimited specific growth rate [33,34]. In this sense, the strategy of medium optimization from flue gas [34] can be schematically presented as shown in Fig. 11.2.

2.5 CO_2 Sequestration From Flue Gas and Addition of Nutrients From Wastewaters in Order to Minimize the Costs

In the light of above commented works, any effort to minimize the cost of nutrients in algae cultivation in any PBR design is a benefit from technical-economical point of view. Borowitzka and Borowitzka [20] for the first time applied a respirometric–titrimetric approach in order to model microalgae kinetics and to calibrate an activated sludge model. It is a well-known fact that during the biotechnological processes (activated sludge, biofilters, etc.) biomass characterization in terms of kinetics is of crucial importance for system design and optimization [20]. Usually, the kinetics is modeled by taking into consideration the main limiting factor (light availability) and inorganic components are assumed to be in excess. However, the authors [20] provided detailed analysis on how to model and use kinetic models in order to minimize the costs of nutrients for different sources of medium components (e.g., source of N, P, and other macro- and microcomponents). This is in accordance with the theoretical approach presented above [19]. It means, the culturing algae on flue gas can use wastewaters as nutrient sources, in order to minimize the cost of substrates. Modeling procedure is from tremendous help and details can be found elsewhere [20].

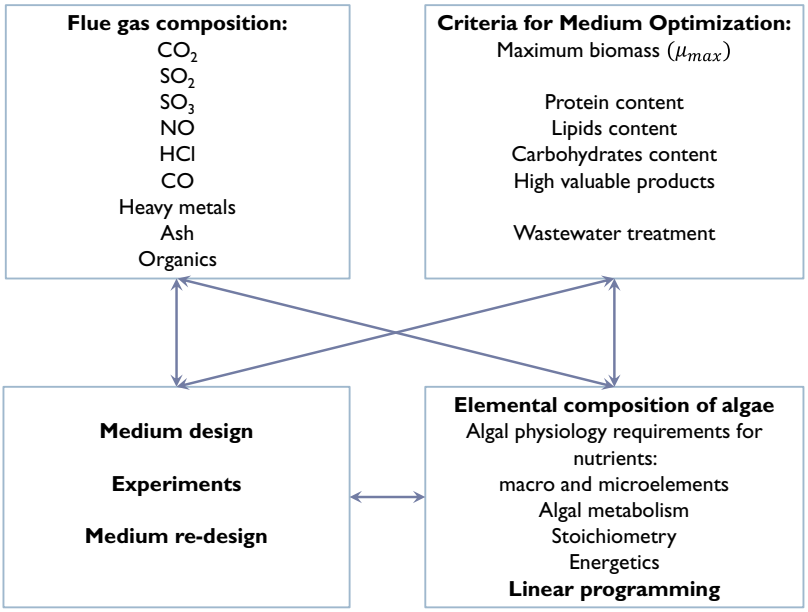


FIGURE 11.2 Graphical representation of the complex theoretical approach for medium optimization of culturing microalgae on flue gas. *Adapted from A.D. Kroumov, A.N. Módenes, D.E.G. Trigueros. A complex theoretical approach for algal medium optimization for CO_2 fixation from flue gas, Acta Microbiol. Bulg. 31 (2015) 61–70.*

2.6 Light Availability—The Most Important State Parameter

Many scientific efforts were directed in the field of PBR design improvement as a function of light illumination, penetration, and distribution into the different PBR constructions. New models and novel techniques were created and verified in real algae cultivation systems where the specific growth rate is a function of light illumination [3,35–37].

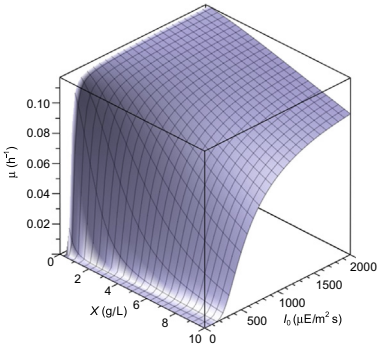
Table 11.1 presents a trial to show the realization of the idea that the cultivation of algae in PBR is a complex system and that a complex approach is required for the description of algae kinetics. Response surface analysis (RSA) was applied on different SGR models as a function of the light intensity alone or coupled with other state parameters of the PBR [38–43] and some of them are shown [44–48]. The RSA helps to determine realistic ranges of state parameters changes and how the algae SGR react. Hence, this methodology supports the search of optimal engineering solutions without performing expensive and time-consuming experiments.

Classical and mostly new developments and findings in the area of modeling SGR as a function of light availability are presented in Table 11.1. The SGR can include the irradiance distribution inside the PBRs (e.g., works of Pruvost et al. [46,49]). An excellent review of Béchet et al. [50] classified the efforts in the area. In order to understand the new

TABLE 11.1 Response Surface Analysis of Light Availability and Its Influence on the Specific Growth Rate of Biomass

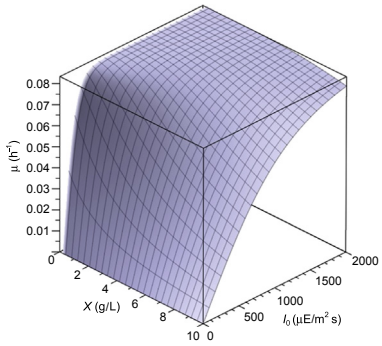
Acién Fernandez et al. [44]

$$\mu = \frac{\left(b+\frac{c}{I_0}\right)\mu_{max} I_{av}}{\left[K_1\left(1+\left(\frac{I_0}{K_2}\right)^{a}\right)\right]^{\left(b+\frac{c}{I_0}\right)} + I_{av}^{\left(b+\frac{c}{I_0}\right)}} \quad (11.16)$$



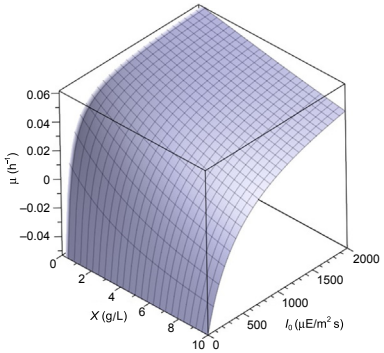
Kurano & Miyachi [45]

$$\mu = \mu_{max}\ tan\ h\left(\frac{I_{av}}{K_L}\right) \quad (11.17)$$



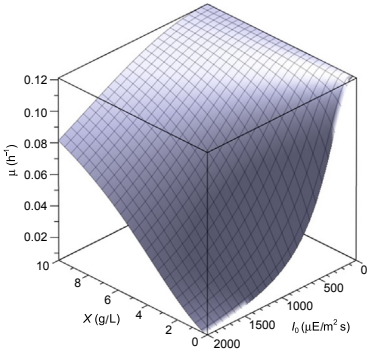
Pruvost et al. [46]

$$\mu = \mu_{max}\frac{I_{av}}{K + I_{av} + \frac{I_{av}^2}{K'}} - \lambda \quad (11.18)$$



Muller-Feuga et al. [47]

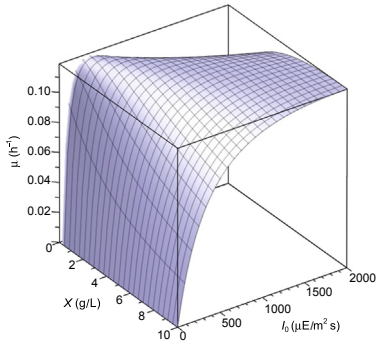
$$\mu = \frac{2\mu_s\left(1-\frac{I_c}{I_s}\right)\left(\frac{I_{av}}{I_s}-\frac{I_c}{I_s}\right)}{\left(1-\frac{I_c}{I_s}\right)^2+\left(\frac{I_{av}}{I_s}-\frac{I_c}{I_s}\right)^2} \quad (11.19)$$



Bernard & Rémond [48]—Light and temperature effect

$$\mu = \mu_{max}\frac{I_{av}}{I_{av} + \frac{\mu_m}{\beta}\left(\frac{I_{av}}{I_{opt}}-1\right)^2}\varphi(T) \quad (11.20)$$

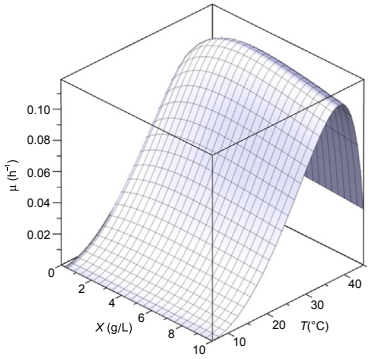
Biomass vs. Incident light (at $T = T_{opt}$):



Where, $\varphi(T) =$

$$\frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})((T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T))} \quad (11.21)$$

Biomass vs. Temperature (at $I_0 = 2000\ \mu E/m^2\ s$):



Parameter values: $\mu_{max} = 0.119\ h^{-1}$, $a = 3.04$, $b = 1.209$, $c = 514.6$, $K_1 = 94.3\ \mu E/m^2\ s$, $K_2 = 768.4\ \mu E/m^2 s$, $K_L = 150\ \mu E/m^2 s$, $K = 65.75\ \mu E/m^2 s$, $K' = 2509.66\ \mu E/m^2\ s$, $\lambda = 0.0531\ h^{-1}$, $\mu_s = 0.06\ h^{-1}$; $I_c = 3.5\ \mu E/m^2\ s$; $I_s = 385\ \mu E/m^2\ s$; $\mu_m = 0.083\ h^{-1}$, $\beta = 2.083 \times 10^{-3}\ h^{-1}$, $I_{opt} = 275\ \mu E/m^2\ s$, $T_{min} = 5.2°C$, $T_{opt} = 38.7°C$ and $T_{max} = 45.8°C$.

Reproduced from A.D. Kroumov, A.N. Módenes, D.E.G. Trigueros, F.R. Espinoza-Quiñones, C.E. Borba, F.B. Scheufele, C.L. Hinterholz, A systems approach for CO_2 fixation from flue gas by microalgae—Theory review, Process Biochem. 51 (2016) 1817–1832. http://dx.doi.org/10.1016/j.procbio.2016.05.019.

developments in modeling SGR vs. light presented in [50] and Table 11.1, three types of kinetic models could be relatively classified:

1. Type I models express the rate of photosynthesis of well-mixed cultures as a function of the average light intensity within the broth I_{av} (see Table 11.1). The rationale behind this approach is that individual algae cells in a well-mixed system are, on average, exposed to the same light intensity and, therefore, have the same average rate of photosynthesis. However, empirical studies have shown that the kinetic parameters associated with these models are actually functions of operational conditions, such as cell concentration, incident light intensity, or system size.
2. Type II models are responsible for the impact of light gradients on the local rate of photosynthesis. These models are constructed by (a) quantifying the light distribution within the broth; (b) selecting the relevant biological model that expresses the local rate of photosynthesis as a function of the local light intensity; and (c) summing the local rates of photosynthesis to obtain the global rate of photosynthesis.
3. Type III models consider that the rate of photosynthesis of an individual algae cell is a function of its "light history" (i.e., the light intensity experienced by an algae cell over time as it moves in the system). Type III modeling thus involves (a) determining the light history of algae cells; (b) based on their light stories, determining the rate of photosynthesis of individual algae cells using a dynamic biological model; and (c) summing the rates of photosynthesis of individual algae cells to calculate the total rate of photosynthesis in the cultivation system.

Therefore, the application and usefulness of these three types of kinetic models depends on the available measurements equipment and goals connected with PBR optimization and scale up for the particular outdoor conditions.

For engineering purposes, such as developing process of complex PBR model and scale up, it is very useful to build the catalog of models SGR vs. light intensity or a combination of light effects by using classical forms (see Refs [50,51]). This will increase our capacity to describe the limitation and inhibition effect of light on the algae growth demonstrated by applied RSA method (see Table 11.1). In our case, the most useful studies can consider the ones that achieve high biomass concentration by using fresh and marine algae on flue gas [1,52,53]. In high biomass concentration, light regime (limitation saturation and/or inhibition) depends on the surface to volume (S/V) ratio of particular PBR type and L/D cycles [54–57], which are determined by PBR mixing conditions [58,59]. Penetration of light occurs only in a very short path (few cm); therefore a vigorous mixing can provide the desired frequency. Many PBR types were extensively studied in the aim of obtaining high biomass concentration of 3 up to 15 g/L [60,61], and a very high reported value was 34 g/L [62]. An ultra-high biomass concentration was reported as 84 g/L [11] for the conditions with light illumination up to 2000 $\mu mol/m^2$ s and 1 cm light path or even lower than 0.75 cm. From a phenomenological point of view, these studies have shown that the limit of photoautotrophic algal growth cannot be a restriction on any industrial application [63]. In our case, it is important to know the correlation of industrial maximal and optimal algal density, which is in the only range of high biomass concentration. Details can be found elsewhere [11].

These authors [11] studied the effect of such high cell density on the chlorophyll a and b content, proteins, lipids, and carbohydrates in *Chlorococcum littorale* cells grown in a 1-cm

flat-plate reactor, with light intensity 2000 μmol/m^2 s and aeration rate 3.0 vvm. The results obtained in this study are consistent with the previous findings [62,64], indicating that the reduced light path (approx. 1 cm) increased the probability of exposure of the average cells in high density culture to an "optimal light regime" as proposed by Pruvost et al. [49], giving the opportunity for a more efficient utilization of light energy.

Previous studies [62,65] have shown that maximum cell density reflects to a higher optimal cell density. As mentioned above, an optimal cell density in the range of 10−20 g/L will support a very high CO_2 fixation rate (15%−20% CO_2 content).

It is obvious that the highest CO_2 fixation rate is attributed to the optimal high biomass concentration. However, it is also important to know how high density of biomass influences the cell's composition (chlorophyll content, proteins, lipids, carbohydrates, etc.) and how this information can be formalized and used to improve the process performance.

Hence, the problem remains on how to select strains tolerant to high CO_2 and light illumination (higher than 2000 μmol/m^2 s) and how to design a cheap PBR with an optimal light path and mixing properties supporting optimal algae growth (maximum L/D cycles frequency). For the conditions under high biomass concentration, the O_2 inhibition effect on SGR cannot be neglected, and engineering solutions for continuous O_2 removal have to be considered.

2.7 Complex Approach for Modeling of Closed PBRs

Various original works were published for development and design of flat plate and other PBRs. Earlier in the chapter, we discussed in detail the works of Wu and Merchuk and ,Vunjak-Novakovic et al. [1,2] connecting with tubular air lift PBRs. Here, we focused the attention of the reader on the work of Slegers et al. [14] on the flat plate, and Pruvost et al. [46,49] on modeling of flat panels, open ponds, and other different PBRs. The authors [14] analyzed in detail the dynamics of biomass growth in a flat plate PBR, as well as in parallel flat plate PBRs. The biomass growth was considered to be the function of height, depth, and time. Two light intensity gradients were analyzed and modeled, as well. Pruvost et al. [46,49] demonstrated in their works a robust and interesting model that integrates radiative transfer, kinetics of photosynthetic growth, and solar illumination database. As a result, they have described the dynamic behavior of different PBRs when exploited in solar conditions. These are two examples of an approach to study and design flat plate and other PBRs where physical and biological principles are combined. Recently, a very complex model was demonstrated for tubular PBR, wherein the gas and liquid phases were taken into consideration as the most important state parameters [66]. This model can be very useful for PBR control, scale up, and optimization.

2.8 Conclusions on Modeling PBRs and CO_2 Sequestration From Flue Gas by Microalgae With the Goal of Cost-Effective Biofuel Production

Theoretical achievements in understanding of CO_2 sequestration from flue gas by microalgae have to be directed to cost-effective technology for biofuel production on the base of microalgae biomass. This task becomes extremely difficult due to the very low petrol market

prices. In order to compete petroleum on the fuel market with renewable energy source such as biofuel, the new innovative solutions in all unit operations have to be developed and applied in order to decrease the costs of production. This cannot be done without using novel mathematical models and tools to describe the PBRs performance and microalgal physiology in particular PBRs design. Moreover, technical-economical analysis (TEA) and life cycle assessment can be applied taking into consideration an integral approach of microalgal biomass use. This requires close collaboration and commitments between lots of scientists, economists, and businessman. Understanding and sharing of knowledge between members of a multidisciplinary team should be a common strategy, which at the end will play a crucial role to present the importance and current state of the art of the subject in front the politicians and governors whose support is undisputedly important for establishing third generation of biofuels as a new future of "Green technologies."

3. TECHNICAL-ECONOMICAL ANALYSIS OF ALGAE FOR BIOENERGY AND COPRODUCTS

Recently, algae bio-oil has been considered as a very challenging alternative energy source for fuel applications. Nevertheless, many current efforts to evaluate economical effectiveness of algae bio-oil showed that a standalone technology concept does not work for algae biodiesel production market. The R&D efforts must be concentrated on total biomass utilization strategy (biorefinery concept) and cost-effective unit operations involved in any stage of biofuels production from algae biomass combined with CO_2 sequestration from flue gases. Utilization of wastewater treatment nutrients is evitable from the economical point of view in the new concepts of algal biomass for energy utilization products besides biodiesel as well as combined with new concepts for downstream strategies.

All cellular components in microalgae biomass definitely should be used to produce valuable liquid fuels in order to reduce overall production costs. Each cellular component of microalgae has its own advantage for producing specific liquid biofuels. It is well known that lipids are suitable for biodiesel and bio-oil production, whereas polysaccharides are appropriate for bioethanol. Recently, sequential production of biodiesel, bioethanol, and bio-oil were successfully conducted for *Dunaliella tertiolecta* biomass [67]. The authors demonstrated a complete use of microalgae biomass for an integrated production of different biofuels. In addition, the suitable extraction of higher-value products (HVP) for novel applications will lead to an enhanced economic overall process for this extensive characterization of the microalgae biomass as well as the produced metabolites.

The second goal of the recent book chapter concerns the technical-economical evaluation of CO_2 biofixation and further biofuel production from algae components through a biorefinery concept. All the requirements of such green technology will be interpreted in the light of cost-effectiveness and market competitiveness. The same approach will be applied for every unit operation in this technological chain.

It has to be noticed that such study varies depending upon used algae strains—there are about 30,000 algae species [68,69]. All the three algae processing techniques (phototrophic, mixotrophic, and heterotrophic) use PBRs together with other involved equipment and downstream steps of desired products. Hence, industrially proven viable algae species and

their metabolite products for bioenergy and some high-value coproducts will be discussed throughout the life cycle assessment and/or TEA approaches.

3.1 CO_2 Sequestration—Life Cycle Assessment

Biofuel production from microalgal biomasses is one of the most promising and challenging clean carbon-neutral sources of energy [70]. The most competitive strategy is multiobjective use of algae biomass where the algae biofuels (e.g., biodiesel, bioethanol, biogas, and biohydrogen) [71] have to be combined with other biomass coproduct applications such as chemicals and pharmaceuticals [72,73], food additives [72,74,75], and many other HVPs [73,76,77].

Current state of the art shows that the only feasible cost-effective industrial production of microalgae biomass can be coupled with the wastewater source of nutrients (i.e., N and P), in addition to free CO_2 from flue gases, resulting in purified water and profits obtained from the wastewater treatment process [78], in addition to the clean environment.

There are many reports on the potential and bioeconomics of algal biomass to generate renewable fuels based on the utilization of CO_2 emitted from traditional fossil-fuelled power stations or other industrial sources of CO_2 (e.g., flue gas from cement production, ethanol industry, among others) [79,80].

The multistep integrated process including the biorefinery concept is as follows:

1. Upstream processes: Development of cost-effective upstream steps for enabling the use of CO_2 from flue gases (direct/indirect use). Development and optimization of a suitable culture medium for a specific microalgae strain and its cost-effective logistics, preparation, and usage according to the regional particularities (e.g., seawater, brackish, freshwater and wastewater, cheap nutrients and fertilizers);
2. Microalgae cultivation techniques and equipment: Use of state of the art of cultivation techniques, metabolism targeting for specific interest products, optimization of operational conditions (i.e., light, pH, temperature, hydrodynamics, nutrients, L/D cycles, etc.), design of PBRs configuration (e.g., open ponds, closed PBRs, etc.).
3. Downstream processes: Use of state of the art of suitable unit operations for each systems configuration (e.g., microalgae species, intended product/fuel, etc.)—harvesting, cell disruption, drying, extraction, etc.
4. Microalgal biomass total conversion: The use of biorefinery concept coupled with HVPs production, aiming:
   a. Different fuel production (e.g., biodiesel, bioethanol, biomethane, biogas) by the several conversion available technologies (i.e., fermentation, transesterification, liquefaction, pyrolysis, anaerobic digestion, etc.) using all components produced by the biomass and also the by-products of each involved processes;
   b. Coproducts: HVP extraction and synthesis (e.g., chemicals, pharmaceuticals, nutraceuticals, food additives, etc.).
5. Transport and logistics: Optimization of the logistics between the raw material and consumer market, integration with the preestablished industry (e.g., cement, ethanol, etc.) (see BOX 11.2A).

In Fig. 11.3, all stages of the overall CO_2 sequestration process are shown, starting from the CO_2 capture from the industrial flue gas up to the biofuel or bioproduct generation through appropriate technology.

BOX 11.2A

CASE STUDY—ETHANOL PRODUCTION

A great example of a well-logistic flue gas recovery would be the use of the CO_2 from ethanol fermentation (e.g., sugarcane, corn, etc.) as a carbon source for microalgae growth and carbohydrate/lipid production aiming bioethanol or biodiesel, since its significant production during the ethanol fermentation process (see Eq. (11.x)), wherein for each gram of glucose 0.51 g of ethanol is produced and 0.49 g of CO_2 is generated.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad (11.x)$$

In such integrated biorefineries the costs of first-, second-, and third-generation biofuel production could be significantly reduced, considering a preestablished transport infrastructure (feedstocks and products), not to mention the closed carbon cycle environmental benefits.

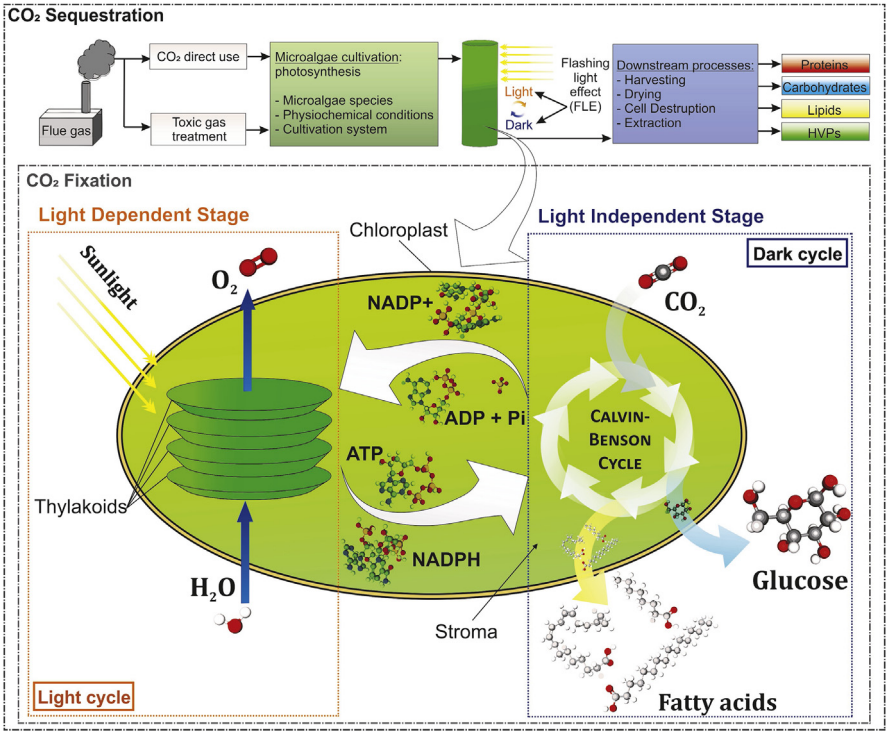


FIGURE 11.3 Strategy for total algal biomass use. The multivariable technical-economical concept including biorefinery concept and utilization of high-value products.

3.2 Upstream Processes

3.2.1 Flue Gas Direct Use or Pretreatment

In the light of the above determined goal of our analysis, the flue gas as a feedstock is a promising source of CO_2 for algae biomass, biofuel, and valuable coproduct production. Despite the abundant large-scale sources of available CO_2 as flue gases (e.g., coal-power plant, chemical plant, baker yeast plant, ethanol plant), the distribution of CO_2 is challenging. The gas transport from any far located flue gas generation plant to the PBR systems or open/raceways ponds by using compressors is not economically beneficial and must be avoided in most of the cases.

Although the common restrictions related to the transportation of flue gas, several efforts have been taken on carbon capture and storage for its transportation. According to Pires et al. [81], not only several technological, economical, and environmental issues, but also safety problems remain to be solved. The increase of CO_2 capture efficiency, reduction of process costs, and verification of environmental sustainability of CO_2 storage should be highlighted.

The carbon capture and storage (CCS) methodologies comprise three steps:

1. CO_2 capture: most common methods are absorption, adsorption, separation by membranes, and cryogenic separation;
2. CO_2 transportation: the captured gas mixture is compressed to a liquid and supercritical fluid has to be transported by pipeline or ship;
3. CO_2 storage: comprises geological storage, ocean storage, and mineralization.

Among these steps, the CO_2 capture stands out as the most costly one, of the CCS, with values ranging from 24 to 52 €/ton of CO_2. The transportation cost varies with the pipeline dimensions (length and diameter), pressure of CO_2, and landscape characteristics, ranging from 1 to 6 €/ton of CO_2 per 100 km of pipeline [81]. Thus, the reduction of such costs is an important issue of integrated biorefinery approach.

Note: The use of compressed CO_2 may imply up to 41% of the total costs of the raw material [25], depending on the CO_2 uptake efficiency (which is a function of algae metabolism and process conditions optimization) and its use in open ponds may lead to significant losses to the atmosphere. In this sense, the direct use of flue gas containing CO_2, when compared to compressed one, presents economic and environmental advantages, particularly when the flue gas generation sector is close to the location of the microalgal cultivation system [25,82].

Note: Chemical and/or physical CO_2 treatment technologies are still relatively costly (22–36 € per ton of C) for the flue gas generating industry and also the energy consumed [25,83].

3.2.2 Culture Medium Development

A feasible culture medium for the microalgae growth will strongly depend on two major factors:

1. The use of low-cost highly available nutrient sources, which can be obtained from accessible wastewater;
2. The rational definition of nutrients of the cultural medium obtained by using the knowledge from elemental composition of the algae cells and calculations on the base of linear

programming approach. Further, maximization of the algae biomass and the desired product accumulation in the cell (e.g., lipids, carbohydrates etc.) can be achieved cost-effectively for the given operational conditions and metabolism of the selected particular strain.

3.2.3 *Low-Cost Residual Nutrient Sources*

By applying the simultaneous wastewater treatment, biofuels production is thus justified by the potential to counterbalance the high costs not only of wastewater treatment. For instance, the US wastewater infrastructure investment requirements range from $13 up to $21 billion annually, as well as $21.4 up to $25.2 billion for annual operation and maintenance costs. Thus, the potential of algal biofuel production based on current technologies is unlikely economically viable without the use of wastewater [84,85].

Several wastewater streams have been frequently used as a readily available and cost-effective substrate for microalgal growth for biomass production and nutrient removal [84,86]. However, it must be emphasized that a major challenge to such integrated systems' implementation related to the large-scale production of algae is the transportation cost (Box 11.2B).

3.2.4 *Development of Nutrient Media for Culturing Algae*

In addition to the use of cost-effective residual nutrient sources, by applying mathematical modeling during the process development and optimization of algae cultivation, some of the economic constraints may be surpassed. For instance, the use of linear programming approach (see Section 2.4), based on mass and energy balances, gives opportunity to precisely calculate nutrients for synthesis of exact amount of algae biomass and metabolites [33,34,89].

3.3 Microalgae Cultivation Techniques

For biodiesel production it is well known that microalgae stand out as one of the most promising feedstock due to the short cell cycle (lower than 24 h), high oil contents productivity (20%–50%), strong adaptive capacity to adverse environment (high salinity, heavy metal

BOX 11.2B

CASE STUDY—ETHANOL PRODUCTION

In this sense, the example of ethanol fermentation case could be once again addressed, considering the presence of the vinasse residual material as a nutrient source (N, P, K, and micronutrients) for the in situ microalgae growth [87,88], in which both CO_2 sequestration and growth medium would have low transportation costs. Also, the use of the glycerol (by-product of the biodiesel production) could be integrated in the carbon supply in the heterotrophic growth of microalgae (see Box 11.3).

BOX 11.3

BIODIESEL PRODUCTION AND GLYCEROL BY-PRODUCT

Modeling the transesterification reaction between triacylglycerol (TAG) and alcohol (e.g., methanol or ethanol) through a catalyzed process (homogeneous or heterogeneous catalysis with alkaline, acid, or even enzymatic catalysts) producing ethyl esters, with crude glycerol as a primary by-product [see Eq. (11.xi)] can be found in details elsewhere [92,93].

Most common industrial process utilizes homogeneous alkali catalysts (e.g., NaOH or KOH), wherein the alkali transesterification is considered as the most economic feasible in industrial scale [68,94].

$$\mathrm{TAG} + 3\mathrm{ReOH} \xrightarrow{\text{Catalyst}} \text{Ethylesters} + \text{Glycerol} \tag{11.xi}$$

In general, for 10 lbs (4.5 kg) of biodiesel produced, approx. 1 lb (0.45 kg) crude glycerol is produced as a coproduct. Due to the increase in biodiesel production over the past decades, the crude glycerol generation (80% purity) significantly increases and consequently leads to a decline in its price (e.g., \$0.25/lb to \$0.025/lb between 2004 and 2005). Thus, this residue production remains as a significant environmental issue. Glycerol has many commercial applications, however not enough to consume its increasing production related to the biodiesel productive chain. Moreover, most of glycerol uses are cost-prohibitive. For instance, the cost to purify this product to commercial pharmaceutical-grade is elevated, approx. \$0.2/lb, thus the refining process is cost-prohibitive for small- and medium-scale biodiesel plants. In spite of that, the low prices of crude glycerol ($\approx$\$0.025/lb) become very competitive carbon source with sugars, such as glucose, for biomass production and lipid storage by microalgae, considering both the advantages of this significant residue conversion to added value products and its surplus/disposal problems solution [95].

ions, toxicants, high CO_2 concentration, etc.), and the possibility of occupation for nonexploited area [69,70,72]. Such characteristics bring to technical, economic, environmental, and social benefits.

In the microalgae cultivation system, not only the growth rate and maximum biomass production of microalgae strains but also the metabolites accumulation will be affected by interdependent parameters as follows:

1. Abiotic parameters (e.g., light, temperature, pH, salinity, O_2, CO_2, nutrient stress, and toxic chemicals);
2. Biotic parameters (e.g., presence of pathogens and competition by other algae);
3. Operational conditions (e.g., shear stress produced by severe mixing, dilution rate, depth, harvest frequency, and addition of bicarbonate). In addition, the cultivation method (i.e., photo-, hetero-, and mixotrophic growth) will affect not only the biomass and metabolites productivity (see Table 11.2) [90], but also the economics of the process.

TABLE 11.2 Cultivation Methods of Microalgae, Reactors Configuration, and Lipids-Carbohydrates Productivity

Cultivation Method	Microalgae Species	Reactor Configuration	Biomass Concentration (g/L)	Biomass Productivity (g/L d)	Lipid Content (% dwb)	Lipid Productivity (mg/L d)	References
Photoautotrophic	*Chlorella vulgaris*	–	–	0.02–0.20	50–58	11.2–40	[90]
	Chlorella protothecoides	–	–	2.00–7.70	14.6–57.8	1214	[90]
	Chlorella vul. CCAP 211/11B	Closed PBR	–	–	19	170	[94]
	Chlorella vul. F&M-M49	Open pond	–	–	18	200	[94]
Heterotrophic	*Chlorella vulgaris*	Tubular reactor	1.2	0.151	23	35	[97]
	Chlorella protothecoides	Shaken flasks[a]	15.3 ± 0.25	3.1 ± 0.05	50	1500	[95]
	Chlorella protothecoides	Shaken flasks[b]	19.2 ± 0.47	3.2 ± 0.07	51	1600	[95]
	Chlorella protothecoides	Shaken flasks[c]	23.5 ± 0.74	3.9 ± 0.12	62	2400	[95]
	Chlorella protothecoides	Fed-batch fermentation[b]	43.3 ± 0.6	–	53	2800	[95]
	Chlorella protothecoides	Fed-batch fermentation[c]	45.2 ± 0.85	–	54	2990	[95]
Mixotrophic	*Chlorella vulgaris*	Tubular reactor	1.7	0.254	21	54	[97]
	Chlorella vulgaris	Shaken flasks[a]	4.85 ± 1.16	1.62 ± 0.39	15.43 ± 8.32	0.25 ± 0.03	[96]
	Chlorella vulgaris	Shaken flasks[b]	1.17 ± 1.34	0.39 ± 0.45	40.10 ± 22.06	0.16 ± 0.10	[96]
	Chlorella vulgaris	–	–	0.25 ± 0.26	20.0–22.0	52.0–56.0	[98]
	Chlorella protothecoides	–	–	23.9	58.4	11,800	[98]

Carbon source: [a]Glucose, [b]Glycerol, [c]Crude Glycerol.

In most cases, phototrophic cultivation method is assumed to be the only commercially feasible method for large-scale microalgae biomass production. In addition, by capturing the atmospheric carbon dioxide the phototrophic microalgae cultures present both environmental and economic advantages [90]. However, it must be considered that phototrophic growth commonly present lower biomass productivity [91], which may result in higher costs of downstream processes (e.g., harvesting and drying). Moreover, the use of residual substrate as carbon source, for example glycerol, which is a relevant by-product of the biodiesel industry and is considered as an environmental concern (see Box 11.3), may bring advantages for mixotrophic culturing method.

In the case of the biodiesel production, the glycerol liberated by the transesterification reaction would be used as a carbon source in a closed cycle recycling by-products, what becomes a great economical and logistic advantage. For instance, Heredia-Arroyo et al. [96] achieved a lipid content of $40.10\% \pm 22.06\%$ and a lipid productivity of 0.16 ± 0.10 mg/L d by using glycerol as a carbon source (see Table 11.2).

In addition to the higher lipid productivity presented by microalgae, which despite the strain-dependence is generally greater than vegetable oils crops, the land use of microalgae systems are considerably lower, which becomes a great economical advantage. For instance, a corn (*Zea mays* L.) culture (with 44% of lipid content) would produce around 172 L oil/ha year, requiring 66 m^2 year/$kg_{bio\text{-}diesel}$ of land in order to reach 152 $kg_{bio\text{-}diesel}$/ha year of biodiesel productivity. Even palm oil (*Elaeis guineensis*) would demand 2 m^2 year/$kg_{bio\text{-}diesel}$ to achieve 4747 $kg_{bio\text{-}diesel}$/ha per year. Those values are significantly inferior when compared to microalgae, which can achieve oil yields (from 58,700 to 136,900 L oil/ha per year) demanding a lowland use (0.1−0.2 m^2 year/$kg_{bio\text{-}diesel}$) and elevated biodiesel productivities (51,927−121,104 $kg_{bio\text{-}diesel}$/ha per year) [68].

In summary, the economic feasibility of the process must be determined by an integrated process approach, i.e., considering the subsequent steps of separation processes and the reuse of by-products and residues in a closed system.

Following these thoughts, the development of new cultivation cost-effective techniques and their applications for bioenergy from microalgae biomass can strongly support and increase the present production capacity. Maximization of synthesis of lipids, carbohydrates, and proteins for the given costs cannot be achieved with standalone approach. Already it is proven that hybrid cultivation techniques will be the optimized solution.

It must be evidenced that independent of the transformation route or conversion process of microalgae biomass to biofuels, each production step must present a positive energy balance, and be also economic feasible, which depends on other well-established market fuels. It is presently accepted that the cost production limit is around of \$0.5/kg biodiesel [78,99], in order to be a competitive alternative to the fossil fuel sources—the current diesel price is approximately \$1.05/kg [94].

In addition, regarding to another possible coproduct from microalgae cultivation systems, such as edible oils, the market value of commodity vegetable oils is around €0.50−1.00/kg [100].

The major biodiesel production costs are related to the vegetable feedstock prices (e.g., palm, corn, soybean, sugarcane, rapeseed); in this sense by comparing microalgae biodiesel to the first-generation fuels, the first one presents the advantage to be a nonfood competitive fuel in terms of not only feedstock, but also arable lands.

