Developments in Applied Phycology 7

Bhaskar Singh
Kuldeep Bauddh
Faizal Bux *Editors*

Algae and Environmental Sustainability

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Bhaskar Singh • Kuldeep Bauddh • Faizal Bux
Editors

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Editors
Bhaskar Singh
Centre for Environmental Sciences
Central University of Jharkhand
Ranchi, Jharkhand, India

Kuldeep Bauddh
Centre for Environmental Sciences
Central University of Jharkhand
Ranchi, Jharkhand, India

Faizal Bux
Institute for Water and Wastewater Technology
Durban University of Technology
Durban, South Africa

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Dedicated to the memories of my late grandfather
Shri Sukhari Garain

Bhaskar Singh

Dedicated to the memories of my late grandfather
Shri Jagannath Prasad

Kuldeep Bauddh

Foreword

The benefits of using algae for biofuels are multifaceted and include the following:

1. Noncompetitive with food crops and land use (as long as ponds are not built on agricultural land).
2. By most, but not all, estimates, prospective/proven oil content from algae biomass is orders of magnitude higher than from other feedstocks like corn, sugar cane, Jatropha, etc.
3. Algae need CO_2 to photosynthesize and can be used to sequester CO_2 from industrial sources of flue and flaring gas. Algae-based fuels are thus C-neutral or even C-positive (i.e., more C-capturing than releasing).
4. Algae can be used to remediate high-nutrient water sources such as sewage treatment plants, agricultural runoff, and landfill leachate basins. This linkage not only cleans up the water but may also lower the cost for nutrients needed to culture algae.
5. End products can be biofuel along with other higher value products such as feed (protein), cosmetics, pharmaceuticals, and health-related products (e.g., omega-3 fatty acids).
6. Different species of algae can be grown in polluted, saline, brackish, and freshwater.
7. Algae can be used as one component of an aquaculture-centered system where fish like Tilapia are grown for food in a recirculating and hydroponic system.
8. Algae do not contribute to acid deposition (i.e., no SO_2 emissions).
9. Some species of blue-green algae utilize N_2 to produce NO_3 (a process called "biological nitrogen fixation"), thus sequestering atmospheric nitrogen and eliminating or at least lessening the need for nitrogen fertilizers. This can help reduce the need for nitrogen fertilizers and, more importantly, help confront the expanding "dead zone" in the Gulf of Mexico.

Bhaskar Singh, Kuldeep Bauddh, and Faizal Bux have successfully compiled a group of 14 scientific papers to discuss most of these benefits. We can dream together that one day (hopefully soon) we will link algae growing in sewage treatment ponds with carbon dioxide emissions from local power plants, cement factories, and oil refineries so that CO_2 and nutrients are reduced while sustainable energy is competitively produced.

Oral Roberts University
Tulsa, OK, USA
July 5, 2015

John Korstad

Preface

Algae have long been an area of interest due to their usefulness as food (for protein, dietary fiber, mineral, antioxidants), other valuable products, and, more recently, as a potential feedstock for biofuels. Because algae grow much faster than terrestrial plants, there is immense potential in harnessing it as a raw material in energy generation. However, there are a few bottlenecks in the commercialization of algal-based biofuels, and thus the present mode of biofuels synthesis is expensive. Growing algae for additional purposes such as treatment of wastewater (phycoremediation) and sequestration of carbon may make the higher cost worthwhile. Through phycoremediation, various pollutants including toxic heavy metals can be removed. *Algae and Environmental Sustainability* aims to address recent advancements in the role of algae in generation of biofuels and their diverse role in enhancing environmental sustainability.

Chapters in the book include discussion on algae as a promising future feedstock for biofuels, phycoremediation potential of algae, role of algal biofilms in treatment of wastewater, mathematical modeling in predicting performance, scaling-up for production of algae biomass, culturing algae for wastewater treatment, remediation of sewage water, role of anaerobic digestion for production of biogas with a biorefinery approach, integration of several technologies in a biorefinery approach that maximizes the benefits and makes the overall process of biofuels production economical, utilization of waste products obtained from biomass that reduces the pollution load in the environment, biohydrogen production and its economical assessment and major bottlenecks, techniques involved in the characterization of bio-oil and biodiesel, remediation of dyes from aquatic environments through biosorption using algae, a case study that reports algal bioremediation and decolorization of biomethanated distillery effluent, application of genetic engineering in the enhancement of algal lipid production and its present scenario and future aspects, phycoremediation of emerging contaminants that find their way in the environment through personal care products, pesticides and endocrine disruptors such as hormonally active agents, potential of algae in carbon dioxide, and role of remote sensing in the monitoring of microalgae and life cycle analysis of algal biofuels to assess their environmental, social, and economic perspectives.

Ranchi, India — Bhaskar Singh
Kuldeep Bauddh
Durban, South Africa — Faizal Bux

Acknowledgment

I acknowledge and thank all those colleagues and professors who so willingly gave their time and expertise contents for the chapters that enriched the edition and helped in launching the book. I am especially grateful to all the people who provided their valuable suggestion and coordination throughout the editing of the book.

I would especially like to thank Prof. John Korstad for doing the plagiarism check of all the fourteen chapters, finding inconsistencies and error and providing his insights and able direction. We are grateful to him for conversation, suggestion, and assistance in all stages of the preparation of the book. I am also grateful to my coeditors, Dr. Kuldeep Bauddh and Prof. Faizal Bux for their support and cooperation throughout in editing the book.

I would like to thank the whole team of Springer for showing patience during the preparation of the book. Without the excellent work of the people, administration, and production team at Springer, it would not have been possible to produce a book of this scope.

Finally I would like to acknowledge, with gratitude, the support and love of my family – my parents (Mrs. Usha Kiran and Mr. S.N. Singh), my brother's family (Mrs. Smriti and Mr. Pawan Kumar), my sister's family (Mrs. Anupama Singh, Mr. S.K. Kashyap, Raj Nandini and Gaurav), and my loving wife (Mrs. Ragini). They all kept me going, and their patience during preparation of book was immeasurable and their encouragement essential.

(Bhaskar Singh)

It was really a challenging assignment for me to complete this book, but with the cooperation, contribution, and patience of potential authors, this dream became a reality. I would like to express my deep sense of gratitude to Prof. Rana Pratap Singh and Dr. Narendra Kumar, Dept. of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, and Dr. Manoj Kumar, Head, Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, for their appreciable direction, motive, and precious advice and their interest which is responsible for the accomplishment of this target. I would like to thank Prof. John Korstad, USA, for his critical and helpful comments on the chapters of the book.

Words are not enough to express my grateful appreciation for my parents (Mr. Suresh Baboo and Mrs. Shyama Devi), elder sister (Mrs. Kalpana Devi), brother-in-law (Mr. Ajay Hans), younger brother (Jagdeep), my niece (Ananya, Komal, and Akshay), and all my relatives. Their dream and ambition made me strong to complete the target. Their endless inspiration, support, affection, and encouragement have made it possible.

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(Kuldeep Bauddh)

Contents

Editors and Contributors

About the Editors

Dr. Bhaskar Singh received his Ph.D. from the Indian Institute of Technology (BHU), Varanasi, UP, India, in 2010. Bhaskar Singh also holds an M. Phil. from Pondicherry (Central) University, India, with a gold medal (2006). He is currently serving as an Assistant Professor at the Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India. Dr. Singh is currently engaged in teaching in the thrust areas of environmental sciences like environmental earth science, natural resources, environmental chemistry, environmental pollution monitoring and control technologies, etc. He is reviewer of several international journals, viz., *Fuel*, *Renewable Energy*, *Renewable & Sustainable Energy Reviews* (Elsevier Publications). Dr. Singh is serving as treasurer of the Ranchi Chapter of the "Professor HS Srivastava Foundation for Science and Society." His current interests lie in the application of algal biomass for biodiesel synthesis, development of heterogeneous and green catalysts, and corrosion aspects of biodiesel fuel. He has published more than 30 research and review papers in peer-reviewed and high-impact international journals and 4 book chapters by international publishers.

Dr. Kuldeep Bauddh received his Ph.D. in environmental science from the Babasaheb Bhimrao Ambedkar University, Lucknow, India, in 2014. He is currently working as an Assistant Professor at the Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India. Dr. Bauddh is currently engaged in teaching the thrust areas of environmental sciences like environmental pollution and management, environmental chemistry, environmental toxicology, soil science, hazardous waste management, etc. He is also working as an associate editor of the journal *Climate Change and Environmental Sustainability* and also co-editor of the magazine *Kahaar*. Dr. Bauddh is serving as secretary of the Ranchi Chapter of the "Professor HS Srivastava Foundation for Science and Society," joint secretary of the "Society for Science of Climate Change and Sustainable Environment," and as executive member of the "Professor HS Srivastava Foundation for Science and Society." His main research areas are phytoremediation, slow release fertilizers, and soil reclamation. He has published more than 25 research papers in national and international journals, 3 book chapters, and 6 popular articles in various magazines.

Professor Faizal Bux is the director of the Institute for Water and Wastewater Technology. As an NRF rated researcher, he has more than 25 years' service at higher education institutes and has received numerous institutional awards including University Top Senior researcher award and NRF award. Prof. Bux's primary research focus includes wastewater treatment, environmental biotechnology, algal biotechnology, and biofuels production from waste substrates. He has supervised over 50 master's and doctoral students (including current), and 15 postdoctoral fellows served their tenure under his guidance. He served as editor for the following ISI journals: *CLEAN – Soil, Air, Water*; *Journal of Environmental Science and Health, Part A*; *Biofuels Research Journal*; and *Water Science and Technology*. He has served as a reviewer for over 45 international journals and contributed to more than 85 ISI journal articles, 9 book

chapters, and over 100 conference presentations (national and international) and is editor/coeditor of 4 books. He has acted as project leader for 10 Water Research Commission projects and enjoys an extensive collaboration base with experts both nationally and globally. Prof. Bux has served as an invited member of the management committee of the International Water Association (IWA) specialist group Microbial Ecology and Water Engineering (MEWE) and serves on the executive committee of IWA-SA. He is a fellow of the Water Institute of Southern Africa. He served on the scientific program committee/advisory board/chair/invited speaker of various national and international conferences and is member of many professional bodies nationally and internationally. He serves as scientific advisor for many NGO's both in South Africa and internationally.

Contributors

Faiz Ahmad Ansari Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

N. Carreras Arroyo Environment Department, Ciemat, Madrid, Spain

Kuldeep Bauddh Center for Environmental Sciences, Central University of Jharkhand, Ranchi, Jharkhand, India

B. Bharathiraja Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai, India

Sudarshan P. Bhat Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

Virthie Bhola Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Faizal Bux Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

M. Chakravarthy Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai, India

Poulomi Chakravarty Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India

Sonal Dixit Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India

C. González-Fernández Biotechnological Processes for Energy Production Unit – IMDEA Energy, Madrid, Spain

Abhishek Guldhe Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Sanjay Kumar Gupta Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Environmental Engineering, Department of Civil EngineeringIndian Institute of Technology Delhi, Delhi, India

Saurabh Kumar Gupta Centre for Land Resource Management, Central University of Jharkhand, Ranchi, India

J. Jayamuthunagai Centre for Biotechnology, Anna University, Chennai, India

N.V. Joshi Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

Maureen Kesaano Department of Biological Engineering, Utah State University, Logan, UT, USA

John Korstad Department of Biology and Chemistry, Oral Roberts University, Tulsa, OK, USA

Richa Kothari Bioenergy and Wastewater Treatment Laboratory, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India

Virendra Kumar Bioenergy and Wastewater Treatment Laboratory, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India

Santhosh Kumar Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa

Dipesh Kumar Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India

R. Ranjith Kumar Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai, India

Manoj Kumar Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India

Sheena Kumari Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Binita Kumari Centre for Land Resource Management, Central University of Jharkhand, Ranchi, India

Durga Madhab Mahapatra Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

Centre for Sustainable Technologies [CST], Indian Institute of Science, Bangalore, India

Anushree Malik Rural Development Technology, Indian Institute of Technology Delhi, New Delhi, India

Pradeep Kumar Mishra Department of Chemical Engineering & Technology, IIT (BHU) Varanasi, Varanasi, India

Arya Pandey Bioenergy and Wastewater Treatment Laboratory, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India

R. Praveenkumar Department of biotechnology, Arunai Engineering College, Tiruvannamalai, India

T.V. Ramachandra Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

Centre for Sustainable Technologies [CST], Indian Institute of Science, Bangalore, India

Centre for Infrastructure, Sustainable Transport and Urban Planning [CiSTUP], Indian Institute of Science, Bangalore, India

J.L. Ramos-Suárez Procycla SL, Manresa, Spain

Ismail Rawat Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Laxmi Kant Sharma Centre for Land Resource Management, Central University of Jharkhand, Ranchi, India

Amritanshu Shriwastav Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Sushil Kumar Shukla Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, India

Ronald C. Sims Department of Biological Engineering, Utah State University, Logan, UT, USA

Poonam Singh Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa

Bhaskar Singh Centre for Environmental Sciences, Central University of Jharkhand, Ranchi, Jharkhand, India

D.P. Singh Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India

Terence Smith Department of Biological Engineering, Utah State University, Logan, UT, USA

V.V. Tyagi School of Energy Management, Shree Mata Vaishno Devi University, Katra, India

Jonathan Wood Department of Biological Engineering, Utah State University, Logan, UT, USA

D. Yogendran Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai, India

Algae: Promising Future Feedstock for Biofuels

1

B. Bharathiraja, J. Jayamuthunagai, M. Chakravarthy, R. Ranjith Kumar, D. Yogendran, and R. Praveenkumar

1 Introduction

Limited availability and critical dependency of global economy on fossil fuel has been the main reason for exploration of alternate fuel resources in order to improve energy security. Food vs fuel debate associated with first generation of biofuels has resulted in the development of second generation of biofuels derived from nonedible biomass feedstocks. First-generation biofuels such as corn bioethanol and soybean biodiesel were brought into reality as a result of early efforts to produce alternate transportation fuels from renewable resources. Initial efforts were mostly concentrating on greenhouse gas mitigation by reducing the carbon dioxide level in atmosphere that has risen tremendously in the past two decades. However, the technological limitations and less production capacities from limited biomass resource, uncertain economic impacts, low net energy balance, food vs fuel dispute of future, etc., have forced the need to shift the technology target to the next generation. Increased net energy balance was found in second-generation biofuels but it was insignificant. Advantages include use of nonfood crops like grass straws, paddy husk, straws, switch grass, and other herbaceous biomass as feedstock for ethanol and other biofuels. Ethanol from above sources had higher net energy than corn ethanol. Second-generation biofuels offer no significant advantage in terms of the need to use agricultural components such as land, water supply, fertilizers, etc. Also, complex fuel processing of second-generation feedstock has resulted in reduced yield and increased process expenditure.

Third-generation biofuel feedstock is selected carefully to avoid the challenges in production, harvesting, and processing of terrestrial biomass. Algal feedstock has proved to be a prominent and sustainable source of renewable fuel. Huge research has been carried out over algal biomass to evaluate its potential as alternative biofuel feedstock, thereby eliminating the concerns about food vs fuel debate. This has involved researchers to develop technologies for production and processing of biodiesel and bioethanol as major fuels from algae. This increased attention toward aquatic biomass has been a great gear shift toward several novel innovations in the field of green fuel production. Continuing efforts since the late 1970s to bring algae as a recognized source for biofuel has still been intensely researched by coupling the normal cultivation techniques with other existing systems such as power plant, textile and sugarcane effluents, sewage water treatment plants, etc. (Fig. 1.1).

The white color in the figure indicates areas closer to the shore and has at least 5 mg m^{-3} chlorophyll concentration (Florentinus et al. 2008).

Global production of micro- and macroalgae is more than 10,000 tons/year as per current records. Researchers have been testing algal population for the extraction of pigments and active pharmaceutical ingredients (APIs). Microalgae have been the preferred research material for assessing biofuel feedstock potential; however, few species of macroalgae have also been exploited recently.

B. Bharathiraja (✉) • M. Chakravarthy • R.R. Kumar
D. Yogendran
Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai 600 062, India
e-mail: btrbio@gmail.com; chakra27031994@gmail.com; ranjithlegend11@gmail.com; yogi757792@gmail.com

J. Jayamuthunagai
Centre for Biotechnology, Anna University, Chennai 600025, India
e-mail: jjnagai81@gmail.com

R. Praveenkumar
Department of biotechnology, Arunai Engineering College, Tiruvannamalai 606603, India
e-mail: praveenramanujam@gmail.com

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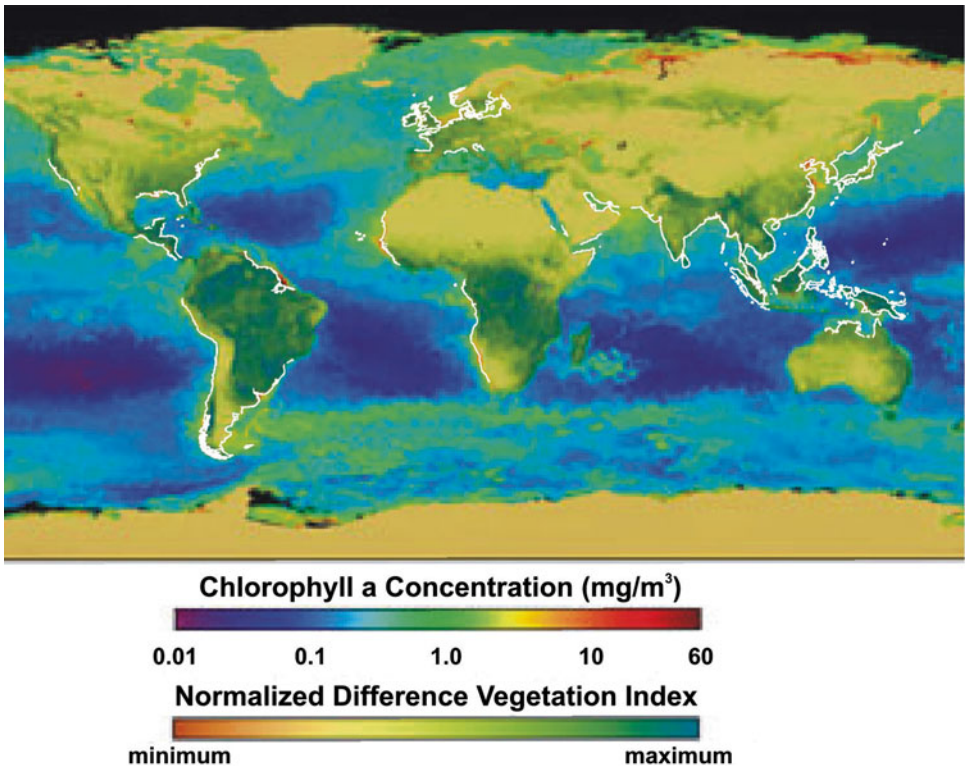

Fig. 1.1 Global chlorophyll concentration and NDVI distribution

2 Micro- and Macroalgal Biomass as Biofuel Feedstock

Microalgae are microscopic plants, growing in a diversity of habitats with varied structural and functional characters. Microalgae are known for their high lipid accumulation capacity (more than 60 %). Selected strains under engineered conditions show a lot more improved performance in growth and lipid production, thereby qualifying them as potential biodiesel feedstock. According to the Aquatic Species Program, a national research activity initiated in 1978 by the Department of Energy (DOE), USA, out of over 3000 collected strains of microalgae, more than 300 were found as promising oil feedstock and showed satisfactory biofuel feedstock traits (Sheehan et al. 1998). Though the project was dropped after 18 years, extended knowledge about algal species was established. Feasibility of selected algal species was tested, and technologies for cultivation and scale-up were established to a greater extent. Methods of algal lipid and cellulose analysis have been established partially during this attempt to collect and study all the microalgal strains. Genetic improvement of wild strains by engineering the genes of lipid metabolism and accumulation proved a greater way out for improved efficiency of algae as biodiesel feedstock. These activities continually inspired the researchers to put further technological advancements in the field of bioreactor engineering, downstream processing, and fuel product processing to push the realization progress toward algal biofuels at a bit faster rate. The advantages of using microalgae as fuel feedstock include:

- Microalgae are highly efficient photosynthetic organisms that have been found to tolerate or adapt in stressful conditions. Multiple pathways possessed by microalgae are shiftable by altering their interdependency of nutrient and other growth factors to facilitate growth and lipid accumulation.
- Promising oil content (>50 wt% triacylglycerides) per unit weight of dry biomass (Miao and Wu 2006).
- Bioreactors for their growth can be vertically or horizontally built to utilize the maximum space possible for hiking the annual biomass productivity per unit area.
- Less use of land and freshwater resource. Unlike terrestrial crops, microalgae are not dependent on cultivable lands since they are not grown directly in the soil like terrestrial biomass. Any wasteland available can be exploited for cultivation purpose and wastewater resource such as industrial effluents, cattle waste effluents, sewage water, and sugar industry effluents that can be fed into microalgal production system to put process expenditure in control.
- Integration technologies such as integration with existing power plants (CO_2 mitigation), sugar industry (carbon-rich sugar recycling as nutrient), textile effluent (pH reduction and accumulation of dye), cattle effluent (removal of organic loading), sewage treatment (water

treatment and recycle), etc., are being explored as a new scope of research.
- Higher CO_2 mitigation capacity of algae resulting in reduction of atmospheric greenhouse gas, thereby attempting to resume environmental stability.

There are three main metabolic pathways present in microalgae – autotrophic, heterotrophic, and mixotrophic kind of metabolisms. Autotrophic algae in the presence of light, CO_2, simple inorganic media, and suitable temperature store significant amount of starch and lipid. Peak growth rate of algae will be as high as 0.2 g dry wt/l/day (Gouveia and Oliveira 2009). Lipid accumulation can be initiated in microalgae by imposing nutrient deficiency like N, P, and K deficiency but also reduces growth rates. In few cases such as *Chlorella* sp., the system works as photoheterotrophic (mixotrophic) in the presence of light and organic carbons whose molecular mechanisms are yet to be determined and defined for lipid accumulation. In the recent past, search over heterotrophic microalgae-based lipid production has been increased considerably than the autotrophic systems to eliminate its light dependency barrier for large-scale production (Miao and Wu 2006).

Macroalgae or seaweeds belong to lower plants, those supposed to have roots, stems, and leaves, but macroalgae do not possess such differentiation. Macroalgae usually have leaflike thallus that floats in water unlike microalgal suspensions. Macroalgae have gas-filled structures to provide buoyancy. Subdivided groups fall into the category of green (Chlorophyceae), red (Rhodophyceae), or brown (Phaeophyceae) algae. Naturally, they grow on seabeds, rocky shores, etc., and are found as multilayered, perennial vegetations growing photosynthetically. These fast-growing multicellular organisms can grow in fresh or salt water and can reach up to 60 m in length (McHugh 2003). We intend to provide a brief summary of the advantages of macroalgae as fuel feedstock:

- Biomass productivity is ten times higher than planktonic species and much higher than terrestrial biomass. Values for maximum productivity can be as high as 1.8 kg C/m^2/y.
- The maximum chlorophyll content is 3 g/m^2 for planktonic species, whereas algal biomass has about 10 kg/m^2, thereby accelerating photosynthetic growth (Luning and Pang 2003).
- Direct uptake of HCO_3 rather than CO_2 for their growth describes its high photosynthetic ability. Hence, on the other hand, carbon mitigation is done at a faster rate to restore balance over the environment (Gao and McKinley 1994).
- Expansive cultivation of exclusive species in seawaters offers promising way for mass algal cultivation as fuel feedstock. The degree of sustainability expected with this kind of cultivation scheme is high and greatly varies with normal design of biomass cultivation (Lobban et al. 1985).
- General requirements of growth such as nutrients, salinity, temperature, light, depth, and ocean currents are readily available as natural resource, and hence several species having a range of specific requirements for their environment appear to be suited for large-scale cultivation.
- Commercial cultivation of seaweeds has long history revealing promising outcomes of about 200 species worldwide that includes *Laminaria*-, *Undaria*-, *Porphyra*-, *Kappaphycus*-, *Gracilaria*-, *Enteromorpha*-, and *Ulva*-like species. However, most of the algae are likely to be tested their efficiency for fuel traits.

The experience of global macroalgal production has left the current state with established cultivation techniques. Of all the macroalgal species reported so far, very few are cultivated for commercial uses (Critchley et al. 1998). Cultivation of seaweeds, growth, and biomass intensification depends on various factors such as nutrient composition, pH, temperature, climate, salinity, etc. The USA, Canada, and European countries such as France, Germany, and the Netherlands are attempting to establish large-scale seaweed cultivation. *Laminaria japonica*, *Undaria pinnatifida*, *Monostroma* sp., *Enteromorpha* sp., *Porphyra* sp., *Eucheuma* sp., *Kappaphycus*, *Gracilaria*, etc., are massively cultivated species (Luning and Pang 2003). *Enteromorpha*, *Ulva*, *Sargassum*, *Gracilaria*, etc., can be cultured in artificial systems by pumping seawater in the pond-like aquatic farms since they are smaller in size. Large kelps are difficult to grow in artificial environment because of their monstrous size which needs enormous surface and higher depths. Potential cultivation of seaweeds coupled with offshore wind farm has already been studied to economize the process (Stanley et al. 2008). Macroalgal biomass harvest may be possible by wild harvest or artificial cultivation in ponds. Possibly, the environmental impacts of mass cultivation of macroalgae may be reduced by coupling it with compatible aquaculture systems (Hughes et al. 2013).