3.3.1 Microalgae Species

In order to increase the desirable target molecules, the microalgae strain selection is a major step in the production of biofuels and coproducts, due to different physical, chemical, and biological properties, which may significantly affect the production process of each potential strain. Consequently, the selection of microalgae strains most likely is specific for its application and operational conditions.

For instance, the search for tolerant strain to the toxic gases from the flue gas and harsh environmental conditions with potential to synthesize desired lipids and carbohydrates may reduce the number of required unit operations such as separation and extraction and as a result the process costs.

3.3.2 Microalgae Strain Selection

Another variable that must be considered in the strain selection is the desirable product accumulation and productivity, such as lipids in case of biodiesel application (see Table 11.2). Our analysis will mainly focus on most robust industrial algae species that adapted their physiology to the extreme environmental conditions during the competitions of survival (e.g., *Dunadelia salina*, resistant to high saline media; *Spirulina platensis*, adaptable to alkaline media; *Chlorella* sp., adaptable to nutrient-rich media) [68,101]; in addition, *Scenedesmus* sp. that is wide employed industrially due to its high lipids contents and fast growth rates [69].

A comprehensive microalgae strain selection for biodiesel production by using an efficient *Multi-Criteria Decision Analysis* (MCDA) methodology was employed elsewhere [69]. The authors selected a *Scenedesmus* sp. strain as the best microalgae strain among the six other alternatives by considering not only the lipid contents/productivity as an independent criterion, but also the growth rate, fatty acid profile (i.e., free fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and saturated fatty acids), and ease of harvesting. According to the authors [69], for the microalgal biomass and biodiesel production several aspects must be taken into account:

1. Technical aspects: energy content, energy efficiency, ease of harvesting, biomass productivity (growth rate), lipid content and productivity, fatty acid profile, reliability, safety, availability of nutrients, etc.;
2. Environmental aspects: land/water body use, water quality/requirement, biodiversity and aquatic life, CO_2 sequestration ability, wastewater utilization, cultivation methods, pollution, chemical usage, light intensity, resistance to contamination, particles emission, impact or mitigation on ecosystems, visual impacts, etc.;
3. Economical aspects: microalgae (raw material) cost, investment cost, cultivation cost and configuration (e.g., open ponds or PBR), nutrient costs, harvesting costs, coutilization of residues and by-products, robustness, storage costs, transportation and logistics costs, competitiveness to other fuels, payback period, etc.;
4. Social aspects: competition for food, technological development, sustainable development, social acceptability, monoculture preventing (e.g., corn, soybean), job creation, other social benefits, etc.

Despite the insufficient information in literature about the application of MCDA methodology in microalgae strain selection for biodiesel (or any other biofuel or bioproduct)

production [69], it must be evidenced that such tool may overcome several economical and technical constraints, since by this approach the dependence between the crucial factors are considered and the process will be integrally evaluated.

Another example of adequate strain selection linked with TEA is presented by Yu et al. [94]. The authors modeled the design of microalgae-based biodiesel production system by considering different production stages and microalgae strain properties. Several steps were considered: (1) cultivation system (open pond and closed PBR); (2) primary (flotation, sedimentation, filtration, and centrifugation) and secondary separation (drying); (3) lipid extraction (CO_2 supercritical extraction and solvent extraction); (4) transesterification (alkaline catalyzed reaction); in addition, the material (i.e., nutrient, CO_2), heat and electricity demand, water reuse and nutrient recycle, and also biogas production from residual biomass by anaerobic digestion were taken into account in order to supply heat for the process (e.g., drying, extraction, etc.).

By using such extensive model Yu et al. [94] determined the optimal process configurations considering the diversified strain properties (e.g., lipid content, effective diameter, productivity, etc.) achieving a minimum production cost of \$2.66/kg, which is above the current limit cost value compared to average fossil diesel price (\$1.05/kg). However, it must be noticed that much more aspects can and must be taken into account. Yu et al. [94] highlighted the need of screening, selection, and genetic improvement of strains with desirable properties in future. In addition, the integration of feedstock sources to other industry sectors—flue gas and wastewater use (Sections 2.3.1 to 2.3.3), as well as the integrated biofuels and coproduct production (i.e., biorefinery concept) would considerably reduce the operational costs, in order to allow a competitive fuel to the fossil ones.

3.3.3 *Metabolic Engineering Techniques and Cultivation Strategies*

Nowadays higher biomass productivity, yields of carbohydrates and lipids, as well as ease of extraction and downstream processes may be achieved through the knowledge of metabolic engineering and genetic modification of algae.

For instance, the lipid accumulation improvement can be accomplished by the understanding of internal metabolic control and more specifically of lipid-biosynthesis pathways. The essential molecular and genetic tools are currently available for a comprehensive characterization of genes coding for enzymes of the lipid-biosynthesis pathways in some algal species [102]. In this context, Khozin-Goldberg and Cohen [102] presented a review about novel emerging information from algal gene identification, such as TAG accumulation.

In addition to an extensive screening of microalgal species for high lipid synthesis for improving biodiesel production, the complete understanding of lipid biosynthesis and metabolism is a cornerstone for developing genetic engineering approaches and cultivation methods to boost algal lipid production (e.g., TAG productivity and TAG content) [100,103], and consequently the economical feasibility of the process.

Since TAG biosynthesis is part of primary cell metabolism, which is integrated with cell membrane lipid assembly and turnover, it must be considered in the full context of cellular metabolism. Microalgae photosynthetic organisms fix CO_2 during the day and convert it into various products (e.g., TAGs), depending on the species of alga or specific conditions such as nutrient deprivation, a process that may be reversed by respiration during the night [103].

Klok et al. [75] experimentally demonstrated and discussed the TAG accumulation increase by nitrogen limitation conditions, which caused an energy imbalance leading to simultaneous cell replication and TAG accumulation. The authors achieved a four-fold increase in TAG productivity (up to 46 mg/L d), which is also dependent on light intensity.

An excellent review regarding complexity of TAG synthesis and accumulation is presented elsewhere [100].

The authors discussed TAG production strategies as follows:

1. Nitrogen starvation: under batch cultivation with a growth phase followed by a nitrogen starvation phase (advantages: high TAG content, productivity and yield, ease of operation; disadvantages: down-time, separate growth phase, limited control over outdoor TAG accumulation rates);
2. Nitrogen limitation: under continuous cultivation with nitrogen limitation in a continuously operated system. (advantages: flexible control over outdoor TAG accumulation rates, customized biomass composition; disadvantages: lower TAG content, productivity and yield, although not yet optimized complex process).

It must be highlighted that the current context of genetic engineering of microalgae still remains in its infancy, due to the fact that reliable nuclear transformation systems are only available to some degree for microalgae, unlike to those that are currently used in other plants. In this context, nuclear transformation has been successfully employed in only a few species (e.g., green algae *Chlamydomonas reinhardtii*, oleaginous diatom *Phaeodactylum tricornutum*, and the well-known robust TAG producer *Nannochloropsis* sp.). Thus considering the limited techniques for altering metabolic pathways in microalgae, examples of targeted overexpression and heterologous expression of genes involved in lipid biosynthesis are scarce. However, some successful manipulations of lipid biosynthesis gene expression were reported, for example, enhanced production of high-value fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [100].

3.4 Bioreactors Configuration

One of the most challenging issues in biofuel production from algae is development of cost-effective large-scale PBR configuration [72]. The recent advances of effective irradiance, hydrodynamics, and mass transfer mechanism has led to high biomass concentrations and elevated productivity values (for closed PBR systems) [58]; however, much R&D efforts still need to be conducted in order to enhance the economics of the process. A comparison and TEA aspects for different industrially important PBRs are given in Table 11.3.

In order for microalgae to reach a status of viable feedstock for biofuel production the overall energy (and carbon balance) must be favorable in a microalgae-based biofuel production chain.

Slade and Bauen [105] by considering a life cycle assessment, wherein the cultivation, harvesting, and oil extraction stages were taken into account, discussed the net energy ratio (NER) of different raceway ponds and PBR systems. The authors concluded that raceway pond systems demonstrate a more attractive energy balance (i.e., NER < 1) than PBRs.

The NER, consequently, showed strong impact over the process costs, in which despite the higher costs related to the downstream processes (i.e., biomass drying and dewatering) of

TABLE 11.3 Advantages and Disadvantages of Open Pond and PBRs

Production System	Advantages	Disadvantages
Raceway pond	Low investment and operational costs	Poor biomass productivity
	Easy to clean	Large area of land required
	Utilizes nonagricultural land	Limited to a few strains of algae
	Low-energy inputs	Poor mixing, light, and CO_2 utilization efficiency
	Easy maintenance	Cultures are easily contaminated
	Low hydrodynamic stress on algae	Difficult temperature control
	Positive energy balance (NER <1)[a]	
Closed PBRs		*Tubular PBR*
	Large illumination surface area	Some degree of wall growth
	Suitable for outdoor cultures	Fouling
	Relatively cheap	Requires large land space
	Good biomass productivities	Gradients of pH, dissolved oxygen and CO_2 along the tubes
		Flat plate PBR
	High biomass productivities	Difficult scale-up
	Easy to sterilize	Difficult temperature control
	Low oxygen build-up readily tempered	Small degree of hydrodynamic stress
	Good light path	Some degree of wall growth
	Large illumination surface area	
	Suitable for outdoor cultures	
		Column PBR
	Compact	Small illumination area
	High mass transfer	Expensive compared to open ponds
	Low energy consumption	Shear stress
	Good mixing with low shear stress	Sophisticated construction
	Easy to sterilize	High investment and operational costs
	Reduced photoinhibition and photooxidation	

[a]*NER (Net energy ratio) is defined as the sum of the energy used for cultivation, harvesting, and drying, divided by the energy content of the dry biomass (i.e., NER < 1—the process produces more energy than it consumes; NER > 1—the process consumes more energy than it produces).*

Based on T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sustain. Energy Rev. 14 (2010) 217–232; S.R. Medipally, F.M. Yusoff, S. Banerjee, M. Shariff, Microalgae as sustainable renewable energy feedstock for biofuel production. Biomed. Res. Int. 2015 (2015) 1–13; L. Brennan, P. Owende, Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. 14 (2010) 557–577; R. Slade, A. Bauen, Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. Biomass Bioenerg. 53 (2013) 29–38.

raceway ponds the costs of algae cultivation in PBRs are expressively higher. They considered two distinct assumptions: (1) base case: it is assumed that CO_2 is purchased from the market and (2) projected case: use of municipal wastewater as the source of all water and nutrient inputs and CO_2 source (assumed to be free) must be close to the algal plant. In this case, raceway pond systems demonstrate a lower cost (estimated costs: $\sim$1.6–1.8 €/kg for the base case and $\sim$0.3–0.4 €/kg for the projected case) of algal biomass production than PBRs

systems (estimated costs: ~9–10 €/kg for a base case and ~3.8 €/kg for a projected case). Most of the production costs in raceway system are associated with operation (labor, utilities, and raw materials); on the other hand the cost of production in PBRs is dominated by the capital cost of the PBRs.

Thus, as previously discussed in Sections 2.3.1 to 2.3.3 (use of flue gas and wastewater), the competitiveness of the microalgae biofuels could only be achieved by using such low-cost sources. Moreover, for closed PBRs much more efforts must be conducted in order to overcome the capital and operating costs.

3.5 Operational Conditions

Operational conditions must be optimized in the way to fully support microalgae physiology and to use all the potential of cells for different wide range of process scenarios and PBRs configurations. In the state of the art in the field, lots of excellent books and original research articles are available. It must be noticed that working/operational conditions are strain dependent and must be optimized by taking into account the algae cell physiological requirements for optimal growth. As a rule, the algal kinetics is studied in a lab-scale closed PBRs fully equipped and controlled in order to understand the relationship between physical, chemical, and biological phenomena taking place during the dynamics changes of process state parameters where no limitations is allowed. This can be called formalization of the algal "pure" kinetics.

State parameters and crucial process design elements can be systematized as follows:

1. Light availability to the microalgal cell culture (delivery of photons);
2. CO_2 availability: mass-transfer CO_2 transport, O_2 removal, and gas exchange;
3. Hydrodynamics: mixing is responsible for cell distribution and trajectories inside the PBR, which influenced light history of the cells. Computer fluid dynamics is an extremely useful tool for process optimization of the productivity and PBR performance;
4. Temperature and pH control;
5. L/D cycles as a function of mixing conditions in PBRs;
6. S/V ratio;
7. Nutrients supply (source of N, P, S, other macro- and microelements);
8. Water loss;
9. Contamination;
10. Scale up: S/V ratio as one of the main scale-up parameters.

3.6 Downstream Processes

Downstream processes may represent a significant fraction of the global cost of biofuels and coproducts; especially for HVPs the downstream processes may represent 80%–90% of the production cost, due to the required high-resolution purification techniques.

Hence, the selection of innovative operation units for the downstream processes is essential for the reduction of the manufacturing costs. Each step in the separation processes,

considering the several target molecules (i.e., lipids, carbohydrates, HVPs, etc.) will depend on several factors such as the systems specificities:

1. Physical-chemical characteristics of the target molecule (as well as the surrounding medium);
2. Product destination (e.g., biofuels, coproducts, food additives, medicines, etc.);
3. Concentration or purity required levels.

3.6.1 Biomass Harvesting and Concentration

Production of biofuels and coproducts from microalgae systems requires many downstream processing steps (see Fig. 11.4), wherein harvesting is the following step after cultivation of microalgae and potentially contributes up to 20%–30% of the total production cost of microalgae biomass. During harvesting step the microalgae suspension requires a concentration method, which is most often done by centrifugation, filtration, and flocculation, wherein microalgae can achieve up to 20%–30% moisture content [67,68,94]; the choosing of the most adequate method will depend not only on the economics of the process but also on the technical characteristics, such as cell dimensions and characteristics. In addition to the traditional methods many efforts are conducted for novel techniques of algae harvesting, for instance, the auto flocculation by change in medium pH [106], which configures a relatively simple method reducing the high capital and operating costs associated to concentration equipment

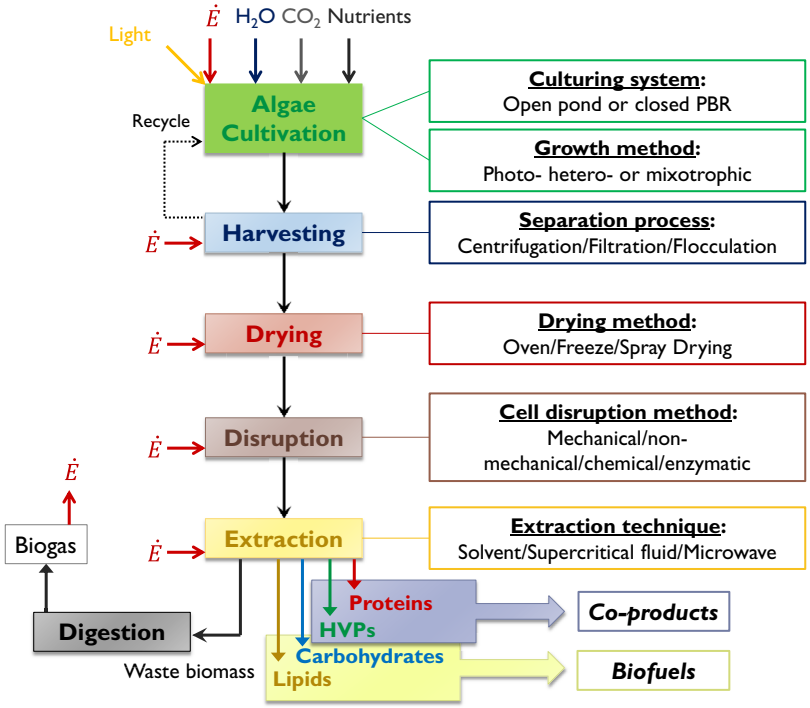


FIGURE 11.4 Schematic structure of the microalgae integrated biofuel and coproducts production system (Ė, energy flux).

(e.g., centrifugation and filtration). Combined methods are also commonly used in order to enhance the efficiency of harvesting [107]. A major criterion for selecting a proper harvesting procedure is the desired product quality [82].

Drying or dewatering of microalgae biomass is generally used prior to various conversion steps. The energy required for dewatering is known to constitute up to 84.9% of total energy consumption [67]. Thus, the energetic demands of the process (i.e., heat, electricity) can be supplied by the anaerobic digestion of waste biomass producing biogas (see Fig. 11.4). It must be evidenced that for thermo sensitive components, mostly for HVPs, the dewatering process must be conducted by less aggressive methods (e.g., freeze drying). For instance, spray drying is frequently used for HVPs (>\$1000 ton^{-1}), but it can cause significant deterioration of some algal components such as pigments. On the other hand, freeze drying or lyophilization has been widely used for drying microalgae in research laboratories; however for use in large-scale is an expensive method [108].

3.6.2 *Processing and Components Extraction*

Extraction of lipid is required for biodiesel production; however in the case of a biorefinery concept the HVP must be firstly extracted. For this purpose, cell disruption is necessary for recovering intracellular products from microalgae.

Likewise many other microbial processes for producing bioactives, the downstream recovery of algal products can be substantially more expensive than the culturing of the algae. Most of the traditional cell disruption methods employed with other microorganisms are also applied for microalgae [108], which includes:

1. Mechanical methods: high-pressure homogenizers, bead milling, ultrasonication; however sonication can be only used to disrupt small amounts of biomass and is not applicable in a large scale;
2. Nonmechanical: autoclave, microwave, and cryogenic grinding (or freezer milling); however those methods require great amounts of energy.
3. Chemical and biological: acid (e.g., hydrochloric acid), alkali (e.g., sodium hydroxide) and enzymatic agents. Nevertheless, chemical ones are aggressive methods and are not generally suitable for sensitive products such as proteins, thus such methods must be carefully applied in order to preserve the HVP characteristics; On the other hand, enzymatic methods commonly present elevated effectiveness on the cell's disruption, however the market price of the enzymes may jeopardize its use.

Regarding HVP extraction and purification, many separation processes have been employed, representing a substantial parcel of the processing cost. Solvent extraction of algal biomass is widely used to extract metabolites, such as astaxanthin, β-carotene, and essential fatty acids. Hexane, ethanol, chloroform, and diethyl ether can extract fatty acids such as EPA, DHA, and arachidonic acid from various microalgae [108].

However, more elaborated methods of purification may be required depending on the metabolite of interest, such as chromatographic methods: supercritical fluid chromatography to recover astaxanthin, polyunsaturated fatty acids, and other compounds; or ion exchange chromatography for proteins [108].

The lipid extraction aiming microalgal biodiesel production is a high-energy consumption step, due to the temperature and pressure conditions of the extraction process. Microalgae

biodiesel can be produced using a two-step method of oil extraction transesterification or one-step transesterification (so-called "in situ or direct transesterification") [67]; however, by considering the coproducts production the one-step method should by discouraged.

The most common method for extracting oil from microalgae is solvent extraction (e.g., hexane, ethanol, methanol, or methanol−chloroform mixture). The extraction efficiency of microalgae oil using n-hexane is rather low. In turn, ethanol is a very good solvent but it can also extract some cellular contaminants such as sugars, amino acids, salts, hydrophobic proteins, and pigments, thus is not desirable for extraction of only lipids. Ultrasonic-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction can be an alternative to organic solvent extraction [67,68,107].

3.7 Biomass Conversion

With regard to valorization, the microalgae biomass produced under high-productivity conditions is mainly composed of proteins (30%−50%), carbohydrates (20%−30%), lipids (10%−30%), and ash (5%−10%) (see Fig. 11.4) [78,109]. Such components of microalgae can be converted into biofuel production by various conversion methods including transesterification, fermentation, pyrolysis, liquefaction, and anaerobic digestion, which are used for production of biodiesel, bioethanol, bio-oil, and methane [67].

Biodiesel can be obtained from the saponifiable lipids (approximately 50% of total lipids) extracted using organic solvent and then transesterified to biodiesel in the presence of base or acid catalysts (see Box 11.3). Bioethanol is produced by microalgae fermentation from fermentable sugars (approximately 30% of total carbohydrates). Thus, only around 20%−30% of the biomass would be used if only biodiesel and bioethanol production were carried out. Thus, the residual biomass waste could be suitable in biogas production. Nonetheless, it must be evidenced that the economical value of biogas is low due to its low calorific value, CO_2 content (up to 45%), and gas nature and could be employed to supply energetic demands of the process (as previously discussed in Section 2.6.); however, in an integrated process it could successfully be used as both energy and as a CO_2 source (carbon recycle). In addition, thermochemical processes such as hydrothermal liquefaction and pyrolysis for bio-oil production have also been proposed [67,78].

Table 11.4 presents an overview of TEA of biodiesel and integrated biofuels production from microalgae.

3.8 Coproducts

At this time, biofuels production from microalgae is still not commercially implemented because "liquid fuel production only" is not economically feasible. Thus, all the components of microalgae biomass should be used to produce multiple products including various liquid biofuels for economically viable option [67].

All microalgae products can be divided in to three groups: low-, medium-, and high-value products. Integrated utilization of microalgae metabolites will significantly decrease the overall biofuel production process. Any new findings in metabolites use and application will increase the competitiveness of the biofuel-based technology from algae. Analysis of Table 11.5 shows that there are lots of potential to use algae metabolites in nutraceuticals and pharmaceuticals and especially in cosmetics.

TABLE 11.4 TEA Analysis of Microalgae-Based Biofuels Production

Overall Process Characteristics/Assumptions	Advances/Conclusions	Unit Production Cost ($/unit)	References
BIODIESEL			
Microalgae species: *Chlorella vul.* CCAP 211/11B **Cultivation:** Closed PBR (photoautotrophic) **Lipid content:** 19% (lipid productivity = 0.17 g/L d) **Separation:** Centrifugation + drying **Extraction:** Dimethyl ester **Biodiesel production:** Transesterification alkali catalyzed Anaerobic digestion of residual biomass Large-scale/subsidized land cost [a]	• Mathematical model for design of microalgae-based biodiesel production system by systematically integration of all production stages and strain properties • Considered optimal downstream processes based on strain properties (e.g., effective diameter) *Conclusions:* • Found strain properties as the key factors for system configuration	3.11 $/kg 2.98 $/kg	[94] [94]
Microalgae species: *Chlorella vul.* F&M-M49 **Cultivation:** Open pond (photoautotrophic) **Lipid content:** 18% (lipid productivity = 0.2 g/L d) **Separation:** Sedimentation + drying **Extraction:** Dimethyl ester **Biodiesel production:** Transesterification alkali catalyzed Anaerobic digestion of residual biomass Large-scale/subsidized land cost[a]			
Microalgae species: *Chlorella vulgaris* **Cultivation:** Open ponds (Paddlewheel) **Lipid content:** 25% **Separation:** Flocculation + lamella clarifier + centrifugation **Cell disruption:** Sonication **Extraction:** Hexane Crude oil production[b]	*Advances*: • CO_2, nitrogen, and phosphorus recovery to culture (50%, 76%, and 50%) *Conclusions*: • Major impacting operating factors on the production cost and sustainability to the process: paddlewheels, nutrients, water, maintenance, and harvesting • Energy, water, CO_2, and nutrient requirements represent the most significant obstacles to the sustainability of algal biodiesel at the industrial scale • Flue-gas and wastewater utilization will help to supplement some of the nutrient demands of algal	$3.21 L^{-1} of crude oil	[110]

(*Continued*)

TABLE 11.4 TEA Analysis of Microalgae-Based Biofuels Production—cont'd

Overall Process Characteristics/Assumptions	Advances/Conclusions	Unit Production Cost ($/unit)	References
	growth and integration with livestock production will provide nutrients to the system that can be used for algal production; however, locating these facilities in close proximity also depends on the available land and resources		
INTEGRATED PROCESS: ETHANOL/BIODIESEL/BIOGAS			
Microalgae species: *Scenedesmus* sp. and *Chlorella* sp. Integrated process: Biomass cultivation for ethanol fermentation and biodiesel **Cultivation:** Open ponds **Pretreatment and conditioning:** Steam and dilute-acid pretreatment of algal biomass (hydrolysis) **Fermentation:** Whole-slurry fermentation of the resulting monomeric hydrolyzed sugars to ethanol (*Saccharomyces cerevisiae*) **Ethanol Distillation:** Dilute ethanol broth distilled to near-azeotropic concentration and purification to 99.5% using vapor-phase molecular sieve adsorption **Lipid extraction:** Hexane extraction of the stillage (from ethanol distillation) to lipid recovering with solvent recovery by distillation stripping column **Product purification and upgrading:** Oil phase purification steps: Degumming, demetallization, and bleaching **Anaerobic digestion:** Aqueous phase anaerobic digestion and combined heat and power (CHP) generation: Biogas combustion in gas turbine to generate electricity employed in facility power demands and excess sold to the grid; flue gas heat recovering by generating steam to satisfy process and utility steam demands. **Utilities, infrastructure and logistics:** Product storage, required utilities and site developing were also considered **nth plant assumption** (i.e., the model assumes that several plants using the same technology have already been built and are operating, not a pioneer plant)	*Advances*: • Integrated processing strategy to selectively recover and convert select algal biomass components to fuels (e.g., carbohydrates to ethanol; lipids to a renewable diesel blendstock; residual stillage to biogas) • CO_2 recycling from the fermentation step to algal cultivation ponds • Analysis considering life-cycle implications: environmental sustainability for the modeled conversion step, including greenhouse gas emissions, fossil energy demand, and consumptive water use *Conclusions*: • Analysis results indicate a future demand in achieving simultaneously high cultivation productivity rates of biomass growth and high lipid contents (41%) otherwise higher costs will be obtained ($5.04/GGE lower for a 27% lipid content) • Analysis indicates that economics are strongly influenced by feedstock costs (contributing 70% to overall selling price) • Seasonality plays a unique and critical role in algal systems	$1.15/LGE ($4.35/GGE)	[111]

GGE, gallon gasoline equivalent; *LGE*, liter gasoline equivalent.

[a]*Authors proposed four different scenarios considering the production scale (i.e., small scale <100 ton of microalgae biodiesel per year; and large scale of 10,000 ton) and land investment (i.e., original land cost = 182.7 S$/m^2 and the subsidies are 90%, for the Singapore's context).*

[b]*Authors performed a TEA from different scenarios of algae farm and biocrude oil refinery of commercially relevant production capacity (1000 bbl/d).*

TABLE 11.5 Microalgae Coproducts, Their Value, and Global Market

Coproducts From Microalgae		Microalgal Species	Price USD/unit	Production kt/year	Global Market USD/year	
HIGH-VALUE PRODUCTS						
Nutraceuticals	Carotenoids	*Spirulina platensis, Chlorella vulgaris, Dunaliella salina, Scenedesmus Haematococcus pluvialis, Crypthecodinium cohnii, Shizochytrium, Nitzschia, Crypthecodinium*	–	2000 [112]	1200 million (2010) [113,114]	151 billion (2011) [112]
	β-Carotene		300–3000/kg [104,115–117]	300–1200 (2010) [118,119]	261 million (2010–2015) [113,114,117,118]	
	Astaxanthin		2000–10,000/kg [104,116,117,120]	100–300 (2004) [118,121]	250 million (2010–2015) [117,118,122]	
	Lutein		30–800/kg [117]	–	Up to 233 million (2010) [113,117]	
	Polyunsaturated fatty acids (Omega-3): EPA, DHA, GLA, AA and PUFA extracts		0.88–60/g (2010) [113,116,117]	10–300 [104,117,119,122]	>700 million [123]	
	Vitamins & supplements		Differs	N/A	68 billion (2010) [113]	
Pharmaceuticals	Pharmaceutical proteins	*Phaeodactylum tricornutum, Haematococcus pluvialis, Euglena viridis, Chlorococcum, Chlorella vulgaris, Spirulina platensis, Chlamydomonas reinhardtii*	0.05–0.2/g [74,124]	–	–	
	Antimicrobials, Antivirals & Antifungals		Differs	N/A	>1.5 billion (2010) (based on given data for lutein, astaxanthin, β-carotene, and PUFA)	
	Neuroprotective products					
Cosmetics	Anti-cellulite	*Spirulina platensis, Chlorella* spp., *Dunaliella salina, Laminaria, Nereocystis, Aphanizomenon flosaquae*	N/A	N/A	2.6 billion (2010) (based on the given data for carotenoids, β-carotene and PUFA)	
	Alguronic acid					
	Sun protection, skin care					

(*Continued*)

TABLE 11.5 Microalgae Coproducts, Their Value, and Global Market—cont'd

Coproducts From Microalgae		Microalgal Species	Price USD/unit	Production kt/year	Global Market USD/year
Phycobiliproteins	C-phycocyanin	*Spirulina platensis, Red microalgae (Porphyridium cruentum), Cynobacteria*	50,000–150,000/kg [104,116,117,125]	5 [115]	>60 million [122]
	B-phycoerythrin			N/A	
	R-Phycoerythrin			N/A	
	Allophycocyanin			N/A	
Stable isotopes			60–38,000/g [74]	–	>13 million [74]
MEDIUM-VALUE PRODUCTS					
Nutraceuticals from dried microalgal biomass		*Spirulina* spp.	10–80/kg [104,112]	Up to 5000 (2010, 2012) [104,112,116]	49 billion (2011) [112]
		Chlorella spp.		2000 (2003) [104,112,116]	
Hydrocolloids	Agar	*Spirulina* spp., *Scenedesmus* spp., *Acanthopeltis japonica, Gelidiella acerosa, Pterocladia capillacea*	18/kg [126]	9.6 [126]	173 million (2010) [126]
	Alginate		12/kg [126]	26.5 [126]	318 million (2010) [126]
	Carrageenan		10.5/kg [126]	50 [126]	525 million (2010) [126]
Other chemicals	Paints	*Chlorophyta, Cyanophyta, Rhodophyta, Euglenophyta*	50–1000/kg [104]	–	49.2 billion (2011)
	Dyes				
	Colorants				
LOW- TO MEDIUM-VALUE PRODUCTS					
Animal feed	Aquaculture	*Haematococcus pluvialis, Chlorella vulgaris, Porphyra* spp.	56/L [104]	300 [104,116]	3–4 billion [118]
	Livestock feed		10–130/kg [112]	~900 [112]	16.1 billion (2010) [112]
Bioplastics		*Spirulina platensis*	–	64 (2010) [113,114]	–
Glycerol		*Spirulina* spp., *Chlorella* spp.	0.3–1 (2010) [113]	1996 (2010) [113,114]	–

AA, arachidonic acid; *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *GLA*, γ-linolenic acid; *PUFA*, polyunsaturated fatty acids.

4. CONCLUSIONS

Industrial production of microalgal biodiesel requires large-scale cultivation and harvesting systems in which costs per unit area have to be minimized. At a large scale, the algal growth working conditions need to be precisely and robustly controlled and optimized. Such processes are most economical viable when combined with sequestration of CO_2 from flue gas emissions, with wastewater remediation processes, and/or with the extraction of high-value compounds for application in other process industries. Current limitations on the use of this feedstock for biodiesel production concern the optimization of the upstream and downstream processes of microalgae production. Hence, light, nutrients, temperature, hydrodynamics, and CO_2 and O_2 levels have to be monitored and controlled precisely in order to ensure optimal upstream process conditions for oil content and biomass yield. Unfortunately, considerable investment in technological development and technical expertise is still needed before algal biodiesel become economically competitive to common fuels. Without political and economic support this goal cannot be accomplished. Further efforts on microalgae production should be focused on the development of integrated approaches for reducing costs of complete microalgae biomass utilization in large-scale systems, aiming a global cost reduction. This can be achieved for example by using cheap sources of CO_2 for culture enrichment (e.g., from a flue gas), use of nutrient-rich wastewaters or inexpensive fertilizers, use of cheaper PBRs design systems with automated process control and with fewer manual labor, and use of greenhouses and heated effluents to increase algal yields. In addition to reducing costs of feedstock (nutrients and fresh water use), such measures will help in reducing greenhouse gases emissions, waste amount, and the feed cost by using nitrogen fertilizers. In addition, the global use of microalgae biomass including HVPs (e.g., food, agriculture, biofuels, and medicine among others) can be connected and linked with the biorefinery concept. Such approach will contribute to the sustainability and market competitiveness of the microalgae industry.

The feasibility of microalgae biomass–based energy will be strongly associated to:

1. Scientific innovations: creation of novel PBRs, mathematical tools, genetic modification methods, selection of microalgae strains with extremely high potential for overproduction of desired metabolites.
2. Overcoming of technological restrictions: process and PBRs design; optimization and control; demanded infrastructure; existence of competitive technologies and other energy sources; standalone technology does not work; biorefinery concept combined with total biomass utilization can be the key of success.
3. Minimizing of economical restrictions (global and regional factors): biomass production costs (feedstock, process, transport, logistics, etc.); energetic efficiency of the productive chain; competitive of the biomass feedstock for other purposes (e.g., industrial, food, etc.);
4. Political willingness: subsides and incentives for the technology;
5. Environmental protection and social impact: related to the preservation of the environment, human health care, and life quality. In fact, these various restrictions are strongly interrelated and time-dependent.

The competition between diversified renewable energy sources with well-established fossil fuels industry, which represent the world's most influential economical and political sector, will certainly confront several technical and economical constraints. However, by considering the urgent need of greener technologies and the fossil fuels depletion is inevitable that those well-established technologies will become more costly and those economical constraints for biomass-based renewable energies, which nowadays are still significant, will be overcome.

5. CONCLUSIONS AND PERSPECTIVES

Biofuels based on the microalgae biomass have a potential to be a main argument for diversification of energy source for human society. Scientific efforts directed to innovative solutions in creation of novel PBRs, mathematical tools, and genetic modification methods; selection of microalgae strains with extremely high potential for overproduction of desired metabolites; downstream processes; and biorefinery integrated concept will make a difference in the state of the art in the near future.

Acknowledgments

The authors are grateful to the CNPq program "Science without borders" under the grant #: 313,737/2014-2 with the CNPq Special Visiting Researcher Fellowship for Alexander Dimitrov Kroumov as well as to the Bulgarian National Science Foundation for partial financial support under the Grant (ДФНИ-Е01/0001—in Bulgarian).

References

[1] X. Wu, J.C. Merchuk, Simulation of algae growth in a bench scale internal loop airlift reactor, Chem. Eng. Sci. 59 (2004) 2899–2912.
[2] G. Vunjak-Novakovic, Y. Kim, X. Wu, I. Berzin, J.C. Merchuk, Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies, Ind. Eng. Chem. Res. 44 (2005) 6154–6163.
[3] P.H.C. Eilers, J.C.H. Peeters, A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton, Ecol. Modell. 42 (1988) 199–215.
[4] X. Wu, J.C. Merchuk, A model integrating fluid dynamics in photosynthesis and photoinhibition processes, Chem. Eng. Sci. 56 (2001) 3527–3538.
[5] Y.K. Lee, S.J. Pirt, Energetics of photosynthetic algal growth: influence of intermittent illumination in short (40 s), Cycles Microbiol. 124 (1981) 43–52.
[6] H.W. Heldt, B. Piechulla, The use of energy from sunlight by photosynthesis is the basis of life on earth, in: Plant Biochem, Elsevier, Amsterdam, 2011, pp. 43–64.
[7] R.E. Lee, Basic characteristics of the algae, in: Phycology, fourth ed., Cambridge University Press, Cambridge, 2008, pp. 3–30.
[8] C. Van Den Hoek, D.G. Mann, H.M. Jahns, Algae: an introduction to phycology, in: Search", first ed., Cambridge University Press, New York, 1995, pp. 1–15.
[9] L. Graham, L. Wilcox, Chapter 6: cyanobacteria, in: Algae, second ed., Prentice-Hall Inc, Upper Saddle River, 2000, pp. 106–117.
[10] C.G. Lee, B.O. Palsson, Photoacclimation of *Chlorella vulgaris* to red light from light-emitting diodes leads to autospore release following each cellular division, Biotechnol. Prog. 12 (1996) 249–256.
[11] Q. Hu, N. Kurano, M. Kawachi, I. Iwasaki, S. Miyachi, Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor, Appl. Microbiol. Biotechnol. 49 (1998) 655–662.
[12] A. Richmond, Principles for attaining maximal microalgal productivity in photobioreactors: an overview, Hydrobiologia 512 (2004) 33–37.