3 Algal Biomass Cultivation

Traditional open pond cultivation of autotrophic cultures is done with shallow raceways (0.15–0.45 m depth) constructed using concrete plastic or silpauline sheets supported by frames. Lower capital for pond construction is greater advantage than photobioreactors (PBRs) (Benemann and Oswald 1996). Paddle wheels or water jets are used to provide mixing. Lower gas transfer with liquid suspension limits biomass productivity. No proper control over temperature, light,

and water loss are limiting factors concerned with quality assurance of biomass. Difficulty in maintaining monocultures is faced due to formation of exotic species microenvironments resulting in contamination of entire pond. pH control can have an upper hand in maintaining algal monocultures to a greater degree in some cases like spirulina. Less process expenditure and easy configuration of cultivation system have made open pond design suitable for algal biofuel production. PBRs can be defined as engineered bioreactors that exist in closed configuration designed to maintain monocultures for higher biomass yield and quality product outcomes. Growing algae in PBR allows having control over all the growth parameters, thus enabling to measure the influence of every parameter on growth and lipid accumulation (Brennan and Owende 2010). Reduced contamination, higher culture densities, lesser water loss, and enhanced control of growth parameters are the advantages concerned with PBRs (Pulz 2001). However, the problems associated with PBR scalability such as fouling, pH gradients, dissolved gases, oxygen buildup, etc., are the challenges to be considered and addressed immediately during commercialization. Advancements in photobioreactor engineering, attempts to succeed in integrated cultivation, equipping existing technologies with quality-ensuring initiatives such as covering ponds with transparent sheets of plastics, glass, etc., can contribute to economic feasibility of algal biomass cultivation for fuel production (Fig. 1.2).

For macroalgal biomass production, submerged cultivation is advised. For offshore culture, sandy clay bottom is made so as to reduce organic load in artificially constructed ponds where cultures are made to grow submerged in seawater. Water should be 0.3–0.5 m deep with specific gravity around 1.010. Optimal growth occurs at alkaline pH (7.0–8.0) and temperature between 15 and 30 °C. Pools, crab ponds, bays, and straights are chosen as algal cultivation sites. Spores are naturally released from the existing cultures, and, on favorable conditions, germination occurs. Shells of oysters and other mollusks can be used to act as cheap substrates for attachment. Culture techniques should be designed as per nature of the seaweed growth. For suspension culture, rope hangs can be chosen, whereas seaweeds requiring bottom supports, off-bottom setups, long lines, etc., can be chosen. Kelp cultivation is usually done on artificial floating rafts. These species have low wet to dry

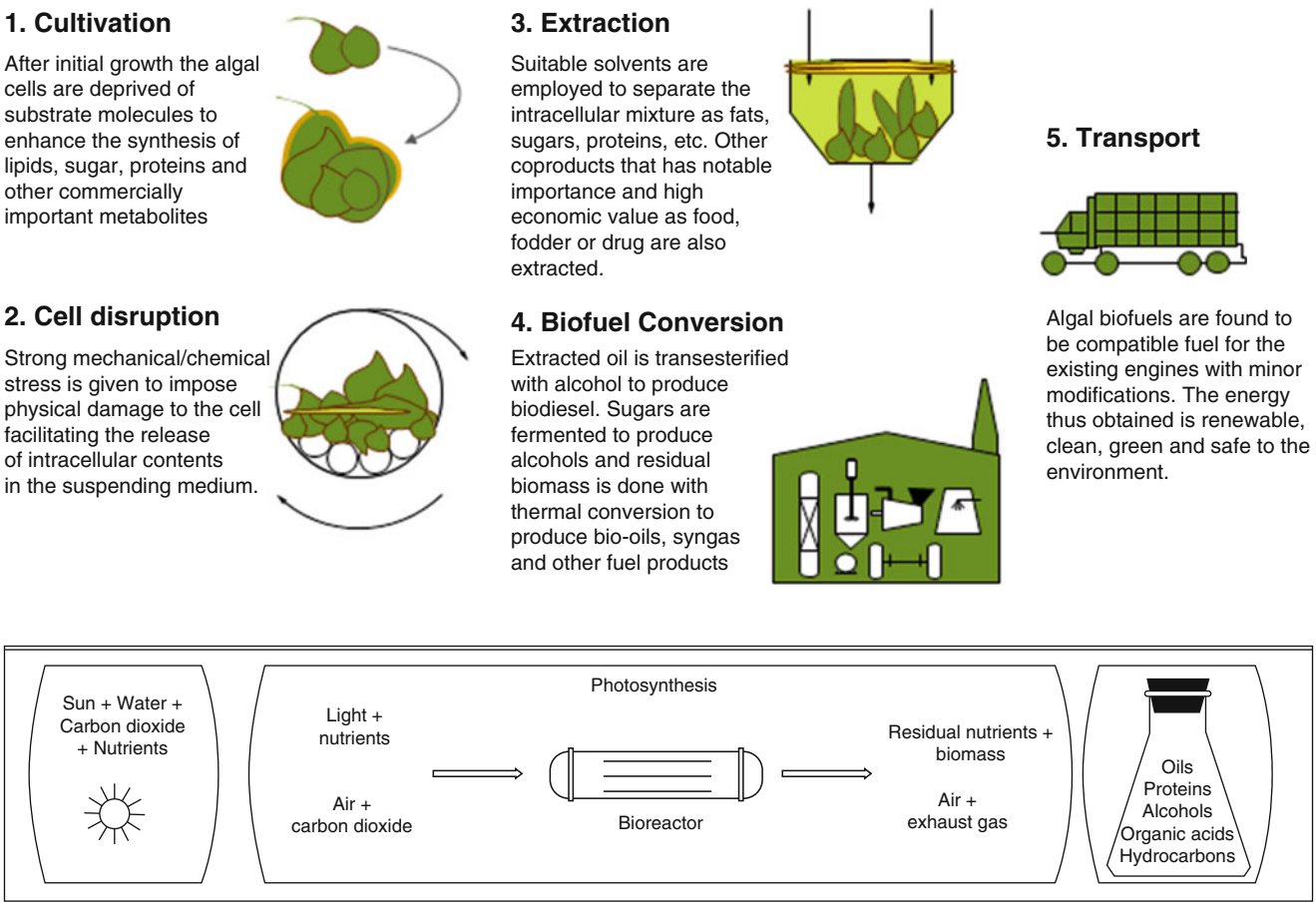

Fig. 1.2 Algal biomass cultivation and its conversion to biofuels

biomass weight ratio. Temperature control has greater effect of growth. Control of culture conditions and barriers against natural calamities are uncontrollable in macroalgal farming. Artificial pond culture of seaweeds in off-marine areas requires artificial seawater. Cultivation techniques focusing enhanced biomass productivity toward biofuel production have not investigated in enough numbers. Economics of macroalgal cultivation for bioenergy remains untested but evidenced as efficient by majority of production in the UK and Ireland. Marine macroalgal farming suffers few drawbacks such as introduction of invasive species, grazing of weeds by fishes, fishing difficulties, changes in nutrient composition at the cost of prolonged cultivation in a same location, etc.

4 Progress and Constraints with Biomass Productivity

Sustainable use of resource for large-scale production has to be regulated to achieve theoretically higher yields. Care must be taken in utilization of resources such as land, water resource, and nutrients.

Resource constraints for macroalgal cultivation are less understood and should progress intensively to extend the scope of macroalgal biofuels. For established microalgal farms, nutrient resource concerns will include consideration of nitrogen, phosphorous, and carbon dioxide consumption per batch algal productivity. All the above macronutrients are conventionally supplied as chemicals that are produced out of energy-intensive processes. The intelligent and easy way of supplying these resources to algae is by obtaining nutrient from cheap resource such as waste streams. Sewage waste and cattle wastes are rich in carbon and nitrogen. Carbon dioxide can be trapped from power plants, petroleum refineries, sugar industries, and other kinds of carbon-rich flue gas-emitting industrial streams. These streams can be treated, diluted/dissolved, and fed as algae feed in growth systems to enhance biomass productivity since these streams appear as natural environment for algae to grow. Concerns regarding the use of value-rich nutrients for fuel feedstock production have necessitated the concept of applying recovered nutrients from cheap streams (Fig. 1.3).

Selection of appropriate land resource for algal cultivation is an uphill task that might seem easy but turn much difficult in reality. Though the use of non-cultivable land is recommended for algal cultivation, the resource is found to be imposing other challenges such as nutrient demand, temperature issues, denying land topography (slopes and hills), and less accessibility because of land localization in isolated geographies. Favorable arable land recommended for establishing a microalgal farm should be a calamity-free area with an average annual temperature of less than 15 °C, <5 % slopes of land, with human density of 250 person/km^{-2}. The particular land satisfying all the above criteria should be accessible easily by transport for making regulated availability of nutrient and water resource for the farm (van Harmelen and Oonk 2006). Large quantity of water is required for cultivation of algae. Post-harvesting techniques may return recovered water to the cultivation system to reduce water loss and enhance nutrient recycle within the system to reduce nutrient addition. PBRs have lesser concern of water and land resource selection since the problem of evaporation, photon scattering, and area covered/GM productivity is much lower than open ponds. The use of freshwater in large quantity for algal cultivation is also a major issue to be considered in microalgal farming for which the desired alternative will be offshore cultivation of marine microalgal species. However, investment, overproduction, processing, and fuel product transportation should result in favorable net energy balance to put algal biomass cultivation for fuel in a profitable path.

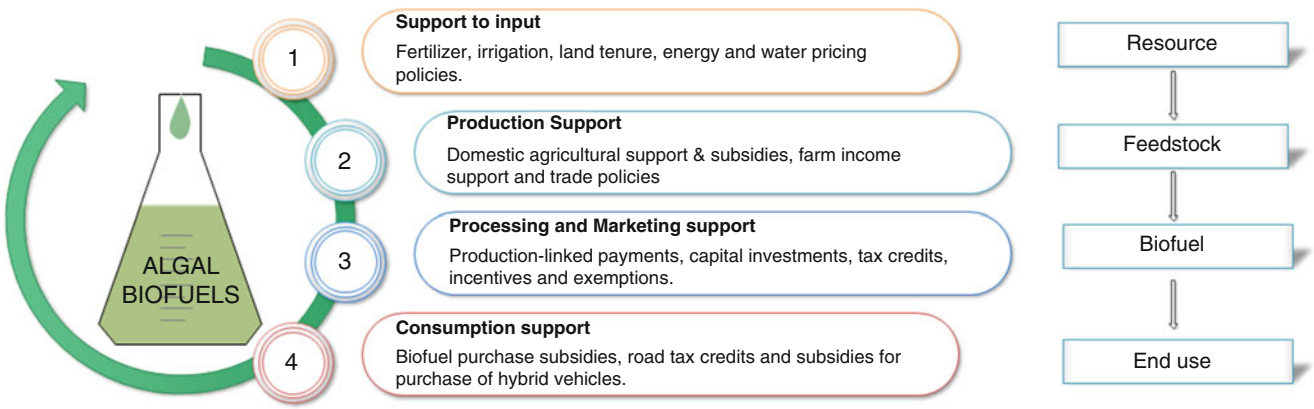

Fig. 1.3 Various support extended by FAO to improve commercial biofuel production

5 Algal Products

A wide variety of products can be obtained from different algal species. Products are categorized in the perspectives of obtaining energy. Algae also produce unique nonfuel bioproducts as coproducts during their growth. Algae can be also used as complete cell biomass to produce heat, electricity, or thermal processing to obtain liquid products that in turn gives energy on combustion. All the biofuel products described in the below sections are obtained as a result of various conversion processes.

5.1 Nonfuel Bioproducts

The cell wall of diatoms is composed of polymerized silica and accumulates oil and chrysolaminarin. The freshwater green algae *Haematococcus pluvialis* is commercially important as a source for astaxanthin, *Chlorella vulgaris* as a supplementary food product, and the halophilic algae *Dunaliella* sp. as a source of β-carotene. Extracted biomass is proposed to be used as fertilizer or animal feed. Coproducts such as pigments, agar, carrageenan, and other bioactive compounds are value-added products that can be removed before fuel conversion of biomass (Spolaore et al. 2006). Spirulina is cultivated for SCP, phycobiliproteins, and γ-linolenic acid. Protein isolates from *Chlorella* are used as feed for aquatic organisms. Dihydroxyacetone oil obtained from *Crypthecodinium cohnii* and *Schizochytrium* sp. finds application in health care and nutrition. *Porphyridium* biomass is a rich source of arachidonic acid that is used as additive nutrient in human diet. The prices of these products fall 11–35€/kg for Single Cell Protein (SCP), 36–50€/kg for *Chlorella* protein, 215–2150€/kg for β-carotene, and an average of 43€/kg for dihydroxyacetone oil. Astaxanthin can cost anywhere between 501 and 7150€/kg. Macroalgae are generally extracted for alginate, agar, and carrageenan. After extraction of these higher value compounds, the second step is to treat the residual biomass with any fuel conversion process.

5.2 Lipids, Hydrocarbons, and Biodiesel

Few species of algae are well known for their ability to produce hydrocarbons. Hydrocarbons are equivalent to gas and oil fractions of crude oil. These hydrocarbons can be converted to gasoline, kerosene, and diesel using appropriate treatments. Generally, algal species usually contain less than 1 % hydrocarbons. Few species like *Botryococcus braunii* typically produce 20–60 % of its dry matter. Hydrocarbon levels up to 80 % dry mass are also obtainable with this particular species (Sheehan et al. 1998). Composition of hydrocarbons depends on the species. Hydrocarbons comprising C30 to C37 alkenes and C23 to C33 alkenes are usually excreted outside the cell, thus making extraction easier. These kinds of algae are naturally growing and have higher doubling time (72 h avg.). Hence, artificial systems with high capital input may not be interesting. Manipulated seawater having optimum salt concentration and pH can be used for hydrocarbon harvesting. Lipids are one of the main components of algae. Oil can be extracted from algae by mechanical pressing and solvent extraction. Cell-disrupted algae are extracted for both intracellular lipids and membranous lipids. Apart from autoclave, bead milling pretreatments, sonication, and microwave treatments are trending cell disruption techniques (Kita et al. 2010; Lee et al. 2010). Milking of live algal cells for lipids is also proposed and found possible with decane and dodecane solvents (DOE 2010). In microalgae, around 60 % of lipids exist as TAG (Tri Acyl Glycerols), FFA (Free Fatty Acids), and phospholipids. Macroalgae have relatively lower lipid content of which majority exist in cell wall lipids. FFA content of algal oil is quite high, and hence pretreatment is necessary before biodiesel conversion. Higher FFA content does not support direct engine combustion of algal oils for driving vehicles. But transesterification with alcohol to produce biodiesel is an intelligent attempt to feed vehicular IC engines that needs capital investment for alcoholic inputs over the process, downstream operations, and maintenance. Microalgal harvest consumes 2915.27 MJ energy/1000 MJ of algal biodiesel. Hence, glycerol coproduct credentials should be coupled with biodiesel production expenditure for economic feasibility of process. Consistent lipid productivity by adopting strategies of nutrient deficient stresses over biomass in production systems can be addressed. From all forms of fuel products obtained from algae, only algal biodiesel has received much attention that is in very near stage of large-scale deployment.

5.3 Carbohydrates and Alcohol

Carbohydrates are the significant components of algae. It can be hydrolyzed and fermented using yeast to produce ethanol. ABE fermentation by *Clostridium* sp. can convert cellulosic fractions to acetone, butanol, and ethanol in the ratio 1:6:3. Carbohydrates in algae exist as starch, cellulose, and other sugar molecules. Carbohydrate fermentation of algae is developed from the existing method of plant carbohydrate fermentation. Such technology extension

toward algal alcoholic fuel production faces some complexities due to molecular differences prevailing with cellulosic raw materials of plant and algal biomass. Hence, hydrolysis efficiency varies, and relatively lower yield of alcoholic production is observed with algae when compared with plant cellulosic fermentation. The advantages of algae over plant biomass will be its consistency, the absence of lignin, high polysaccharide content, etc. Hence, this area has potential scope of improvement and currently lies in the stage of basic research. Analysis of molecular structure, identifying the technology to catalyze conversion of carbohydrates coupled with novel pretreatment steps, may bring solution to challenges identified with algal alcohol production.

5.4 Hydrogen

Biohydrogen production is coupled with fuel cells to harvest energy. Electrolysis of water for hydrogen production is not suitable for large-scale production. Biological production of hydrogen from carbon substrates is possible by non-sulfur purple bacteria. This option is only viable if wastewater is used as raw material for algal growth. Few algae produce hydrogen in the absence of oxygen by consuming carbohydrates in the presence of light and water. Current research does not prove algal hydrogen production a profiting attempt. However, biological route of solar energy conversion to hydrogen can be enhanced by genetic modification of algae to make hydrogen production renewable (Melis and Happe 2001). Possible use of hydrogen in fuel cell vehicles and hydrogen internal combustion engines is under investigation. Hydrogen with a wide range of flammability draws much attention toward safer storage and handling. High flame speed, lower density, high diffusivity, and low ignition energy provide increased fuel/power ratio improving fuel economic credentials. Biohydrogen production strategies are still on the verge of exploitation and offer improvement opportunities.

6 Conclusions

Diverse pathways for algal biofuel production are available, and all the pathways are needed to be evaluated for absolute conclusion by performing multiple numbers of case studies. Diverse input sources; their combination of use in algal farming such as seawater, freshwater, waste effluent, exhaust gases, etc.; and their availability influence the validation of algal biofuel concept. Land features such as climatic conditions, topography, solar light energy, and temperature strongly influence the design and feasibility of algal farming. Algal cultivation can be customized to suit many land and water types with widely varying opportunities. Technological advancements in cultivation designs such as covered artificial ponds, bag culture, column reactors, fat PBR, alveolar PBR, tubular reactor systems, etc., potentially contribute the progress in algal biomass productivity (Table 1.1).

Economic viability is yet to be addressed in this concept of algal biofuels. No commercialized illustrations defining the bioenergy production are available as far as now, and coproduction of fuel and algal biomass as feed or nutrient-rich fertilizer is recommended for supportive income generation to put the process in a profitable path. Biomass production is potentially high, and conversion of this technology to a large-scale process for sustainable production is a challenge that requires high capital input. Attempts to discover other pathways that help in parallel production of valuable coproducts are the need for hour to address economic viability. Opportunities that are unique to algal cultivation include the use of low-economic arable lands, seawater, and recycled nutrient source as feed and simultaneous GHG mitigation. However, commercialization of this technology may find difficulties of limited social and ecological impact. Knowledge gap due to lack of immediate industry scale experiments, standardized data sheets, compilation of existing references, energy balance, and net energy studies may reduce the probability of immediate implementation of algal biofuel concept. Still, the possible way out to solve existing fuel demand lies with the algal biofuel concept when compared with first- and second-generation biofuels.

Table 1.1 Comparison of different generation biofuels

Issues	Biofuels from edible biomass	Biofuels from nonedible biomass	Biofuels from algal biomass
Vehicular compatibility	+	+	+
Quality	+	+	+
Energy security	+	+	+
Food vs fuel dispute	+	–	–
Agricultural inputs	+	–	–
Net energy balance research	–	+	+
Environmental impact	–	–	–
Arable land and water	–	–	+
Seawater	–	–	+

7 Future Perspectives: Scope, Challenges, and Opportunities

Versatility of algae makes localization of farms in many geographical locations and possible use of diverse water source. Technical feasibility of algal biofuel processes is well explained by a significant number of research work. Economic viability of large-scale production is yet to be ascertained. Automation systems for controlled cultivation, harvest, and product extraction are opportunities that could be exploited immediately to address the process industrialization. Manipulation of existing technologies and their integration with algal farming can cut down production cost and product cost. Technologies for coproduct extraction and processing should be demonstrated with the target of obtaining significant quantities. Utilization of entire biomass to make the concept as zero waste schemes will bring fortunate improvements favoring commercialization. Strategies for simultaneous utilization of algal fractions such as carbohydrates, proteins, and lipids for alcoholic fuels and ester fuels are to be developed. Mapping the resource availability, compatibility of algal farms with corresponding resources, accessibility, technology incorporation, boundary definitions, and interdependency of these parameters can provide a standard long-term solution that will be much significant so as to overcome the existing technological limitations.

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Phycoremediation: Future Perspective of Green Technology

2

Sonal Dixit and D.P. Singh

1 Introduction

The unrestricted developmental activities such as rapid industrialization and urbanization carried out during the past few decades have given rise to serious problems of environmental contamination. The load of pollutants including toxic metals is ever rising in the environment as a result of enhanced industrial activities. These heavy metals enter the environment through a variety of human activities, such as mining, refining, electroplating industries, etc. (Micheletti et al. 2007). Thus, the deposition of toxic metals in the environment and their speciation between abiotic and biotic components of ecosystem is posing toxicity in the latter group as it is impossible to degrade these pollutants by any means; the only way to overcome the effects of toxic heavy metals is their physical removal from the contaminated sites (Volesky 1997). The accumulation of toxic heavy metals in the aquatic environment has become a significant problem worldwide, and, therefore, it is a matter of great concern (Khoshmanesh et al. 1996; Dönmez et al. 1999; Gupta et al. 2006). At present, heavy metals are one of the most widespread pollutants, and their continuous accumulation in water bodies, soil and water sediments constitutes a serious hazard to both the environment and human health (Wase and Forster 1997). To avoid the adverse effects of metal-contaminated wastewater, it is necessary to treat them prior to their discharge into the environment.

The conventional physicochemical techniques for the removal of inorganic pollutants from wastewater involve lime precipitation, chemical oxidation or reduction, ion exchange, electrochemical treatment, filtration, reverse osmosis, membrane technologies and evaporative recovery (Barakat 2011). However, these techniques have significant shortcomings, for instance, low efficiency at lower concentrations of individual metal pollutants, high capital investment and operational costs and production of toxic sludge (Khoshmanesh et al. 1996). Therefore, it is imperative to have new technologies for reduced contamination of environmental components, which are not only environment friendly but also cost-effective.

The removal of toxic inorganic environmental pollutants has received an ever-increasing attention in recent years, and various biomaterials such as bacteria, fungi, algae and plants have been employed as bioremedial agents to decontaminate the metal-polluted environment (Kotrba and Ruml 2000; Kiran et al. 2008). This chapter mainly emphasizes the removal of certain deadly inorganic heavy metals by using algal strains and their phycoremediation potential.

2 Pollution in the Aquatic Environment

There are several ways in which we can classify contaminants of a water body. Broadly there are two classes of pollutants: organic pollutants and inorganic pollutants (Fig. 2.1).

2.1 Organic Pollutants

These are the compounds which consist of mainly carbon and hydrogen. The toxicity of organic pollutants depends upon the functional groups present in them. There are several subgroups of organic pollutants, as follows.

2.1.1 Hydrocarbons

They can be divided into two classes: aliphatic hydrocarbons (alkanes, alkenes and alkynes) and aromatic hydrocarbons which contain carbon ring. Aromatic hydrocarbons such as poly aromatic hydrocarbons (PAHs) are much more reactive than any other class of aliphatic hydrocarbons.

S. Dixit • D.P. Singh (✉)
Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Vidhya Vihar, Raebareli Road, Lucknow, UP, India
e-mail: dpsingh_lko@yahoo.com

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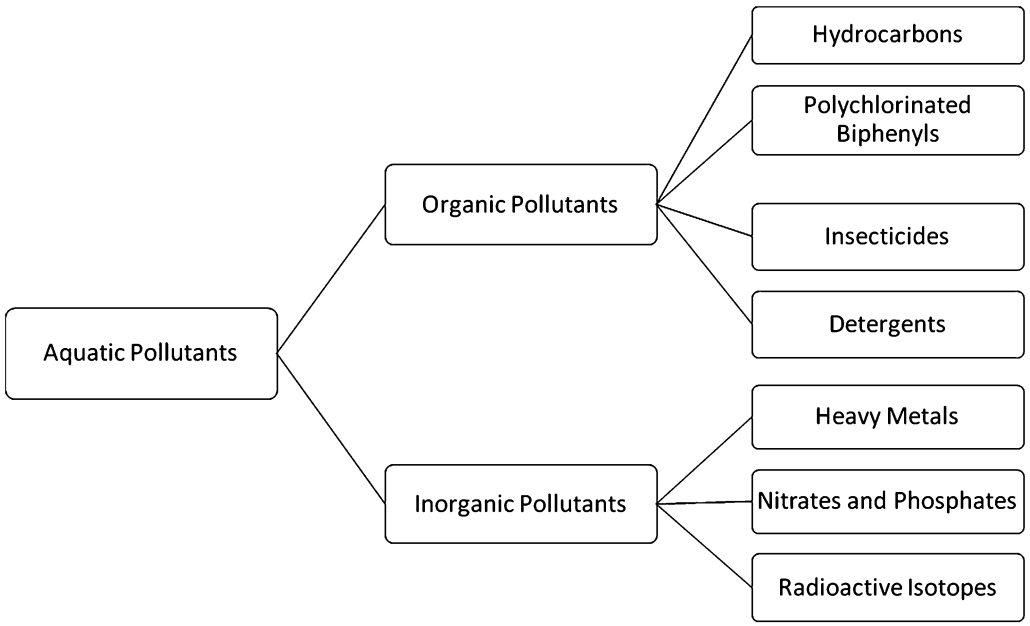

Fig. 2.1 Classification of the water pollutants

2.1.2 Polychlorinated Biphenyls (PCBs)

These are stable and unreactive fluids, relatively insoluble in water. They are mostly used as hydraulic fluids, coolants/insulation fluids and plasticizers in paints.

2.1.3 Insecticides

Some of the insecticides are found to be highly dangerous for living tissues as they accumulate in fat tissues and enter in the food chain. Examples are DDT, lindane, carbamate, azadirachtin, etc.