[13] F. García-Camacho, A. Sánchez-Mirón, E. Molina-Grima, F. Camacho-Rubio, J.C. Merchuck, A mechanistic model of photosynthesis in microalgae including photoacclimation dynamics, J. Theor. Biol. 304 (2012) 1–15.
[14] P.M. Slegers, R.H. Wijffels, G. van Straten, A.J.B. van Boxtel, Design scenarios for flat panel photobioreactors, Appl. Energy 88 (2011) 3342–3353.
[15] A. Sánchez Mirón, A. Contreras Gómez, F. Garcıa Camacho, E. Molina Grima, Y. Chisti, Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae, J. Biotechnol. 70 (1999) 249–270.
[16] J.C. Merchuk, X. Wu, Modeling of photobioreactors: application to bubble column simulation, J. Appl. Phycol. 15 (2003) 163–169.
[17] J.C. Ogbonna, H. Tanaka, Night biomass loss and changes in biochemical composition of cells during light/dark cyclic culture of *Chlorella pyrenoidosa*, J. Ferment. Bioeng. 82 (1996) 558–564.
[18] L. Nedbal, M. Trtílek, J. Červený, O. Komárek, H.B. Pakrasi, A photobioreactor system for precision cultivation of photoautotrophic microorganisms and for high-content analysis of suspension dynamics, Biotechnol. Bioeng. 100 (2008) 902–910.
[19] A. Richmond, CRC Handbook of Microalgal Mass Culture, Taylor & Francis Group, 1986.
[20] M. Borowitzka, L. Borowitzka, Micro-algal Biotechnology, Cambridge University Press, Cambridge, 1988.
[21] J.V. Moroney, R.A. Ynalvez, Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*, Eukaryot. Cell 6 (2007) 1251–1259.
[22] M. Giordano, J. Beardall, J.A. Raven, CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution, Annu. Rev. Plant Biol. 56 (2005) 99–131.
[23] M.R. Badger, G.D. Price, CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution, J. Exp. Bot. 54 (2003) 609–622.
[24] J.V. Moroney, S.G. Bartlett, G. Samuelsson, Carbonic anhydrases in plants and algae, Plant Cell Environ. 24 (2001) 141–153.
[25] S. Van Den Hende, H. Vervaeren, N. Boon, Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities, Biotechnol. Adv. 30 (2012) 1405–1424.
[26] S. Ebrahimi, C. Picioreanu, R. Kleerebezem, J.J. Heijnen, M.C.M. van Loosdrecht, Rate-based modelling of SO_2 absorption into aqueous $NaHCO_3/Na_2CO_3$ solutions accompanied by the desorption of CO_2, Chem. Eng. Sci. 58 (2003) 3589–3600.
[27] A. Concas, G.A. Lutzu, M. Pisu, G. Cao, Experimental analysis and novel modeling of semi-batch photobioreactors operated with *Chlorella vulgaris* and fed with 100% (v/v) CO_2, Chem. Eng. J. 213 (2012) 203–213.
[28] E. Molina-Grima, F.G. Acién-Fernández, F. García-Camacho, Y. Chisti, Photobioreactors: light regime, mass transfer, and scaleup, J. Biotechnol. 70 (1999) 231–247.
[29] M. Marsullo, A. Mian, A.V. Ensinas, G. Manente, A. Lazzaretto, F. Marechal, Dynamic modeling of the microalgae cultivation phase for energy production in open raceway ponds and flat panel photobioreactors, Front. Energy Res. 3 (2015) 1–18.
[30] M.A. Borowitzka, Vitamins and fine chemicals, in: M. Borowitzka, L. Borowitzka (Eds.), Micro-algal Biotechnol, Cambridge University Press, Cambridge, 1988, pp. 153–196.
[31] J.U. Grobbelaar, Algal nutrition − mineral nutrition, in: Handbook of Microalgal Culture, Blackwell Publishing Ltd, Oxford, 2007, pp. 95–115.
[32] J. Cundiff, K. Mankin, Microbial systems, in: Dynamics of Biological Systems, ASAE Press, St. Joseph, 2003, pp. 5–7.
[33] R.K. Mandalam, B.Ø. Palsson, Elemental balancing of biomass and medium composition enhances growth capacity in high-density *Chlorella vulgaris* cultures, Biotechnol. Bioeng. 59 (1998) 605–611.
[34] A.D. Kroumov, A.N. Módenes, D.E.G. Trigueros, A complex theoretical approach for algal medium optimization for CO_2 fixation from flue gas, Acta Microbiol. Bulg. 31 (2015) 61–70.
[35] P.H.C. Eilers, J.C.H. Peeters, Dynamic behaviour of a model for photosynthesis and photoinhibition, Ecol. Modell. 69 (1993) 113–133.
[36] N. Kurano, H. Ikemoto, H. Miyashita, T. Hasegawa, H. Hata, S. Miyachi, Fixation and utilization of carbon dioxide by microalgal photosynthesis, Energy Convers. Manag. 36 (1995) 689–692.
[37] J.C. Ogbonna, H. Tanaka, Light requirement and photosynthetic cell cultivation -development of processes for efficient light utilization in photobioreactors, J. Appl. Phycol. 12 (2000) 207–218.
[38] H. Tamiya, E. Hase, K. Shibata, A. Mituya, T. Iwamura, T. Nihei, T. Sasa, Kinetics of growth of *Chlorella*, with special reference to its dependence on quantity of available light and on temperature, in: J. Burlew (Ed.), Algal Cult. From Lab. To Pilot Plant, Carnegie Institution of Washington, Washington, 1976, pp. 204–234.

[39] J.L.P. Van Oorschot, Conversion of Light Energy in Algal Culture, 55, Veenman, Wageningen, 1955, pp. 225–277.
[40] J. Steele, Microbial kinetics and dynamics, in: L. Lapidus, N. Amundson (Eds.), Chem. React. Theory, Prentice-Hall Inc, Englewood Cliffs, 1977, pp. 405–483.
[41] S. Aiba, Growth kinetics of photosynthetic microorganisms, in: Adv. Biochem. Eng, Springer Berlin Heidelberg, Berlin, Heidelberg, 1982, pp. 85–156.
[42] E. Molina-Grima, F. Garcia Camacho, J.A.S. Pérez, J.M.F. Sevilla, F.G. Acién-Fernández, A.C. Gómez, A mathematical model of microalgal growth in light-limited chemostat culture, J. Chem. Technol. Biotechnol. 61 (1994) 167–173.
[43] E. Molina Grima, J. Fernandez Sevilla, J. Sánchez Pérez, F. Garcia Camacho, A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances, J. Biotechnol. 45 (1996) 59–69.
[44] F.G. Acién Fernández, F. García Camacho, J.A. Sánchez Pérez, J.M. Fernández Sevilla, E. Molina Grima, Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: effects of dilution rate, tube diameter, and solar irradiance, Biotechnol. Bioeng. 58 (1998) 605–616.
[45] N. Kurano, S. Miyachi, Selection of microalgal growth model for describing specific growth rate-light response using extended information criterion, J. Biosci. Bioeng. 100 (2005) 403–408.
[46] J. Pruvost, J.F. Cornet, J. Legrand, Hydrodynamics influence on light conversion in photobioreactors: an energetically consistent analysis, Chem. Eng. Sci. 63 (2008) 3679–3694.
[47] A. Muller-Feuga, R. Le Guédes, J. Pruvost, Benefits and limitations of modeling for optimization of *Porphyridium cruentum* cultures in an annular photobioreactor, J. Biotechnol. 103 (2003) 153–163.
[48] O. Bernard, B. Rémond, Validation of a simple model accounting for light and temperature effect on microalgal growth, Bioresour. Technol. 123 (2012) 520–527.
[49] J. Pruvost, B. Le Gouic, O. Lepine, J. Legrand, F. Le Borgne, Microalgae culture in building-integrated photobioreactors: biomass production modelling and energetic analysis, Chem. Eng. J. 284 (2016) 850–861.
[50] Q. Béchet, A. Shilton, B. Guieysse, Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation, Biotechnol. Adv. 31 (2013) 1648–1663.
[51] J.F. Andrews, A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates, Biotechnol. Bioeng. 10 (1968) 707–723.
[52] S.Y. Chiu, C.Y. Kao, C.H. Chen, T.C. Kuan, S.C. Ong, C.S. Lin, Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor, Bioresour. Technol. 99 (2008) 3389–3396.
[53] I. Douskova, J. Doucha, K. Livansky, J. MacHat, P. Novak, D. Umysova, V. Zachleder, M. Vitova, Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs, Appl. Microbiol. Biotechnol. 82 (2009) 179–185.
[54] Q. Liao, L. Li, R. Chen, X. Zhu, A novel photobioreactor generating the light/dark cycle to improve microalgae cultivation, Bioresour. Technol. 161 (2014) 186–191.
[55] E. Sforza, D. Simionato, G.M. Giacometti, A. Bertucco, T. Morosinotto, Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors, PLoS One 7 (2012) e38975.
[56] J.C. Merchuk, F. Garcia-Camacho, E. Molina-Grima, Photobioreactor design and fluid dynamics, Chem. Biochem. Eng. Q. 21 (2007) 345–355.
[57] M. Janssen, J. Tramper, L.R. Mur, R.H. Wijffels, Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects, Biotechnol. Bioeng. 81 (2003) 193–210.
[58] G. Olivieri, A. Marzocchella, P. Salatino, L. Gargiulo, P. Lettieri, L. Mazzei, Photobioreactors for microalgal cultures: a model for photosynthesis rate assessment, Chem. Eng. Trans. 32 (2013) 1039–1044.
[59] H. Takache, J. Pruvost, H. Marec, Investigation of light/dark cycles effects on the photosynthetic growth of *Chlamydomonas reinhardtii* in conditions representative of photobioreactor cultivation, Algal Res. 8 (2015) 192–204.
[60] M.R. Tredici, R. Materassi, From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms, J. Appl. Phycol 4 (1992) 221–231.
[61] D. Chaumont, Biotechnology of algal biomass production: a review of systems for outdoor mass culture, J. Appl. Phycol. 5 (1993) 593–604.

[62] Q. Hu, H. Guterman, A. Richmond, A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs, Biotechnol. Bioeng. 51 (1996) 51–60.
[63] A. Richmond, Biological principles of mass cultivation, in: Handbook of Microalgal Culture, Blackwell Publishing Ltd, Oxford, 2007, pp. 125–177.
[64] E. Cohen, S. Arad, A closed system for outdoor cultivation of *Porphyridium*, Biomass 18 (1989) 59–67.
[65] Q. Hu, A. Richmond, Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor, J. Appl. Phycol. 8 (1996) 139–145.
[66] G.A. Andrade, M. Berenguel, J.L. Guzmán, D.J. Pagano, F.G. Acién, Optimization of biomass production in outdoor tubular photobioreactors, J. Process. Control 37 (2016) 58–69.
[67] O.K. Lee, D.H. Seong, C.G. Lee, E.Y. Lee, Sustainable production of liquid biofuels from renewable microalgae biomass, J. Ind. Eng. Chem. 29 (2015) 24–31.
[68] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sustain. Energy Rev. 14 (2010) 217–232.
[69] E. Nwokoagbara, A.K. Olaleye, M. Wang, Biodiesel from microalgae: the use of multi-criteria decision analysis for strain selection, Fuel 159 (2015) 241–249.
[70] S.M. Abdo, S.A. Abo El-Enin, K.M. El-Khatib, M.I. El-Galad, S.Z. Wahba, G. El Diwani, G.H. Ali, Preliminary economic assessment of biofuel production from microalgae, Renew. Sustain. Energy Rev. 55 (2016) 1147–1153.
[71] N. Rashid, M.S.U. Rehman, S. Memon, Z. Ur Rahman, K. Lee, J.I. Han, Current status, barriers and developments in biohydrogen production by microalgae, Renew. Sustain. Energy Rev. 22 (2013) 571–579.
[72] J. Singh, S. Gu, Commercialization potential of microalgae for biofuels production, Renew. Sustain. Energy Rev. 14 (2010) 2596–2610.
[73] M.F. De Jesus Raposo, R.M.S.C. De Morais, A.M.M.B. De Morais, Health applications of bioactive compounds from marine microalgae, Life Sci. 93 (2013) 479–486.
[74] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2006) 87–96.
[75] A.J. Klok, D.E. Martens, R.H. Wijffels, P.P. Lamers, Simultaneous growth and neutral lipid accumulation in microalgae, Bioresour. Technol. 134 (2013) 233–243.
[76] M.S. Chauton, K.I. Reitan, N.H. Norsker, R. Tveterås, H.T. Kleivdal, A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities, Aquaculture 436 (2015) 95–103.
[77] M. Koller, A. Muhr, G. Braunegg, Microalgae as versatile cellular factories for valued products, Algal Res. 6 (2014) 52–63.
[78] F.G. Acién, J.M. Fernández, E. Molina-Grima, Economics of microalgae biomass production, in: Biofuels from Algae, Elsevier, 2014, pp. 313–325.
[79] M. Packer, Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy, Energy Policy 37 (2009) 3428–3437.
[80] M. Schneider, M. Romer, M. Tschudin, H. Bolio, Sustainable cement production-present and future, Cem. Concr. Res. 41 (2011) 642–650.
[81] J.C.M. Pires, F.G. Martins, M.C.M. Alvim-Ferraz, M. Simões, Recent developments on carbon capture and storage: an overview, Chem. Eng. Res. Des. 89 (2011) 1446–1460.
[82] A. Richmond, Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Blackwell Science Ltd, Oxford, 2004.
[83] B. Wang, Y. Li, N. Wu, C.Q. Lan, CO_2 bio-mitigation using microalgae, Appl. Microbiol. Biotechnol. 79 (2008) 707–718.
[84] L. Christenson, R. Sims, Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts, Biotechnol. Adv. 29 (2011) 686–702.
[85] J.K. Pittman, A.P. Dean, O. Osundeko, The potential of sustainable algal biofuel production using wastewater resources, Bioresour. Technol. 102 (2011) 17–25.
[86] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Appl. Energy 88 (2011) 3411–3424.
[87] S.S.I. Marques, I.A. Nascimento, P.F. De Almeida, F.A. Chinalia, Growth of *Chlorella vulgaris* on sugarcane vinasse: the effect of anaerobic digestion pretreatment, Appl. Biochem. Biotechnol. 171 (2013) 1933–1943.

[88] C.A. Christofoletti, J.P. Escher, J.E. Correia, J.F.U. Marinho, C.S. Fontanetti, Sugarcane vinasse: environmental implications of its use, Waste Manag. 33 (2013) 2752–2761.
[89] R. Dillschneider, C. Posten, A linear programming approach for modeling and simulation of growth and lipid accumulation of *Phaeodactylum tricornutum*, Energies 6 (2013) 5333–5356.
[90] S.R. Medipally, F.M. Yusoff, S. Banerjee, M. Shariff, Microalgae as sustainable renewable energy feedstock for biofuel production, Biomed. Res. Int. 2015 (2015) 1–13.
[91] C.Y. Chen, K.L. Yeh, R. Aisyah, D.J. Lee, J.S. Chang, Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review, Bioresour. Technol. 102 (2011) 71–81.
[92] B. Wenzel, M. Tait, A. Módenes, A. Kroumov, Modelling chemical kinetics of soybean oil transesterification process for biodiesel production: an analysis of molar ratio between alcohol and soybean oil temperature changes on the process conversion rate, Bioautomation 5 (2006) 13–22.
[93] A.D. Kroumov, A.N. Módenes, B.M. Wenzel, Development of the enzymatic kinetics model of vegetable oils transesterification for biodiesel production (in Portuguese), Acta Sci. Technol. 29 (2007) 9–16.
[94] N. Yu, L.T.J. Dieu, S. Harvey, D.Y. Lee, Optimization of process configuration and strain selection for microalgae-based biodiesel production, Bioresour. Technol. 193 (2015) 25–34.
[95] Y.H. Chen, T.H. Walker, Biomass and lipid production of heterotrophic microalgae *Chlorella protothecoides* by using biodiesel-derived crude glycerol, Biotechnol. Lett. 33 (2011) 1973–1983.
[96] T. Heredia-Arroyo, W. Wei, R. Ruan, B. Hu, Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials, Biomass Bioenerg. 35 (2011) 2245–2253.
[97] Y. Liang, N. Sarkany, Y. Cui, Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions, Biotechnol. Lett. 31 (2009) 1043–1049.
[98] J. Wang, H. Yang, F. Wang, Mixotrophic cultivation of microalgae for biodiesel production: status and prospects, Appl. Biochem. Biotechnol. 172 (2014) 3307–3329.
[99] C. Song, G. Chen, N. Ji, Q. Liu, Y. Kansha, A. Tsutsumi, Biodiesel production process from microalgae oil by waste heat recovery and process integration, Bioresour. Technol. 193 (2015) 192–199.
[100] A.J. Klok, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, Edible oils from microalgae: insights in TAG accumulation, Trends Biotechnol. 32 (2014) 521–528.
[101] S.A. Scott, M.P. Davey, J.S. Dennis, I. Horst, C.J. Howe, D.J. Lea-Smith, A.G. Smith, Biodiesel from algae: challenges and prospects, Curr. Opin. Biotechnol. 21 (2010) 277–286.
[102] I. Khozin-Goldberg, Z. Cohen, Unraveling algal lipid metabolism: recent advances in gene identification, Biochimie 93 (2011) 91–100.
[103] B. Liu, C. Benning, Lipid metabolism in microalgae distinguishes itself, Curr. Opin. Biotechnol. 24 (2013) 300–309.
[104] L. Brennan, P. Owende, Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sustain. Energy Rev. 14 (2010) 557–577.
[105] R. Slade, A. Bauen, Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects, Biomass Bioenerg. 53 (2013) 29–38.
[106] S.B. Ummalyma, A.K. Mathew, A. Pandey, R.K. Sukumaran, Harvesting of microalgal biomass: efficient method for flocculation through pH modulation, Bioresour. Technol. 213 (2016) 216–222, http://dx.doi.org/10.1016/j.biortech.2016.03.114.
[107] J. Kim, G. Yoo, H. Lee, J. Lim, K. Kim, C.W. Kim, M.S. Park, J.W. Yang, Methods of downstream processing for the production of biodiesel from microalgae, Biotechnol. Adv. 31 (2013) 862–876.
[108] E. Molina Grima, E.H. Belarbi, F.G. Acién Fernández, A. Robles Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics, Biotechnol. Adv. 20 (2003) 491–515.
[109] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306.
[110] J.N. Rogers, J.N. Rosenberg, B.J. Guzman, V.H. Oh, L.E. Mimbela, A. Ghassemi, M.J. Betenbaugh, G.A. Oyler, M.D. Donohue, A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales, Algal Res. 4 (2014) 76–88.
[111] R. Davis, C. Kinchin, J. Markham, E.C.D. Tan, L.M.L. Laurens, "Process Design and Economics for the Conversion of Algal Biomass to Biofuels: Algal Biomass Fractionation to Lipid and Carbohydrate – Derived Fuel Products", Technical Report NREL/TP–5100–62368, 2014.
[112] J. Egardt, Ø. Lie, J. Aulie, P. Myhre, Microalgae: a market analysis carried out as part of the Interreg KASK IVA project: Blue Biotechnology for sustainable innovations, Technical Report Blue Bio, (January 2013).

[113] G. Markou, E. Nerantzis, Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions, Biotechnol. Adv. 31 (2013) 1532–1542.
[114] K.R. Hakeem, M. Jawaid, U. Rashid, Biomass and bio-energy, Fuel Energ. Abstr. 44 (2003) 158.
[115] A.C. Guedes, H.M. Amaro, F.X. Malcata, Microalgae as sources of carotenoids, Mar. Drugs 9 (2011) 625–644.
[116] J.N. Rosenberg, G.A. Oyler, L. Wilkinson, M.J. Betenbaugh, A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution, Curr. Opin. Biotechnol. 19 (2008) 430–436.
[117] S. Van Iersel, A. Flammini, "Algae-based Biofuels: Applications and Co-products" No. 44, FAO Environmental and Natural Resources Service Series, Rome, 2010.
[118] R.H. Wijffels, Potential of sponges and microalgae for marine biotechnology, Trends Biotechnol. 26 (2008) 26–31.
[119] C. Enzing, M. Ploeg, M. Barbosa, L. Sijtsma, Technical Report EUR 26255, in: M. Vigani, C. Parisi, E.R. Cerezo (Eds.), Microalgae-based Products for the Food and Feed Sector: An Outlook for Europe, Publications Office of the European Union, Luxembourg, 2014.
[120] K.D. Nguyen, Astaxanthin: A Comparative Case of Synthetic vs. Natural Production, Chemical and Biomolecular Engineering Publications and Other Works, Knoxville, 2013.
[121] M. Vigani, C. Parisi, E. Rodríguez-Cerezo, M.J. Barbosa, L. Sijtsma, M. Ploeg, C. Enzing, Food and feed products from micro-algae: market opportunities and challenges for the EU, Trends Food Sci. Technol. 42 (2015) 81–92.
[122] M.A. Borowitzka, High-value products from microalgae—their development and commercialization, J. Appl. Phycol. 25 (2013) 743–756.
[123] P. Clough, Marketing Omega-3 products as nutraceuticals, Nutr. Health 19 (2008) 233–234.
[124] D. Barrera, S. Mayfield, High-value recombinant protein production in microalgae, in: A. Richmond, Q. Hu (Eds.), Handbook of Microalgal Culture: Applied Phycology and Biotechnology, second ed., Blackwell Publishing Ltd, Oxford, 2013, pp. 532–544.
[125] R. Bermejo Román, J.M. Alvárez-Pez, F.G. Acién Fernández, E. Molina Grima, Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*, J. Biotechnol. 93 (2002) 73–85.
[126] S. Kraan, Algal polysaccharides, novel applications and outlook, in: C. Chang (Ed.), Carbohydrates – Comprehensive Studies on Glycobiology and Glycotechnology, Intech, Rijeka, 2013.

CHAPTER

12

Polyamines: Stress Metabolite in Marine Macrophytes

M. Kumar[1,a], U. Kuzhiumparambil[1,a], P.J. Ralph[1], L. Contreras-Porcia[2]

[1]University of Technology Sydney (UTS), Sydney, NSW, Australia; [2]Universidad Andres Bello, Santiago, Chile

OUTLINE

1. INTRODUCTION

Marine macrophytes including seaweeds and sea grasses are considered as marine ecosystem engineers delivering a range of ecologically and economically valuable biological

[a]Equally contribution.

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00012-6

services. Seaweeds also called as marine macroalgae are rich source of metabolites and natural products including proteins, furanones, polyunsaturated fatty acids, phenolics, pigments, phlorotannins, phycocolloids (carrageenan and agar), and minerals [1]. Therefore, seaweeds hold a great promise in nutraceuticals, pharmaceuticals, biofuels, and phycocolloid industries with annual market value of US$7 billion [2]. Sea grasses are monocotyledonous marine flowering plants widely distributed across the global coastline covering an area of 30−60 million km^2. They are descendants of terrestrial plants that returned to marine aquatic life million years ago, support 50% of the world's fisheries, and provide essential nutrition for almost 3 billion people. Their nutrient cycling value alone is estimated to be three times higher than that of tropical rainforests, worth US$2 trillion per year [3]. Both marine macrophytes are equally important in delivering a range of biological services in aquatic environments including nutrient cycling, carbon sequestration, sediment stabilization, and habitat provision to a range of dependent marine fauna.

Marine macrophytes often experience extreme climatic and anthropogenic disturbances in their ecological niche. The major stressful conditions in their aquatic habitat include fluctuations in salinity, light, and temperature; desiccation together with eutrophication; hypoxia and/or anoxia; heavy metals; and industrial effluents. The impact of all these stresses tends to be similar because they all directly or indirectly exert considerable pressure on osmotic balance of cells leading to perturbation of various physiological functions at cellular level and eventually affect the productivity of aquatic ecosystems [4]. Adaptation and acclimation to such environmental stress are therefore of particular importance in intertidal benthic organisms such as seaweeds and sea grasses. These marine macrophytes rapidly accumulate reactive oxygen species (ROS) in response to these stressful environments, a response commonly known as oxidative burst [5]. Although, ROS production has an important role in inducing signaling events, the production of ROS must be tightly regulated. The excess ROS accumulation results in membrane lipid peroxidation, oxidation of biomolecules, DNA damage, pigments and protein degradation, and eventually cell death. To alleviate such damaging effects, marine macrophytes reprogram their metabolic machinery as an acclimation/adaptive strategy. During acclimation, marine macrophytes express a battery of antioxidant enzymes together with maintaining a higher level of nonenzymatic antioxidants and a diverse array of primary and secondary metabolites [including proline, betaine, polyamines (PAs), low molecular weight carbohydrates, polyols, oxylipins, polyunsaturated fatty acids, among others] [4]. These metabolites contribute to control the cellular ROS levels and maintain cellular redox status and homeostasis.

In terrestrial plants, the role of PAs in regulating the cellular signaling and metabolism during stress has been proposed in two mechanisms—(1) they act as ROS scavengers by modulating antioxidant system and (2) they promote ROS production through their catabolism [6]. In marine macrophytes, PAs are primarily known to regulate morphogenesis and developmental processes; however, their involvement in response to abiotic stress has been recently recognized [7]. Here, we attempted to provide a comprehensive knowledge of PA research in marine macrophytes, although it is just a scratch on window when compared to land plant, but this research area holds a great promise in exploring novel stress tolerance mechanism in marine macrophytes.

2. POLYAMINE METABOLISM AND BIOLOGICAL ROLE IN MARINE MACROPHYTES

PAs, the low molecular weight aliphatic amines, are ubiquitous in all living organisms except the archeal methanogens and halophiles. They are found in the chloroplasts, mitochondria, cell wall fractions, and vacuoles of higher plants in millimolar concentrations. In marine macrophytes, the diamine putrescine (Put), triamine spermidine (Spd), and tetramine spermine (Spm) are the major PAs; however, some brown and red seaweeds are reported to have norspermine and norspermidine, and cadeverine PAs in sea grasses [7]. These are produced in free and conjugated forms (bound soluble and bound insoluble) bound to small molecules such as hydroxycinnamic acid or to larger molecules such as proteins or nucleic acids.

In plants, PAs as compatible solutes have shown to modulate a diverse range of biological processes including cell division and elongation, root and shoot architecture, flower and fruit development, replication, transcription, translation, membrane and cell wall stabilization, chromatin organization, ribosome biogenesis, and responses to various biotic and abiotic stresses [8,9]. So far, PA research in marine macrophytes has primarily addressed their involvement in maturation of reproductive structures and morphogenesis/callus induction [7]. However, their function as stress alleviators against salinity, desiccation stress, and metal toxicity has only recently been recognized [10–17]. In marine macrophytes, PA level varies significantly ranging from 160 μg to 30 mg/g^1 fresh weight (FW), between the species and also with the development stage of tissue analyzed [18].

The biosynthesis pathways of PAs begin either directly from ornithine catalyzed by ornithine decarboxylase (ODC) or indirectly from arginine catalyzed by arginine decarboxylase (ADC) with two intermediates, agmatine and N-carbamoylputrescine, which are catalyzed by two corresponding biosynthetic enzymes, agmatine iminohydrolase and N-carbamoylputrescine amidohydrolase resulting in the synthesis of Put (Fig. 12.1) [5,7]. Later, Put is converted to Spd via spermidine synthase with the addition of aminopropyl group provided by decarboxylated S-adenosylmethionine (dcSAM), which is catalyzed by S-adenosylmethionine decarboxylase (SAMDC) using S-adenosylmethionine (SAM) as a substrate. Similarly, Spm is formed from Spd via spermine synthase (SPMS) with the same aminopropyl group rendered by dcSAM [7]. Furthermore, Spm and Spd can also be synthesized from methionine pathway with the involvement of methionine adenosyltransferase (MAT) and decarboxylase. The PA catabolism involves its oxidative deamination by diamine oxidase (DAO, a Cu-containing enzyme) and polyamine oxidase (PAO, a FAD-dependent enzyme) that results in the production of 4-aminobutanal, H_2O_2, and ammonia (NH_3). The resulting 4-aminobutanal is further converted to γ-aminobutyric acid (GABA), which serves as a source of succinic acid, an intermediary of Krebs cycle [19]. Interestingly, the Krebs and GS/GOGAT cycle are linked directly to urea cycle, members of which are direct precursors of PAs (Fig. 12.1). It appears that marine plants share with land plants similar PA metabolic pathways. However, our understanding of the functionality of PAs in marine macrophytes when compared with that of land plants has merely dented the surface, although the existence of PAs in marine plants was reported since 1994.

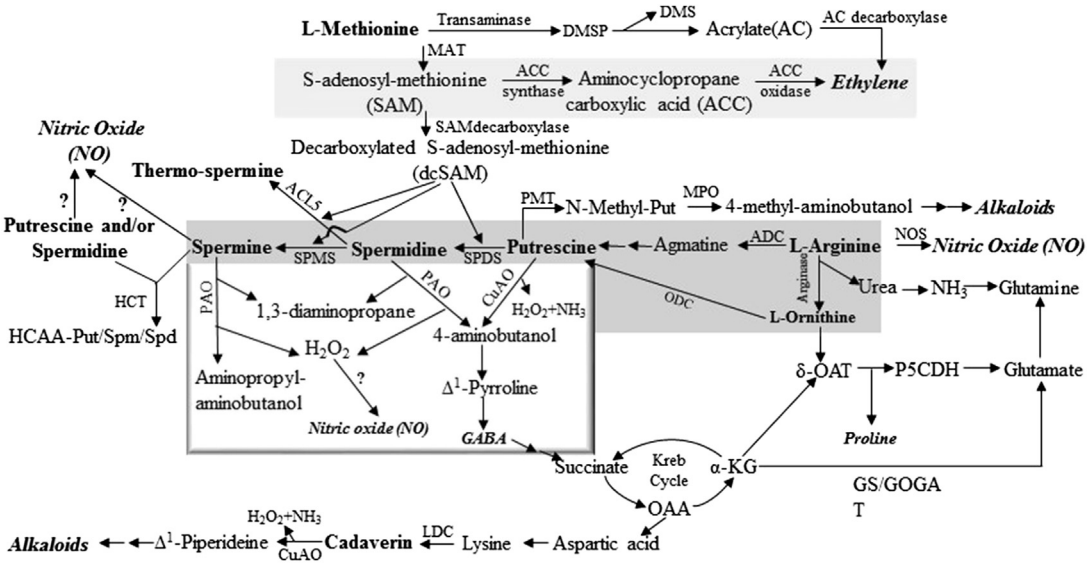


FIGURE 12.1 Schematic representation of polyamine (PA) metabolism with special reference to marine macrophytes. *Dark gray shaded* area represents two major biosynthetic pathways of PAs via ADC and ODC. *Bevel shaded* area represents the PA oxidation pathway. *Light gray shaded* area represents the ethylene biosynthesis pathway, which has been lost in sea grass during their evolution to marine aquatic life. Metabolites in italics are the biomolecules possibly linked to PA metabolism. *ACL5*, thermospermine synthase; *ADC*, arginine decarboxylase; *CuAO*, Cu-based amine oxidase; *DMS*, dimethyl sulfide; DMSP, dimethylsulfoniopropionate; *GABA*, Y-aminobutyric acid; *GS/GOGAT*, glutamine synthetase/glutamine oxoglutarate aminotransferase; *HCAA*, hydroxycinnamic acid amide; *HCT*, hydroxycinnamoyl transferase; *KG*, ketoglutarate; *LDC*, lysine decarboxylase; *MAT*, methionine adenosyltransferase; *MPO*, N-methyl-putrescine oxidase; *NOS*, nitric oxide synthase; OAA, oxaloacetate; *ODC*, ornithine decarboxylase; *P5CDH*, △-1-pyrroline-5-carboxylate dehydrogenase; *PAO*, polyamine oxide; *PMT*, putrescine N-methyl transferase; *SPDS*, spermidine synthase; *SPMS*, spermine synthase. (?) unknown mechanism.

3. POLYAMINE ANALYSIS IN MARINE MACROPHYTES

PAs are the secondary metabolites in plants and have been analyzed in terrestrial and marine plants using a targeted metabolite approach with a reversed-phase high-performance liquid chromatography (RP-HPLC)- or thin-layer chromatography (TLC)-based analytical system [4]. The levels of PAs vary considerably between the species, with the highest level of PAs in seaweeds belonging to Rhodophyta compared to Chlorophyta [18]. Separation of PAs, positively charged aliphatic compounds with two or more amino groups, with the HPLC or TLC requires either incorporation of an ion-pair reagent in the solvent system or derivatization of the amino groups with a derivatizing reagent. The derivatizing reagent enhances the detection of PAs by UV absorbance or fluorescence. Various reagents such as dansyl chloride, dabsyl chloride, fluorescamine, fluorenyl-methyl chloroformate, *o*-phthalaldehydes, and benzoylchloride have been used to derivatize PAs for HPLC separation based on fluorescence [4]. However, dansyl chloride—based derivatization has been preferred for PA analysis because dansyl chloride reacts with both primary and secondary amines; dansyl

derivatives are highly stable and can be detected at very low concentrations in the pmol range. However, it is worth to mention here that because thermospermine (tSpm) is usually undistinguishable from its isomer Spm in the standard analysis of PAs. A recently developed method based on ion-pair extraction and gas chromatography mass spectrometry has been the most promising in clarifying the ambiguity with the existence and separation of tSpm from Spm [20] and thus can also be used in marine plants. Nonetheless, many published analytical methods suffer from at least one of the following drawbacks: (1) long chromatographic run times, (2) PA determination using one specific matrix resulting in higher limit detection, and (3) time incurred in derivatization step. In recent years, ultrahigh-performance liquid chromatography (UHPLC) is the method of choice for metabolomics research rather than RP-HPLC because it provides greater resolution, peak capacity, and lower detection limits with the use of small-particle-size columns (1.7 μm). However, it does require high pressure (10,000−15,000 psi) compared to 6000 psi in RP-HPLC [4].

Recently, we have developed a UHPLC-based method for PA detection in sea grasses namely *Zostera muelleri* and *Posidonia australis*. This method followed the procedure of sample preparation and derivatization using dansyl chloride as described by Kumar et al. [10]. However, the chromatographic separation and detection of PAs was carried out on an Agilent Zorbax Eclipse XDB-C18 column (50 mm × 2.1 mm ID, 1.8 μm particle size) equipped with an RRLC inline filter (0.2 μm). Samples were eluted from the column with a programmed water (A) and acetonitrile (B) solvent gradient over 15 min. The gradient elution of (A) water and (B) acetonitrile is as follows: 0−1 min, 90%A → 40%A; 1−4 min, 40%A → 20%A; 4−7 min, 20%A → 5%A; 7−10 min, 5%A; 10−11 min, 5%A → 90%A; 11−15 min, 90%A (Fig. 12.2A). The fluorescence detector was operated at excitation 340 nm and emission at 500 nm, flow rate was maintained at 0.3 mL/min, and column temperature was maintained at 25 C. UPLC analysis of PAs was performed on an Agilent 1200 Series Rapid Resolution LC system. The system was equipped with binary pumps, a thermostat column compartment, a microvacuum degasser, a high-performance autosampler, and a fluorescence detector. Data processing was performed using chemstation for LC 3D systems (Agilent Technologies). 1 mM PA standard stock solutions were made in 5% perchloric acid and they were also dansylated at working concentration of 8 μM following the procedure of Kumar et al. [10], using 1,7-heptanediamine (Htd, 50 μM) as internal standard. Finally, the dansylated standards were filtered through a 0.45-μm-pore-sized syringe filter, mixed together at a concentration of 2 μM, and were injected to the UHPLC. PA analysis using UHPLC resulted in highly reproducible peaks, with consistent retention times and peak areas. Retention times of different dansylated standard PAs were as follows: 5.93 (Cad), 6.12 (Put), 6.67 (Htd), 7.49 (Spd), and 8.65 (Spm) (Fig. 12.2B). With this UHPLC-based method, the level of total PAs in sea grass leaves were found to be 260.47 $\mu M/g^1$ FW in *P. australis* and 357.95 $\mu M/g^1$ FW in *Z. muelleri*. Total PAs in sea grass *Cymodocea nodosa* using TLC-based approach has been reported to be 13.8 mg/g^1 FW with BS-Put as the highest PA [27]. In sea grass *Z. muelleri*, BS-PAs specifically BS-Spd registered the highest level (142.5 ± 14.2 $\mu M/g^1$ FW) followed by free PAs (with high level of Spm, 42.6 ± 4.2 $\mu M/g^1$ FW) and BI-PAs (Put, 28.9 ± 4.2 $\mu M/g^1$ FW) (Fig. 12.2C). However, in *P. australis*, the level of PAs followed the order as BS > BI > free PAs, with free Spm as the richest PA with concentration 72.6 ± 6.0 μM $/g^1$ FW (Fig. 12.2D).

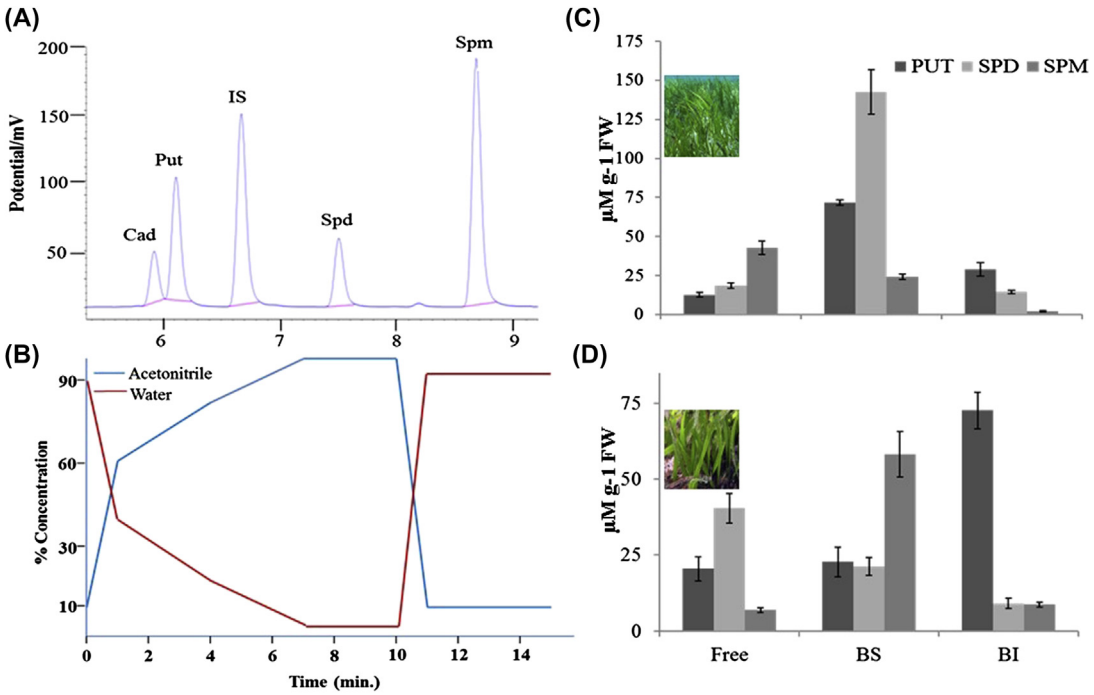


FIGURE 12.2 (A) Schematic diagram of the gradient used in ultrahigh-performance liquid chromatography (UHPLC) method for polyamine analysis in sea grasses; (B) chromatogram of dansylated standard polyamines (Put, putrescine; Spd, spermidine; Spm, spermine; Cad, cadaverin; IS, internal standard); (C) and (D) represent polyamine levels in seagrass Zostera muelleri and Posidonia australis, respectively. BS and BI represent bound-soluble and bound-insoluble PAs.

4. INVOLVEMENT OF POLYAMINE IN MARINE MACROPHYTES UNDER STRESS CONDITIONS

Endogenous and/or exogenous PAs have been shown to modulate stress-triggered ROS homeostasis and oxidative damage by enhancing the antioxidant enzyme activities and pools of nonenzymatic antioxidants in marine plants [10–17]. Their biological activities have been attributed to their cationic nature, which enables them to interact with all the negatively charged cellular components such as DNA, RNA, proteins, phospholipids, and others. In addition, stabilization of PSII proteins in the thylakoid membranes (D1, D2, cytb6/f) with their covalent binding to PAs catalyzed by transglutaminases and/or by electrostatic interaction owing to their polycationic nature has also been suggested [21]. PAs have been suggested to protect cells by forming binary or tertiary complexes with the phospholipid polar heads that impede autooxidation of Fe^{2+} and phospholipids, and subsequently reducing the generation of ROS [22]. PAs as stress alleviators in marine plants have been evident with the accumulation of free Put, Spd, and Spm together with a significant decrease in transglutaminase activity and an increase in arginine-dependent PA synthesis in red seaweed

Grateloupia doryphora exposed to hyposalinity stress [14]. Furthermore, pretreatment of *Ulva lactuca* with Spd or Spm, in contrast to Put, resulted in constantly higher expression of protein disulfide isomerase (UfPDI) when exposed to hypersalinity, suggesting the potential of PAs to alleviate salinity stress and restore the growth rate [16]. Furthermore, the elevated expression of MAT (catalyzes Spm and Spd synthesis from dcSAM and Put) in *Undaria pinnatifida* under hypersalinity, low temperature, desiccation, and hormone exposure further suggested the role of PAs as stress alleviators [23]. Recently, exogenous Spm supplementation (1 mM) was found to regulate the stabilization of DNA methylation by reducing the cytosine demethylation, which alleviated the cadmium-induced stress in *Gracilaria dura* [12]. Spm supplementation, in contrast to Put, efficiently ameliorated the Cd toxicity by decreasing the accumulation of ROS and membrane damage, while restoring or enhancing the level of enzymatic (SOD, APX, GR) and nonenzymatic (AsA, GSH) antioxidants and their redox ratio, phycobiliproteins and phytochelatins [12]. However, understanding the precise mechanism of PAs and enzymatic antioxidant system connection needs further studies. Moreover, a significant increase in the content of both free and bound-soluble Put was also noticed without any change in spermidine in *U. lactuca* exposed to 0.4 mM Cd^{+2} for 4 days [10]. Moreover, a noticeable increase in the level of free and bound-insoluble Put and Spm in desiccated thalli of *G. dura* suggested the possible involvement of PAs in stress response [11]. In terrestrial plants, the response of PAs to salt stress has been suggested to be ABA-dependent because ADC and SPMS are induced by abscisic acid (ABA)-responsive elements (ABRE and/or ABRE-related motifs), which are present in the promoters of PA biosynthetic genes [6]. However, the existence of such phenomenon has not been explored so far in marine plants. In this regard, the upregulation of cytochrome P450 such as ABA 8′-hydroxylase during hydration–desiccation cycle in *Pyropia orbicularis* opened a new avenue to explore ABA involvement in transcription of regulatory networks of desiccation stress signals and gene expression [24,25]. Moreover, ABA in *P. orbicularis* was suggested to be involved in the upregulation of several compounds such as prolines and PAs. Thus, it is also possible to hypothesize the participation of ABA-dependent and/or ABA-independent pathways in the regulatory response to desiccation stress in marine plants [26].

The red seaweed *Pyropia cinnamomea* has been shown to thrive under intense UV radiations with PA accumulation, especially bound-soluble and bound-insoluble PAs synthesized via ADC biosynthesis pathway [13]. Similarly, the higher ratio of Put/Spd, together with high level of phenolics in *Sargassum cymosum* when exposed to UVA + UVB radiation, suggested the binding of PAs to phenolic molecules that result in stabilizing the primary cell wall by cross-linking it to cellular components, such as polysaccharide-bound phenols [17]. So far, *C. nodosa* is the only sea grass examined for the presence of PAs, wherein the total PA level was shown to decrease significantly in the embryonic stage, but increased during the seedling development stages [15,27]. Furthermore, exogenous application of Spm has been shown to protect *C. nodosa* from hyposalinity stress with significant accumulation of Cad, which maintains the photosynthetic apparatus under long-term hypoosmotic stress [15]. Increase in PA concentration within the cell can be due to their de novo synthesis or due to reduction in PA degradation pathways although the exact mechanisms still remain unelucidated. For example, accumulation of GABA possibly from Put degradation via DAO and/or PAO activities during hypersalinity stress in *Ectocarpus siliculosus* has also been documented (Fig. 12.1)

[28]. This study provided the first indication of GABA synthesis from PA degradation with the fact of nonexistence of genes required for GABA synthesis in *E. siliculosus*.

Earlier perceptions of passive PA uptake in seaweeds (an aspect not studied in sea grasses yet) also need to readdress with the recent demonstration for the existence of L-type amino acid transporter called *resistant to methylviologen 1* (*RMV1*) responsible for PA uptake in land plants. This evidence essentially emphasizes that in prokaryotes and eukaryotes, PA transport is not a passive mechanism, and selectivity should be achieved through specific recognition and translocation [29]. However, the recent genomic data of *Zostera marina* indicated the presence of putative PA transporter with >70% protein sequence similarly to *Arabidopsis thaliana* PA transporters (At1g31830, At1g31820; http://www.uniprot.org/uniprot/?query=proteome:UP000036987).

The importance of PAs in acclimation/adaptation to light stress has been documented well in unicellular green algae *Scenedesmus obliquus* (see references in review [18]). Studies have suggested that light-harvesting complexes contain mainly Put, while Spm are primarily localized in reaction centers. The existence of Put, Spd, and Spm in all photosynthetic subcomplexes but Spm only in PSII core has also been demonstrated. Moreover, not only individual PA level but contribution of PA ratio has also been suggested crucial to examine the stress response. Therefore, it will be very interesting to study if PAs in plastids play a crucial role in protecting the thylakoid membranes against the deleterious effects of extreme environments of marine ecosystem. Recently, a novel role of PA in chemiosmosis for regulating ATP synthesis and subsequent fine-tuning the antenna regulation to photoprotect the chloroplast has been suggested [30]. This mechanism may provide novel insight into the regulation of photosynthetic machinery by PAs in marine macrophytes to make them more tolerant to stress.

5. METABOLITES' CROSS TALK WITH POLYAMINES NEEDS EXPLORATION IN MARINE MACROPHYTES

Plants employed multilevel signal transduction to induce stress response. The interaction of diverse metabolites, belonging to different but interconnected metabolic pathways, in an orchestic manner results in cellular homeostatic condition under stress conditions. For example, coordinated action of PAs with other phytohormones, such as ethylene (ET), jasmonate, and other signaling molecules such as Ca^{2+}, cyclic nucleotides, ROS, and reactive nitrogen species (RNS), forming a complex signaling network, has been demonstrated in terrestrial plants [6,19].

PAs and prolines both share a common substrate ornithine for their biosynthesis. The ornithine formed by arginase activity can be utilized for PA synthesis via ODC pathway. Alternatively, ornithine can be converted into proline through the action of ornithine-δ-aminotransferase (δ-OAT), pyrroline-5-carboxylate dehydrogenase (P5CDH), and/or pyrroline-5-synthase and reductases (P5CS and P5CR) [19]. Therefore, the endogenous accumulation of PAs or their exogenous application might result in more substrate for proline biosynthesis, especially under stress conditions. Furthermore, SAM, which is involved in

PA biosynthesis via SAMDC that generates dcSAM for Spm and Spd synthesis, is also a universal methyl donor in many enzymatic reactions involving *O*-, *N*-, and *C*- methyltransferases in primary and secondary metabolism. SAM is also a substrate for ET biosynthesis via 1-aminocyclopropane-1-carboxylate (ACC) synthase that converts SAM to ACC, which is later oxidized by ACC oxidase to ET [6,7,19] (Fig. 12.1). Hence, PAs and ET compete for the common substrate SAM to execute their biological role in a cell. In marine macrophytes, ET biosynthesis from methionine via DMSP and acrylate pathway has also been suggested (Fig. 12.1). PA and ET act in an antagonistic manner; PAs inhibit senescence while ET promotes it. Moreover, PA-Spm has been shown to regulate ET biosynthesis by inhibiting ACC synthase at transcriptional level. ET is also equally effective in inhibiting ADC and SAMDC and PA biosynthesis inhibition that suggest a delicate connection between PA and ET regulation [31]. Functionality of ET in marine macrophytes has been addressed to influence the reproductive process in red seaweed *Pterocladiella capillacea* and in acclimation response to high-light stress in *Ulva intestinalis* [32,33]. García-Jiménez et al. [32] suggested that ET induces tetrasporogenesis not directly but via upregulation of Put biosynthesis in red seaweed *P. capillacea*. These findings point a synergistic action and a possible cross talk of ET and PAs in regulating reproductive processes and also offer to explore the functional cross talk between these metabolites and ET receptor responses at genetic level. Moreover, ET in *U. intestinalis* under high-light stress and in *Gelidium arbuscular* on white light and salinity stress has shown to accumulate without any change in the activities of acrylate decarboxylase and DMS lyase, respectively [33,34]. These findings in marine macrophytes (particularly in seaweeds) suggest ET biosynthesis under stress conditions via SAM to ACC pathway and thus may be linked to PA metabolism indirectly. However, it needs further investigation before coming to any firm conclusions. Surprisingly, this is not the case in marine sea grasses, as the recent genomewide survey analysis of sea grasses suggests the loss of ET biosynthesis and signaling pathway genes in *Zostera* species during their evolution from terrestrial to marine aquatic life [35].

Recently, it has been shown that PAs can induce the production of nitric oxide (NO) that serves as a signal-inducing salinity resistance in land plants by stimulating plasma membrane H^+-ATPase, and Na^+/H^+ ion channels involve in ion homeostasis under stress [6]. However, it is not clear whether or not NO production induced by PAs is mediated by H_2O_2, a product of PA oxidation, or by some unknown mechanisms. Moreover, NO and ROS reaction under stress conditions generates peroxynitrites, which affect the cofactor required for ACS and ACO involved in ET synthesis. Therefore, NO may directly affect ET production or its downstream signaling and may also indirectly influence other phytohormones (such as salicylic acid, jasmonic acid, abscisic acid) and signaling molecules such as cADP ribose, cGMP, and Ca^{2+}, which are intricately connected to ET biosynthesis [6,31]. NO signaling in land plants is also mediated through posttranslational modifications, mainly nitration and S-nitrosylation of various proteins and metabolites such as phytochelatins and fatty acids in plant cells [36]. Moreover, Fe-superoxide dismutase, dehydroascorbate reductase, and monodehydroascorbate reductase are PA-induced S-nitrosylation target proteins in higher plants and suggest NO-modulated S-nitrosylation is a possible link between PA and NO [37]. Enzyme P5CS, catalyzing the proline synthesis, was identified as S-nitrosylation target in Spd-treated plants, with the strong induction of *P5CS1* expression by Spd application providing evidence for NO-dependent functional link between PAs and prolines in response

to abiotic stress [38]. Inhibition of MAT through S-nitrosylation and subsequent reduced turnover of SAM has also been demonstrated to downregulate the ET production in *Arabidopsis* [39]. However, such phenomenons are yet to be explored in marine macrophytes.

NO has been proposed long back to play a role in the adaptation of the intertidal green macroalga *U. lactuca* to desiccation stress [40]. Recently, NO production in *Ulva fasciata* on exposure to high-light conditions and the subsequent upregulation of methionine sulfoxide reductase expression has been suggested for acquisition of full tolerance to high-light stress [41]. An increased activity of Δ′-pyrroline-5-carboxylate reductase (*P5CR*) together with a decrease in Ca^{2+} ions concentration and the blockage of calmodulin action resulted in elevated levels of proline in *Ulva* sp., and *E. siliculosus* under hypersalinity conditions. This suggest the functionality of proline as an osmolyte for osmotic adjustment, in stabilizing subcellular structures (membranes and proteins) and scavenging free radicals, as a nitrogen-storage compound, and as an energy source after the release of stress and buffering cellular redox potential under stress [28,42]. Furthermore, Zhang et al. [43] reported that copper-induced proline synthesis is associated with NO generation, which subsequently resulted in higher activity and transcript level of *P5CS* and *P5CR* in *Chlamydomonas reinhardtii*. The accumulation of GABA during anoxic conditions in sea grass and in hypersalinity stress in *E. siliculosus* has been suggested to provide an alternative route for pyruvate metabolism via alanine-GABA shunt (Ala-GABA). Furthermore, Ala and GABA are known to be important metabolites allowing carbon and nitrogen storage during adverse conditions and provide energy for metabolism upon reestablishment of normal conditions.

All these findings together suggest a possible link among PAs-NO-ET-Proline-GABA and thus need a detailed investigation in this regard in fresh and/or marine flora subjected to environmental or anthropogenic perturbations such as high light, salinity, desiccation, heavy metals, warming, and acidic oceanic conditions. Exploring the interconnection of PAs with diverse linked metabolic pathways may provide novel insights into their signaling role during stress response in marine macrophytes.

6. CONCLUSION AND FUTURE PERSPECTIVE

It is quite apparent from the aforementioned studies that PAs are involved in stress responses of marine macrophytes to natural and anthropogenic perturbation. However, the knowledge on PAs as stress alleviators in marine macrophytes compared to land plants is merely a scratch on window. Moreover, their involvement in morphogenesis and developmental process (carposporogenesis, sporulation, and tetrasporogenesis) has also been well recognized in seaweeds but not in sea grasses. The lack of extensive genomic information in both seaweeds and sea grasses, and the absence of authoritative data based on molecular gene expression studies, has been the major constrain to establish the functions of PAs as stress busters.

With the recent influx of whole-genome sequencing information of seaweeds namely *E. siliculosus* (brown seaweed), *Chondrus crispus* and *Porphyra umbilicalis* (red seaweed), and sea grass *Z. marina*, new suites of opportunities have emerged to investigate the PA metabolism [4]. With this information, the expression of genes encoding PA metabolism and other

associated metabolic networks can be studied in detail, confirming their role as a biomarker of stress severity or an indicator of stress tolerance. At present, there is a need to address several crucial aspects of PA metabolism in marine macrophytes: (1) which metabolic pathway is primarily involved for PA synthesis (ADC/ODC) in marine macrophytes under stress (Fig. 12.1); (2) identification of cellular components mediating links between ROS/RNS generation, signaling, and PAs (if any); (3) exploration of PA interaction with diverse interconnected metabolic networks; (4) how PAs transport from source to sink; and (5) how PA interactions with photosynthetic complexes, such as LHCs, influence stress response in marine macrophytes. As such there are bulks of questions than answers in regard to PA involvement in response to abiotic and biotic stress in marine macrophytes.

Unprecedented opportunities with the availability of genome sequences of marine macrophyte models enabled the use of reverse genetics, transgenic and omic approaches to generate exceptional knowledge in understanding the role of PAs in stress response in future. Recently, cloning of ODC gene from red seaweed *Grateloupia imbricata* (*GiODC*) and its expression studies throughout the reproductive process is an excellent breakthrough and lay foundation to undertake research aiming at cloning and transform genes encoding for PA biosynthesis in marine macrophytes. The identification of PAs regulated downstream targets and exploring the connections between PAs and other stress responsive molecules will open new opportunities to investigate the functionality of individual PAs at various omic platforms to understand the systems biology of marine macrophytes under stress environments.

Acknowledgment

The first author (Manoj Kumar) gratefully acknowledges the Australian Research Council for awarding him Discovery Early Career Research Award (DECRA Fellowship, DE150100461-2015).

References

[1] S.L. Holdt, S. Kraan, Bioactive compounds in seaweed: functional food applications and legislation, J. Appl. Phycol. 23 (2011) 543–597.
[2] Food and Agriculture Organization of the United Nations, Fisheries and Aquaculture Information and Statistics Services, 2014, [WWW document] URL http://www.fao.org/figis/.
[3] M. Waycott, C.M. Duarte, T.J. Carruthers, R.J. Orth, W.C. Dennison, S. Olyarnik, A. Calladine, J.W. Fourqurean, K.L. Heck, A.R. Hughes, Accelerating loss of seagrasses across the globe threatens coastal ecosystems, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 12377–12381.
[4] M. Kumar, U. Kuzhiumparambil, M. Pernice, Z. Jiang, P.J. Ralph, Metabolomics: an emerging frontier of systems biology in marine macrophytes, Algal Res. 16 (2016) 76–92.
[5] M. Kumar, P. Kumari, C.R.K. Reddy, B. Jha, Salinity and desiccation induced oxidative stress acclimation in seaweeds, in: B. Nathalie (Ed.), Advances in Botanical Research, Academic Press, 2014, pp. 91–123.
[6] J. Saha, E.K. Brauer, A. Sengupta, S.C. Popescu, K. Gupta, B. Gupta, Polyamines as redox homeostasis regulators during salt stress in plants, Front. Environ. Sci. 3 (2015) 21.
[7] M. Kumar, C. Reddy, P.J. Ralph, Polyamines in morphogenesis and development: a promising research area in seaweeds, Front. Plant Sci. 6 (2015) 1–4.
[8] A. Sengupta, M. Chakraborty, J. Saha, B. Gupta, K. Gupta, Polyamines: osmoprotectants in plant abiotic stress adaptation, in: N. Iqbal, R. Nazar, N.A. Khan (Eds.), Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies, Springer India, New Delhi, 2016, pp. 97–127.
[9] A.F. Tiburcio, T. Altabella, M. Bitrián, R. Alcázar, The roles of polyamines during the lifespan of plants: from development to stress, Planta 240 (2014) 1–18.

[10] M. Kumar, P. Kumari, V. Gupta, P. Anisha, C. Reddy, B. Jha, Differential responses to cadmium induced oxidative stress in marine macroalga *Ulva lactuca* (Ulvales, Chlorophyta), BioMetals 23 (2010) 315–325.
[11] M. Kumar, V. Gupta, N. Trivedi, P. Kumari, A. Bijo, C. Reddy, B. Jha, Desiccation induced oxidative stress and its biochemical responses in intertidal red alga *Gracilaria corticata* (Gracilariales, Rhodophyta), Environ. Exp. Bot. 72 (2011) 194–201.
[12] M. Kumar, A. Bijo, R.S. Baghel, C. Reddy, B. Jha, Selenium and spermine alleviate cadmium induced toxicity in the red seaweed *Gracilaria dura* by regulating antioxidants and DNA methylation, Plant Physiol. Biochem. 51 (2012) 129–138.
[13] K. Schweikert, C.L. Hurd, J.E. Sutherland, D.J. Burritt, Regulation of polyamine metabolism in *Pyropia cinnamomea* (W.A. Nelson), an important mechanism for reducing UV-B-induced oxidative damage, J. Phycol. 50 (2014) 267–279.
[14] P. García-Jiménez, P.M. Just, A.M. Delgado, R.R. Robaina, Transglutaminase activity decrease during acclimation to hyposaline conditions in marine seaweed *Grateloupia doryphora* (Rhodophyta, Halymeniaceae), J. Plant Physiol. 164 (2007) 367–370.
[15] M. Zarranz Elso, P. García-Jiménez, R.R. Robaina, Endogenous polyamine content and photosynthetic performance under hypo-osmotic conditions reveal *Cymodocea nodosa* as an obligate halophyte, Aquatic Biol. 17 (2012) 7–17.
[16] L.-C. Li, Y.-T. Hsu, H.-L. Chang, T.-M. Wu, M.-S. Sung, C.-L. Cho, T.-M. Lee, Polyamine effects on protein disulfide isomerase expression and implications for hypersalinity stress in the marine alga *Ulva lactuca* Linnaeus, J. Phycol. 49 (2013) 1181–1191.
[17] L.K. Polo, M.R.L. Felix, M. Kreusch, D.T. Pereira, G.B. Costa, C. Simioni, R.D.P. Martins, A. Latini, E.S.I. Floh, F. Chow, F. Ramlov, M. Maraschin, Z.L. Bouzon, É.C. Schmidt, Metabolic profile of the brown macroalga *Sargassum cymosum* (Phaeophyceae, Fucales) under laboratory UV radiation and salinity conditions, J. Appl. Phycol. 27 (2015) 887–899.
[18] K. Schweikert, D.J. Burritt, Polyamines in macroalgae: advances and future perspectives, J. Phycol. 51 (2015) 838–849.
[19] M. Bitrián, X. Zarza, T. Altabella, A.F. Tiburcio, R. Alcázar, Polyamines under abiotic stress: metabolic crossroads and hormonal crosstalks in plants, Metabolites 2 (2012) 516–528.
[20] J.L. Rambla, F. Vera-Sirera, M.A. Blázquez, J. Carbonell, A. Granell, Quantitation of biogenic tetraamines in *Arabidopsis thaliana*, Anal. Biochem. 397 (2010) 208–211.
[21] S. Shu, S.R. Guo, L.Y. Yuan, A review: polyamines and photosynthesis, in: M. Najafpour (Ed.), Advances in Photosynthesis – Fundamental Aspects, InTech, 2012, http://dx.doi.org/10.5772/26875. ISBN 978-953-307-928-8.
[22] H. Shi, Z. Chan, Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway, J. Integr. Plant Biol. 56 (2014) 114–121.
[23] K. Qiao, S.G. Li, H.Y. Li, S.M. Tong, H.S. Hou, Molecular cloning and sequence analysis of methionine adenosyltransferase from the economic seaweed *Undaria pinnatifida*, J. Appl. Phycol. 25 (2013) 81–87.
[24] L. Contreras-Porcia, C. López-Cristoffanini, C. Lovazzano, M.R. Flores-Molina, D. Thomas, A. Núñez, C. Fierro, E. Guajardo, J.A. Correa, M. Kube, R. Reinhardt, Differential gene expression in *Pyropia columbina* (Bangiales, Rhodophyta) under natural hydration and desiccation conditions, Lat. Am. J. Aquat. Res. 41 (2013) 933–958.
[25] M.R. Flores-Molina, D. Thomas, C. Lovazzano, A. Núñez, J. Zapata, M. Kumar, J.A. Correa, L. Contreras-Porcia, Desiccation stress in intertidal seaweeds: effects on morphology, antioxidant responses and photosynthetic performance, Aquat. Bot. 113 (2014) 90–99.
[26] E. Guajardo, J.A. Correa, L. Contreras-Porcia, Role of abscisic acid (ABA) in activating antioxidant tolerance responses to desiccation stress in intertidal seaweeds species, Planta 243 (2016) 767–781.
[27] F.D. Marián, P. Garcia-Jimenez, R.R. Robaina, Polyamine levels in the seagrass *Cymodocea nodosa*, Aquat. Bot. 68 (2000) 179–184.
[28] S.M. Dittami, A. Gravot, D. Renault, S. Goulitquer, A. Eggert, A. Bouchereau, C. Boyen, T. Tonon, Integrative analysis of metabolite and transcript abundance during the short-term response to saline and oxidative stress in the brown alga *Ectocarpus siliculosus*, Plant Cell Environ. 34 (2011) 629–642.
[29] M. Fujita, K. Shinozaki, Identification of polyamine transporters in plants: paraquat transport provides crucial clues, Plant Cell Physiol. 55 (2014) 855–861.
[30] N.E. Ioannidis, K. Kotzabasis, Polyamines in chemiosmosis *in vivo*: a cunning mechanism for the regulation of ATP synthesis during growth and stress, Front. Plant Sci. 5 (2014).

[31] M. Pál, G. Szalai, T. Janda, Speculation: polyamines are important in abiotic stress signaling, Plant Sci. 237 (2015) 16–23.
[32] P. Garcia-Jimenez, R.R. Robaina, Effects of ethylene on tetrasporogenesis in *Pterocladiella capillacea* (Rhodophyta), J. Phycol. 48 (2012) 710–715.
[33] P. Ina, S. Michael, M. Gill, Ethene (ethylene) production in the marine macroalga *Ulva (Enteromorpha) intestinalis* L. (Chlorophyta, Ulvophyceae): effect of light-stress and co-production with dimethyl sulphide, Plant Cell Environ. 28 (2005) 1136–1145.
[34] P. Garcia-Jimenez, O. Brito-Romano, R.R. Robaina, Production of volatiles by the red seaweed *Gelidium arbuscula* (Rhodophyta): emission of ethylene and dimethyl sulfide, J. Phycol. 49 (2013) 661–669.
[35] J.L. Olsen, P. Rouzé, B. Verhelst, Y.-C. Lin, T. Bayer, J. Collen, E. Dattolo, E. De Paoli, S. Dittami, F. Maumus, G. Michel, A. Kersting, C. Lauritano, R. Lohaus, M. Töpel, T. Tonon, K. Vanneste, M. Amirebrahimi, J. Brakel, C. Boström, M. Chovatia, J. Grimwood, J.W. Jenkins, A. Jueterbock, A. Mraz, W.T. Stam, H. Tice, E. Bornberg-Bauer, P.J. Green, G.A. Pearson, G. Procaccini, C.M. Duarte, J. Schmutz, T.B.H. Reusch, Y. Van de Peer, The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea, Nature 530 (2016) 331–335.
[36] F.J. Corpas, J.C. Begara-Morales, B. Sánchez-Calvo, M. Chaki, J.B. Barroso, Nitration and S-Nitrosylation: two post-translational modifications (PTMs) mediated by reactive nitrogen species (RNS) and their role in signalling processes of plant cells, in: J.K. Gupta, U.A. Igamberdiev (Eds.), Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants, Springer International Publishing, Cham, 2015, pp. 267–281.
[37] R. Wimalasekera, F. Tebartz, G.F.E. Scherer, Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses, Plant Sci. 181 (2011) 593–603.
[38] G. Tanou, V. Ziogas, M. Belghazi, A. Christou, P. Filippou, D. Job, V. Fotopoulos, A. Molassiotis, Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress, Plant Cell Environ. 37 (2014) 864–885.
[39] C. Lindermayr, G. Saalbach, G. Bahnweg, J. Durner, Differential inhibition of *Arabidopsis* methionine adenosyltransferases by protein *S*-nitrosylation, J. Biol. Chem. 281 (2006) 4285–4291.
[40] M.S. Murthy, A.S. Rao, E.R. Reddy, Dynamics of nitrate reductase activity in two intertidal algae under desiccation, Bot. Mar 29 (1986) 471–474.
[41] Y.-T. Hsu, T.-M. Lee, Nitric oxide up-regulates the expression of methionine sulfoxide reductase genes in the intertidal macroalga *Ulva fasciata* for high light acclimation, Plant Cell Physiol. 53 (2012) 445–456.
[42] T.-M. Lee, C.-H. Liu, Correlation of decreased calcium contents with proline accumulation in the marine green macroalga *Ulva fasciata* exposed to elevated NaCl contents in seawater, J. Exp. Bot. 50 (1999) 1855–1862.
[43] L.P. Zhang, S.K. Mehta, Z.P. Liu, Z.M. Yang, Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*, Plant Cell Physiol. 49 (2008) 411–419.

CHAPTER

13

Microalgal Biomass Cultivation

P. Geada[1], *V. Vasconcelos*[2], *A. Vicente*[1], *B. Fernandes*[1]

[1]University of Minho, Braga, Portugal; [2]University Porto, Porto, Portugal

OUTLINE

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00013-8

1. INTRODUCTION

Microalgae are thallophytes (plants lacking roots, stems, and leaves) that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells [1]. In a taxonomic approach, cyanobacteria cannot be considered to be part of the algae as they are prokaryotes. However, in applied phycology, cyanobacteria are included in the microalgae group together with microscopic algae because they share a significant number of characteristics [2]. Some of the current systems of microalgae classification are based on type of pigments, chemical nature of storage products, and cell wall constituents. Additional criteria take into consideration the following cytological and morphological characters: occurrence of flagellate cells, structure of the flagella, nuclear and cell division characteristics, presence of an envelope of endoplasmic reticulum around the chloroplast, and possible connection between the endoplasmic reticulum and the nuclear membrane [3]. There are two basic types of microalgae cells: prokaryotic and eukaryotic.

1.1 Prokaryotic Cells

Autotrophic prokaryotic cells lack membrane-bounded organelles (plastids, mitochondria, Golgi bodies, and flagella), and the DNA is not structured in chromosomes lying free in the cytoplasm [3]. They have a four-layered gram-negative cell wall, with a lipopolysaccharide layer involving a murein (peptidoglycan) layer. The lack of cellulose in cyanobacteria is responsible for a high digestibility of these cells (e.g., *Spirulina*) when compared to eukaryotic cells (e.g., *Chlorella vulgaris*). The cell wall may be delimited by mucilaginous envelopes (glycocalyx, sheath, capsule, or slime), perforated by small pores or having appendages such as fimbriae and pili [3]. The plasma membrane is $\sim$8 nm thick.

In prokaryotic cells, the photosynthetic apparatus is enclosed in the thylakoid, which is a membrane system that may be organized in concentric rings, in parallel bundles, or dispersed in the cytoplasm. Glycogen, polyphosphate, and cyanophycin granules, as well as lipid droplets, carboxysomes, gas vacuoles, and ribosomes are some of the most common cell inclusions of cyanobacteria cells.

In cyanobacteria, cell division can occur through binary fission, multiple fission, and fragmentation (hormogonia); additionally, some filamentous genera produce akinetes. Genetic recombination by transformation or conjugation can occur despite the absence of evident sexual reproduction [3].

1.2 Eukaryotic Cells

Autotrophic eukaryotic cells have organelles [4] and cell walls (secreted by the Golgi apparatus) with a microfibrillar layer of cellulose that can be delimited by an amorphous layer. However, some species are naked, lacking the cell wall. Often, there is a plasma membrane that bounds the cytoplasm, which contains the nucleus and different organelles (e.g., Golgi apparatus, chloroplast, mitochondria, lipid globules, vacuoles). The nucleus contains the nucleolus and several DNA molecules distributed among the chromosomes. The chloroplast contains a series of thylakoids, containing the chlorophylls and associated pigments.

Among eukaryotic microalgae, there is a wide variety of reproduction strategies, from vegetative reproduction by cell division to fragmentation and production of spores. Sexual reproduction also occurs in most of the species.

1.3 Biodiversity and Adaptation

Microalgae are present in all existing Earth ecosystems from deserts to polar seas living in a wide range of environmental conditions because they have developed different physiological systems, which allow them to adapt to various, some of them extreme, conditions, e.g., temperature, pH, salinity, or light intensity. Although they are mostly present in aquatic environments (freshwater and salt water), they are also found in the surface of soils and stone both as free-living and in symbiotic association.

Although the mechanism of photosynthesis in microalgae is similar to that of higher plants, they are generally more efficient converters of solar energy because of their simple cellular structure. In addition, because the cells usually grow in aqueous suspension, they have more efficient access to water, CO_2, and other nutrients [5].

It is estimated that more than 50,000 species exist, but only a limited number (around two-third) have been studied and analyzed and kept in collections all over the world. Some of the largest collections of microalgae are the Collection of Freshwater Algae at the University of Coimbra, Portugal; the Culture Collection of Algae of the Göttingen University, Germany; the Culture Collection of Algae in the University of Texas, USA; the National Institute for Environmental Studies, Japan; and the LEGE-CIIMAR Culture Collection (WDCM 1089), Porto, Portugal [6,7].

Despite of these numbers, only a limited number is cultivated in industrial quantities for commercial purposes.

1.4 Biochemical Composition

This diversity is reflected also in a biochemical diversity, which includes the production of a wide range of carbohydrates, lipids, and proteins (three main microalgae components) that in many cases are of commercial interest. In addition to a remarkable diversity of biomolecules there is an extraordinary metabolic plasticity allowing the manipulation of the biochemical composition of microalgal cells through the control of growth conditions (cf. Section 5.4). In most of the microalgae species, starch or starchlike compounds are the typical carbohydrate storage product. A wide range of microalgae produce lipids as storage products being frequently observed as oil droplets in cells, mainly in the form of polyunsaturated fatty acids (PUFAs), including arachidonic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid. Fatty acids and sterols are also found in cellular membranes, being more difficult to extract than the storage lipids.

Microalgal proteins are exceptionally diverse and are present in high concentrations both in prokaryotic (e.g., 40–60% of *Spirulina*'s dry weight) and eukaryotic (e.g., 30–60% *Porphyridium*'s dry weight) cells, making microalgae an excellent alternative protein source. In opposition to carbohydrates and lipids that reach their maximum concentration at stationary growth phase, protein-rich cells are usually in the exponential growth phase, i.e., actively growing cells.

2. NUTRITION

2.1 Nutritional Modes

In a broad sense, microalgae can be either autotrophic or heterotrophic, being autotrophy clearly the most important and the most used in microalgae mass cultivation.

Autotrophic microalgae convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2 by the oxidation of substrates (e.g., water), which is then used in respiration to produce energy to support growth [1]. Photoautotrophic microalgae use light as a source of energy and thus only require inorganic mineral ions for growth, while chemoautotrophics are a small group that oxidizes inorganic compounds for energy. Most microalgae are photoautotrophic; however, it is known that trace amounts of organic compounds (e.g., vitamins) are necessary for their growth [8].

Heterotrophic microalgae use organic compounds produced by other organisms to satisfy their material and energy needs. They are photoheterotrophs if they use light as energy source to use organic compounds as nutrients, or chemoheterotrophs if they oxidize organic compounds for energy, instead of light. Heterotrophic microalgae cultivations have been successfully used in closed bioreactors (e.g., Martek company cultivated *Crypthecodinium cohnii* for DHA production in heterotrophic conditions) [9].