2.1.4 Detergents

These are classified as phosphate detergents and surfactants. Phosphate detergents are used to soften the hard water and they have caustic property. Surfactants are used to enhance the foaming and emulsifying properties of detergents and are very toxic.

2.2 Inorganic Pollutants

Mostly inorganic pollutants include highly toxic metals (lead, cadmium, zinc, mercury, etc.), while some of these are non-metallic inorganic substances such as nitrates and phosphates but still dangerous for the quality deterioration of aquatic system and partly toxic for the living system. An excessive input of nitrates and phosphates in aquatic bodies received through inorganic fertilizers is the major cause of algal blooms in surface waters, leading to eutrophication of water bodies.

2.2.1 Heavy Metals

The heavy metals, commonly defined as metals having a specific density of more than 5 g/cm^{-3} (Hawkes 1997), include Fe, Mn, Cu, Mo, Zn and Co, which are required in traces as nutrients by the living organisms, but they become toxic at higher concentrations. The other group of metals like Cd, Hg and Pb exert their potential toxic effects even at extremely lower concentrations. These metals are found in surface water bodies in their stable ionic forms. They mostly interfere with electron transfer reactions, and their interaction with oxygen often leads to the formation of toxic oxyradicals. Metalloids can bind with organic compounds, leading to the formation of lipophilic substances which are highly toxic, and can be stored in the fat tissues of animals including humans. Since the heavy metals cannot be broken down into less harmful components as they are non-biodegradable, they can only be remediated to reduce their toxic effects.

2.2.2 Radioactive Isotopes

Radioactive isotopes are either present in nature or created by the humans in the nuclear industry. The decay of radioactive isotopes and their half-life determines the potential danger of these elements to humans. Different kinds of radiations can cause damage to the living tissues, depending upon the type of radiation and its energy level.

3 Bioremediaton

Bioremediation is a biological process used to clean up the hazardous chemicals present in the environment (Gianfreda and Rao 2004). It has several obvious advantages over physicochemical remediation methods in terms of cost-effectiveness, convenience, complete removal of organic

pollutants and lack of collateral destruction of the site materials or its impact on indigenous flora and fauna (Timmis and Pieper 1999). With the advances in the field of biotechnology, bioremediation has become one of the major developing fields applied for environmental restoration. The bioremediation technique involves the use of microorganisms to reduce the concentration and toxicity of various chemical pollutants such as heavy metals, dyes, pesticides, etc. A considerable effort is devoted for developing a low-cost environmental friendly bioremediation technology that can effectively immobilize the dissolved toxic metals; a variety of living biomass has been tested (Say et al. 2001; Adhiya et al. 2002; Sheng et al. 2004) for the removal and/or recovery of metals for their probable reuse potential. Because of high metal-adsorbing capacity, low cost and widespread abundance, the algal biomass has attracted the attention of scientists all over the world (Davis et al. 2003). Various types of either living or dead microalgal biomass have been employed to absorb the dissolved toxic metals.

3.1 Phycoremediation

Phycoremediation is a part of bioremediation where macroalgae or microalgae are being used for the removal or biotransformation of pollutants, including nutrients, xenobiotics and CO_2. It simply offers cleanup technology, which is cost-effective, nonintrusive and safe.

3.1.1 Algae

Algae represent a large group of aquatic, most primitive photoautotrophic organisms that include around 30,000 species, ranging from unicellular (microalgae) to more complex multicellular organisms (macroalgae). Cyanobacteria (blue-green algae) were also included under the microalgae by some authors (Priyadarshani et al. 2011). Algae possess chlorophyll and are able to transform light energy into chemical energy in a similar way to higher plants but lack true roots, stems and leaves. They grow comparatively faster, which results in fixation of CO_2 being 10–50 times faster than in plants (Subashchandrabose et al. 2013). When compared to plants, microalgae have a simple cell structure, and they are also often surrounded by fluid allowing easier uptake of water and nutrients (Chacoón-Lee and González-Mariño 2010). Algae are taxonomically divided based on their pigments, storage compounds and the main compounds present in their cell wall. The major classes are Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Euglenophyta, Pyrrophyta, Chrysophyta and Cyanophyta (blue-green algae).

Advantages of Using Algae

- The blue-green alga (cyanobacteria) uses light energy source and CO_2 for its growth and survival. This way it helps in carbon sequestration and mitigation of global warming.
- They are economically more viable and an eco-friendly tool.
- They are capable of not only photosynthesis but also fix up atmospheric nitrogen, and they can survive better under the nutrient-limited conditions.
- Microalgae cultures can be cultivated in open ponds or in large-scale water reservoirs. At the same time, the algal growth under laboratory conditions provides reliable and consistent supply of biomass.
- They have regenerative and metal recovery potentiality.
- They generate lesser volume of chemical and/or biological sludge to be disposed off.
- They have high efficiency in dilute effluents and have large surface area to volume ratio.
- They have the potential to treat sites polluted with more than one type of pollutant.

4 Removal of Heavy Metals by Algae

Microalgae are sensitive indicators of environmental changes, and their ubiquitous presence serves as the basis of most freshwater and marine ecosystems, widely being used in the assessment of risk and development of environmental regulations for metals (Levy et al. 2007). Algae are known to accumulate heavy metals and bind with metal ions in uncomplicated aquatic environment in a short period of time by biosorption without any problem of toxicity (Afkar et al. 2010). Algae have many features that make them an ideal tool for the selective removal and concentration of heavy metals, which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation (Cai et al. 1995). An important biochemical function of algae is their involvement in the shaping of proper ecological relationships and interaction between organisms in the aquatic environment (Wilde and Benemann 1993; Sandau et al. 1996; Bajguz 2000) by way of accumulating high concentration of heavy metals depending on their concentration in the external environment. The threshold level of heavy metals varies greatly for different algal species, but it increases as the metal concentration in the water decreases (Kelly 1988; Sharma and Azeez 1988). However, little attention has been paid to metal removal and detoxification by algae in the natural environment.

The studies on biosorption of metals by marine algae revealed an interesting adsorption potential of some algal species such as *Ascophyllum nodosum*, *Sargassum baccularia* (Volesky 1994; Chong and Volesky 1995; Holan et al. 1998), *Scenedesmus abundans* (Terry and Stone 2002), *Ecklonia radiata* (Matheickal and Yu 1996) and *Sargassum fluitans* (Fourest and Volesky 1996). Marine alga *Dunaliella tertiolecta* has been shown to have high phytochelatin (PC) content attributed to its capability to hyperaccumulate Zn

and Cd (Tsuji et al. 2002, 2003). Similarly, a periphytic green alga *Stigeoclonium tenue* is also known to produce high amounts of PC-related peptides when adapted to high Zn concentrations (Pawlik-Skowronska 2003). Ettajani et al. (2001) reported the hyperaccumulation of Cd in microalgae *Skeletonema costatum* and *Tetraselmis suecica*. The use of both marine and freshwater algae for adsorption and elution of gold, silver and cobalt has been reported (Fujita et al. 1992; Hamdy 2000).

The growth of large amounts of algae due to eutrophication of water body is commonly seen in most of the water bodies. Such eutrophic algae may help to eliminate the toxicity of heavy metals and exert a major influence on the behaviour and fate of trace metals entering freshwaters. Metal accumulation capacity of algal biomass is either comparable or sometimes higher than chemical sorbents (Mehta and Gaur 2005). It has also been reported that metal uptake capacities of certain algae are much higher than the activated carbon, natural zeolite and synthetic ion-exchange resin (Volesky 1992). It provides a cost-effective solution for industrial wastewater management. Algae have been used for pharmaceutical reasons for detoxification of heavy metals in the human body due to a very efficient adsorption of the toxic ions (David and Volesky 1998). Moreover, algae possess high metal-binding capacities, because polysaccharides, proteins, or lipids on the surface of their cell walls contain some functional groups such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals (Holan and Volesky 1994) (Table 2.1).

4.1 Factors Affecting the Removal of Heavy Metals

Biosorption of metals by algae may be affected by several factors, including concentration of metals and biomass, pH, temperature, the presence of competing ions, etc.

4.1.1 Effect of pH

Most of the studies have shown that sorption of metal ions is a function of pH of the solution. Earlier studies have indicated that ambient pH condition is an important parameter affecting the biosorption of heavy metal ions (Matheickal et al. 1991; Fourest et al. 1994; Matheickal and Yu 1996). pH affects the chemistry of the metal, the activity of the metal-binding functional groups in the biomass and the competition of metallic ions (Selatina et al. 2004). pH strongly influences the speciation and biosorption ability of the metal ions (Esposito et al. 2001). Since a majority of metal-binding groups of algal cell are acidic (e.g. carboxyl), their availability is pH dependent. These groups generate negatively charged surface groups at acidic pH, and electrostatic interactions

Table 2.1 Some work on algae as heavy metal removing agent

Algae	Metal removed	Description of metal-rich surrounding	References
Anacystis nidulans	Cu	Metal solution	Singh and Yadava (1986)
Tolypothrix tenuis	Cd	Aqueous solution	Inthorn et al. (1996)
Chlorella vulgaris, *Scenedesmus obliquus* and *Synechocystis* sp.	Cu, Ni, Cr (VI)	Aqueous solution	Dönmez et al. (1999)
Nostoc muscorum	Cu and Cd	Multimetal solution	Prasad and Pandey (2000)
Nostoc linckia and *N. rivularis*	Zn and Cd	Sewage water	El-Enany and Issa (2000)
Chlorella vulgaris	Cu and Ni	Single and binary metal solution	Mallick (2003)
Spirulina sp.	Trace element	Copper smelter and refinery effluent	Chojnacka et al. (2005)
Aulosira fertilissima	Cr and Ni	Free-cell condition	Banerjee et al. (2004)
Nostoc muscorum and *Anabaena subcylindrica*	Cu, Pb, Co and Mn	Sewage and industrial wastewater	El-Sheekh et al. (2005)
Cladophora fascicularis	Cu and Pb	Aqueous solution	Liping et al. (2006)
Spirulina platensis (*Spi*SORB)	Cu	Column reactor system	Vannela and Verma (2006)
Nostoc calcicola and *Chroococcus* sp.	Cr	Metal-contaminated soil	Anjana et al. (2007)
Lyngbya and *Gloeocapsa*	Cr	Contaminated sites	Kiran et al. (2008)
Gloeocapsa sp.	Pb	Residue of cyanobacterial EPS	Raungsomboon et al. (2008)
Spirulina platensis and *Aphanothece flocculosa*	Hg	Wet biomass	Cain et al. (2008)
Gloeothece sp. strain PCC 6909	Cu	Wastewater	Micheletti et al. (2008)
Hapalosiphon welwitschii Nägel	Cd	Metal solution	Guzman and Cao (2010)
Oscillatoria sp. NTMS01 and *Phormidium* sp. NTMS02	Cr (VI)	Aqueous solution	Kamaraj et al. (2011)
Sargassum wightii and *Caulerpa racemosa*	Cr (III and VI), Pb, Cd	Aqueous solution	Tamilselvan et al. 2012
Nostoc muscorum	Pb and Cd	Metal solution	Dixit and Singh (2014)

between cationic species and the cell surface are responsible for metal biosorption.

There are numerous studies showing an increase in metal sorption with increasing pH of the solution (Gupta et al. 2006; Solisio et al. 2008; Liping et al. 2008). A decreased metal sorption by algae has been frequently observed at extremely acidic pH (<2) (Özer et al. 1994; Mehta and Gaur 2001a). The marine algae *Sargassum* sp., *Padina* sp., *Ulva* sp. and *Gracilaria* sp. were investigated for their biosorption performance in the removal of lead, copper, cadmium, zinc and nickel from dilute aqueous solutions. Maximum biosorption was found to be at pH 5.0 for lead and copper and at pH 5.5 for cadmium, zinc and nickel (Sheng et al. 2004).

There is also a great variability in optimum pH for sorption of a particular metal ion by different algal species. For example, the optimum pH for sorption of Cu by cyanobacteria (*Microcystis aeruginosa* and *Spirulina platensis*) is far greater than that for Cu sorption by green algae (*Cladophora prolifera*, *Chlorella vulgaris*, *C. kessleri*). This variability may be related to the differences in chemical composition of cell surface of various algal species. Chojnacka et al. (2005) found a distinct relationship between pH of aqueous metal solution and involvement of functional group in binding of Pb onto *Spirulina maxima*; for pH ranging between 2–5, 5–9 and 9–12, respectively, the functional groups involved in the binding of Pb were carboxyl, carboxyl and phosphate and carboxyl, phosphate and hydroxyl.

4.1.2 Effect of Contact Time

The contact time is of great importance in adsorption for the assessment of the suitability of microbes to serve as biosorbents in a continuous flow system. The biosorption consists of two phases: a primary rapid phase that accounts for the major part in the total metal biosorption and a second slow phase that contributes to a relatively small part. Metal uptake increases with an increase in contact time but remains constant after equilibrium time period (Murugesan et al. 2006). Equilibrium time varied with metals due to the difference in initial metal concentration and affinity of the biosorbent for a particular metal ion.