Some microalgae are mixotrophic (or amphitrophic), combining heterotrophy and autotrophy, using both organic compounds and CO_2 for growth. Except in conditions of total darkness (where heterotrophy dominates), both autotrophy and heterotrophy are simultaneously present. In comparison with photoautotrophic microalgae, cells grown in heterotrophic and mixotrophic conditions are known to grow much [10–12] being, for that reason, proposed as feasible alternatives for the production of microalgal biomass. This characteristic makes mixotrophic growth suitable for the production of compounds characteristic from autotrophic and heterotrophic metabolism at high production rates and less dependent of light limitation issues [12]. Both heterotrophic and mixotrophic cultivations present a higher contamination risk due to the presence of organic carbon sources, making this trophic routes unfeasible in open-air reactors. Additionally, heterotrophic and mixotrophic microalgae cultivation require organic carbon sources such as glucose or acetate for growth, which can be responsible for 80% of the costs with growth media [13]. To reduce microalgal production costs, it is thus imperative to find cheap organic substrates that meet the nutritional requirements of microalgae (cf. Section 5.5).

2.2 Nutritional Needs

Many elements have to be provided for the growth of microalgae, such as carbon (C), oxygen (O), hydrogen (H), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sulfur (S), phosphorus (P), and trace elements such as copper (Cu), manganese (Mn), selenium (Se), or zinc (Zn).

In autotrophic growth while C, O, and H are usually obtained from water and air, N, P, and K have to be absorbed from the culture medium [14], their uptake being highly dependent on various factors such as species, nutrient ratios, temperature, or pH. These variations

in the uptake rate led to a great amount of studies to determine the optimal nutrient concentrations for various species under different growth conditions. C, N, and P are usually regarded as the most important nutrients for microalgae cultivation.

Carbon—The trophic route to be used during the cultivation will determine whether inorganic carbon, organic carbon, or both will be supplied to the culture. Glucose, acetic acid, and fructose are some of the most used organic carbon sources. Because atmospheric CO_2 cannot satisfy the carbon requirements of intensive autotrophic microalgae cultivations, the supply of CO_2 and HCO_3 is of utmost importance.

Additionally, it is common to reach pH values as high as 11 during microalgae cultivations due to gradual accumulation of OH^- during active photosynthesis. The pH values can be controlled via direct sparging of CO_2 into the media. Similarly to CO_2, acetic acid can be used both as carbon source and to control pH, being the most convenient method for pH control.

Nitrogen (N)—Although the N content of the biomass varies (1 to >10%) according to the supply and availability, its importance and influence is critical in microalgal cultures, being considered as the second most important nutrient (after carbon) for microalgae cultivation [15]. The N concentration, mainly its limitation, is often used as strategy to control cell composition (Section 5.4), usually resulting in the accumulation of PUFAs, polysaccharides, and carotenoids and the decrease in chlorophylls resulting in cell discoloration [16]. For other purposes, to ensure that N never becomes the rate-limiting factor, an adequate supply is vital and therefore culture media are often formulated with an excess of N. This oversupply should be controlled to avoid toxic effects. By using the enzyme nitrogenase, some cyanobacteria are capable of utilizing elemental nitrogen reducing N_2 to NH_4^+; however, N is typically supplied as nitrate (NO_3^-), ammonia (NH_4^+), and urea. When used isolated, ammonia leads to a pH decrease and nitrate to a pH increase. Ammonia is often lost during the cultivation due to volatilization phenomena.

Phosphorus (P)—Due to its role in microalgal growth and in different cellular processes of energy transfer and DNA biosynthesis, P (preferentially in the form of orthophosphate) is one of the key nutrients in microalgal cultivation. It is very common to verify the precipitation of P because it bounds very easily to other ions (e.g., iron). This makes P unavailable for uptake and therefore P is frequently referred as one of the most common growth-limiting factors in microalgal cultivation. This problem is usually overcome by the addition of metal chelators such as EDTA disodium salt and EDTA. Similarly to N, P availability influences biomass composition, specially the carbohydrate and lipid content [8]. Another parameter to consider to maximize the biomass productivity is the N:P ratio. Microalgae can store P in polyphosphate bodies, to be used when the supply of this nutrient is a limiting factor [8].

2.3 Growth Media

The first microalgae cultivation solutions were based on soil water extracts and, since then, a lot of work has been done through the design of new media and the optimization of previously used media, to boost the potential of microalgae cultivation. Some of the variables that are usually manipulated are overall nutrient concentration; salinity; concentration of major ionic components; and nitrogen and carbon sources.

The selection of the growth medium will depend primarily on the microalgae of interest (e.g., if it is a salt water or freshwater species) and the cultivation purpose, namely (1) culture maintenance; (2) obtaining maximum biomass productivities; (3) optimal metabolite production (e.g., through nitrogen deprivation conditions); and (4) physiological studies. The trophic route to be favored will also extensively influence the medium composition, specially the carbon source.

All these different options require different cultivation media or, at least, different formulations of the recipes, but in almost all media macronutrients are supplied in the order of g/L and the micronutrients in mg/L. The decision on the purity grade of the chemicals used in the medium preparation will be influenced by the growth purpose and price. If the microalgae culture will be used for physiologic studies, the highest purity available should be used to avoid medium contamination with trace metals that are frequently present in some chemicals. On the other hand, the utilization of these highly pure chemicals is not feasible in microalgae mass cultivation, where purity is often not a problem but the price is. Most of the recipes used in microalgae mass production are not reported by the companies to maintain their competitive advantage. However, it is known that most of these companies cultivate microalgae under autotrophic conditions using commercial fertilizers diluted in natural waters (groundwater or seawater). The utilization of wastewaters is also increasing as an option to obtain the required nutrients for microalgae growth (Section 5.5). Some of the most commonly used media (for both freshwater and seawater microalgae) are described in Table 13.1. These media suffered some modifications during the years, being the basis for almost all of the currently used microalgae cultivation media. For more information please refer to the paper by Andersen et al. [17], where an extensive list of cultivation media is described in detail.

2.4 Nutrient Supply

The availability of low-cost water and nutrients supply source is one of the critical challenges of microalgae mass cultivation. Culturing of microalgae at industrial scale requires substantial amount of nutrients that are normally obtained from inorganic fertilizers. With the expansion of the commercial microalgae growing industry, competition with the agricultural sector for inorganic fertilizers is expected to increase, which could result in inorganic fertilizers becoming an economically unviable source of nutrients for microalgal production systems [46]. The use of chemical fertilizers has the advantage of reducing contamination in culturing media and thus promotes water reutilization to reculture microalgae. However, lifecycle assessment studies have pointed out that ~50% of the overall energy use and greenhouse gases emission were associated with utilization of chemical fertilizers [47]. Thus, in a long run, using chemical fertilizers to culture microalgae for biofuel production might be not sustainable. Thus, it is necessary to find alternatives to the use of fertilizers in microalgae mass cultivation.

Microalgae mass cultivation can be more environmentally sustainable, cost-effective, and profitable, if combined with bioremediation processes such as wastewater and flue gas treatments. Microalgae cultivation using wastewater and flue gases as source of nutrients has proven to be a very feasible strategy [6]. For more details regarding the utilization of waste as nutrient source, please refer to Section 5.5.

TABLE 13.1 Commonly Used Microalgae Cultivation Media

	Medium	References	Notes
Freshwater	Chu #10	[18]	Without chelator, vitamins, and trace metals (except iron)
	Bold's Basal	[19,20]	Without vitamins and trace metal concentrations are high
	Sorokin/Krauss	[21]	Especially formulated for *Chlorella* cultivation
	N-11	[22]	Designed for freshwater, soil, thermal, and marine cyanobacteria
	Volvox	[23]	Designed for culturing *Volvox globator*
	D	[24]	Designed for growing thermophilic cyanobacteria from hot springs
	Zarrouck	[25]	Bicarbonate-rich growth medium specially designed for *Spirulina* cultivations
	BG-11	[26]	Designed for freshwater, soil, thermal, and marine cyanobacteria
	Carefoot	[27]	Developed to grow freshwater dinoflagellates
	C (*Closterium*)	[28]	Derivative of *Volvox* medium
	CA	[29]	Created for culturing oligotrophic desmids
	DyIII	[30]	Based on a pond water composition during exponential growth of *Dinobryon*
	MA	[31]	Developed for culturing *Microcystis* strains
	URO	[32]	Based on the nutrients concentrations of Lake Biwa
	AF6	[33]	Designed for freshwater microalgae requiring slightly acidic medium
	D11	[34]	Developed for culturing freshwater microalgae
	Ben-Amotz and Avron	[35]	Especially formulated for *Dunaliella* cultivation
	Spirulina	[36]	Designed for *Spirulina* cultivation
	Z8	[37]	Designed for cyanobacteria, can also be used for marine species by adding NaCl
Seawater	ASP-2	[38]	–
	Aquil	[39,40]	Designed for critical trace metal experimental work
	ESAW	[41,42]	Designed for coastal and open ocean phytoplankton
	ASN-III	[43]	–
	CCAP	[44]	–
	YBC-II	[45]	Developed to culture nitrogen-fixing *Trichodesmium* (no nitrogen source in medium)

3. CULTIVATION MODE

3.1 Batch

In batch cultivations before the cultivation starts, all the nutrients (except the carbon source in autotrophic cultivations) needed for growth and the desired product formation are added to the cultivation medium.

During batch cultivations, growth ceases due to limiting substrate depletion and/or growth-inhibiting products accumulation. The main consequences from these continuous changes in the cultivation environment and also the cell aging are (1) variation in cell composition during the cultivation period and (2) different growth phases (i.e., lag, exponential, stationary, and death phases) with consequent productivity fluctuations during the cultivation period. The biomass productivity of a batch cultivation is determined by dividing the total amount of biomass/product obtained by the total time of batch operation (microalgae cultivation plus turnaround time, i.e., draining the cultivation broth, cleaning the bioreactor, sterilizing the bioreactor, and refill with fresh culture medium).

Most of the current microalgae mass cultivations are based on batch cultivation systems [48–50] mainly due to its simplicity, reduced risk of contamination (when compared with continuous cultivations), and reduced need for maintenance.

3.2 Fed-Batch

Within the continuous flow cultivation techniques, fed-batch is probably the most used at industrial scale [51]. In these fed-batch microalgae cultivations, fresh medium is continuously or discontinuously added; however, in opposition to continuous cultivations, the cells and the products remain in the bioreactor. The culture is harvested periodically at the end of the cultivation cycle, which results in a cultivation system with variable volume and dilution rate (D) (see definition in Eq. (13.1), Section 3.3). Fed-batch, by definition, is placed between continuous and batch modes (Fig. 13.1) and is specially suited for the production of metabolites that are not associated with microalgae growth or to avoid substrate inhibition.

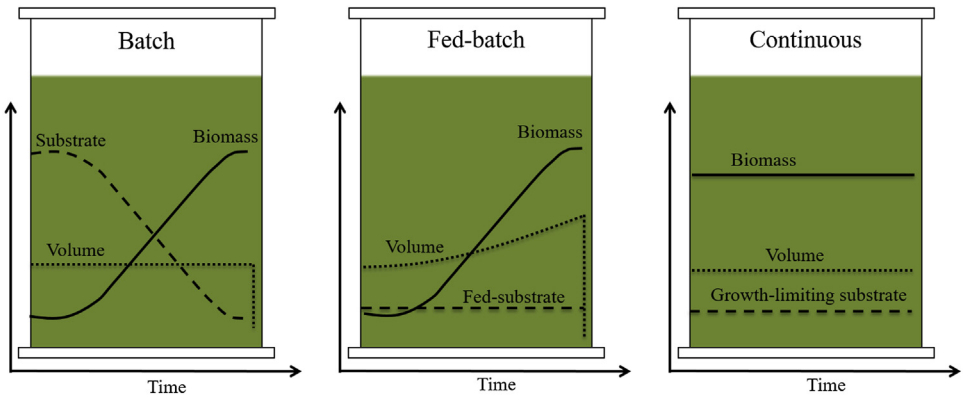


FIGURE 13.1 Indicative variations in working volume, biomass, and substrate concentrations under different operation modes.

One possible strategy to obtain specific products is to increase the biomass concentration until a desirable value (using a batch mode) and then change the microalgae metabolism by feeding product precursors or just nutrients to meet the demand for maintenance and product synthesis.

When a state is achieved where parameters vary in a cyclic pattern, it is considered that a quasi steady state is reached [51]. Fed-batch mode is used under different approaches. For example, it can be used with or without feedback/feedforward control or it can be used in a variable volume (most common) approach or, in opposition, in a "fixed" volume mode, which is achieved by adding a very concentrated nutrient stream that creates a negligible volume variation. With minor changes in the equipment, when compared with batch cultivations, it is possible to obtain an enhanced control over substrate and biomass concentration by simply adopting the fed-batch technique.

3.3 Continuous

In opposition to batch cultivations, in continuous systems there is a control of cultivation conditions/environment, which enables obtaining tailor-made biomass composition at a fixed rate [52–54]. Continuous cultivation of microalgae has been extensively described by Fernandes et al. [54], who described other advantages assigned to continuous system such as higher volumetric productivities; reduced space required; lower labor, investment, and operational costs; and reduced "unprofitable" periods. In continuous microalgae cultivations, the productivity is calculated by multiplying the biomass/product concentration in the outflow stream by the dilution rate (D), which is reciprocal of the residence time (average time that an element of volume spends inside the bioreactor). Comparing with batch cultivations, in continuous mode the turnaround time represents a much lower fraction of the total time [54].

Continuous cultivation systems are open systems with continuous feeding of fresh medium and continuous culture broth removal, usually with an unchanged volume of medium inside the bioreactor and a constant cultivation environment that maintains cells (Fig. 13.1), after some generations, in a balanced growth state or steady state [55]. In theory, and due to the relationship between the availability of the limiting substrate and microbial growth, it should be possible to keep the culture indeterminately in the exponential phase. However, in reality, cell aging–related problems will eventually stop the growth [54].

In a bioreactor operating in continuous mode and assuming steady-state conditions, no biomass in the substrate inflow, and constant volume, it is considered that:

$$\mu = \frac{F}{V} = D \tag{13.1}$$

where μ—specific growth rate (h^{-1}); F—volumetric flow (m^3/h); V—working volume of the bioreactor (m^3); and D—dilution rate (h^{-1}). In Eq. (3.1) specific death rate is considered to be negligible comparing to μ. If $D > \mu_{max}$ the biomass concentration will tend to be zero, due to the so-called washout process.

However, photoautotrophic continuous cultivation of microalgae greatly differs from the cultivation of other microorganisms under the same cultivation mode due to light-related constraints such as light attenuation due to the suspension and photosynthetic light capture [56–58].

Therefore, in microalgae continuous cultivations, maximum productivities occur at high cell densities and low to moderate μ at light-limited conditions (all energy is absorbed by the biomass during a defined small time interval). Maximum growth rate (μ_{max}), on the other hand, is regularly achieved in the absence of light-limited conditions and in the presence of low cell concentrations [15,54].

The concept of optimal cell density (OCD) is often used in microalgae continuous cultivations. The OCD is the cell concentration that allows maximum biomass productivity being μ, under these conditions, far (around 50%) from μ_{max} [15,59].

Several modes of operation for continuous cultivation of microalgae, namely turbidostat, luminostat, chemostat, cyclostat [60], or A-stat [61] have been proposed. However in this chapter only the most used will be described in the following sections.

3.3.1 Turbidostat

In a turbidostat, the turbidity is maintained constant in the cultivation system by using a system composed of an optical sensor that measures turbidity, connected to a pump that delivers the fresh medium at a variable rate that is controlled by a feedback control loop [54,62]. Despite being a more challenging and technically complex approach than the chemostat [63], in microalgae mass cultivation the turbidostat is the most commonly used approach to implement a continuous photoautotrophic microalgae cultivation [54]. According to Lee and Shen [64] the turbidostat method is the best option when the objective is to maintain the cell density at a certain constant value or in situations where near-maximum growth rate is achieved. Turbidostat is also appropriate when inhibitory substrates are present or when microalgae species with slow growth rates and/or intricate cell cycle are cultivated [54].

Alternative turbidostat systems are employed where cell density is indirectly controlled through the monitoring of growth-related parameters such as O_2 production, CO_2 consumption, pH variations, or substrate concentration [64].

3.3.2 Luminostat

Luminostat technique is specially suited for cultivations where a near-constant light regime is required to increase the photosynthetic efficiency and productivity through the maximization of light absorption, reducing dark volumes as well as photolimitation and photosaturation phenomena [57]. Therefore, the luminostat approach has been created to overcome light availability constraints generated by the fluctuations in light (e.g., period of the day, climate conditions) and cell concentration. In this technique (mainly used as a research tool), the light transmission is continuously controlled by light sensor that measure the light reaching the rear of the bioreactor. According to the light intensity measured the cell concentration is manipulated using a pump to reach the desired light regime [54].

3.3.3 Chemostat

The chemostat technique is used mostly as a research tool in studies involving the investigation of the effect of pH, temperature, or substrate concentration at constant μ due to its ability to maintain a constant chemical composition of the growth medium and a constant μ [53,54]. The control of these variables is done by feeding, at a predetermined and fixed D, the culture with fresh medium containing the limiting nutrients, which will determine μ—Eq. (13.1).

3.4 Performance Comparison—Batch Versus Continuous

Several authors [6,54,65–67] described previously that, in theory and without considering the cleaning and restart steps in batch cultivations, the biomass productivity is 2.3–5 times higher in continuous cultivation systems.

However, despite that and despite all the advantages enumerated in Section 3.3, batch cultivations are still the most common cultivation mode in microalgae cultivation [50,54,68]. The reasons for this preference are, according to Ref. [54]: (1) technoeconomic (complexity, need for skilled labor, special equipment, and higher contamination potential) and (2) "emotional" (accommodation to well-known and well-established batch processes that generate resistance to change combined with some suspicion caused by the lack of information and successful case studies).

The comparison between biomass productivity of batch and continuous systems for microalgae cultivation has been extensively discussed [52,54,69–71]. The results of these studies lead to the conclusion that the relative performance of continuous and batch systems is highly variable and dependent on the microorganisms, operational conditions, and application/product of interest. It was also concluded that to achieve all the full potential of continuous cultivations regarding biomass productivity, a near-optimal value of D must be used.

Regarding the performance in terms of delivery of a constant product (i.e., cell or metabolite characteristics), continuous mode is undoubtedly the most suitable option to attain this objective.

To decide either to use batch, fed-batch, or continuous systems a thorough study should be made to determine the best operation mode for that particular case and which are the best operational conditions.

4. CULTIVATION SYSTEMS

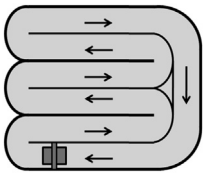
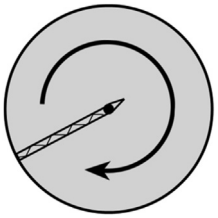
After selecting a suitable microalgae strain to obtain the product of interest, all the process details from biomass production to product extraction and purification must be taken into account so that a viable commercialization strategy is achieved. As a result, the choice of an adequate cultivation method, whether by open or closed systems, must be intimately related to the product of interest and its application.

4.1 Open Systems

Despite being intensively studied in later years of the 20th century, open-air cultivation systems have been used since 1950s and include lakes and natural ponds, circular ponds, raceway ponds, and inclined or cascade systems. These are the most common and widespread systems applied for large-scale commercial growths because open-air culture structures involve lower costs, are easier to build, and usually present longer lifetime and larger production capacity when compared to closed systems. Still, several drawbacks arise from the use of open-air systems, as shown in Table 13.2.

For instance, open ponds are more susceptible to regional climate conditions such as annual rainfall and rainfall pattern when compared to closed systems, representing significant

TABLE 13.2 Summarized Advantages and Limitations of Open Cultivation Systems

Open Systems	Advantages	Limitations
Raceway ponds 	• Relatively economical • Easy maintenance and cleaning • Low energy consumption	• Poor control over culture parameters • Ineffective light and CO_2 utilization • Difficult to grow algal cultures for long periods • Poor mixing • Low productivity • Limited to few strains • High risk of contamination
Circular ponds 		

Adapted from L. Brennan, P. Owende, Biofuels from microalgae — a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sust. Energy Rev. 14 (2010) 557–577; G. Dragone, B.D. Fernandes, A.A. Vicente, J.A. Teixeira, Third generation biofuels from microalgae, in: A.M. Vilas (Ed.), "Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology", Formatex Research Center, Badajoz, Spain, 2010, pp. 1355–1366.

technical challenges for a proper control of crucial growth parameters such as evaporation. Another disadvantage of the open culture systems concerns to the lack of a sterile or controlled environment, which may give rise to contaminations by predators and other fast-growing heterotrophs. In that sense, commercial production of algal species using natural and artificial ponds is limited to fast-growing, naturally occurring, or extremophilic organisms such as *Dunaliella* (adaptable to very high salinity), *Spirulina* (adaptable to high alkalinity), and *Chlorella* (adaptable to nutrient-rich media) thus ensuring the existence of a monoculture [73,74]. Due to these constraints, the most interesting applications for microalgae grown in open-air systems are aquaculture feed, biofuels production, and wastewater or flue gas treatment. However, successful examples employing microalgae grown in open systems in other biotechnological fields are also known. Betatene Ltd, probably the major β-carotene producer worldwide, makes use of very large ponds (~250 ha and an average depth of 0.2–0.3 m) containing extremely halophilic waters to obtain high amounts of green alga *Dunaliella salina* using no other mixing mechanism than wind and convection themselves.

Among open-air cultivation systems, circular ponds are widely used in Asia to produce *Chlorella* genus. They are usually characterized by a central rotating agitator and a depth around 0.3 m. Due to mechanical restrictions found in rotating arm design, the area of such systems must not exceed 10,000 m^2. The additional concrete construction costs and high energy input for mixing make circular ponds an unfeasible primary option for commercial plants [75].

Raceway ponds are the most commonly used artificial systems presenting an oval-shaped closed loop, generally between 0.2 and 0.5 m deep. Mixing is induced by the continuous movement of a paddle wheel, which avoids algae sedimentation. Using low-depth (0.15–0.2 m)

raceway ponds, productivities of 10–25 g/m^{2}day and biomass concentrations up to 1 g/L might be achieved [76]. The largest facility using this kind of ponds, 440,000 m^2, is used to grow *Spirulina* and it is located in California, USA [74,77].

Cascade systems are the only open-air systems able to reach high cell densities (up to 10 g/L) and are suitable for repeated pumping-tolerating microalgae such as *Chlorella* and *Scenedesmus*. Culture suspension flows throughout a sloping surface in which turbulence is generated by gravity [78,79]. This highly turbulent regime allows the existence of very thin culture layers (<2 cm), which provide higher cell concentrations and surface-to-volume ratios when compared to raceway ponds.

4.2 Closed Systems

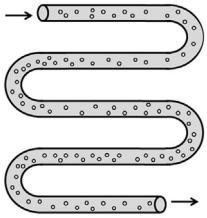
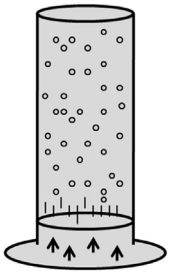
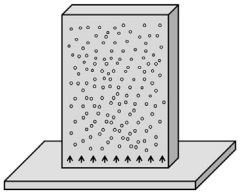
The term "photobioreactor" (PBR) is sometimes applied to describe open ponds and channels, but in this chapter this expression will be used to refer to closed systems. Due to a good regulation and control of nearly all the important biotechnological parameters, the risk of contamination inside PBRs is low thus allowing the growth of monoseptic cultures. Furthermore, PBRs are also characterized by a flexible technical design, no major CO_2 losses, reproducible cultivation conditions, and reliable control over temperature and hydrodynamics [54,76].

Despite the relative success of open systems, enclosed PBRs present the following advantages: (1) better control over pH, temperature, light, CO_2 concentration, and gas transfer; (2) large surface-to-volume ratio; (3) low or none CO_2 loss and reduced growth medium evaporation; (4) more uniform temperature; (5) reduced outside contamination risk; (6) high cell densities; and (7) allows the production of complex biopharmaceuticals [80,81]. Because some of the new microalgae and microalgal high-value products are employed in pharmaceutical and cosmetics industries, biomass growth in PBRs becomes mandatory to reach a culture free of pollution, as required in those biotechnological fields. Furthermore, some microalgae need a culture environment that is not highly selective being the use of closed systems a safe manner to avoid potential contaminants such as other microorganisms without compromising the final product quality [82].

Despite the great variety of designs that have been developed so far, there are three main PBR categories: (1) tubular (e.g., helical, manifold, serpentine, and α-shaped); (2) flat (e.g., alveolar panels and glass plates); and (3) column (e.g., bubble columns and airlift). Table 13.3 summarizes the advantages and limitations related to these systems.

Closed horizontal continuous-run tubular loop PBRs are generally applied in commercial microalgal monocultures. Biomass circulation throughout the tubes is promoted by a pump system or preferably using airlift technology. These are the largest closed PBRs, as the 25 m^3 plant from Mera Pharmaceuticals, Hawaii (USA), and the 700 m^3 plant located nearby Wolfsburg (Germany) can prove it [54,76,83]. Maximum productivities between 25 and 30 g/m^{2}day have been achieved using serpentine and two-plane tubular PBRs [84,85]. Another successful example of tubular PBR was regarding the employment of a 300 L α-shaped bioreactor for cultivation of *Chlorella pyrenoidosa* [86]. Despite having notable advantages such as relatively low costs associated, large illumination surface area, and fairly reasonable biomass productivities, tubular systems also comprise serious limitations. Large land surface occupation, difficult temperature control, fouling and foaming formation, dissolved O_2 and CO_2 gradients along with pH gradients that jointly cause growth inhibition, and the need for frequent

TABLE 13.3 Summarized Advantages and Limitations of Closed Cultivation Systems [Tubular, Column, and Flat photobioreactors PBRs]

Closed Systems	Advantages	Limitations
Tubular PBRs 	• Reasonably economic • Great illumination surface area • Suitable for outdoor cultures • Good biomass productivities	• pH, dissolved O_2 and CO_2 gradients generation along the tubes • Fouling formation • Considerable wall growth • Makes use of large land space • Risk of photoinhibition effect
Column PBRs 	• Low energy consumption • Good mass transfer and mixing • Best exposure to light–dark cycles • Low shear stress, photoinhibition, and photooxidation effects • High potential for scalability • Easy to sterilize • High photosynthetic efficiency	• Low illumination surface area • Expensive and sophisticated construction materials • Possible shear stress to algal cultures under certain growth conditions • Decreasing illumination surface area associated with scale-up • Scalability
Flat PBRs 	• Relatively cheap • Easy to clean up • Large illumination surface area • Reduced power consumption • Good biomass productivities • Good light path • Low oxygen buildup	• Difficult to scale-up • Difficult temperature control • Moderate wall growth • Hydrodynamic stress to some microalgal strains • Low photosynthetic efficiency

Adapted from L. Brennan, P. Owende, Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sust. Energy Rev. 14 (2010) 557–577; G. Dragone, B.D. Fernandes, A.A. Vicente, J.A. Teixeira, Third generation biofuels from microalgae, in: A.M. Vilas (Ed.), "Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology", Formatex Research Center, Badajoz, Spain, 2010, pp. 1355–1366.

recarbonation of the cultures are some of the disadvantages that make these PBRs only justifiable for the production of high-value products [87,88]. To overcome these constraints, the use of vertical PBRs might pose a good alternative.

Vertical orientation has been proposed to enhance microalgae productivity because sunlight incidence is spread over a larger reactor surface area, which results in the exposure of cells to lower light intensities and consequently a maximized photosynthetic efficiency. On the other hand, a reduced photosaturation effect is also observed [89,90]. Vertical column PBRs are usually cylinders characterized by a diameter up to 0.4 m and a height up to 4 m. To increase the surface–volume ratio, these PBRs should present relatively small diameters. Height restriction must be taken into account to avoid gas transfer limitations that might result in CO_2 and pH gradients and O_2 accumulation and to choose a resistant transparent material suitable for

building these PBRs. Column PBRs are characterized by high volumetric gas transfer coefficients. They have a constant bubbling of gas from the bottom of the reactor that enables an efficient CO_2 utilization and optimal O_2 removal [91]. Additionally, gas bubbles provoke a gentle culture mixing with little shear stress for cells and thus almost inexistent cell damage is associated with these PBRs, except when high superficial gas velocities are employed [91,92]. However, microalgal growth is often limited by other parameters such as efficient utilization of light. Airlift PBRs are probably the only vertical reactors that are capable of providing regular light–dark cycles and sustaining better biomass production of different microalgae probably due to the excellent and nonchaotic mixing they can offer [91]. The efficient mixing of swirling flows ensures a good homogenization of nutrients inside the culture as well as the algal displacement along the column. In addition, high shear stress generated at walls restricts the biofilm formation at the PBR surfaces [68,93,94]. The existence of some other advantages such as low power input, well-defined fluid flow patterns, simplicity in design and construction, and low capital and operating costs make these the most required vertical PBRs [95,96]. Some limitations such as difficult temperature control and large fraction of dark zones inside the reactor, mainly due to the presence of the internal column that limits light penetration, could though be found when performing microalgae growth using airlift PBRs.

Some of the first closed systems forms were flat PBRs that have received special attention mainly due to the large light exposure surface area and high cell densities, higher than 80 g/L, revealed during photoautotrophic growth [97,98]. A thin layer of very dense culture is mixed or moved across a flat transparent panel allowing radiation absorbance in the first few millimeters near the PBR surface. These panels are usually irradiated by direct sunlight from one side and can be placed either vertically or inclined at an optimum incident angle to present the most efficient energy absorption possible from the sun. Due to the low accumulation of dissolved oxygen and high photosynthetic efficiency obtained comparing to tubular PBRs, flat designs are suitable for large microalgal cultures [1]. Nevertheless, microalgae cultivation using flat PBRs might face some limitations including difficult temperature control of the culture, certain degree of wall growth, possible hydrodynamic stress to some algal strains, and the fact of implying many compartments and support materials when scale-up is attempted.

5. STRATEGIES TO INCREASE COST-EFFECTIVENESS

Natural resources management, such as the use of microorganisms to fulfill human needs, is based on a key principle: sustainability. To reach such equilibrium, there must be a continuous seek for efficient operational conditions, minimum environmental risk, and the respect of socioeconomic considerations [1]. Despite of the increasing interest in microalgae as a source of multiple products of interest, companies will not be interested to proceed with large investments unless improved productivity (either for biomass and/or metabolites) and/or process cost reduction are achieved [99]. Numerous variables such as strain selection, cultivation system localization and costs, nutrients supply, final products' concentration, biorefinery strategy implementation, and the use of genetic engineering tools should be taken into account so that we manage to attain high-productivity microalgal growth in a cost-effective way.

5.1 Microalgae Selection

Microalgae species and strains vary greatly in terms of growth rate, productivity, nutrient and light requirement, and ability to accumulate different desirable compounds and to adapt to adverse conditions. Therefore, usually, the first step in mass cultivation of microalgae is to find or engineer the right species and strains for specific purposes and cultivation systems [100]. There are many screening programs around the world studying microalgae species in different locations for suitable strains. However, most of the research work is focused on a small number of fast-growing microalgal species, which have been found to accumulate substantial quantities of lipids. Typical species include *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species, as well as *Botryococcus braunii*. Other important algal groups include *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Nannochloropsis*, and *Isochrysis* spp. There are four main features that should be taken into account during microalgae selection process:

- **Growth physiology**—evaluated based on features such as maximum specific growth rate, maximum cell density, tolerance to environmental variables (temperature, pH, salinity, oxygen levels, and CO_2 levels). The amenability for heterotrophic/mixotrophic growth and to grow until high cell density is also an interesting feature.
- **Metabolite production**—assessed for both the unit concentration as well as the total yield of the metabolites useful for commercialization. The variety, type, and amount of metabolites produced may be important for the implementation of a biorefinery approach. The ability of a microalgal species to secrete metabolites in liquid or volatile forms is another feature of potential significance taking into consideration the harvesting step.
- **Robustness**—high culture consistency, reasonable resilience, high community stability, low susceptibility to external predators, contaminants, and extreme abiotic factors are very important characteristics to be considered. The selection of extremophile strains can be a strategy to select more robust strains.
- **Amenability to genetic manipulation**—strains that are susceptible to optimization of its performance through genetic manipulation can be considered as preferential due to its higher potentialities and flexibility. In this particular, cyanobacteria are the ones with most promising developments in genetic engineering, which is mainly due to its prokaryotic nature.

5.2 Location Selection

The choice of a suitable location to build up a cultivation system is a major consideration to increase the overall process cost-effectiveness. Choosing the appropriate location can ensure higher system productivity and lower construction and operation costs, for example. This can be achieved if the cultivation system is built in a location where climatic conditions are ideal for the selected microalgae (optimizing the growth and reducing the temperature control costs), and/or a location nearby CO_2 and nutrients sources at residual cost (e.g., CO_2-emitting industries that produce wastewaters with nitrogen and phosphorus).

According to Ref. [6] the site selection has to be performed considering several criteria:

- water supply/demand, its salinity and chemistry;
- land topography, geology, and ownership;
- climatic conditions, temperature, insulation, evaporation, precipitation;
- easy access to nutrients and carbon supply sources.

The decisions made in terms of location will always be determined by the microalgae species selected, the product of interest (e.g., human consumption or production of biofuels), and type of cultivation (e.g., photoautotrophic or mixotrophic growth). The selected microalgae will determine the type of water used (fresh, marine, or even wastewater); the existence of special nutritional requirements (e.g., mixotrophy); growth temperature range; resistance to contamination; if the microalgae is extremophile or not. One of the most interesting possibilities is to combine the microalgae growth with a pollution control strategy of other industry, for example, for the removal of CO_2 from flue gas emissions or the removal of nitrogen and phosphorus from a wastewater effluent [6,101,102]. In general, every large CO_2-producing industry such as power plants, wastewater treatment plants, cement industries, or others involving fermentation processes (e.g., breweries) stands as a good potential candidate to receive this kind of biotechnological systems to diminish construction and operational costs. Among these, power plants and wastewater treatment plants are probably the most attractive locations for microalgae cultivation systems construction. Power plants are inexpensive CO_2 sources, with availability of heat that can be used in downstream processes (e.g., drying), and (usually) present large quantities of water. Wastewater treatment plants have large amounts of nutrient-rich water and usually produce CO_2 that can be used in microalgae production. Intensive livestock and food industries might also provide nutrient-rich sources that are necessary to meet nutritional requirements of heterotrophic and mixotrophic microalgae systems at residual costs. Geographical areas with high irradiances along the year and moderate temperatures are optimal for microalgae cultivation. Because of the average amount of sunlight hours per day (10−12 h), and the mean solar irradiance ranging from 400 $\mu E/m^2s$ (winter time) to 1800 $\mu E/m^2s$ (summer time), southern Spain [103] and southern Portugal are considered especially suitable for outdoor cultivation of microalgae.

In most of the developed countries, there are seasonal variations in temperatures and solar light energy throughout the year, which make it difficult to carry out outdoor microalgae cultivation all year round, in a sustainable manner, in such regions [98]. Oppositely, in most tropical developing countries, it is possible to maintain outdoor cultivations for a relatively long period. For that reason, and according to Ref. [98], tropical developing countries are probably the best choice for commercial exploitation of microalgae.

5.3 Cultivation System Selection

As previously demonstrated, a wide variety of cultivation systems are currently available for microalgal biomass growth; however, their selection, design, and construction should consider the microalgae strain, desired product, geographical location, as well as the overall cost of production.

Efficient and cost-effective microalgae culture systems should, ideally, comprise high areal and volumetric productivities, high illumination and mass transfer rates, inexpensiveness (either as investment and maintenance costs), easy control of the growth parameters

(temperature, pH, O_2, turbulence), and reliability to obtain high biomass yields [98,104]. There are practical points in this context that must be addressed during the development and design of a cost-effective microalgae cultivation system [105]:

- Is the microalgae culture system illuminated through all surfaces, providing a high area:volume ratio, and thus allowing an efficient utilization of the incident radiation?
- Does the microalgae culture system allow maintaining continuous monocultures with little risk of contamination or deterioration?
- Is the detection and control of biofouling formation quick enough to reduce the chances of contamination?
- Is CO_2 efficiently supplied to reach high mass transfer rates?
- Are there any mechanisms to prevent the excessive O_2 concentration that might cause stress to the cells?
- Is the cooling system suitable for local climatic conditions?
- Is the mixing system efficient so that significant cell stress is avoided?