The metal removal rates were rapid, with 90 % of the total adsorption taking place within 60 min (Sheng et al. 2004). Some authors observed that at the initial stage (0–12 min), the adsorption rate of Pb was so rapid that 74 % of the metal was biologically adsorbed by *Spirulina* (Chen and Pan 2005). Heavy metal ion (Cr^{3+}, Cu^{2+} and Cd^{2+}) removal from solutions by *Spirulina* species showed that the equilibrium reached after 10 min (Chojnacka et al. 2005). An increase in the biosorption of Cu by *Spirogyra* species was observed with increase in contact time from 0 to 120 min and after that becomes almost constant up to 180 min (Gupta et al. 2006).

4.1.3 Effect of Temperature

Owing to the dependence on metabolism, metal uptake by live cells is considerably affected by variation in temperature. There are reports showing altered metal uptake by live organisms with change in temperature regime (Skowron'ski 1986; Mehta and Gaur 2001a; Mehta et al. 2002a), with maximum uptake occurring at specific temperature optima. Higher temperature usually enhances sorption due to the increased surface activity and kinetic energy of the solute (Sag and Kutsal 2000; Vijayaraghavan and Yun 2007). An increase in metal sorption with increasing temperature (Tsezos and Volesky 1981; Kuyucak and Volesky 1989; Aksu and Kutsal 1991; Aksu 2002) suggests that metal biosorption by algae is an endothermic process. On the contrary, some studies indicate exothermic nature of metal sorption by algae (Cruz et al. 2004; Aksu 2001; Benquell and Benaissa 2002). Due to the exothermic nature of some adsorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass (Mameri et al. 1999; Suhasini et al. 1999). A temperature change affects the number of factors which are important in heavy metal biosorption. Some of the factors include (i) the stability of the metal ion species initially placed in solution, (ii) microorganism-metal complex depending on the biosorption sites, (iii) the effect of temperature on the cell wall of microorganisms and (iv) the ionization of the chemical moieties on the cell wall (Sag and Kutsal 2000). Increased biosorption of heavy metals with increasing temperature has been ascribed to bond rupture, which perhaps enhances the number of active sites involved in metal sorption or higher affinity of sites for metals. There are also some reports which show no effect of temperature on metal sorption (Norris and Kelly 1979; Zhao et al. 1994). It is always desirable to conduct biosorption at room temperature, as this condition is easy to replicate.

4.1.4 Effect of Biomass Concentration

The dosage of a biosorbent strongly influences the extent of biosorption, and also the amount of metal ion recovered from a solution is affected by biomass concentration. Biomass concentration in solution seems to influence the specific uptake: for lower value of biomass concentrations, there is an increase in the specific uptake (Gadd et al. 1988; Fourest and Roux 1992). An increase in biomass concentration leads to interference between the binding sites (Gadd et al. 1988). Hence, this factor needs to be taken into consideration in any application of microbial biomass as biosorbent. Conversely the quantity of biosorbed solute per unit weight of biosorbent decreases with increasing biosorbent dosage, which may be due to the complex interaction of several factors.

An increase in the biomass concentration generally increases the amount of solute biosorbed due to the increase in the surface area of biosorbent, which in turn increases the

availability of metal-binding sites (Esposito et al. 2001; Mehta and Gaur 2001c). However, there is no straightforward relationship between biomass concentration and metal removal as some workers have noticed a decrease in sorption of heavy metals by different algae with increasing biomass concentration (Hamdy 2000; Nuhoglu et al. 2002; Gong et al. 2005). This may be due to the limited availability of metal, increased electrostatic interactions, interference between binding sites and poor mixing at higher biomass concentrations (Meikle et al. 1990; Fourest et al. 1994).

4.1.5 Effect of Initial Metal Ion Concentration

Sorption and removal of heavy metals largely depend on the initial metal ion concentration in the solution. Several workers have reported that metal sorption initially increases with an increase in the metal ion concentration in solution and then becomes saturated after a certain concentration of metal (Da Costa and Leite 1991; Aloysius et al. 1999; Mehta and Gaur 2001a, b, c; Mehta et al. 2002a, b). This is because at lower initial metal concentrations, the ratio of the initial moles of metal to the available surface area is low; subsequently, the fractional sorption becomes independent of the initial concentration. However, at higher concentrations, the sites available for sorption become fewer compared to the moles of metal present, and hence, the binding of the metal is strongly dependent upon the initial metal concentration. It is always necessary to identify the maximum saturation potential of a biosorbent, for which experiments should be conducted at the highest possible initial metal concentration. Algal cell surface has several kinds of functional groups with varying affinity for an ionic species. Low- and high-affinity functional groups are involved in sorption of metal ions at high and low concentration of metal ions, respectively.

4.1.6 Effect of the Presence of Anions and Cations

Actual industrial wastewaters contain different kinds of impurities, which may significantly affect metal biosorption (Ho and McKay 2000). Some studies indicated that cations and anions in addition to the ion of interest have a generally detrimental impact on metal accumulation (Suh and Kim 2000). Among such impurities, cations such as Na^+, K^+, Mg^+ and Ca^+ and anions like sodium salts of chloride, nitrate, acetate and EDTA exist in most of the industrial effluents, and they greatly interfere with the metal sorption potential of biosorbents (Chen and Yiacoumi 1997; Lee and Volesky 1997; Low et al. 2000). Earlier low level of accumulation of Co and Cu by algae in the presence of carbonate, orthophosphate, sulphate, nitrate, EDTA and chloride ions has been observed (Rai et al. 1981). However, the accumulation of ionic species like nitrate and ammonium also increased in the presence of Cu and Fe in *Anabaena doliolum* (Rai and Mallick 1992). The presence of other cations including metal ions significantly affects metal sorption by algae (Mehta and Gaur 2001a, b; Mehta et al. 2002a, b). Reduced heavy metal uptake in the presence of light metals is attributed to competition for cellular-binding sites or precipitation or complexation by carbonates, bicarbonates, or hydroxides of Ca and Mg (Rai et al. 1981). High concentrations of salts like NaCl in solution can also decrease the rate of metal sorption by algae (Cho et al. 1994). The inhibitory effect of Na is more pronounced with weakly bound metals such as Zn or Ni. It is important to note that Na^+ and K^+, being monovalent cations, do not compete directly with covalent binding of heavy metals by the biosorbent. Other compounds that could be considered as impurities in metal removal process are surfactants and some chelating agents. The nature of impurities differs depending on the type of effluent to be treated. Whereas most of the studies reported the inhibitory effect of light metal ions on sorption of heavy metals by biosorbent, a few of them showed no effect (Pawlik and Skowronski 1994; Adhiya et al. 2002; Axtell et al. 2003; Jalali-Rad et al. 2004).

4.2 Mechanism of Heavy Metal Removal

The mechanism of metal biosorption is a complicated process. The status of biomass (living or nonliving), types of biomaterials, properties of metal solution chemistry and environmental conditions such as pH influence the mechanism of metal biosorption. The uptake of heavy metal ions by algae was found to occur in two principal ways: passive uptake due to surface adsorption (metabolism independent) followed by cellular uptake (metabolism dependent) via intracellular transport and chelation (absorption) (Khummongkol et al. 1982; Cho et al. 1994; Yee et al. 2004). Some metals such as Pb and Sr may be passively adsorbed by charged polysaccharides in cell wall and intracellular matrix (El-Sheekh et al. 2003; Osman et al. 2004; Fathi et al. 2000; 2005); other metals (e.g. Zn, Cd) are taken up actively against large intracellular concentration gradients. As passive biosorption mainly depends on the binding to functional surface ligands, the cell wall structure is the most important for rapid metal ion uptake.

The probable sites of an algal cell for the binding of metal ions are shown in Fig. 2.2. Adsorption occurs directly onto the cell wall in some algae, but the presence of various amounts of mucilage or extracellular polymeric substances (EPS) (Leppard 1995; Lee 1997) in others (e.g. Cyanophyta) may play a key role in metal binding (Weckesser et al. 1988). The algal cell wall contains many functional groups, such as hydroxyl (–OH), phosphoryl, amino ($–NH_2$), carboxyl (–COOH), sulphydryl (–SH), etc., which confer a negative charge on the cell surface. Since metal ions in water are generally in the cationic forms, they are adsorbed onto the cell surface. The functional group involved in the metal sorption

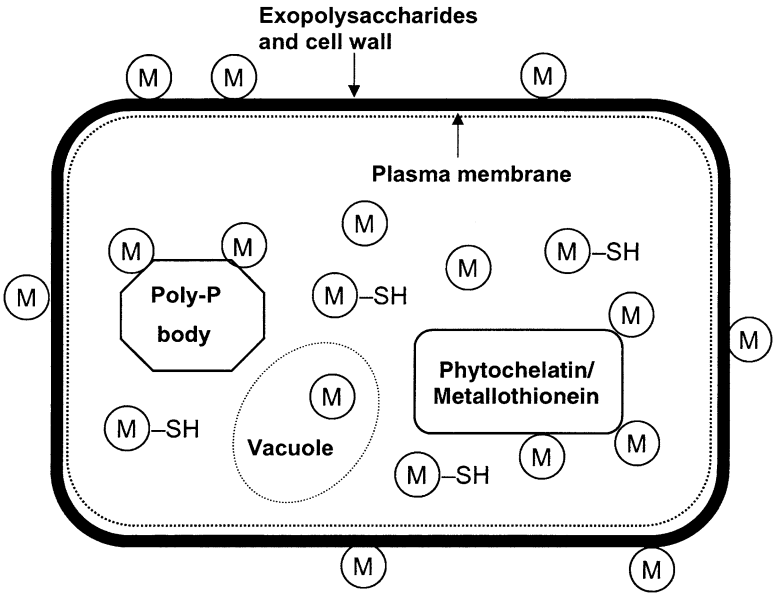

Fig. 2.2 Metal-binding sites of a typical algal cell. The alphabet M represents the metal species (independent of its oxidation state) (Source: Mehta and Gaur 2005)

by algae have been identified by FTIR spectroscopy, pH titration, potentiometric and conductimetric titration techniques and also after blocking of functional groups by certain chemicals. Each functional group has a specific dissociation constant (pKa), and it dissociates into corresponding anion and proton at a specific pH (Niu and Volesky 2000). The cell wall functional groups are found linked with various cell wall components, e.g. peptidoglycan, teichoic acid, polysaccharides and proteins. Among different cell wall constituents, polysaccharides and proteins have most of the metal-binding sites (Kuyucak and Volesky 1989). Since the distribution and abundance of the cell wall components vary among different algal groups, the number and kind of functional group also vary among them.

Hamdy (2000) reported that metal uptake is dependent on the type of biosorbent, with different accumulation affinities towards the tested elements, and the amount of metal uptake increased steeply by increasing the weight of the biomass. Fathi et al. (2005) reported that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water. Verma and Singh (1990) reported Cu uptake in a diazotrophic cyanobacterium *Nostoc calcicola* to be biphasic. During the first 10 min, there was a rapid binding of cations to the cell wall, followed by subsequent metabolism-dependent intracellular uptake for at least 1 h.

5 Potential Applications of Algae in Biotechnology

Algae are one of the potential organisms, which are useful to mankind in several ways. Algal cells constitute a vast potential resource in various applications as follows.

5.1 Food and Feed

Blue-green algal protein has received worldwide attention either as a supplement or as an alternative source of food. Some strains of *Spirulina* and *Nostoc* are consumed as human food in Chile, Mexico, Peru and the Philippines. *Spirulina* is used as a food supplement because of its excellent nutrient composition (60–70 % protein, 20 % carbohydrate, 5 % lipids, 7 % minerals and 6 % moisture) and digestibility. It is also a rich source of β-carotene, thiamine and riboflavin and is one of the richest sources of vitamin B12. *Nostoc commune* has high amount of fibre and moderate protein; therefore, this species is used as a new dietary fibre source in human diet (Jeraci and Vansoest 1986). Halotolerant marine algal species *Dunaliella* is also a rich source of β-carotenoid (Gudin and Chaumont 1991). Green alga *Chlorella vulgaris* is used as a food supplement in many countries including China, Japan, Europe and the USA (Yamaguchi 1997).

5.2 Fine Chemicals

A variety of fine chemicals such as pigments, vitamins and enzymes with various applications can be obtained commercially from different algal strains. Some marine algae are a potential source for commercial production of vitamins such as vitamin B complex and vitamin E (Borowitzka 1988). *Haematococcus pluvialis* accumulate the highest level of astaxanthin which is a potent radical scavenger and a singlet oxygen quencher that surpasses the antioxidant benefits of β-carotene, vitamin C and vitamin E (Lorenz and Cysewski 2000). A number of cyanobacteria are also rich in vitamins and many excrete them into the surrounding environment (Borowitzka 1988). The

products obtained from cyanobacteria like carotenoids and phycobiliproteins are used as natural food colourants and also as food additives and have high commercial value (Emodi 1978). *Dunaliella salina*, a halotolerant microalga, is able to accumulate very large amount of β-carotene and also a valuable chemical, glycerol (Avron 1992; Oren 2005). Feed grade *Phormidium valderianum* is an excellent source of phycocyanin, a blue natural colourant useful as a phycofluor in diagnostics. Cyanobacteria being photoautotrophs have the ability to photosynthetically transform simple, labelled compounds such as $14CO_2$, $13CO_2$, $33H_2O$ and $15NO_3$ into complex organic compounds. In addition, cyanobacteria are a rich source of polysaccharides, lipids, amino acids, fatty acids, halogenated compounds, etc., which are used as flocculants, surfactants and others (Patterson 1996).

Enzymes that can be exploited commercially such as chitinase, L-asparaginase, L-glutaminase, amylase, protease, lipase, cellulase, urease and superoxide dismutase have been reported from several algal strains (Prabhakaran et al. 1994; Wikstrom et al. 1997). Several common and unique sequence-specific endonucleases are known from *Anabaena cylindrica* (Acy I), *A. flos-aquae* (Afl I and Afl III), *A. variabilis* (Ava I and Ava II), *A. variabilis UW* (Avr II), *Microcoleus* sp. *UFEX* 2220 (Mst II) and *Nostoc* sp. *PCC* 7524 (Nsp C I), which can be marketed at low cost since the relative biomass production of cyanobacteria is much less expensive than bacteria (Elhai and Wolk 1988). Marine microalgae *Isochrysis galbana* and *Diacronema vlkianum* produce long-chain fatty acids, mainly eicosapentaenoic acid (EPA, 20:53) and docosahexaenoic acid (DHA, 22:6ω3) (Liu and Lin 2001).

5.3 Pharmaceuticals

Algae are one of the richest sources of known and novel bioactive compounds with wide pharmaceutical applications (Raghavan et al. 2002). The reported biological activities comprise cytotoxic, antitumor, antibiotic, antimicrobial, antiviral (e.g. anti-HIV) activities as well as biomodulatory effects like immunosuppressive and anti-inflammatory properties (Burja et al. 2001). *Chlorella vulgaris* has been used as an alternative medicine in the Far East. It is considered an important curing agent for many kinds of health disorders such as gastric ulcers, wounds, constipation, anaemia, hypertension, diabetes, infant malnutrition and neurosis (Yamaguchi 1997). A preventive action of *Chlorella* against atherosclerosis and hypercholesterolemia is attributed to glycolipids and phospholipids and antitumor actions assigned to glycoproteins, peptides and nucleotides (Yamaguchi 1997). However, the most important substance in *Chlorella*, i.e. beta-1,3-glucan, is an active immunostimulator, a free radical scavenger and a reducer of blood lipids (Spolaore et al. 2006). The anti-HIV activity of marine cyanobacterial compounds from *Lyngbya lagerheimii* and *Phormidium tenue* was also reported (Gustafson et al. 1989). Halophilic marine algae *Dunaliella* has anticancerous, anti-atherosclerosis, anti-inflammatory, anti-allergic, antidiabetic, antibacterial and antiviral properties (Hennekens et al. 1996; Fujitani et al. 2001; Ayelet et al. 2008; Francisco et al. 2009; Nakazawa et al. 2009). Medically important gamma-linolenic acid (GLA) is relatively rich in cyanobacteria *Spirulina platensis* which is easily converted into arachidonic acid in the human body and then into prostaglandin E2, which has lowering action on blood pressure and contracting function of the smooth muscle, thus playing an important role in lipid metabolism (Thajuddin and Subramanian 2005).

5.4 Biofertilizer

Several cyanobacterial strains colonize paddy fields where heterocystous species are capable to fix atmospheric nitrogen (Mishra and Pabbi 2004). However, a variety of non-heterocystous cyanobacteria are also able to fix atmospheric nitrogen under microaerophilic conditions. The role of N2-fixing cyanobacteria in maintenance of the fertility of rice fields has been well substantiated and documented all over the world (Saadatnia and Riahi 2009). In India alone, the beneficial effects of cyanobacteria on yield of many rice varieties have been demonstrated in a number of field locations (Venkataraman 1981). Beneficial effects of cyanobacterial inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Kaushik and Venkataraman 1979; Thajuddin and Subramanian 2005).

Red marine algae *Laurencia obtusa*, *Corallina elongata* and *Jania rubens* were also used as biofertilizers by some workers to enhance the growth of maize (*Zea mays* L.) plants, and it was reported that the mixture of these algae is more suitable for the growth of maize in the field (Safinaz and Ragaa 2013). Lozano et al. (1999) stated that the application of an extract from algae to soil or foliage increased ash, protein and carbohydrate content of potatoes.

5.5 Wastewater Treatment

Algal species have been used for many decades in wastewater treatment because of its high capacity to uptake inorganic nutrients (Talbot and De la Noue 1993; Bajguz 2000; Ettajani et al. 2001; Tsuji et al. 2002, 2003; Afkar et al. 2010). The importance of microalgae in wastewater treatment has increased in recent years due to the biotechnological potential for producing valuable substances for biofuel production and animal feed (Pulz and Gross 2004; Spolaore et al. 2006). Marine cyanobacteria *Oscillatoria* sp. BDU 10742 and

Table 2.2 Important microalgal species, their products and applications

Species	Product	Application areas	References
Spirulina sp.	Phycocyanin, biomass	Health food, cosmetics, wastewater treatment	Lee (2001) and Costa et al. (2003)
Chlorella vulgaris	Biomass	Health food, food supplement, feed surrogates, wastewater treatment	Lee (2001)
Dunaliella salina	Carotenoids, β-carotene	Health food, food supplement, feed	Jin and Melis (2003) and Del Campo et al. (2007)
Haematococcus pluvialis	Carotenoids, astaxanthin	Health food, feed additives, pharmaceuticals	Del Campo et al. (2007)
Odontella aurita	Fatty acids	Pharmaceuticals, cosmetics, baby food	Pulz and Gross (2004)
Porphyridium cruentum	Polysaccharides	Pharmaceuticals, cosmetics, nutrition	Fuentes et al. (1999)
Isochrysis galbana	Fatty acids	Animal nutrition	Molina Grima et al. (2003) and Pulz and Gross (2004)
Phaeodactylum tricornutum	Lipids, fatty acids	Nutrition, fuel production	Yongmanitchai and Ward (1991)
Lyngbya majuscula	Immune modulators	Pharmaceuticals, nutrition	Singh et al. (2005)
Muriellopsis sp.	Carotenoids, lutein	Health food, food supplement, feed	Blanco et al. (2007) and Del Campo et al. (2007)

Aphanocapsa sp. BDU 16 were able to treat a factory effluent rich in calcium and chloride and enabled 100 % survival of *Tilapia* fish with only cyanobacteria as the feed source (Uma and Subramanian 1990). Shashirekha et al. (1997) found that *Phormidium valderianum* BDU 30501 was able to treat phenol-containing effluents. Studies at the National Facility for Marine Cyanobacteria (NFMC) have identified suitable cyanobacteria for treating a number of noxious effluents containing organophosphorus pesticides, detergents, antibiotics and other molecules (Subramaninan and Uma 1996) and also for the degradation of solid wastes like coir pith by their lignolytic action (Malliga et al. 1996). There are several reports for the treatment of heavy metal-contaminated wastewater by using marine and freshwater algae and also sorption/desorption of heavy metal for the recovery of this valuable resource (Hamdy 2000; Ettajani et al. 2001; Terry and Stone 2002; Dixit and Singh 2013, 2014) (Table 2.2).

6 Conclusion

During the last few decades, the researchers have started viewing the algal cells as photobioreactor which can be exploited by many ways in different spheres of biotechnology at minimal cost. The algal cells require only water, minimum quantity of nutrients, sunlight and CO_2 for their growth and survival. Phycoremediation using these algal cells is finding favour for the treatment of variety of wastewaters due to their minimal need for space and easy-to-grow characteristics. The resulting biomass after bioremediation (phycoremediation) has the potential to be used as animal feed, production of biofuel and other industrially relevant bioproducts. Thus, phycoremediation technology of wastewater treatment is considered as a low-cost technology which promises a more sustainable and environment-friendly way of life.

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Applications of Algal Biofilms for Wastewater Treatment and Bioproduct Production

3

Maureen Kesaano, Terence Smith, Jonathan Wood, and Ronald C. Sims

1 Introduction and Current State of Technology

Microalgae-based systems in wastewater treatment present an inexpensive biotechnology, which can remediate wastewater, reduce energy consumption via O_2 aeration, sequester CO_2 from the atmosphere, and provide biomass for bioproducts production (Kumar et al. 2010). Cultivation of microalgae occurs mostly in suspension using open ponds and photobioreactor systems (Chaumont 1993). However, algae concentrations from suspended growth platforms are low (0.5–4 g/L cells dry weight) with high water content (about 99 %), making recovery of the microscopic cells difficult (Eriksen 2008; Murphy and Allen 2011). A dewatering step is often required to separate the algae from water, which can be energy intensive, time consuming, and sometimes cost prohibitive (Mata et al. 2010). The dewatering techniques commonly applied in suspended growth algal systems include sedimentation, centrifugation, filtration, and flocculation.

There is a growing interest in attached algal growth systems that stems from the need to simplify harvesting and minimize downstream processing costs. With attached growth, the microalgae cells are immobilized on a surface either naturally or artificially using polymeric substances (Chevalier and de la Noüe 1985; Hoffmann 1998; Mallick 2002; Boelee et al. 2013). The already concentrated biomass then can simply be scraped off the surface for further processing. Algal biofilms, also known as phototrophic biofilms, will naturally attach on a surface in the presence of light, moisture, and nutrients with the help of extracellular polymeric substances (EPS) (Roeselers et al. 2008; Kesaano and Sims 2014). Natural biofilm communities comprise of a number of microorganisms including microalgae (green algae, diatoms, and cyanobacteria species), bacteria, fungi, and protozoa (Callow 2000).

In natural marine or fresh water environments, algal biofilms represent an important source of autochthonous carbon for other trophic levels and influence nutrient recycling at the ecosystem level (Barranguet et al. 2000; Kanavillil et al. 2012). The same concept can be applied to wastewater treatment where algal biofilms are utilized for nutrient recycling (removal of nutrients or heavy metals through plant uptake) and as sources of carbon and O_2 for enhanced bacterial activity (nitrification or BOD removal). Research on algal biofilm-based wastewater treatment systems is still limited; however, the commercial success of these systems can be realized through integration of wastewater remediation with bioproduct production (Adey et al. 2011; Sturm and Lamer 2011; Beal et al. 2012; Olguín 2012; Dalrymple et al. 2013).

Bioreactor types currently used to grow algal biofilms in wastewater environments include enclosed photobioreactors (Abe et al. 2008; De Godos et al. 2009; Kumar et al. 2010) and open photobioreactors, i.e., rotating cultivation systems (Christenson and Sims 2011; Orandi and Lewis 2013; Gross et al. 2013) and horizontal, inclined, or vertical sheets and plates (Zippel et al. 2007; Guzzon et al. 2008; Johnson and Wen 2010; Ozkan et al. 2012; Schnurr et al. 2013) (Fig. 3.1).

The purpose of this chapter is to illustrate applications of algae biofilms for municipal and industrial wastewater treatment and for sustainable bioproduct production through the implementation of an algae-based biorefinery. Specific objectives include describing algal biofilms, including challenges for scale-up, exploring the functions of algal biofilms in municipal and industrial wastewater treatment, highlighting bioproducts that have been generated from algae, and describing the concept of an algae-based biorefinery that integrates wastewater treatment with bioproduct production.

M. Kesaano (✉) • T. Smith • J. Wood • R.C. Sims
Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, UT 84322, USA
e-mail: maureen.kesaano@aggiemail.usu.edu

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Fig. 3.1 Pilot and laboratory-scale rotating algal biofilm reactors (RABR) for treating wastewater effluent from Logan City sewage lagoons and providing biomass for bioproducts (Courtesy of the Sustainable Waste-to-Bioproducts Engineering Center (SWBEC), Utah State University)

1.1 An Algal-Based Biorefinery for Transforming Wastes into Bioproducts

Using algae to simultaneously treat wastewater and produce bioproducts (Fig. 3.2) has positive benefits related to the potential to offset the high costs of wastewater treatment. The United States of America (USA) has wastewater infrastructure investment requirements of $13 to $21 billion annually with an additional $21.4 to $25.2 billion required for annual operation and maintenance (US CBO 2002). Revenues from biodiesel and other bioproducts including bioplastics; feed for fish and animals; biofuels including acetone, butanol, and ethanol (ABE); and phycocyanin, produced using wastewater-grown algae, could be used toward wastewater infrastructure requirements. In addition, anaerobic digestion of the algae biomass can produce biomethane for heat and power and electricity generation for supporting wastewater treatment operations and energy requirements for bioproducts production. Therefore the potential for integrating wastewater treatment and bioproduct production offers economic, environmental, industrial, and sustainability benefits for communities that can culture algae using wastewater.