5.4 Inducing an Increase in Metabolite Productivity

Under natural photosynthetic conditions, many microalgae strains have the ability to produce diverse metabolites including lipids, carbohydrates, proteins, pigments, and antioxidants along with biomass growth [1]. Synthesis of major intracellular membrane compounds as glycerol-based polar lipids is though favored in this situation. Despite this fact, the optimization of growth-affecting parameters may lead to increased accumulation of products of interest. A wide range of studies were therefore conducted to assess the influence of light quality and quantity, nutrients level, CO_2 concentration, temperature, and salinity on microalgal growth and metabolite production [106–110]. However, this rationale is only valid when metabolites productivity is correlated with biomass proliferation. In some cases, optimal growth conditions might not meet the proper environment to obtain maximum product amount. To overcome this limitation and, at the same time, balance the biomass growth and metabolites accumulation, the implementation of a two-stage growth strategy arises as a promising alternative to conventional methods. The first phase is thus based on optimal conditions for microalgal growth, whereas the second stage relies on a shift in cultivation conditions to decrease or even stop the primary metabolism. The use of carbon and energy is then directed toward the production of secondary metabolites such as reserve materials (e.g., starch or oil) leading to higher intracellular contents of those added-value products [111,112]. To achieve this overproduction state, which can induce an increasing amount up to 10 times, a variety of strategies such as macronutrients limitation [113], high light intensity [114], or the utilization of heterotrophic and mixotrophic culture conditions [115] might be applied. Such strategy becomes especially important to make microalgae biofuels cost-competitive because it provides larger energy-rich material productivities and, consequently, lower costs in biodiesel and bioethanol production processes [113].

5.5 Utilization of Waste as Nutrient Source

Low-cost water and CO_2 requirement, in addition to high nutrients availability, are certainly among the challenges currently faced by microalgal industrial scale cultivation.

To tackle these limitations and produce biofuels or other value-added products from algae using an environmentally sustainable, cost-effective, and profitable process, biomass growth might be combined with pollution control strategies as the treatment of wastewater effluents (nutrient-rich source) or flue gas emissions (CO_2-rich source) [6].

The excessive anthropogenic activities performed since the Industrial Revolution have resulted in significant increase of atmospheric CO_2 reaching 390.9 ppm in 2011, which represents ~140% of the existing amount in preindustrial era [116]. The use of plants or photosynthetic microorganisms in CO_2 sequestration is a sustainable manner to mitigate the global warming caused by disproportionate generation of CO_2, converting it into O_2 and biomass [1,117]. In addition, the fast growth rate and high photosynthetic efficiency presented by microalgae together with the production using nonarable land, thus not competing with food and feed crops, place them as a better solution when compared to terrestrial plants [118,119]. Phototrophic growth demands a continuous supply of CO_2 as carbon source although this essential chemical compound may also play an important role to control the pH of growth medium or even to promote a thorough mixing and consequent solution homogenization when aerated in cultivation systems. The importance of this last feature includes a balanced exposure of all cells to light minimizing the self-shading and phototoxicity effects, which is especially important when working with high-density cultures [120]. The ability to efficiently utilize CO_2 from numerous sources such as atmosphere, industrial exhaust gases (e.g., flue and flaring gases, might contain more than 15% of CO_2), and soluble carbonate salts (e.g., $NaHCO_3$ and Na_2CO_3) results in a high carbon content, commonly representing 40–50% of the overall microalgal biomass composition [120,121]. According to this, about 1.5–2 kg of CO_2 is necessary to produce 1 kg of biomass [121]. Therefore, an economic analysis considering the feasibility of large-scale microalgae cultivation must definitely take into account the cost of CO_2 supply. In that sense, an approach combining the growth of algae and their use as an additional treatment step of flue gas, to reduce CO_2 emission levels of exhaust gases, seems to be a successful employment. This has been supported by studies performed with flue gas emitted from municipal waste incinerators [122], coal-fired power plants [123], industrial heater using kerosene as fuel [124], and gas boiler [125]. Although CO_2 culture supply can be done by previous separation from flue gases, direct injection into the growth medium arises as the only affordable strategy for continuous provision of CO_2. Otherwise, the inexistence of cheap CO_2 sources to cells forces the use of a discontinuous supply approach [126].

Together with CO_2, the amount of nutrients, namely nitrogen and phosphorus, required for microalgal production at industrial scale is very significant. Considering the large amount of waste derived from intensive livestock and food industries worldwide, the use of their nutrient-rich effluents as supplement to microalgae growth medium could result in a reliable solution to revert such destructive environmental impact.

Furthermore, taking advantage of the significant organic carbon content that some waste streams present by employing them as rich-carbon sources might be a promising feature to explore in heterotrophic and mixotrophic microalgal cultures. In spite of growing faster and reaching higher cellular oil contents when compared to photoautotrophic cells, which is very important if used as biofuel feedstock, heterotrophic and mixotrophic algae growths demand for organic carbon sources such as glucose that represent 80% of the entire medium cost [10–13]. As a result of the ceaseless seek for cheaper organic carbon sources to reduce

growth media costs, without compromising the potential commercial applications, several studies were performed envisioning the use of crude glycerol from biodiesel production, acetate from anaerobic digestion, and carbohydrates from agricultural and industrial wastes in microalgae heterotrophic and mixotrophic cultivations, proving that these inexpensive organic substrates are viable options [127–129]. A serious environmental problem dairy industries have to deal with nowadays is cheese whey management, which could be substantially minimized by coupling this with microalgal cultivation. This liquid by-product derived from cheese production process contains high amounts of organic matter that might be employed in heterotrophic and mixotrophic growths [12].

The use of algae for nutrient removal from municipal wastewater has also been extensively investigated and in general this nutrient stream provides a good microalgal growth medium. From an environmental and economic point of view, utilization of municipal or industrial wastewaters is a very attractive strategy because it allows waste streams to be used to generate alternative renewable biofuels to mitigate the current energy crisis.

5.6 Biorefinery Concept

Because of the high production and/or downstream processes costs, the number of microalgal-based biotechnological products launched in the market is still low when compared to its broad potential. A significant market shift is though expected especially due to recent advances found in PBR engineering, systems biology, genetic engineering, and biorefining [130]. The biorefinery approach is a robust and economically viable strategy since, like in petroleum refineries, a single raw material is converted in different products and therefore resulting into different revenue sources. Thus, this scheme aims at maximizing the use of microalgal biomass to produce a wide range a relevant products causing a considerable drop of overall costs for any given product if compared with a single product–based system [100,131]. Although different approaches might be employed considering the selected microalgae, Fig. 13.2 represents a possible generic biorefinery scheme for microalgal biomass as well as the diversity of products that might be obtained from these microorganisms. Notice that the heat and energy generated during some biorefinery subprocesses could be integrated in energy-needed stages making this a (partially) self-sustainable process [100].

To make the microalgae biorefinery concept economically feasible, downstream processes must be improved so that high yields are achieved and microalgae metabolites functionality is maintained [131,132]. The conventional disruption and extraction methods usually utilize chemicals or very high pressure optimized conditions to obtain a certain product but might result in severe damage for several other product fractions [132]. In spite of very effective techniques to apply in a specific product, there is a need for mild, inexpensive, and low–energy consumption methodologies to meet the maximal exploitation of microalgal metabolites according to the biorefinery concept. The use of mild-processing techniques—such as pulsed electric fields, enzymes, ultrasonication (for cell disruption purposes), and ionic liquids or surfactants (for separation and extraction purposes)—might represent an appropriate alternative to overcome this major bottleneck [131,132].

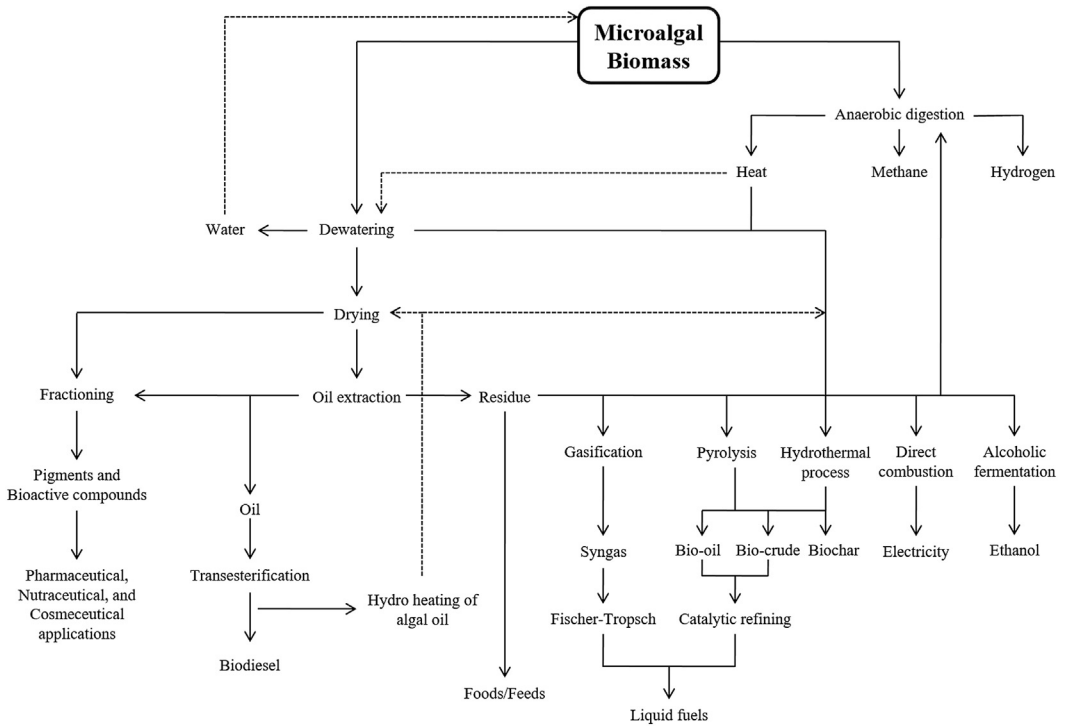


FIGURE 13.2 Biorefinery approach for microalgal biomass. *Adapted from P. Chen, M. Min, Y. Chen, L. Wang, Y. Li, Q. Chen, C. Wang, Y. Wan, X. Wang, Y. Cheng, S. Deng, K. Hennessy, X. Lin, Y. Liu, Y. Wang, B. Martinez, R. Ruan, Review of the biological and engineering aspects of algae to fuels approach, Int. J. Agric. Biol. Eng. 2 (2009) 1–30; T. Suganya, M. Varman, H.H. Masjuki, S. Renganathan, Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach, Renew. Sus. Energy Rev. 55 (2016) 909–941.*

5.7 Genetic Manipulation

Genetic manipulation techniques have been developed for some species (e.g., *Chlamydomonas reinhardtii*, *Volvox carteri*, and the diatom *Phaeodactylum tricornutum*) and are increasingly being applied to optimize the production of varied high-value products in several microalgal systems [133].

Many researchers are seeking to overcome the production and harvesting challenges through genetic and metabolic engineering of microalgae. Using nutrient deprivation or other stresses to induce a natural metabolite trigger is not always beneficial because productivity and product of interest accumulation are often inversely related, as mentioned in Section 5.4.

Thus, it is reasonable to conclude that genetic and metabolic engineering are likely to have a great impact on improving the economics of microalgal mass production [133–135]. According to Ref. [5], molecular level engineering can be used to potentially:

- maximize biomass productivity by increasing photosynthetic efficiency;
- optimize microalgae growth rate;
- increase the content of the metabolite of interest;

- reduce the need for cooling systems and risk of contamination by improving cells' temperature tolerance;
- potentiate that growth continues to increase in response to increasing light level by eliminating the light saturation phenomenon;
- restrain the cells' susceptibility to photooxidation.

The stability of engineered strains and methods to achieve a steady production in industrial microbial processes are known to be important issues [136], but have not been thoroughly examined for microalgae. In combination with increasingly refined genetic manipulation tools, the ability of scientists to engineer algae for the accumulation of specific metabolites is entering a new era [137].

6. CONCLUSIONS AND PERSPECTIVES

Microalgae exhibit enormous biodiversity presenting a huge potential for producing large quantities of biomass that can contain high concentrations of different (some of them are of high added-value) metabolites. However, to take advantage of all this potential, microalgae cultivation, harvesting, and subsequent product extraction must be cost-effective. As presented in this chapter a cost-effective microalgae mass cultivation system can be pursued through different strategies, for example, by the selection of the most suitable cultivation mode, induction of metabolite accumulation, or through the utilization of wastes as nutrient source. All the strategies presented in this chapter need to be refined and studied. However, the approach that will probably make microalgae cultivation a highly attractive business will be the utilization of highly rationally engineered microalgae production schemes by incorporating state-of-the-art knowledge about microalgae physiology of high-performance strains, metabolic network models, genetic manipulation, and improved bioinformatics resources probably in a biorefinery concept.

Acknowledgments

This research work was supported by the grant SFRH/BD/52335/2013 (Pedro Geada) and SFRH/BPD/98694/2013 (Bruno Fernandes) from Fundação para a Ciência e a Tecnologia (Portugal). The authors thank the FCT Strategic Project of UID/BIO/04469/2013 unit and UID/Multi/04423/2013.

References

[1] L. Brennan, P. Owende, Biofuels from microalgae − a review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sust. Energy Rev. 14 (2010) 557−577.
[2] O. Pulz, K. Scheibenbogen, W. Groß, Biotechnology with cyanobacteria and microalgae, in: H.-J. Rehm, G. Reed (Eds.), Biotechnology Set, second ed., Wiley-VCH Verlag GmbH, Weinheim, Germany, 2001, pp. 105−136.
[3] L. Tomaselli, The microalgal cell, in: A. Richmond (Ed.), Handbook of Microalgal Culture, Blackwell Publishing Ltd, Oxford, UK, 2004, pp. 1−19.
[4] R.E. Lee, Phycology, fourth ed., Cambridge University Press, Cambridge, UK, 2008.
[5] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294−306.
[6] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sust. Energy Rev. 14 (2010) 217−232.

[7] V.T. Duong, Y. Li, E. Nowak, P.M. Schenk, Microalgae isolation and selection for prospective biodiesel production, Energies 5 (2012) 1835.
[8] J.U. Grobbelaar, Inorganic algal nutrition, in: A. Richmond, Q. Hu (Eds.), Handbook of Microalgal Culture, John Wiley & Sons, Ltd, Oxford, UK, 2013, pp. 123–133.
[9] A. Mendes, A. Reis, R. Vasconcelos, P. Guerra, T. Lopes da Silva, *Crypthecodinium cohnii* with emphasis on DHA production: a review, J. Appl. Phycol. 21 (2009) 199–214.
[10] M.C. Cerón Garcí, J.M. Fernández Sevilla, F.G. Acién Fernández, E. Molina Grima, F. García Camacho, Mixotrophic growth of *Phaeodactylum tricornutum* on glycerol: growth rate and fatty acid profile, J. Appl. Phycol. 12 (2000) 239–248.
[11] X. Miao, Q. Wu, High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*, J. Biotechnol. 110 (2004) 85–93.
[12] A.P. Abreu, B. Fernandes, A.A. Vicente, J. Teixeira, G. Dragone, Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source, Biores. Technol. 118 (2012) 61–66.
[13] X. Li, H. Xu, Q. Wu, Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors, Biotechnol. Bioeng. 98 (2007) 764–771.
[14] J. Zhu, J. Rong, B. Zong, Factors in mass cultivation of microalgae for biodiesel, Chin. J. Catal. 34 (2013) 80–100.
[15] A. Richmond, Biological principles of mass cultivation of photoautotrophic microalgae, in: A. Richmond, Q. Hu (Eds.), Handbook of Microalgal Culture, John Wiley & Sons, Ltd, Oxford, UK, 2013, pp. 169–204.
[16] B. Fernandes, J. Teixeira, G. Dragone, A.A. Vicente, S. Kawano, K. Bišová, P. Přibyl, V. Zachleder, M. Vítová, Relationship between starch and lipid accumulation induced by nutrient depletion and replenishment in the microalga *Parachlorella kessleri*, Biores. Technol. 144 (2013) 268–274.
[17] R.A. Andersen, J.A. Berges, P.J. Harrison, M.M. Watanabe, Recipes for freshwater and seawater media, in: R.A. Andersen (Ed.), Algal Culturing Techniques, Elsevier Academic Press, London, UK, 2005, pp. 429–538.
[18] S.P. Chu, The influence of the mineral composition of the medium on the growth of planktonic algae, J. Ecol. 30 (1942) 284–325.
[19] H.C. Bold, The morphology of *Chlamydomonas chlamydogama* Sp. Nov. Bull. Torrey Bot. Club 76 (1949) 101–108.
[20] H.W. Bischoff, H.C. Bold, Phycological Studies. IV. Some Soil Algae from Enchanted Rock and Related Algal Species, The University of Texas Publication No. 6318, 1963.
[21] C. Sorokin, R.W. Krauss, The effect of light intensity on the growth rates of green algae, Plant Physiol. 33 (1956) 109–113.
[22] E.O. Hughes, P.R. Gorham, A. Zehnder, Toxicity of a unialgal culture of *Microcystis aeruginosa*, Can. J. Microbiol. 4 (1958) 225–236.
[23] L. Provasoli, I.J. Pintner, Artificial media for freshwater algae: problems and suggestions, in: C.A. Tyron Jr., R.T. Hartman (Eds.), The Ecology of Algae, University of Pittsburgh, Pittsburgh, USA, 1960, pp. 84–96. Special Publication No. 2.
[24] R.P. Sheridan, Photochemical and Dark Reduction of Sulfate and Thiosulfate to Hydrogen Sulfide in *Synechococcus lividus*, PhD. Thesis, University of Oregon, Eugene, USA, 1966.
[25] C. Zarrouck, Contribution a l'etude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima*, PhD Thesis, University of Paris, France, 1966.
[26] M.M. Allen, R.Y. Stanier, Growth and division of some unicellular blue-green algae, J. Gen. Microbiol. 51 (1968) 199–202.
[27] J.R. Carefoot, Culture and heterotrophy of the freshwater dinoflagellate, *Peridinium cinctum* fa. *ovoplanum* Lindeman, J. Phycol. 4 (1968) 129–131.
[28] T. Ichimura, Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum*, in: K. Nishizawa (Ed.), Seventh International Seaweed Symposium, University of Tokyo Press, Tokyo, Japan, 1971, pp. 208–214. August 8–12, Sapporo, Japan.
[29] T. Ichimura, M. Watanabe, The *Closterium calosporum* complex from the Ryukyu Islands: variation and taxonomic problems, Mem. Natl. Sci. Mus. Tokyo 7 (1974) 89–102.
[30] J.T. Lehman, Ecological and nutritional studies on *Dinobryon* Ehrenb.: seasonal periodicity and the phosphate toxicity problem, Limnol. Oceanogr. 21 (1976) 646–658.

[31] T. Ichimura, Sôrui no bunri to baiyôhô: Tansui sôrui (Isolation and culture methods of algae: freshwater algae), in: K. Nishizawa, M. Chihara (Eds.), "Sôrui Kenkyûbô" (Methods in Phycological Studies), Kyorutsu Shuppan, Tokyo, Japan, 1979, pp. 294–305.
[32] H. Nakahara, Urogurena no seikatusi (Life cycle of *Uroglena americana*), in: G. Kadota (Ed.), "Biwako Ni Okeru Purankuton No Ijôhasseikikô Ni Kansuru Tyôsa" (Survey on Red Tide Outbreaks in Lake Biwa), Biwako purankuton ijôhassei tyôsadan, Otsu, Japan, 1981, pp. 137–144 [in Japanese without English titles].
[33] S. Kato, Laboratory culture and morphology of *Colacium vesiculosum* Ehrb. (Euglenophyceae), Jpn. J. Phycol. 30 (1982) 63–67 (in Japanese with English summary; recipe in English).
[34] J.M. Graham, M.T. Auer, R.P. Canale, J.P. Hoffmann, Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 4. Photosynthesis and respiration as functions of light and temperature, J. Great Lakes Res. 8 (1982) 100–111.
[35] A. Ben-Amotz, M. Avron, The biotechnology of mass culturing *Dunaliella* for products of commercial interest, in: R.C. Cresswell, T.A. Rees, N. Shah (Eds.), Algal and Cyanobacterial Biotechnology, Longman Scientific & Technical, Harlow, UK, 1989, pp. 91–114.
[36] U.G. Schlösser, SAG–Sammlung von Algenkulturen at the University of Göttingen. Catalogue of Strains, Botanica Acta 107 (1994) 113–186.
[37] J. Kotai, Instructions for Preparation of Modified Nutrient Solution Z8 for Algae. NIVA Publication B-11/69, Norwegian Institute for Water Research, Oslo, Norway, 1972.
[38] L. Provasoli, J.J.A. McLaughlin, M.R. Droop, The development of artificial media for marine algae, Archiv für Mikrobiologie 25 (1957) 392–428.
[39] F.M.M. Morel, J.G. Rueter, D.M. Anderson, R.R.L. Guillard, Aquil: a chemically defined phytoplankton culture medium for trace metal studies, J. Phycol. 15 (1979) 135–141.
[40] N.M. Price, G.I. Harrison, J.G. Hering, R.J. Hudson, P.M.V. Nirel, B. Palenik, F.M.M. Morel, Preparation and chemistry of the artificial algal culture medium Aquil, Biol. Oceanogr. 6 (1989) 443–461.
[41] P.J. Harrison, R.E. Waters, F.J.R. Taylor, A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton, J. Phycol. 16 (1980) 28–35.
[42] J.A. Berges, D.J. Franklin, P.J. Harrison, Evolution of an artificial seawater medium: improvements in enriched seawater, artificial water over the past two decades, J. Phycol. 37 (2001) 1138–1145.
[43] R. Rippka, Isolation and purification of cyanobacteria, Methods Enzymol. 167 (1988) 3–27.
[44] J. Tompkins, M.M. DeVille, J.G. Day, M.F. Turner, Culture Collection of Algae and Protozoa, Catalogue of Strains, Ambleside, UK, 1995.
[45] Y.-B. Chen, J.P. Zehr, M. Mellon, Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: evidence for a circadian rhythm, J. Phycol. 32 (1996) 916–923.
[46] O. Fenton, D. Ó hUallacháin, Agricultural nutrient surpluses as potential input sources to grow third generation biomass (microalgae): a review, Algal Res. 1 (2012) 49–56.
[47] A.F. Clarens, E.P. Resurreccion, M.A. White, L.M. Colosi, Environmental life cycle comparison of algae to other bioenergy feedstocks, Environ. Sci. Technol. 44 (2010) 1813–1819.
[48] F. Camacho, E. Molina, M.E. Martínez, S. Sánchez, F. García, Continuous culture of the marine microalga *Tetraselmis* sp. — productivity analysis, Aquaculture 90 (1990) 75–84.
[49] K.A. Rusch, J. Michael Christensen, The hydraulically integrated serial turbidostat algal reactor (HISTAR) for microalgal production, Aquacult. Eng. 27 (2003) 249–264.
[50] J. Marchetti, G. Bougaran, L. Le Dean, C. Mégrier, E. Lukomska, R. Kaas, E. Olivo, R. Baron, R. Robert, J.P. Cadoret, Optimizing conditions for the continuous culture of *Isochrysis affinis galbana* relevant to commercial hatcheries, Aquaculture 326–329 (2012) 106–115.
[51] A. Richmond, Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Blackwell Publishing Ltd, Oxford, UK, 2004.
[52] Y.-H. Zhu, J.-G. Jiang, Continuous cultivation of *Dunaliella salina* in photobioreactor for the production of β-carotene, Eur. Food Res.Technol. 227 (2008) 953–959.
[53] A.J. Klok, D.E. Martens, R.H. Wijffels, P.P. Lamers, Simultaneous growth and neutral lipid accumulation in microalgae, Biores. Technol. 134 (2013) 233–243.
[54] B.D. Fernandes, A. Mota, J.A. Teixeira, A.A. Vicente, Continuous cultivation of photosynthetic microorganisms: approaches, applications and future trends, Biotechnol. Adv. 33 (2015) 1228–1245.

[55] A. Catarina Guedes, N.G. Katkam, J. Varela, F. Xavier Malcata, Photobioreactors for cyanobacterial culturing, in: N.K. Sharma, A.K. Rai, L.J. Stal (Eds.), Cyanobacteria: An Economic Perspective, John Wiley & Sons, Ltd, Chichester, UK, 2014, pp. 270–292.
[56] E. Molina Grima, F.G.A. Fernández, F. Garcıa Camacho, Y. Chisti, Photobioreactors: light regime, mass transfer, and scaleup, J. Biotechnol. 70 (1999) 231–247.
[57] M. Cuaresma, M. Janssen, E.J. van den End, C. Vílchez, R.H. Wijffels, Luminostat operation: a tool to maximize microalgae photosynthetic efficiency in photobioreactors during the daily light cycle? Biores. Technol. 102 (2011) 7871–7878.
[58] E. Sforza, M. Enzo, A. Bertucco, Design of microalgal biomass production in a continuous photobioreactor: an integrated experimental and modeling approach, Chem. Eng. Res. Des. 92 (2014) 1153–1162.
[59] J. Myers, J.-R. Graham, On the mass culture of algae. II. Yield as a function of cell concentration under continuous sunlight irradiance, Plant Physiol. 34 (1959) 345–352.
[60] G.-Y. Rhee, I.J. Gotham, S.W. Chisholm, Use of cyclostat cultures to study phytoplankton ecology, in: P.C. Calcott (Ed.), Continuous Culture of Cells, CRC Press, Cleveland, USA, 1981, pp. 159–186.
[61] M.J. Barbosa, J.W. Zijffers, A. Nisworo, W. Vaes, J. van Schoonhoven, R.H. Wijffels, Optimization of biomass, vitamins, and carotenoid yield on light energy in a flat-panel reactor using the A-stat technique, Biotechnol. Bioeng. 89 (2005) 233–242.
[62] H.L. MacIntyre, J.J. Cullen, Using cultures to investigate the physiological ecology of microalgae, in: R.A. Andersen (Ed.), Algal Culturing Techniques, Elsevier Academic Press, London, UK, 2005, pp. 287–326.
[63] J.M. Sandnes, T. Ringstad, D. Wenner, P.H. Heyerdahl, T. Källqvist, H.R. Gislerød, Real-time monitoring and automatic density control of large-scale microalgal cultures using near infrared (NIR) optical density sensors, J. Biotechnol. 122 (2006) 209–215.
[64] Y.-K. Lee, H. Shen, Basic culturing techniques, in: A. Richmond (Ed.), Handbook of Microalgal Culture, Blackwell Publishing Ltd, Oxford, UK, 2004, pp. 40–56.
[65] F.G. Acién Fernández, J.M. Fernández Sevilla, E. Molina Grima, Principles of photobioreactor design, in: C. Posten, C. Walter (Eds.), Microalgal Biotechnology: Potential and Production, De Gruyter, Berlin, Germany, 2012, pp. 151–180.
[66] Y.-K. Lee, W. Chen, H. Shen, D. Han, Y. Li, H.D.T. Jones, J.A. Timlin, Q. Hu, Basic culturing and analytical measurement techniques, in: A. Richmond, Q. Hu (Eds.), Handbook of Microalgal Culture, John Wiley & Sons, Ltd, Oxford, UK, 2013, pp. 37–68.
[67] J. Fernández-Sevilla, F. Acién-Fernández, E. Molina-Grima, Photobioreactors design for hydrogen production, in: D. Zannoni, R. De Philippis (Eds.), Microbial BioEnergy: Hydrogen Production, Springer, Dordrecht, Netherlands, 2014, pp. 291–320.
[68] K. Loubière, E. Olivo, G. Bougaran, J. Pruvost, R. Robert, J. Legrand, A new photobioreactor for continuous microalgal production in hatcheries based on external-loop airlift and swirling flow, Biotechnol. Bioeng. 102 (2009) 132–147.
[69] E. Cohen, A. Koren, S. Arad, A closed system for outdoor cultivation of microalgae, Biomass Bioenergy 1 (1991) 83–88.
[70] A. Chetsumon, I. Maeda, F. Umeda, K. Yagi, Y. Miura, T. Mizoguchi, Continuous antibiotic production by an immobilized cyanobacterium in a seaweed-type bioreactor, J. Appl. Phycol. 7 (1995) 135–139.
[71] D. Kumar, J. Rai, J.P. Gaur, Removal of metal ions by *Phormidium bigranulatum* (cyanobacteria)-dominated mat in batch and continuous flow systems, Biores. Technol. 104 (2012) 202–207.
[72] G. Dragone, B.D. Fernandes, A.A. Vicente, J.A. Teixeira, Third generation biofuels from microalgae, in: A.M. Vilas (Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Formatex Research Center, Badajoz, Spain, 2010, pp. 1355–1366.
[73] P. Das, S.S. Aziz, J.P. Obbard, Two phase microalgae growth in the open system for enhanced lipid productivity, Renew. Energy 36 (2011) 2524–2528.
[74] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2006) 87–96.
[75] M.A. Borowitzka, Culturing microalgae in outdoor ponds, in: R.A. Andersen (Ed.), Algal Culturing Techniques, Elsevier Academic Press, London, UK, 2005, pp. 205–218.
[76] O. Pulz, Photobioreactors: production systems for phototrophic microorganisms, Appl. Microbiol. Biotechnol. 57 (2001) 287–293.

[77] A. Demirbas, Use of algae as biofuel sources, Energy Convers. Manage. 51 (2010) 2738–2749.
[78] I. Šetlík, V. Šust, I. Málek, Dual purpose open circulation units for large scale culture of algae in temperate zones. I. Basic design considerations and scheme of a pilot plant, Algol. Stud. 1 (1970) 111–164.
[79] T. de Marchin, M. Erpicum, F. Franck, Photosynthesis of *Scenedesmus obliquus* in outdoor open thin-layer cascade system in high and low CO_2 in Belgium, J. Biotechnol. 215 (2015) 2–12.
[80] F. Chen, High cell density culture of microalgae in heterotrophic growth, Trends Biotechnol. 14 (1996) 421–426.
[81] R.N. Singh, S. Sharma, Development of suitable photobioreactor for algae production – a review, Renew. Sust. Energy Rev. 16 (2012) 2347–2353.
[82] D. Benson, K. Kerry, G. Malin, Algal biofuels: impact significance and implications for EU multi-level governance, J. Clean. Prod. 72 (2014) 4–13.
[83] M. Olaizola, Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors, J. Appl. Phycol. 12 (2000) 499–506.
[84] G. Torzillo, B. Pushparaj, F. Bocci, W. Balloni, R. Materassi, G. Florenzano, Production of Spirulina biomass in closed photobioreactors, Biomass 11 (1986) 61–74.
[85] G. Torzillo, P. Carlozzi, B. Pushparaj, E. Montaini, R. Materassi, A two-plane tubular photobioreactor for outdoor culture of Spirulina, Biotechnol. Bioeng. 42 (1993) 891–898.
[86] Y.-K. Lee, S.-Y. Ding, C.-S. Low, Y.-C. Chang, W. Forday, P.-C. Chew, Design and performance of an α-type tubular photobioreactor for mass cultivation of microalgae, J. Appl. Phycol. 7 (1995) 47–51.
[87] A. Sánchez Mirón, M.-C. Cerón Garcıa, F. Garcıa Camacho, E. Molina Grima, Y. Chisti, Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture, Enzym. Microb. Technol. 31 (2002) 1015–1023.
[88] J.C. Weissman, R.P. Goebel, J.R. Benemann, Photobioreactor design: mixing, carbon utilization, and oxygen accumulation, Biotechnol. Bioeng. 31 (1988) 336–344.
[89] M. Cuaresma, M. Janssen, C. Vílchez, R.H. Wijffels, Horizontal or vertical photobioreactors? How to improve microalgae photosynthetic efficiency, Biores. Technol. 102 (2011) 5129–5137.
[90] C. Posten, G. Schaub, Microalgae and terrestrial biomass as source for fuels—a process view, J. Biotechnol. 142 (2009) 64–69.
[91] B. Wang, C.Q. Lan, M. Horsman, Closed photobioreactors for production of microalgal biomasses, Biotechnol. Adv. 30 (2012) 904–912.
[92] J. Vega-Estrada, M.C. Montes-Horcasitas, A.R. Domínguez-Bocanegra, R.O. Cañizares-Villanueva, *Haematococcus pluvialis* cultivation in split-cylinder internal-loop airlift photobioreactor under aeration conditions avoiding cell damage, Appl. Microbiol. Biotechnol. 68 (2005) 31–35.
[93] J. Pruvost, J. Legrand, P. Legentilhomme, A. Muller-Feuga, Simulation of microalgae growth in limiting light conditions: flow effect, AIChE J. 48 (2002) 1109–1120.
[94] J. Pruvost, J. Legrand, P. Legentilhomme, A. Muller-Feuga, Effect of inlet type on shear stress and mixing in an annular photobioreactor involving a swirling decaying flow, Can. J. Chem. Eng. 82 (2004) 495–503.
[95] B.D. Fernandes, G. Dragone, J.A. Teixeira, A.A. Vicente, Light regime characterization in an airlift photobioreactor for production of microalgae with high starch content, Appl. Biochem. Biotechnol. 161 (2010) 218–226.
[96] K. Loubiere, J. Pruvost, F. Aloui, J. Legrand, Investigations in an external-loop airlift photobioreactor with annular light chambers and swirling flow, Chem. Eng. Res. Des. 89 (2011) 164–171.
[97] Q. Hu, N. Kurano, M. Kawachi, I. Iwasaki, S. Miyachi, Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor, Appl. Biochem. Biotechnol. 49 (1998) 655–662.
[98] C.U. Ugwu, H. Aoyagi, H. Uchiyama, Photobioreactors for mass cultivation of algae, Biores. Technol. 99 (2008) 4021–4028.
[99] J. Singh, S. Gu, Commercialization potential of microalgae for biofuels production, Renew. Sust. Energy Rev. 14 (2010) 2596–2610.
[100] P. Chen, M. Min, Y. Chen, L. Wang, Y. Li, Q. Chen, C. Wang, Y. Wan, X. Wang, Y. Cheng, S. Deng, K. Hennessy, X. Lin, Y. Liu, Y. Wang, B. Martinez, R. Ruan, Review of the biological and engineering aspects of algae to fuels approach, Int. J. Agric. Biol. Eng. 2 (2009) 1–30.
[101] I.T.D. Cabanelas, J. Ruiz, Z. Arbib, F.A. Chinalia, C. Garrido-Pérez, F. Rogalla, I.A. Nascimento, J.A. Perales, Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal, Biores. Technol. 131 (2013) 429–436.

[102] S. Cho, N. Lee, S. Park, J. Yu, T.T. Luong, Y.-K. Oh, T. Lee, Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources, Biores. Technol. 131 (2013) 515–520.
[103] M. García-González, J. Moreno, J.P. Cañavate, V. Anguis, A. Prieto, C. Manzano, F.J. Florencio, M.G. Guerrero, Conditions for open-air outdoor culture of *Dunaliella* salina in southern Spain, J. Appl. Phycol. 15 (2003) 177–184.
[104] M. Olaizola, Commercial development of microalgal biotechnology: from the test tube to the marketplace, Biomol. Eng. 20 (2003) 459–466.
[105] A. Richmond, Principles for attaining maximal microalgal productivity in photobioreactors: an overview, Hydrobiologia 512 (2004) 33–37.
[106] K. Black, M. Yilmaz, E. Phlips, Growth and toxin production by *Microcystis aeruginosa* PCC 7806 (Kutzing) Lemmerman at elevated salt concentrations, J. Environ. Prot. 2 (2011) 669–674.
[107] Y. Liu, L. Li, R. Jia, The optimum resource ratio (N: P) for the growth of *Microcystis aeruginosa* with abundant nutrients, Procedia Environ. Sci. 10 (2011) 2134–2140.
[108] S.P. Singh, P. Singh, Effect of CO_2 concentration on algal growth: a review, Renew. Sus. Eng. Rev. 38 (2014) 172–179.
[109] A. Vadiveloo, N.R. Moheimani, J.J. Cosgrove, P.A. Bahri, D. Parlevliet, Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp. (Eustigmatophyceae), Algal Res. 8 (2015) 121–127.
[110] Z. Yang, L. Geng, W. Wang, J. Zhang, Combined effects of temperature, light intensity, and nitrogen concentration on the growth and polysaccharide content of *Microcystis aeruginosa* in batch culture, Biochem. Sys. Ecol. 41 (2012) 130–135.
[111] I. Douskova, J. Doucha, J. Machat, P. Novak, D. Umysova, M. Vitova, V. Zachleder, Microalgae as a means for converting flue gas CO_2 into biomass with a high content of starch, in: "Bioenergy: Challenges and Opportunities. International Conference and Exhibition on Bioenergy", April 6th – 9th, University of Minho, Guimarães, Portugal, 2008.
[112] X. Wang, Z. Ruan, P. Sheridan, D. Boileau, Y. Liu, W. Liao, Two-stage photoautotrophic cultivation to improve carbohydrate production in *Chlamydomonas reinhardtii*, Biomass Bioenergy 74 (2015) 280–287.
[113] G. Dragone, B.D. Fernandes, A.P. Abreu, A.A. Vicente, J.A. Teixeira, Nutrient limitation as a strategy for increasing starch accumulation in microalgae, Appl. Energy 88 (2011) 3331–3335.
[114] I. Brányiková, B. Maršálková, J. Doucha, T. Brányik, K. Bišová, V. Zachleder, M. Vítová, Microalgae—novel highly efficient starch producers, Biotechnol. Bioeng. 108 (2011) 766–776.
[115] T. Heredia-Arroyo, W. Wei, B. Hu, Oil accumulation via heterotrophic/mixotrophic *Chlorella protothecoides*, Appl. Biochem. Biotechnol. 162 (2010) 1978–1995.
[116] World Meteorological Organization, The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2011, Annual Greenhouse Gas Bulletin, 2012, pp. 1–4.
[117] K. Kumar, C.N. Dasgupta, B. Nayak, P. Lindblad, D. Das, Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria, Biores. Technol. 102 (2011) 4945–4953.
[118] L.M. Brown, K.G. Zeiler, Aquatic biomass and carbon dioxide trapping, Energy Convers. Manage. 34 (1993) 1005–1013.
[119] T. Suganya, M. Varman, H.H. Masjuki, S. Renganathan, Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach, Renew. Sus. Energy Rev. 55 (2016) 909–941.
[120] A. Kumar, S. Ergas, X. Yuan, A. Sahu, Q. Zhang, J. Dewulf, F.X. Malcata, H. van Langenhove, Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions, Trends Biotechnol. 28 (2010) 371–380.
[121] T.M. Sobczuk, F.G. Camacho, F.C. Rubio, F.G.A. Fernández, E.M. Molina, Carbon dioxide uptake efficiency by outdoor microalgal cultures in tubular airlift photobioreactors, Biotechnol. Bioeng. 67 (2000) 465–475.
[122] I. Douskova, J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umysova, V. Zachleder, M. Vitova, Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs, Appl. Microbiol. Biotechnol. 82 (2009) 179–185.
[123] P. McGinn, K. Dickinson, S. Bhatti, J.-C. Frigon, S. Guiot, S.B. O'Leary, Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations, Photosynth. Res. 109 (2011) 231–247.

[124] S.R. Chae, E.J. Hwang, H.S. Shin, Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photo-bioreactor, Biores. Technol. 97 (2006) 322–329.
[125] J. Doucha, F. Straka, K. Lívanský, Utilization of flue gas for cultivation of microalgae *Chlorella* sp.) in an outdoor open thin-layer photobioreactor, J. Appl. Phycol. 17 (2005) 403–412.
[126] A.P. Carvalho, L.A. Meireles, F.X. Malcata, Microalgal reactors: a review of enclosed system designs and performances, Biotechnol. Prog. 22 (2006) 1490–1506.
[127] A. Bhatnagar, S. Chinnasamy, M. Singh, K.C. Das, Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters, Appl. Energy 88 (2011) 3425–3431.
[128] T. Heredia-Arroyo, W. Wei, R. Ruan, B. Hu, Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials, Biomass Bioenergy 35 (2011) 2245–2253.
[129] S. Abad, X. Turon, Valorization of biodiesel derived glycerol as a carbon source to obtain added-value metabolites: focus on polyunsaturated fatty acids, Biotechnol. Adv. 30 (2012) 733–741.
[130] R.H. Wijffels, M.J. Barbosa, An outlook on microalgal biofuels, Science 329 (2010) 796–799.
[131] E. Günerken, E. D'Hondt, M.H.M. Eppink, L. Garcia-Gonzalez, K. Elst, R.H. Wijffels, Cell disruption for microalgae biorefineries, Biotechnol. Adv. 33 (2015) 243–260.
[132] M. Vanthoor-Koopmans, R.H. Wijffels, M.J. Barbosa, M.H.M. Eppink, Biorefinery of microalgae for food and fuel, Biores. Technol. 135 (2013) 142–149.
[133] R.H. Wijffels, O. Kruse, K.J. Hellingwerf, Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae, Curr. Opin. Biotechnol. 24 (2013) 405–413.
[134] P.G. Roessler, L.M. Brown, T.G. Dunahay, D.A. Heacox, E.E. Jarvis, J.C. Schneider, S.G. Talbot, K.G. Zeiler, Genetic engineering approaches for enhanced production of biodiesel fuel from microalgae, in: M.E. Himmel, J.O. Baker, R.P. Overend (Eds.), Enzymatic Conversion of Biomass for Fuels Production, American Chemical Society, Washington, USA, 1994, pp. 255–270.
[135] T. Dunahay, E. Jarvis, S. Dais, P. Roessler, Manipulation of microalgal lipid production using genetic engineering, Appl. Biochem. Biotechnol. 57, 58 (1996) 223–231.
[136] Z. Zhang, M. Moo-Young, Y. Chisti, Plasmid stability in recombinant *Saccharomyces cerevisiae*, Biotechnol. Adv. 14 (1996) 401–435.
[137] L.L. Beer, E.S. Boyd, J.W. Peters, M.C. Posewitz, Engineering algae for biohydrogen and biofuel production, Curr. Opin. Biotechnol. 20 (2009) 264–271.

CHAPTER

14

Algal Biofilms and Their Biotechnological Significance

S. Thapa, A. Bharti, R. Prasanna

ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

OUTLINE

1. INTRODUCTION

Algal biofilms are complex assemblages of microalgae and other microorganisms that proliferate by forming submerged thick mats on aquatic water bodies and terrestrial surface where damp conditions prevail [1,2], such as submerged stones and pebbles, rivers, wet wall surfaces, and the bark of trees. Algal biofilms are often found on manmade structures like drainage pipes, building structures, ship bodies, water pools etc. and are recognized for the nuisance, in terms of aesthetics, safety, and economics [3]. However, they exhibit excellent promise for biotechnological interventions in wastewater treatment,

Algal Green Chemistry
http://dx.doi.org/10.1016/B978-0-444-63784-0.00014-X

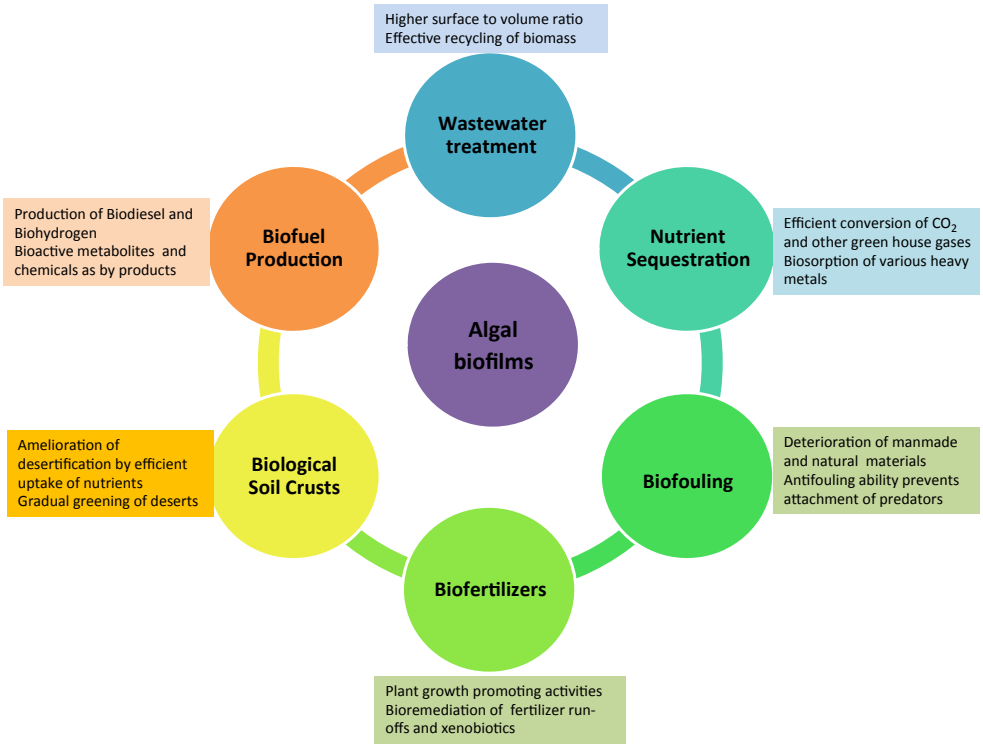


FIGURE 14.1 Overview of the areas of applications of algal biofilms.

bioremediation, and biofouling, besides having applications in production of biofuels, biopolymers, and bioactive metabolites (Fig. 14.1). They are now being increasingly explored as biofertilizers and for their role in biological soil crust formation and ameliorating desertification [4–6].

Several cyanobacteria and eukaryotic algae form complex and colored mats of intricate and entangled microbial structures on the illuminated surface of terrestrial as well as aquatic environments [7–9]. The top layer of microbial mats is predominantly occupied by oxygenic phototrophs, such as cyanobacteria [10] and diatoms, along with the amalgamated layers of anoxygenic phototrophic bacteria, such as green and purple sulfur bacteria [11–13]. Confocal laser scanning microscopy, which allows the in situ observations of living hydrated biofilms, revealed that biofilms in reality have a complex 3-D structure made up of variously shaped cell stacks and streamers permeated by channels, instead of being a planar structure as previously thought [14].

The major component of phototrophic biofilms is microalgae. Microalgae or microphytes are unicellular organisms that have diverse morphology from macro to minute microscopic sizes, existing as single cell, filamentous, or aggregate form dwelling in marine and freshwater bodies and play a role in ecological succession [15]. These organisms mainly belong to the classes *Bacillariophyceae*, *Cyanophyceae*, and *Chlorophyceae*. Microalgae utilize CO_2 through phototrophic metabolism and yield oxygen gas (O_2), crucial for life on

earth [16]. Microalgae have specialized storage bodies known as carboxysomes containing enzymes that drive CO_2 assimilation through Calvin cycle. Moreover, they avoid photorespiration by accumulating more carbon in their cells. Microalgae are comparatively more productive in terms of photosynthetic efficiency that avoid water requirement obligatory for land plants [17,18]. It has been reported that 1 g biomass of algae can capture up to 2 g of CO_2 [19].

Algal biofilms in aquatic bodies are mainly dominated by diatoms and they are abundantly found in marine water as plankton and through their photosynthetic activity contribute to 45% of primary production in marine ecosystems [20]. The major characteristics of diatoms are silica laden frustules and fucoxanthin, which imparts a characteristic brown color [21]. They form an important component of the algal biofilms in submerged substratum having mucilaginous substance exterior to the plasma membrane, which may be occasionally carboxylated or sulfated because of certain functional groups present [22,23]. Biofilms of Cyanophyta and Chlorophyta are found mainly in terrestrial surfaces. Green biofilm algae are predominantly unicellular, colonial, or filamentous [21]. The common green alga *Enteromorpha* and the brown alga *Ectocarpus*, are known to be adaptable to averse surroundings such as antifouling paint on a ship's hull by converting themselves to diminutive forms. Many aquatic green and brown algae reproduce through the production of large numbers of motile spores, which leads to rapid colonization of the substratum.

Cyanobacteria are simple prokaryotic organisms, [24,25] which are blue-green, sometimes red in color, due to the presence of phycobilin pigments that mask chlorophyll. Many species are capable of fixing atmospheric nitrogen, which facilitates growth in nutrient-deficient environments [26,27]. Cyanobacteria occur in a variety of morphological forms, as unicellular, colonial, and unbranched/branched filamentous forms that flourish in the environments such as spray zone, shallow water and sediments of freshwater environments, and in the upper intertidal and muddy or shallow sediments of salt marshes and estuaries. One of the most important roles that cyanobacterial biofilms play is N_2-fixation in paddy fields [24]. In common with eukaryotic algae, cyanobacteria are able to perform oxygenic (oxygen-evolving) photosynthesis. Cyanobacteria-dominated biofilms are also associated with hostile environments [28].

The various roles performed by algal biofilms have immense significance in the environment, which are given in detail, in the further sections.

2. ROLE IN WASTEWATER TREATMENT OPTIONS FOR BIOREMEDIATION AND NUTRIENT SEQUESTRATION

The first and foremost purpose of wastewater treatment is to get clean water and it involves the triad of physical, chemical, and biological sciences to achieve this, among which, biological activity is the most crucial part of this process. Although there are several advanced technologies available for the treatment of wastewater, a reliable, economically feasible, and environment-friendly approach is still a challenge. There is a great potential of exploiting intrinsic capacity of microbes to clean up water by degradation of pollutants (such as industrial waste, pesticides, heavy metals etc.) and its removal. Microalgae are

among the potential microflora suitable for growth even in low-quality water augmented with inorganic substance present in the waste water, after removal of organic waste through the action of heterotrophic bacteria during secondary treatment. Hence, tertiary treatment processes are being done through biological means by microalgae that replace the use of hazardous chemicals in this process. Through their photosynthetic mode of metabolism they reduces BOD with liberation of O_2, removes heavy metals as well, and their huge biomass provide high-value products in the form of clean energy fuels, nutraceuticals, fertilizers etc.

From last two or three decades, algae in the form of suspensions are being used for treatment of waste water, but concerns of costlier downstream processing of biomass leads to a new dynamic approach of using biofilmed microalgae where they adhere to walls of treatment plants [29] or exist in the form of immobilized matrix [30], irrespective of free cells. The use of attached growth/fixed film systems in wastewater treatment dates from 1893 with the introduction of trickling filters in England. Wastewater treatment using fixed film systems is achieved by providing favorable growth conditions to a desired consortium of microorganisms that have the ability to metabolize the pollutants of concerns. Biofilms ensure higher biomass productivity and its immobilization provides greater surface to volume ratio as compared to suspended cultures [31]. This aggregated biomass recovery is less costly as flocculation is not required and biofilms or microalgal mats on the side walls can be easily scrapped and removed.

Microalgae are involved in nutrient remediation through both direct and indirect modes. They directly take up inorganic nitrogen sources such as nitrate or nitrite, and phosphate through luxury uptake pathway, and store them as polyphosphate granules, as in the case of cyanobacteria. Microalgae can efficiently take up nitrogenous compounds as compared to macro algae and frequently they accumulate the phosphorous in the form of phosphate [32,33]. Ammonia is indirectly stripped from the solution due to increase in pH as a result of proton uptake by cell converting NH_4^+ ions into NH_3 gas. The greater efficiency of indirect removal of ammonium has been reported for cyanobacterium *Phormidium bohneri*.

Though many current technologies are being devised for biofilm-based microalgae systems in wastewater treatment, they are still not adequately addressed in the available literature, which hinders design and scale up of effective systems for applications to municipal, industrial, and agricultural waste streams. Unlike the established heterotrophic attached growth systems used in wastewater treatment, there is a need to understand more about the performance and processes involved with algal biofilm-based wastewater treatment technologies. This will help to design and scale up water treatment processes and facilitate biomass utilization effectively.

The last decade recorded highest greenhouse gas emissions in history that have adversely impacted global climate change, posing a threat to equitable and sustainable development across the continent. According to fifth IPCC (The Intergovernmental Panel on Climate Change) report 2014, emission of greenhouse gases such as CO_2 have been elevated due to anthropogenic activities during last three decades and concentration of CO_2 as green house gas has increased to about 78%, which has resulted in increased sea level by 0.19 m. Negative impacts on agricultural crop yield due to climate change have also been reported. Hence, there is need to address adaptations and mitigation strategies for reducing and managing the risks of climate change.

One of the useful mitigation approaches is biological sequestration that involves biological agents such as plants and microorganisms to fetch carbon from atmosphere through their metabolic activity and act as carbon sink in various forms like forest bodies, grassland, peat bogs, and microbial biomass. Bioenergy with carbon dioxide capture and storage (BECCS) is one of the ways that would reduce the source or enhance the sinks of greenhouse gas from the environment in a multipurpose way [34]. According to OECD environmental outlook to 2050 (2011), BECCS has been remarked as carbon negative process that can achieve lower concentration targets (450 ppm). In this regard microalgae-based biofilms can be effective BECCS system where CO_2 is converted to biomass from their emission source in engineered system such as photobioreactor with concomitant removal of inorganic nitrogenous and phosphatic nutrients present in water bodies with the generation of organic biomass that can be employed in generation of useful fuel byproducts [35]. Microalgal biofilms are present in both aquatic as well as terrestrial ecosystems such as damp environments and contribute as a major source of carbon sinks by fixing CO_2 through their phototrophic metabolism.

Microalgal biofilms are a promising approach that promote adherence of microbial cells in an exopolysaccharide matrix along with water, forming surplus biomass that can be easily separated with ease at reliable cost and can be employed in generation of various forms of biofuels or bioactive substances. These biofilmed microalgal communities are also an effective means of removal of heavy metals and radioactive elements by means of intracellular uptake, surface adsorption, or precipitates of sulfides or phosphates. They have been reported in the uptake of certain metal ions (Cd, Pb, Hg, As, Cr, Fe, etc.) either intracellularly or through surface adsorption. Generally, immobilized cyanobacteria have more potential in metal removal than their free-living counterpart, as reported with immobilized *Anabaena doliolum* [36]. The treated wastewater through algal biofilms can meet the growing demand of irrigation water for agriculture sector (requires 80% of total water) in country like India where most of the water requirement is through monsoon fed rain water, which is totally unpredictable in current scenario of global climate change [37].

Microalgal biomass generally contains lipids, proteins and carbohydrates that are modified and modulated to obtain a wide spectrum of fuel products that includes biofuels (e.g., biohydrogen, biodiesel, and bioethanol), animal feeds, chemical feedstock, and high-value bioactive compounds (e.g., Docosahexanoic acid) [38–40]. In addition, this biomass can also be converted to biochar by pyrolysis, which is currently investigated for negative carbon dioxide emissions. Biochar is a form of charcoal, obtained from biological sources that increases soil fertility, improves water retention capacity, and helps in land reclamation [41]. Hence biofuels and biochar are excellent sources of carbon negative processes, where intake of CO_2 is there with zero carbon release, enabling long-term sequestration. Such a technology of carbon sequestration along with wastewater treatment infrastructures can be more sustainable [42].

More intensive investigations need to be addressed as only small-scale work has been done toward efficacy of biofilm-based formulations of microalgae and their deployment as nutrient sequestering options. By studying the minute details about the performance and processes involved with algal biofilm-based wastewater treatment technologies, generating improved design, and scale-up carbon-negative technology, devoid of hazardous chemicals, they can be deployed effectively.

3. ALGAL BIOFILMS IN AGRICULTURE

In order to meet the agricultural demands and the needs of the increasing population, the use of biofertilizers in agriculture as nutrient-providing options is promising [43,44]. Cyanobacterial inoculants are extensively utilized in rice, and in the last decade, found to have promise as biofertilizers, biofortifying and biocontrol agents in several cereal, leguminous, and vegetable crops [5,45]. These photosynthetic prokaryotes are known to adapt to various environmental conditions, which includes extreme temperatures, high salinity, drought conditions etc. and are often the primary colonizers of inhospitable habitats [27].

Growing as assemblages on the surface of water, cyanobacterial biofilms are often the major inhabitants of wetland rice ecosystems. These cyanobacteria have been widely studied for their agronomic significance in free living as well as symbiotic association with water fern *Azolla* [46]. The effectiveness of using cyanobacteria as nitrogen fixers in rice cultivation is successfully reported [47–49]. The metabolism of cyanobacteria allows them to readily switch over to other available sources of nitrogen in addition to biological nitrogen fixation using atmospheric N_2 as their sole N source.

The mucilaginous matrix of cyanobacteria represents a nutrient-rich niche for several heterotrophs in nature, which leads to biofilm-like growth of cyanobacteria in soil and aquatic bodies (Fig. 14.2). Significant accomplishment of cyanobacteria as biofertilizers in paddy cultivation widens their scope of utilization in other crops as well. Biofilmed inoculants were developed using a heterocystous cyanobacterium *Anabaena torulosa* and agriculturally useful bacteria and fungi [4]. Such biofilmed inoculants were able to exhibit superior plant growth promoting and biocontrol properties, when used as inoculants in wheat, rice, cotton, and maize [5,43,50]. They are able to enhance the nutrient levels in soil, improve their uptake, and elicit resistance to pathogen attack or other types of stress [43,50,51].

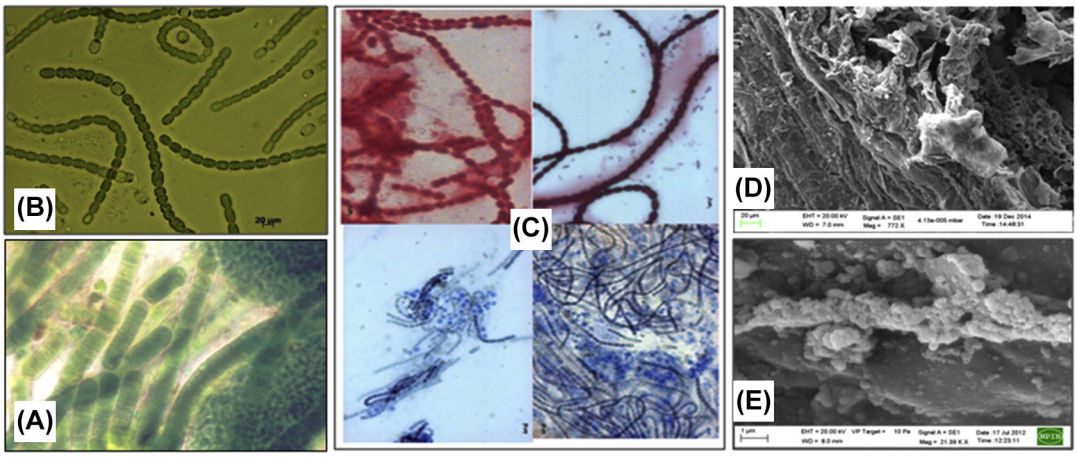


FIGURE 14.2 Microphotographs of cyanobacterial biofilms (clockwise from bottom left corner). (A) Naturally occurring biofilm from paddy fields comprising unicellular, colonial, and filamentous cyanobacteria. (B) *Anabaena* filaments with distinct mucilage envelope. (C) *Anabaena*-based biofilms with agriculturally important bacteria and fungi. (D, E) Scanning electron images of colonization of *Anabaena*-based biofilms in root tissues of maize and tomato plants.

4. EXPLORING ALGAL BIOFILMS AS SOURCES OF EXOPOLYSACCHARIDES

In biofilms, extracellular polymeric substances (EPS) are biomolecules and inert solids that bind cells to each other and to solid materials. Under natural conditions, they are a common feature of any microbial biofilms, where they predominantly play key protective and structural roles [52]. Extracellular polymeric substances are generally present at the exterior of cells, generated through active secretion, cell lysis, shedding of cell surface material, and also adsorption from the environment [53,54]. The predominant EPS are polysaccharides and proteins; however, nucleic acids, lipids, and suspended solids can also make up the EPS matrix [54].

Due to the massive and, in some cases, hyper-production, and the rheological features of these polymers, cyanobacterial EPSs have gained increasing scientific attention due to their possible biotechnological applications. EPSs have a putative physical protective role against several harmful factors, both chemical and physical, representing a boundary between cells and the immediate outer environment, and preserve the cells from antibacterial agents and protozoan predation [55]. Complex biofilm structures, which occur as multilayers, show the predominance of cyanobacteria in the production of exopolysaccharides that constitutes the exocellular polymeric matrix (EPM). It has been reported that in the soil system, EPM contributes to biofilm structural stability, adhesion to the substrate, nutrient and metal ion uptake, and the provision of moisture for the constitution of an optimal microenvironment [56].

The control of aeration, temperature, and salinity are important for optimal EPS productivity, while pH, the presence of metal ions, and dilution rate were reported as possible conditioners [57]. The availability/amount and possible limitations of nitrogen, phosphate, sulfate, and carbon can influence EPS production [58]. In particular, the C/N ratio is an important parameter [59]. Phototrophic biofilms can be found growing on exposed lithic substrates, and cyanobacterial EPSs play key roles in protecting from UV irradiation and drought [60].

Cyanobacteria isolated from very dry environments, such as desert soils or the lithic surfaces of monuments, display the capacity of excreting large amounts of EPSs [8,61,62], a trait underlining adaptation to drought. Water stress leads to the loss of membrane structural integrity and the loss of macromolecule functioning [63], which may be associated with cell death [64,65]. EPS helps in maintaining gelatinous envelope around the cells that regulates water uptake and water loss processes [66]. EPS also protects from the UV radiations by forming an envelope all around the cell, thereby providing a longer path length for the radiations to cover. The presence of UV absorbing compounds like scytonemin [67] and mycosporine-like amino acids play a protective role [68]. The synthesis of these pigments is also elicited by UV exposure [60,69].

In phototrophic biofilms, the surface-attached community draws its energy and carbon source almost entirely from light energy and CO_2 fixation due to cyanobacteria [8]. The production of EPS also helps in the adhesion of algal filaments on the surface thereby forming an important component in the biofilm formation. Cyanobacterial biofilms helps in the attachment of the cells to the surface and with the passage of time, it increases in thickness thereby allowing other microbes to grow and flourish. EPSs provide for the structuring of

the biofilms, creating preferential flows of water and nutrients. In addition, EPM creates hydrated microenvironments in which the cells are protected from harmful solar radiation, physical harm, and represent a source of carbon for heterotrophs. In marine environments, cyanobacterial and diatom-produced EPSs form a matrix that affords stability to mudflat sediments against erosion [70] and enriching sediments with organic matter and nutrients [71].

Cyanobacterial growth as epiphytes is mostly in the biofilm-like growth on plant parts or on the interface of soil and water in flooded wetlands, including rice. Venkatachalam et al. [72] observed the presence of biofilm-like growth of cyanobacteria, green algae, and diatoms, on the surface of leaf sheaths of rice plants, and the predominant cyanobacteria were of the nitrogen-fixing types, notably *Nostoc*, *Anabaena*, *Calothrix*, and *Gloeotrichia*. One of the earliest reports in this context was of Martinez and Catling [73] who reported the presence of nitrogen-fixing cyanobacteria in the deepwater rice and of the photo-dependent nitrogen-fixing activity associated with their nodal roots. In the rice fields, globose or gelatinous colonies of *Gloeotrichia* are often observed to be attached to rice plants, which show a gradual decrease in terms of abundance from the seedling to tillering stages. The plant-like green alga *Chara* is a common inhabitant in deepwater rice. Colonies of *Nostoc*, *Calothrix*, and *Anabaena* sp. grow more firmly attached to the plant surface [74].

5. BIOLOGICAL SOIL CRUSTS AND ALGAL BIOFILMS

Biological soil crusts (BSCs) are formed from colonization of living entities such as cyanobacteria lichens, fungi, and few plants like mosses that leads to formation of crumbs over deserted soil surfaces, thus improving the soil productivity and fertility. These BSCs are also known as biocrusts or microphytic crusts. Cyanobacteria are the dominant group of microbes involved in improving soil structure and stability through their filamentous growth such as *Nostoc*, *Microcoleus* [75] that form complex aggregates ensheathed in exopolysaccharide. The biological soil crust comprises of almost three fourths of the living biomass [76]. It has also been shown that biological soil crust comprising lichens and mosses are more protective in nature than the cyanobacteria alone [77]. Exopolysaccharide production by cyanobacteria in crusts helps to check soil erosion as they hold soil particles together [78] and also shield the biocrusts from desiccation [79]. This type of crust formation is an essential aid in the flourishing of flora and fauna in barren lands and thereby land reclamation.

Cyanobacteria and cyanobacteria containing soil lichens provide a major share of fixed carbon and nitrogen in many desert ecosystems [80–82]. The major functional feature of biological soil crusts is its mucilaginous matrix, which also regulates soil water relations by altering the topography of the soil surfaces as is evidenced by the infiltration rates, which is a function of water residence time and surface permeability, which increases where the crusts create rough surfaces and decreases where the surfaces are flat [83,84]. Some of the soil mosses and lichens are harbored by epiphytic biofilms of cyanobacteria, forming a consortium that has nitrogen-fixing activity [85]. Cyanobacterial crusts significantly modulate the uptake of essential nutrients by plants thus improving nutrient quality of associated plants that may provide good-quality forage.

BSCs is one of the effective mode of land reclamation in desert areas where major constraints in vegetation and crop production is due to erosion of surface layer of productive soil. Biological soil crusts are stable forms that do not dissolve in rain and persist even in drought conditions unlike vascular plants, thus they are potent soil surface protectors. The ecological importance of crusts in arid and semiarid environments is immense and undeniable; unfortunately, biocrusts and the ecosystem services they provide may be extremely fragile, taking decades for a fully developed crust to regenerate following disturbance [86].

Bhatnagar et al. [87] discovered the potential hemostatic abilities of exopolymers of four desert cyanobacteria, including three species of *Anabaena* sp. and *Tolypothrix tenuis* and explored their role as novel clotting agents for wound management. Certain substance, such as scytonemin, carotenoids, and mycosporine-like amino acid, produced by cyanobacteria protects biocrusts from UV damage [88].

6. BIOFOULING AND ANTIFOULING

The negative aspect of biofilms is biofouling in industrial water systems where microbial biofilm development exceeds a given threshold of interference [89]. Algal biofilms have severe implications on submerged structures particularly in the marine ecosystems. Algal biofilms due to their metabolic activities result in fouling of those structures where they develop. These include petroleum pipelines, platforms surroundings, water channels, swimming pools, aquaculture tanks, as well as oceanic vehicles such as boats, ships, and submarines. Algal biofilms also deteriorate historical buildings, fences, signposts, and architectural monuments/statues, posing a major threat to the aesthetic value. Economic losses due to biofouling are being faced by industry in spite of their better understanding of industrial water systems [90]. The biofilm formation is a sequential process that starts with the adsorption of organic macromolecules (proteins, glycoproteins, and polysaccharides) followed by adhesion [91]. Biofouling films are similar to biological filtration where nutrient sequestration from water source by microbial colony supplements them with new biomass and metabolites [92]. Algal biofilms in such cases are complex, frequently highly productive facets of these ecosystems, enclosed in a mucilaginous sheath of exopolysaccharide.

Impaired efficiency of filtration and cooling systems, increased propulsive fuel consumption in shipping, and rapid corrosion are some of the undesirable effects caused by biofouling marine algae on artificial surfaces [93]. Algal communities constitute a major contribution to marine biofouling. The attachment and settlement of different algal spores may be sequential, with spores of green and brown algae attaching first followed by spores of red algae. Most green and brown algae have motile spores as a result of hair like flagella. It has been proposed that motility and the hair-like flagella mediate site selection and initial adherence. Red algae on the other hand have nonmotile spores, and the dispersal of spores is a passive process where size and density determines their sinking rate [94]. The attachment of spores consists of two different phases: an initial attachment and a permanent attachment. With respect to the permanent stage it has been demonstrated that all algal spores produce an adhesive material (probably a polysaccharide-protein complex), which strengthens spore attachment to the surface. Furthermore, algal spores have been shown to display contact-dependent recognition of specific signals [95].

Much of these algal biofilms are dominated by diatoms, which plays considerable role in nutrient dynamics of the biofilm through syntrophic interactions [96]. Cross-feeding in biofilms may involve much more than exchange of organic molecules. It could, for instance, involve the detoxification of the biofilm environment by bacterial respiration of photosynthetically produced oxygen [97]. In *Craspedostauros australis*, glycans associated with extracellular glycoproteins serve as mucin-like functions on the cell surface and helps in adhesion of diatoms [23]. The productivity of fouling algal biofilms can be stimulated with increased availability of nitrogen alone or phosphorus and nitrogen together [98]. Incorporation of silt particles in sedimentary diatom biofilms exhibits positive feedback between diatom growth and silt accumulation [99].

Cyanobacterial exopolysaccharides may result in the detoxification of antifouling paint, enhanced corrosion of steel surfaces, and the conditioning of surfaces for subsequent colonizing organisms [100]. Cohesive and adhesive forces are vital constraints that need to be overcome in removal of fouling biofilms [101]. The EPS in biofilms are capable of forming various types of interaction with their surroundings due to various forces such as hydrogen bonds, electrostatic interactions, and Van der waals interactions, which magnifies their binding capacity with their substrates and hence stabilizes them [101].

7. ROLE IN BIOREMEDIATION

From the era of industrialization till date, there has been tremendous proliferation of chemical industries, which has been a source of contamination and accumulation of toxic wastes [102]. The tenacity of chemical pollutants has adverse impact on environment causing long-term environmental disasters in the public domain [103]. Bioremediation is an emerging in situ technology for the clean-up of environmental pollutants using microorganisms. The biological processes for treating toxic effluents are better than chemical and physical methods in terms of their efficiency and economy [102], and the potential of biofilm communities for bioremediation processes has recently been realized. Fertilizer or pesticide application, which is intended to maximize agricultural production or pest control, has contributed to soil degradation and aggravates soil pollution by the accumulation of toxic substances like cadmium, lead, and several organic pollutants. Biofilm-mediated bioremediation presents a proficient and safer alternative to bioremediation with planktonic microorganisms because cells in a biofilm have a better chance of adaptation and survival (especially during periods of stress) as they are protected within the matrix [104]. Due to beneficial physical and biochemical interactions among the organisms in the biofilms, immobilization and degradation of pollutants is facilitated. Biosorption of heavy metals can take place through a variety of ways like ion exchange, chelation, adsorption, and diffusion through cell walls. These are passive modes of remediating heavy metals. Whereas actively metals could be taken inside the cell and concentrated through a process known as bioaccumulation, which is dependent on cellular metabolism. Successful application of a bioremediation process relies upon an understanding of interactions among microorganisms, organic contaminants, and soil or aquifer materials.

Cyanobacteria being photosynthetic in nature provide a favorable condition for removal of heavy metals from the environment because their interior pH is almost two units higher than surrounding liquid [105], and hence it provides resistance to mass transfer of

products out of the biofilm [106]. Immobilized cyanobacteria are potent in removing metal than their free-living forms [107]. Immobilized *Anabaena doliolum* showed an increased uptake of Cu and Fe, i.e., in the order of 45% and 23% higher than that of free-living cells, respectively [36].

Extracellular polysaccharides that are negatively charged at elevated pH levels generated by oxygenic photosynthesis may account for the metal-binding properties of phototrophic biofilms [108,109]. The metal-binding properties are probably due to a high density of anionic charges, especially carboxyls, identified in the capsular polymer. Parker et al. [110] showed that mucilage sheaths isolated from the cyanobacteria *Microcystis aeruginosa* and *Aphanothece halophytica* exhibit strong affinity for heavy metal ions such as copper, lead, and zinc. In addition to biosorption and bioaccumulation, the elevated pH inside photosynthetically active biofilms may favor removal of metals by precipitation [106]. Factors affecting the metal uptake efficiency include the presence of cations in water, light intensity, pH, biofilm density, and the presence of metal-binding humic substances, size of the particle, and the tolerance of individual algal species to specific heavy metals [111,112].

Pesticides form an important component among the chemical substances that are used in agriculture. Pesticides directly applied either as soil treatment or as the "fallout" after aerial application to crops invariably reach soil and water resources with their harmful effects on nontarget species such as the beneficial cyanobacteria [113,114]. The growth of cyanobacteria as biofilms in aquatic bodies is reported to accumulate very high concentration of insecticides. *Synechococcus elongatus*, *Anacystis nidulans*, and *Microcystis aeruginosa* have been able to degrade many organophosphorus and organochlorine insecticides from the aquatic system. Thus it seems that cyanobacteria can be mass cultured in waste water lagoon to degrade organic matter, to remove pollution load, and to meet the requirement of nitrogenous fertilizers with minimal investment compared to the conventional wastewater treatment plant. Simple growth requirements and versatile metabolic capabilities make cyanobacteria an excellent biological option for developing low-cost and low-maintenance biocatalysts, even by genetic modification, for detoxification of pesticides in agricultural fields. *Synechococcus elongatus*, *Phormidium tenue*, and *Nostoc linckia* were found to degrade the organophosphorous insecticides like monocrotophos and quinalphos at 5–50 ppm within 30 days [115]. Ibrahim et al. [116] found that *Nostoc muscorum* degraded malathion due to its capability to use it as a sole phosphorus source. Chungjatupornchai and Fa-Aroonsawat [117] even genetically engineered *Synechococcus* PCC7942 for the surface and intracellular expression of *opd* gene that encodes organophosphorus hydrolase from *Flavobacterium* sp., a bacterium capable of degrading organophosphorus parathion isolated from the rice fields of the Philippines [118].

It has been shown that, in particular, *Oscillatoria* spp. growing as biofilmed aggregates is able to deal with heavy oil pollution [119,120]. Although there is no direct evidence that cyanobacteria are directly involved in the degradation of petroleum products, they probably facilitate degradation by sulfate reducing bacteria [121] and aerobic heterotrophs [122,123]. Previous studies have shown that the addition of nitrogen supplements enhances microbial assimilation of carbon from oil [124]. Cyanobacterial N_2 fixation in such biofilms could provide sufficient nitrogen compounds for heterotrophic oil degradation. Radwan et al. [125] reported the association of oil-utilizing bacteria with 10 different macroalgae isolated from the Arabian Sea. These bacteria helped in the attenuation of hydrocarbon from the sea water.

Phototrophic biofilms are ubiquitous and dominant primary producers forming the base of aquatic food webs. Therefore, phototrophic biofilms can be promising as sensitive bio-indicators of petrochemical pollution and for ecotoxicology tests [126].

8. OTHER APPLICATIONS OF ALGAL BIOFILMS

Clean and environment friendly biofuel in the form of biodiesel is one of the renewable sources of energy that is provided by algal biomass, as a valuable alternative to depleting nonrenewable petroleum energy source [127]. The growth efficiency of microalgae, which is 100 times faster than terrestrial plants and doubling time of less than one day [128], makes them suitable for biomass production. Capability of some algal strains to accumulate huge amount of lipids inside their cells makes them suitable candidates to harness biodiesel [129]. This amount is very high as compared to oils from soybean, rapeseed, oil palm, and *Jatropha.* Culturing microalgae for biodiesel production requires less land area.

Biofilms were little studied for the production of biofuel due to difficulties in maintenance, or problems in the growth environment to achieve a homogenous suspension. However, studies have revealed that biofilm mode of growth offers the ease of simple mechanical harvesting such as scraping them from the surface [42]. Also, biomass production was significantly high at respective light conversion efficiencies. Algal lipids can also be enhanced by exposing the cultures to a range of environmental stresses most commonly being the nutrient starvation stresses. However, Schnurr et al. [130] reported that nutrient starvation is not a desirable method of lipid production for algal biofilm biofuel production. But the lipid content increases in case of the suspension cultures.

Harvested algal biomass can be utilized as a source of biogas production such as biomethane and biohydrogen. Waste biomass of microalgae is an important source of biogas. Decomposing algal biomass releases methane to the atmosphere and degrades water quality in the pond systems [131]. As an alternative, the algal biomass can either be employed for biodiesel production or for biogas production through their anaerobic digestion [132]. Anaerobic digestion is hopefully an appropriate use of algal biomass in connection with wastewater treatment plants in coming years. However, methane production is less than wastewater sludge by algae ($\sim$0.3 vs. 0.4 L CH_4/g volatile solids introduced) [133].

Culturing of microalgae at industrial scale for biofuels production requires substantial amount of nutrients, typically nitrogen (usually in the form of nitrate) and phosphorus (usually in the form of orthophosphate). These nutrients are normally derived from chemical or inorganic fertilizers that are used to achieve promising growth rate of microalgae and to obtain bulk quantity of biomass. The use of chemical fertilizer has the advantage of reducing contamination in culturing medium and thus promotes water reutilization to reculture microalgae. However, a recent Life Cycle Assesment study has pointed out that 50% of the overall energy use and GHG emission were associated with utilization of chemical fertilizers [134]. Chemical fertilizer production has been categorized as an energy intensive industry, in which 37–40 GJ of low heating value natural gas will be consumed to produce 1 tonne of ammonia (inorganic nitrogen sources, N-fertilizer) [135]. Furthermore, 1.2 kg of carbon dioxide (CO_2) will be emitted for every 1 kg of ammonia produced [136].

Hydrogen is a clean and ideal substitute for fossil fuels because of its high density and water as a byproduct. Biological production of hydrogen could provide a renewable source

of energy. Cyanobacterial enzymes have been found to be capable of biological photohydrogen production. The bidirectional hydrogenase complex can either produce or oxidize H_2 in the presence of suitable electron donor or acceptor. The physiological role of bidirectional hydrogenases is under review, as the enzyme is not present ubiquitously in all the strains. Hydrogen-generating cyanobacteria should have some specific characteristics like having high metabolic rate, no restrictions regarding place, and minimum amount of adenosine triphosphate for growth. Biofilms containing cyanobacteria are of great significance as they fulfill these specifications. The efficiency of hydrogen production from cyanobacterial biofilms can be enhanced by exploiting cyanobacterial species or genetically modified strains with a reduced uptake of hydrogenase activity [137,138]. In order to achieve or optimize the conditions necessary for hydrogen production, the diversity of algal species conferring this property has to be thoroughly searched out and potential strains can be further designed for genetically engineered cyanobacteria. This technology is still in experimental trials and its commercialization needs intensive research.

9. FUTURE PROSPECTS

Biofilms are now becoming a subject of interest globally, as their "nuisance value" in medical and industrial sciences is now being explored for their unique capabilities and advantages for use in agriculture and environmental biology. The science of microbiology is now looking at various processes from a biofilm perspective. Researchers from all around the world are now working together to gain insights into the molecular basis of the biofilms, their pervasiveness, and adaptability to diverse niche. Although tremendous progress has been made in terms of their structural and molecular features, serious efforts need to be made to combine the knowledge from chemical and physical sciences with physiology, genetics, and ecology of the biofilms. Multidimensional "omics" and nanoscale studies can help to gain deeper insights into the unique phenomenon of biofilm formation and its applications for the welfare of humankind.

Acknowledgments

The authors are thankful to the Post Graduate School, ICAR Indian Agricultural Research Institute, New Delhi for the fellowships to ST and AB, and to the Network Project on Microorganisms "Application of Microorganisms in Agricultural and Allied Sectors" (AMAAS) granted by Indian Council of Agricultural Research (ICAR), New Delhi for funds to RP. The facilities provided by the Division of Microbiology, ICAR Indian Agricultural Research Institute, New Delhi are gratefully acknowledged.

References

[1] M.E. Callow, Algal biofilms, in: L.V. Evans (Ed.), Biofilms: Recent Advances in Their Study and Control, Harwood Academic Publishers, Amsterdam, 2000, pp. 189–209.

[2] B.S.C. Leadbeater, M.E. Callow, Formation, composition and physiology of algal biofilms, in: L.F. Melo, T.R. Bott, M. Fletcher, B. Capdeville (Eds.), Biofilms – Science and Technology, Kluwer Academic Publishers, Amsterdam, Netherlands, 1992, pp. 149–162.

[3] M.L. Grbic, J. Vukojevic, G.S. Simic, J. Krizmanic, M. Stupar, Biofilm forming cyanobacteria, algae and fungi on two historic monuments in Belgrade, Serbia, Arch. Biol. Sci. 62 (3) (2010) 625–631.

[4] R. Prasanna, S. Pattnayak, T.C.K. Sugitha, L. Nain, A.K. Saxena, Development of cyanobacterium based biofilms and their in vitro evaluation for agriculturally useful traits, Folia Microbiol. 56 (2011) 49–58.
[5] R. Prasanna, S. Triveni, N. Bidyarani, S. Babu, K. Yadav, A. Adak, S. Khetarpal, M. Pal, Y.S. Shivay, A.K. Saxena, Evaluating the efficacy of cyanobacterial formulations and biofilmed inoculants for leguminous crops, Arch. Agron. Soil Sci. 60 (3) (2014) 349–366.
[6] K.T. Harper, J. Belnap, The influence of biological soil crusts on mineral uptake by associated vascular plants, J. Arid Environ. 47 (2001) 347–357.
[7] R. Guerrero, M. Piqueras, M. Berlanga, Microbial mats and the search for minimal ecosystems, Inter. Microbiol. 5 (2002) 177–188.
[8] G. Roeselers, T.B. Norris, R.W. Castenholz, S. Rysgaard, R.N. Glud, M. Kuhl, G. Muyzer, Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland), Environ. Microbiol. 9 (2007) 26–38.
[9] D.M. Ward, M.J. Ferris, S.C. Nold, M.M. Bateson, A natural view of microbial biodiversity within hot spring cyanobacterial mat communities, Microbiol. Mol. Biol. Rev. 62 (1998) 1353–1370.
[10] R.W. Castenholz, Phylum BX. Cyanobacteria. Oxygenic photosynthetic bacteria, in: D.R. Boone, R.W. Castenholz, G.M. Garrity (Eds.), Bergey's Manual of Systematic Bacteriology, Springer, New York, 2001, pp. 474–487.
[11] M. Martinez-Alonso, J. Van Bleijswijk, N. Gaju, G. Muyzer, Diversity of anoxygenic phototrophic sulfur bacteria in the microbial mats of the Ebro Delta: a combined morphological and molecular approach, FEMS Microbiol. Ecol. 52 (2005) 339–350.
[12] R.W. Castenholz, Class I: Chloroflexi, in: D.R. Boone, R.W. Castenholz, G.M. Garrity (Eds.), Bergey's Manual of Systematic Bacteriology, Springer, New York, 2001, p. 427.
[13] A.L. Ruffroberts, J.G. Kuenen, D.M. Ward, Distribution of cultivated and uncultivated cyanobacteria and Chloroflexus-like bacteria in hot-spring microbial mats, Appl. Environ. Microbiol. 60 (1994) 697–704.
[14] Z. Lewandowski, Structure and function of biofilms, in: L.V. Evans (Ed.), Biofilms: Recent Advances in Their Study and Control, Harwood Academic Publ, Amsterdam, 2000, pp. 1–18.
[15] A. Burns, D.S. Ryder, Potential for biofilms as biological indicators in Australian riverine systems, Ecol. Manag. Restor. 2 (1) (2001) 53–64.
[16] P. Hunter, The tide turns towards microalgae, EMBO Rep. 11 (8) (2010) 583–586.
[17] R. Sayre, Microalgae: the potential for carbon capture, BioScience 60 (9) (2010) 722–727.
[18] M. Hannon, J. Gimpel, M. Tran, B. Rasala, S. Mayfield, Biofuels from algae: challenges and potential, Biofuels 1 (5) (2010) 763–784.
[19] H. Herzog, D. Golomb, Carbon capture and storage from fossil fuel use, Encycl. Energy 1 (2004) 1–11.
[20] A. Yool, T. Tyrrell, Role of diatoms in regulating the ocean's silicon cycle, Glob. Biogeochem. Cycles 17 (4) (2003) 1103–1124.
[21] C. Van den Hoek, D.G. Mann, H.M. Jahns, Algae: An Introduction to Phycology, Cambridge Univ Press, USA, 1995.
[22] R. Wetherbee, J.L. Lind, J. Burke, The first kiss: establishment and control of initial adhesion by raphid diatoms, J. Microbiol. 34 (1998) 9–15.
[23] A. Chiovitti, A. Bacic, J. Burke, R. Wetherbee, Heterogeneous xylose-rich glycans are associated with extracellular glycoproteins from the biofouling diatom *Craspedostauros australis* (Bacillariophyceae), Euro. J. Phycol. 38 (2003) 351–360.
[24] G.E. Fogg, W.D.P. Stewart, P. Fay, A.E. Walsby, The Blue-Green Algae, Academic Press, London, 1973.
[25] N.G. Carr, B.A. Whitton, The Biology of Cyanobacteria, Blackwell Scientific Publications, Oxford, 1982.
[26] G. Toledo, Y. Bashan, A. Soeldner, In vitro colonization and increase in nitrogen fixation of seedlings of black mangrove inoculated by a filamentous cyanobacterium, Can. J. Microbiol. 41 (1995) 1012–1020.
[27] V. Gupta, S.K. Ratha, A. Sood, V. Chaudhary, R. Prasanna, New insights into the biodiversity and applications of cyanobacteria (blue-green algae)-prospects and challenges, Algal Res. 2 (2) (2013) 69–97.
[28] W.F. Vincent, R.W. Castenholz, M.T. Downes, C. Howard-Williams, Antarctic cyanobacteria, light, nutrients and photosynthesis in the microbial mat environment, J. Phycol. 29 (1993) 745–755.
[29] C. Gonzalez, J. Marciniak, S. Villaverde, C. Leon, P.A. Garcia, Efficient nutrient removal from swine manure in tubular biofilm photo-bioreactor using algae-bacteria consortia, Water Sci. Technol. 58 (1) (2008) 95–102.
[30] L.L. Zhuang, H.Y. Hu, Y.H. Wu, T. Wang, T.Y. Zhang, A novel suspended-solid phase photobioreactor to improve biomass production and separation of microalgae, Biores. Technol. 153 (2015) 399–402.

[31] A.N. Shilton, N. Powell, B. Guieysse, Plant based phosphorus recovery from wastewater via algae and macrophytes, Curr. Opin. Biotechnol. 23 (6) (2012) 884–889.
[32] N. Renuka, A. Sood, S.K. Ratha, R. Prasanna, A.S. Ahluwalia, Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production, J. Appl. Phycol. 25 (2013) 1529–1537.
[33] N. Renuka, A. Sood, S.K. Ratha, R. Prasanna, A.S. Ahluwalia, Nutrient sequestration, biomass production by microalgae and phytoremediation of sewage water, Inter. J. Phytorem. 15 (8) (2012) 789–800.
[34] N. Murado, Liberating Energy from Carbon: Introduction to Decarbonization, Springer Science and Business Media, 2014, 2014.
[35] S. Selosse, O. Ricci, Achieving negative emissions with BECCS (bioenergy with carbon capture and storage) in the power sector: new insights from the TIAM-FR (TIMES Integrated Assessment Model France) model, Energy 76 (2014) 967–975.
[36] L.C. Rai, N. Mallick, Removal and assessment of toxicity of Cu and Fe to *Anabaena doliolum* and *Chlorella vulgaris* using free and immobilized cells, World J. Microbiol. Biotechnol. 8 (1992) 110–114.
[37] S.K. Shah, Towards adopting nanotechnology in irrigation: Micro irrigation systems, India Water Portal, National Knowledge Commission, India, 2011.
[38] D.J. Pyle, R.A. Garcia, Z. Wen, Producing docosahexaenoic acid (DHA)-rich algae from biodiesel-derived crude glycerol: effects of impurities on DHA production and algal biomass composition, J. Agric. Food Chem. 56 (11) (2008) 3933–3939.
[39] R. Harun, M. Singh, G.M. Forde, M.K. Danquah, Bioprocess engineering of microalgae to produce a variety of consumer products, Renew. Sust. Energ. Rev. 14 (3) (2010) 1037–1047.
[40] O. Pulz, W. Gross, Valuable products from biotechnology of microalgae, Appl. Microbiol. Biotechnol. 65 (6) (2004) 635–648.
[41] N.P. Gurwick, L.A. Moore, C. Kelly, P. Elias, A systematic review of biochar research, with a focus on its stability in situ and its promise as a climate mitigation strategy, PLoS One 8 (9) (2013) e75932.
[42] L.B. Christenson, R.C. Sims, Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts, Biotechnol. Adv. 29 (6) (2011) 686–702.
[43] R. Prasanna, N. Bidyarani, S. Babu, F. Hossain, Y.S. Shivay, L. Nain, Cyanobacterial inoculation elicits plant defense response and enhanced Zn mobilization in maize hybrids, Cogent Food Agric. 1 (2015) 995807.
[44] J.S. Singh, V.C. Pandey, D.P. Singh, Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development, Agric. Ecosyst. Environ. 140 (3–4) (2011) 339–353.
[45] R. Prasanna, D.W. Dhar, Y.S. Shivay, Significance of biofertilizers in integrated nutrient management, in: R. Prasad, D. Kumar, D.S. Rana, Y.S. Shivay, R.K. Tewatia (Eds.), Textbook of Plant Nutrient Management, Indian Society of Agronomy, New Delhi, India, 2015, pp. 231–248.
[46] P.A. Roger, Reconsidering the utilization of cyanobacteria in wetland rice cultivation, in: S. Dutta, C. Sloger (Eds.), Biological N_2 Fixation Associated with Rice Production, Oxford and IBH, New Delhi, 1991, pp. 119–141.
[47] Y. Ariosa, A. Quesada, J. Aburto, D. Carrasco, R. Carreres, F. Leganes, E.F. Valiente, Epiphytic cyanobacteria on *Chara vulgaris* are the main contributors to N_2 fixation in rice fields, Appl. Environ. Microbiol. 70 (2004) 5391–5397.
[48] M. Habte, M. Alexander, Nitrogen fixation by photosynthetic bacteria in lowland rice culture, Appl. Environ. Microbiol. 39 (1980) 342–347.
[49] N.W. Lem, B.R. Glick, Biotechnological uses of cyanobacteria, Biotechnol. Adv. 3 (1985) 195–208.
[50] R. Prasanna, S. Babu, N. Bidyarani, A. Kumar, S. Triveni, D. Monga, A.K. Mukherjee, S. Kranthi, N. Gokte-Narkhedhar, A. Adak, K. Yadav, L. Nain, A.K. Saxena, Prospecting cyanobacteria fortified composts as plant growth promoting and biocontrol agents in cotton, Exp. Agric. 51 (2015) 42–65.
[51] M. Kumar, N. Karthikeyan, R. Prasanna, Priming of plant defense and plant growth in disease challenged crops using microbial consortia, in: D.K. Choudhary, A. Varma (Eds.), Microbial-Mediated Induced Systemic Resistance in Plants, Springer Science plus Business Media, Singapore, 2016, pp. 39–56.
[52] F. Rossi, R.D. Philippis, Role of cyanobacterial exopolysaccharides in phototrophic biofilms and in complex microbial mats, Life 5 (2015) 1218–1238.
[53] H.C. Flemming, T.R. Neu, D. Wozniak, The EPS matrix: the "house of biofilms cells", J. Bacteriol. 189 (2007) 1–6.
[54] J. Wingender, T. Neu, H.C. Flemming, What are bacterial extracellular polymeric substances?, in: Microbial Extracellular Polymeric Substances, Springer, Heidelberg, Berlin, 1999, pp. 1–19.

[55] R. De Philippis, M. Vincenzini, Exocellular polysaccharides from cyanobacteria and their possible applications, FEMS Microbiol. Rev. 22 (1998) 151–175.
[56] S. Ozturk, B. Aslim, Relationship between chromium (VI) resistance and extracellular polymeric substances (EPS) concentration by some cyanobacterial isolates, Environ. Sci. Pollut. Res. 15 (2008) 478–480.
[57] B. Nicolaus, A. Panico, L. Lama, I. Romano, M.C. Manca, A. de Giulio, A. Gambacorta, Chemical composition and production of exopolysaccharides from representative members of heterocystous and non-heterocystous cyanobacteria, Phytochemistry 52 (1999) 639–647.
[58] J. Moreno, M.A. Vargas, H. Olivares, J. Rivas, M.G. Guerrero, Exopolysaccharide production by the cyanobacterium *Anabaena* sp. ATCC 33047 in batch and continuous culture, J. Biotechnol. 60 (1998) 175–182.
[59] A. Otero, M. Vincenzini, *Nostoc* (Cyanophyceae) goes nude: extracellular polysaccharides serve as a sink for reducing power under unbalanced C/N metabolism, J. Phycol. 40 (2004) 74–81.
[60] F. Rossi, E. Micheletti, L. Bruno, S.P. Adhikary, P. Albertano, R. de Philippis, Characteristics and role of the exocellular polysaccharides produced by five cyanobacteria isolated from phototrophic biofilms growing on stone monuments, Biofouling 28 (2012) 215–224.
[61] F. Rossi, R.M. Potrafka, F.G. Pichel, R. de Philippis, The role of the exopolysaccharides in enhancing hydraulic conductivity of biological soil crusts, Soil Biol. Biochem. 46 (2012) 33–40.
[62] C. Hu, Y. Liu, B.S. Paulsen, D. Petersen, D. Klaveness, Extracellular carbohydrate polymers from five desert soil algae with different cohesion in the stabilization of fine sand grain, Carbohydr. Polym. 54 (2003) 33–42.
[63] E.J. Knowles, R.W. Castenholz, Effect of exogenous extracellular polysaccharides on the desiccation and freezing tolerance of rock-inhabiting phototrophic microorganisms, FEMS Microbiol. Ecol. 66 (2008) 261–270.
[64] M.A. Redmile-Gordon, P.C. Brookes, R.P. Evershed, K.W.T. Goulding, P.R. Hirsch, Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms, Soil Biol. Biochem. 72 (2014) 163–171.
[65] J. Wierzchos, A. De Los Ríos, L.G. Sancho, C. Ascaso, Viability of endolithic micro-organisms in rocks from the McMurdo Dry Valleys of Antarctica established by confocal and fluorescence microscopy, J. Microsc. 216 (1) (2004) 57–61.
[66] S. Pereira, A. Zille, E. Micheletti, P. Moradas-Ferreira, R. de Philippis, P. Tamagnini, Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly, FEMS Microbiol. Rev. 33 (2009) 917–941.
[67] F. Garcia-Pichel, R.W. Castenholz, Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment, J. Phycol. 27 (1991) 395–409.
[68] F. Garcia-Pichel, R.W. Castenholz, Occurrence of UV-Absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity, Appl. Environ. Microbiol. 59 (1993) 163–169.
[69] M. Ehling-Schulz, W. Bilger, S. Scherer, UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*, J. Bacteriol. 179 (1997) 1940–1945.
[70] M.L. Yallop, B. de Winder, D.M. Paterson, L.J. Stal, Comparative structure, primary production and biogenic stabilization of cohesive and non-cohesive marine sediments inhabited by microphytobenthos, Estuarine Coastal Shelf Sci. 39 (1994) 565–582.
[71] L.J. Stal, Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments, Geomicrobiol. J. 20 (2003) 463–478.
[72] S. Venkatachalam, K. Ranjan, R. Prasanna, B. Ramakrishnan, S. Thapa, A. Kanchan, Diversity and functional traits of culturable microbiome members, including cyanobacteria in the rice phyllosphere, Plant Biol. 18 (2016) 627–637.
[73] M.R. Martinez, H.D. Catling, Algae living on deepwater rice in Bangladesh, Int. Rice Res. Newsletters 3 (3) (1978) 12.
[74] S.A. Kulasooriya, P.A. Roger, W.L. Barraquio, I. Watanabe, Biological Nitrogen Fixation by Epiphytic Microorganisms in Rice Fields, IRRI Research Paper Series 47, 1980, pp. 1–10.
[75] C. Bu, S. Wu, Y. Yang, M. Zheng, B. Neilan, Identification of factors influencing the restoration of cyanobacteria-dominated biological soil crusts, PLoS One 9 (3) (2014) e90049.
[76] J. Belnap, Biological soil crusts in deserts: a short review of their role in soil fertility, stabilization, and water relations, Algol. Stud. 109 (2003) 113–126.
[77] J. Belnap, D.A. Gillette, Disturbance of biological soil crusts: impacts on potential wind erodibility of sandy desert soils in southeastern Utah, Land Degrad. Dev. 8 (1997) 355–362.

[78] C. McKenna-Neuman, C.D. Maxwell, J.W. Boulton, Wind transport of sand surfaces with photoautotropic microoganisms, CATENA 27 (1996) 229–247.
[79] J. Belnap, Potential role of cryptobiotic soil crust in semiarid rangelands, in: S.B. Monsen, S.G. Kitchen (Eds.), Proceedings-Ecology and Management of Annual Rangelands, Intermountain Research Station, Ogden, 1994, pp. 179–185. General Technical Report INT-GTR-313 USDA Forest Service.
[80] R.J. Beymer, J.M. Klopatek, Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands, Arid Soil Res. Rehab. 5 (1991) 187–198.
[81] R.D. Evans, J.R. Ehleringer, A break in the nitrogen cycle in arid lands? Evidence from $\delta^{15}N$ of soils, Oecologia 94 (1993) 314–317.
[82] J. Belnap, Surface disturbances: their role in accelerating desertification, Environ. Monitor. Assess. 37 (1995) 39–57.
[83] J. Belnap, O.L. Lange, Biological Soil Crusts: Structure Function and Management, in: Ecological Studies, vol. 150, Springer-Verlag, Berlin, Heidelberg, 2003.
[84] M.D. Madsen, D.G. Chandler, J. Belnap, Spatial gradients in ecohydrologic properties within a pinyon–juniper ecosystem, Ecohydrology 1 (2008) 349–360.
[85] G.A. Peters, R.E. Toia Jr., H.E. Calvert, B.H. Marsh, Lichens to *Gunnera* – with emphasis on *Azolla*, Plant Soil 90 (1986) 17–34.
[86] J. Belnap, D. Eldridge, Disturbance and recovery of biological soil crusts, in: J. Belnap, O.L. Lange (Eds.), Biological Soil Crusts: Structure, Function, and Management, Springer-Verlag, Berlin, 2001, pp. 363–383.
[87] M. Bhatnagar, L. Parwani, V. Sharma, J. Ganguly, A. Bhatnagar, Exopolymers from *Tolypothrix tenuis* and three *Anabaena* sp. (Cyanobacteriaceae) as novel blood clotting agents for wound management, Carbohyd. Polym. 99 (2014) 692–699.
[88] M.A. Bowker, S.C. Reed, J. Belnap, S.L. Phillips, Temporal variation in community composition, pigmentation, and Fv/Fm of desert cyanobacterial soil crusts, Microb. Ecol. 43 (2002) 13–25.
[89] P.S. Murthy, R. Venkatesan, Industrial biofilms and their control, in: H.C. Flemming, P.S. Murthy, R. Venkatesan, K.C. Cooksey (Eds.), Marine and Industrial Biofouling, Springer, Heidelberg, 2009, pp. 65–101.
[90] T.E. Cloete, Biofouling control in industrial water systems: what we know and what we need to know, Mater. Corros. 54 (7) (2003) 520–526.
[91] S. Fazi, I. Pizzetti, F. Di Pippo, Initial Phase of Biofouling: The Microbial Biofilm Formation, Energia Ambiente e Innovazione, Rome, Italy, 2014.
[92] H.C. Flemming, Why microorganisms live in biofilms and the problem of biofouling, in: H.C. Flemming, P. Sriyutha Murthy, R. Venkatesan, K. Cooksey (Eds.), Marine and Industrial Biofouling, Springer Verlag, Berlin, Heidelberg, 2008, pp. 3–12.
[93] L.V. Evans, Marine biofouling, in: C.A. Lembi, J. Robert Waaland (Eds.), Algae and Human Affairs, Cambridge University Press, USA, 1988, pp. 433–453.
[94] T. Okuda, M. Neushul, Sedimentation studies on red algal spores, J. Phycol. 17 (1981) 113–118.
[95] C. Holmstrom, S. Kjelleberg, Bacterial interactions with marine fouling organisms, in: Biofilms: Recent Advances in Their Study and Control, Harwood Academic Publisher, Amsterdam, 2000, pp. 101–115.
[96] P.D. Redekar, A.B. Wagh, Studies on fouling diatoms from the Zuari estuary Goa (west coast of India), Seaweed Res. Util. 22 (2000) 113–119.
[97] K.E. Cooksey, B. Cooksey, Adhesion of fouling diatoms to surfaces: some biochemistry, in: L.V. Evans, K.D. Hoagland (Eds.), Algal Biofouling, Elsevier, Amsterdam, 1986, pp. 41–53.
[98] S.L. Loeb, Algal biofouling of oligotrophy lake Tahoe: causal factors affecting production, in: L.V. Evans, K.D. Hoagland (Eds.), Algal Biofouling, Elsevier, Amsterdam, 1986, pp. 159–173.
[99] L.N. Mueller, J.F. De Brouwer, J.S. Almeida, L.J. Stal, J.B. Xavier, Analysis of a marine phototrophic biofilm by confocal laser scanning microscopy using the new image quantification software PHLIP, BMC Ecol. 6 (1) (2006) 1.
[100] P. Li, S.E. Harding, Z. Liu, Cyanobacterial exopolysaccharides: their nature and potential biotechnological applications, Biotechnol. Genet. Eng. Rev. 18 (1) (2001) 375–404.
[101] H.C. Flemming, J. Wingender, T. Griegbe, C. Mayer, Physico-chemical properties of biofilms, in: L.V. Evans (Ed.), Biofilms: Recent Advances in Their Study and Control, Harwood Academic Publishers, Amsterdam, 2000, pp. 19–34.
[102] D. Paul, G. Pandey, J. Pandey, R.K. Jain, Accessing microbial diversity for bioremediation and environmental restoration, Trends Biotechnol. 23 (2005) 135–142.

[103] G. Pandey, R.K. Jain, Bacterial chemotaxis toward environmental pollutants: role in bioremediation, Appl. Environ. Microbiol. 68 (2002) 5789–5795.
[104] A.W. Decho, Microbial biofilms in intertidal systems: an overview, Cont. Shelf Res. 20 (2000) 1257–1273.
[105] J.G. Kuenen, B.B. Jongensen, N.P. Revsbech, Oxygen MICROPROFILES of trickling filter biofilms, Water Res. 20 (1986) 1589–1598.
[106] S.K. Liehr, H.J. Chen, S.H. Lin, Metals removal by algal biofilms, Water Sci. Technol. 30 (1994) 59–68.
[107] H.J. Gijzen, T.J.M. Schoemakers, C.G.M. Caerteling, G.D. Vogels, Anaerobic degradation of paper mill sludge in two phase digester containing rumen micro-organisms and colonized polyurethane foam, Biotechnol. Lett. 10 (1988) 61–66.
[108] P.V. Bhaskar, N.B. Bhosle, Bacterial extracellular polymeric substance (EPS): a carrier of heavy metals in the marine foodchain, Environ. Int. 32 (2006) 191–198.
[109] Y. Liu, M.C. Lam, H.H. Fang, Adsorption of heavy metals by EPS of activated sludge, Water Sci. Technol. 43 (2001) 59–66.
[110] D.L. Parker, J.E. Mihalick, J.L. Plude, M.J. Plude, T.P. Clark, L. Egan, J.J. Flom, L.C. Rai, H.D. Kumar, Sorption of metals by extracellular polymers from the cyanobacterium *Microcystis aeruginosa f.flos-aquae* strain C3-40, J. Appl. Phycol. 12 (2000) 219–224.
[111] C. Fortin, F.H. Denison, J. Garnier-Laplace, Metal-phytoplankton interactions: modeling the effect of competing ions (H^+, Ca^{2+}, and Mg^{2+}) on uranium uptake, Environ. Toxicol. Chem. 26 (2007) 242–248.
[112] A. Leusch, Z.R. Holan, B. Volesky, Biosorption of heavy metals (Cd, Cu, Ni, Pb, Zn) by chemically-reinforced biomass of marine algae, J. Chem. Technol. Biotechnol. 2 (1995) 279–288.
[113] K. Venkateswarlu, Pesticide interactions with cyanobacteria in soil and culture, in: J.-M. Bollag, D. Stotzky (Eds.), Soil Biochemistry, vol. 8, Marcel Dekker Inc., New York, 1993, pp. 137–179.
[114] B. Ramakrishnan, M. Megharaj, K. Venkateswarlu, N. Sethunathan, R. Naidu, Mixtures of environmental pollutants: effects on microorganisms and their activities in soils, Rev. Environ. Contam. Toxicol. 211 (2011) 63–120.
[115] M. Megharaj, K. Venkateswarlu, A.S. Rao, Metabolism of monocrotophos and quinalphos by algae isolated from soil, Bull. Environ. Contam. Toxicol. 39 (1987) 251–256.
[116] W.M. Ibrahim, M.A. Karam, R.M. El-Shahat, A.A. Adway, Biodegradation and utilization of organophosphorus pesticide malathion by cyanobacteria, BioMed. Res. Inter. 2014 (2014).
[117] W. Chungjatupornchai, S. Fa-Aroonsawat, Biodegradation of organophosphate pesticide using recombinant cyanobacteria with surface and intracellular expressed organophosphorus hydrolase, J. Microbiol. Biotechnol. 18 (5) (2008) 946–951.
[118] N. Sethunathan, T. Yoshida, *Flavobacterium* sp. that degrades diazinon and parathion, Can. J. Microbiol. 19 (1973) 873–875.
[119] R.M. Abed, A. Al-Thukair, D. de Beer, Bacterial diversity of a cyanobacterial mat degrading petroleum compounds at elevated salinities and temperatures, FEMS Microbiol. Ecol. 57 (2006) 290–301.
[120] J. Van Bleijswijk, G. Muyzer, Genetic diversity of oxygenic phototrophs in microbial mats exposed to different levels of oil pollution, Ophelia 58 (2004) 157–164.
[121] E.A. Edwards, L.E. Wills, M. Reinhard, D. Grbic-Galic, Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions, Appl. Environ. Microbiol. 58 (1992) 794–800.
[122] M. Benthien, A. Wieland, T. García de Oteyza, J.O. Grimalt, M. Kuhl, Oil-contamination effects on a hypersaline microbial mat community (Camargue, France) as studied with microsensors and geochemical analysis, Ophelia 58 (2004) 135–150.
[123] Y. Cohen, Bioremediation of oil by marine microbial mats, Inter. Microbiol. 5 (2002) 189–193.
[124] R.B. Coffin, L.A. Cifuentes, P.H. Pritchard, Assimilation of oil derived carbon and remedial nitrogen applications by intertidal food chains on a contaminated beach in Prince William Sound, Alaska, Marine Environ. Res. 44 (1997) 27–39.
[125] S.S. Radwan, R.H. Al-Hasan, S. Salamah, S. Al-Dabbous, Bioremediation of oily sea water by bacteria immobilized in biofilms coating macroalgae, Int. Biodeter. Biodegr. 50 (2002) 55–59.
[126] S. Nayar, B.P.L. Goh, L.M. Chou, The impact of petroleum hydrocarbons (diesel) on periphyton in an impacted tropical estuary based on in situ microcosms, J. Exp. Marine Biol. Ecol. 302 (2004) 213–232.
[127] A. Demirbas, Progress and recent trends in biodiesel fuels, Energy Convers. Manage. 50 (2009) 14–34.

[128] M.R. Tredici, Photobiology of microalgae mass cultures: understanding the tools for the next green revolution, Biofuels 1 (2010) 143–162.
[129] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306.
[130] P.J. Schnurr, G.S. Espie, D.G. Allen, Algal biofilm growth and the potential to stimulate lipid accumulation through nutrient starvation, Biores. Technol. 136 (2013) 337–344.
[131] P. Chaiprasert, Biogas production from agricultural wastes in Thailand, J. Sus. Energ. Environ (Special Issue) (2011) 63–65.
[132] D.E. Brune, T.J. Lundquist, J.R. Benemann, Microalgal biomass for greenhouse gas reductions: potential for replacement of fossil fuels and animal feeds, J. Environ. Eng. 135 (2009) 1136–1144.
[133] N. Abdel-Raouf, A.A. Al-Homaidan, I.B.M. Ibraheem, Microalgae and waste water treatment, Saudi J. Biol. Sci. 19 (2012) 257–275.
[134] A.F. Clarens, E.P. Resurreccion, M.A. White, L.M. Colosi, Environmental life cycle comparison of algae to other bioenergy feedstocks, Environ. Sci. Technol. 44 (2010) 1813–1819.
[135] I. Rafiqul, C. Weber, B. Lehmann, A. Voss, Energy efficiency improvements in ammonia production – perspectives and uncertainties, Energy 30 (2005) 2487–2504.
[136] S. Kim, B.E. Dale, Environmental aspects of ethanol derived from no-tilled corn grain: nonrenewable energy consumption and greenhouse gas emissions, Biomass Bioenergy 28 (2005) 475–489.
[137] P. Lindberg, P. Lindblad, L. Cournac, Gas exchange in the filamentous cyanobacterium *Nostoc punctiforme* strain ATCC 29133 and its hydrogenase-deficient mutant strain NHM5, Appl. Environ. Microbiol. 70 (2004) 2137–2145.
[138] A.A. Tsygankov, V.B. Borodin, K.K. Rao, D.O. Hall, H_2 photoproduction by batch culture of *Anabaena variabilis* ATCC 29413 and its mutant PK84 in a photobioreactor, Biotechnol. Bioengineer. 64 (1999) 709–715.

Index

'*Note:* Page numbers followed by "f" indicate figures, "t" indicate tables and "b" indicates boxes.'

B

D

H

I

P