The use of wastewater has a major advantage in supplying nutrients for algae cultivation for large-scale algae biomass production. Micronutrients required in trace amounts that are readily supplied by wastewater include silica, calcium, magnesium, potassium, iron, manganese, sulfur, zinc, copper, and cobalt (Knud-Hansen et al. 1998). Macronutrients include nitrogen, phosphorus, and inorganic carbon, which are present in wastewater in mg/l quantities, with concentrations dependent on the wastewater source (Christenson and Sims 2011). Therefore nutrients in wastewaters can offset the cost of commercial fertilizers otherwise needed for algae cultivation. Also, wastewater treatment revenues can offset algae cultivation costs.

The US Department of Energy (DOE) has specifically recognized the potential synergy of wastewater treatment and algae biomass production, stating, "Inevitably, wastewater treatment and recycling must be incorporated with algae biofuel production" (US EIA 2010). Pittman et al. (2011) has stated that, based on current technologies, algae cultivation without the use of wastewater for a bioproduct like biofuels is unlikely to be economically viable or provide a positive energy return. Lundquist et al. (2010) analyzed several different scenarios of algae-based wastewater treatment coupled with biofuel production and concluded that only those cases that emphasized wastewater treatment were able to produce cost-competitive biofuels. They concluded that without wastewater treatment as the primary goal, the near-term outcome for large-scale algae biofuels production is not favorable.

Challenges for algae feedstocks include production or cultivation and logistics, followed by conversion to bioproducts, and all of these with regard to scale-up and integration. In addition to research and development strategies that focus on algal genetics, strain development, and cultivation strategies as described by the US DOE (2012), evaluation of mixed culture algae cultivation on wastewaters generated by municipalities and industries provides additional and potentially significant inputs for the production of abundant, cost-effective, and sustainable algae biomass supplies (Christenson and Sims 2011).

The challenges with regard to large-scale production of algae biomass using wastewater include the following gas transfer and exchange, photosynthetically active radiation (PAR) delivery, environmental control, land and water availability, and harvesting and processing of the biomass. To prevent limitation of cultivation by nitrogen or phosphorus, recycling algal nutrients released during anaerobic digestion to increase the concentration of macronutrients provides a process for increasing algae productivity. The molar ratio of the wastewater supply must match the stoichiometric ratio of the algae biomass. A common N:P ratio often used for average algae biomass is the Redfield ratio of 16:1 (Stumm and Morgan 1981); however, the specific ratio for a given wastewater may vary from 8 to 45 (Klausmeier et al. 2004).

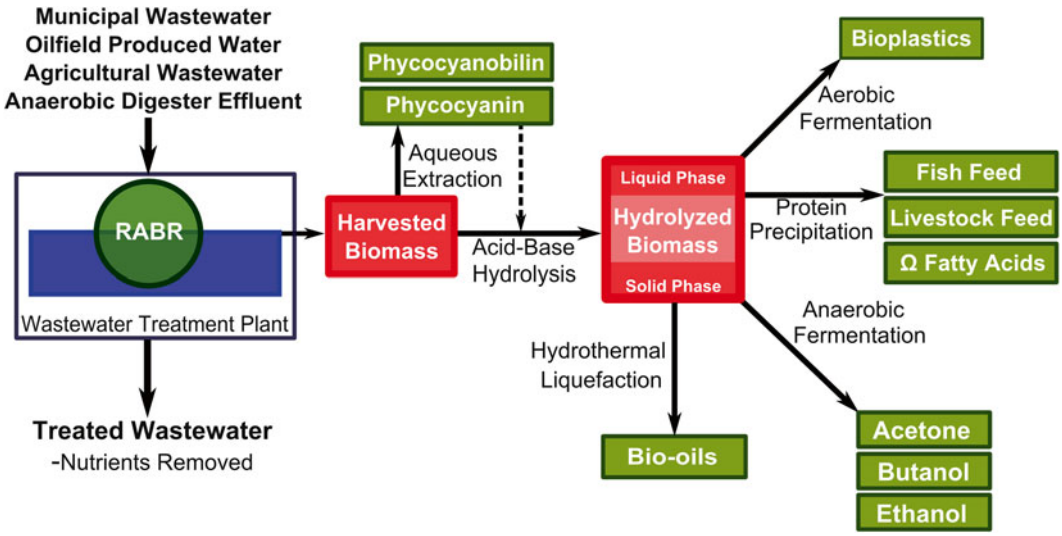

Fig. 3.2 Algal-based biorefinery coupled with wastewater treatment

With regard to conversion to bioproducts after cultivation and harvesting of algal feedstocks, algal biomass may require processing or fractionation into lipids, carbohydrates, and/or proteins before these individual components can be converted or further processed into the desired fuel or product. Logistical challenges include developing and optimizing cost-effective integrated systems for harvesting, collecting, storing, preprocessing, handling, and transporting algae. There is also a focus on developing effective ways to process or fractionate algae biomass directly into different fuel or product precursors (US DOE 2012).

Feedstock quality and conversion specifications for specific types of bioproducts need to be addressed. Current technologies for algal fractionation and product extraction are not commercially viable, scalable, or sustainable. Options to circumvent or improve these processes exist; for example, conversion of whole algal biomass or secretion or direct production of the desired fuel or product in culture can be used, but little data exist on the cost, sustainability, and efficiency of these processes (US DOE 2012). Finally, algal biomass production, harvesting, and processing scenarios will require techno-economic and life cycle analyses to evaluate materials, energy, and economic costs and benefits in order to determine the potential impact nationally with regard to transportation and power supplies.

2 Mathematical Modeling of Algal Biofilm Growth

Mathematical models are commonly used to predict the growth and behavior of microorganisms. One of the key reasons for modeling the growth of microorganisms is that an accurate model can be used to optimize the growth of the organisms (Murphy and Berberoğlu 2011). In order to maximize the growth of an algal biofilm, it is important to control for critical variables such as temperature and light, as well as minimize potential limiting factors, such as insufficient bioavailable nutrients. If biofilms are being grown for wastewater treatment, then an accurate mathematical model is important to ensure sufficient treatment (Polprasert and Agarwalla 1994).

There is a growing amount of available research that addresses different methods for mathematically modeling photosynthetic biofilms (Graba et al. 2014; Wolf et al. 2007). Research on photosynthetic biofilms has typically focused on two different applications. The first application focuses on the kinetics and biochemistry of the biofilm, with the goal of understanding and modeling the growth and internal dynamics of the biofilm (Flora et al. 1995). The second application is on algal biofilms grown under natural environmental conditions and is additionally focused on how the biofilm interacts with the local environment (Son and Fujino 2003). However, research on the modeling of photosynthetic biofilms is far from comprehensive, and there are significant gaps in knowledge including modeling efforts involving the large-scale implementation of biofilms for wastewater treatment purposes.

For both abovementioned applications, there is a consensus that several key variables are central in their influence on the growth of biofilms. Light, temperature, and nutrient availability are crucial for the growth of algal biofilms and thus must be considered in any successful modeling effort. However, there are additional variables which may affect the growth of an algal biofilm and may be incorporated in proposed models depending on the growth conditions and desired model results.

Light is the central variable in the growth of photosynthetic biofilms. Specifically, the wavelengths of light between 400 and 700 nm, commonly called photosynthetic active

radiation (PAR), are used in the photosynthetic process (Graba et al. 2014). Since photosynthetic biofilms are typically under a certain depth of water, ambient PAR may need to be adjusted for its passage through the water column, which is commonly accomplished using the Beer-Lambert equation (Cerucci et al. 2010). Light may also be adjusted for its passage through the biofilm; the topmost algal cells shade the cells deeper in the biofilm and thus limit light available to cells deeper within biofilm. This adjustment is mostly performed using the Beer-Lambert equation and an extinction coefficient (Liehr et al. 1990). The measured light value is typically attenuated using either the Steele equation or the Monod equation (Flora et al. 1995; Murphy and Berberoglu 2014). The premise behind these equations is that there is an optimum light value. Below this optimum value, there is insufficient light, and the biofilm's growth becomes light limited. Above the optimum value, there is too much light, and the biofilm can become photoinhibited (Liehr et al. 1990). Estimates of this optimum value have been found to range between 90 and 700 uE/m^2/s and will differ depending on the biofilm's composition (Liehr et al. 1990).

Temperature is also critical for growth of photosynthetic biofilms. Under controlled environments, such as in a laboratory, the temperature may be held constant and the effects thus excluded from consideration within a model. In an uncontrolled environment, a common method for incorporating temperature changes is to adjust growth rates using a reference temperature and an Arrhenius constant (Son and Fujino 2003).

Nutrients are also essential for the growth of algal biofilms. Most modeling efforts focus on how insufficient quantities of bioavailable carbon, nitrogen, or phosphorus (commonly known as macronutrients) can limit biofilm growth. To attenuate for the effects of nutrient limitation, Monod-style kinetics have traditionally been employed (Liehr et al. 1990). Droop kinetics, which includes the luxury uptake of nutrients by the biofilm, have also recently been applied (Cerucci et al. 2010). The depth of the biofilm can also affect the availability of nutrients. The biofilm itself can act as a diffusional barrier for the passage of ions (nutrients), and thus the flux of nutrient ions through the biofilm may need to be calculated to accurately determine potential nutrient limitation (Flora et al. 1995).

A common measurement of biofilm growth characteristics involves the loss due to shear stress or detachment. Essentially, the biofilm may be lost due to shear stress as a result of the velocity of the growth medium; this loss may be treated as a chronic loss or as the result of a catastrophic event like flooding. Biomass may also be lost when a biofilm loses internal cohesion due to increasing depth of the biofilm and bacterial activity (Graba et al. 2014; Rutherford et al. 2000). The formulas used to calculate this loss vary depending on the model (Graba et al. 2014; Rutherford et al. 2000).

There are a number of organisms which graze on algal biofilms. This is a common consideration when biofilms are grown under natural environmental conditions, but less commonly considered when the biofilms are grown under highly controlled laboratory conditions. Examples of organisms that prey on algal biofilm biomass include snails, nematodes, and the larva of insects such as Chironomidae and Trichoptera. Losses due to predation are calculated as a function of grazer biomass (Graba et al. 2014; Rutherford et al. 2000).

There are additional variables, which may be considered in the growth of algal biofilms. One example is the biochemical interaction between algae and bacteria within the biofilm. Algae produce oxygen as they photosynthesize, which is used by heterotrophic bacteria as they metabolize. Heterotrophs, in turn, produce carbon dioxide as they metabolize, which is taken up during photosynthesis in phototrophic organisms (Wolf et al. 2007). This uptake of carbon dioxide can produce different environmental conditions within the biofilm than those existing externally, which should be taken into account when modeling growth. Removing soluble carbon dioxide from solution increases the pH of the solution in a carbon-buffered environment, which may affect the bioavailability of inorganic nutrients (Liehr et al. 1990). This can lead to an internal nutrient limitation, when externally it appears that there are sufficient nutrients (Liehr et al. 1990).

The substratum the biofilm grows on has also been demonstrated to affect the growth of the biofilm. Although algal biofilms can grow on a wide variety of surfaces, the growth rates have been shown to differ depending on the substratum material (Christenson and Sims 2012). Different algal species also have different biofilm growth characteristics (Graba et al. 2014). Under natural environmental conditions, the biofilms will naturally form a consortium of different species; however, a species or group of species may be dominant and the model based on the growth characteristics of that species (Graba et al. 2014). In summation, there are many variables that potentially affect the growth of algal biofilms; the variables that are specifically considered depend on the growth conditions of the biofilm and the intended results of the model.

3 Culturing Algal Biofilms for Wastewater Treatment

3.1 Municipal and Animal Wastewater Types

Nitrogen (N), phosphorus (P), carbon (C), and silicon (Si), in the case of diatoms, are potential limiting nutrients in mass algal cultivation systems. In contrast, wastewater treatment facilities are strictly regulated to limit the amount of nutrients

(N and P) discharged into the environment because of eutrophication concerns in the receiving waters. As a result, integration of microalgae into a wastewater treatment process takes advantage of the inexpensive source of nutrients for microalgae growth while simultaneously treating the wastewater. Effluents from confined animal feeding operations (CAFOs) usually have higher nutrient and solid concentrations compared to municipal wastewaters, and so the application of an algal biofilm-based system to treat a wide variety of wastewaters, including CAFOs, is desirable. The robustness of the system can be determined from experiments using different nutrient loading rates and environmental parameters such as light; temperature; nutrient forms including ammonia, total kjeldahl nitrogen, nitrate, and nitrite; and pH. Research on algal biofilm-based biotechnology for wastewater treatment can be categorized as follows:

(1) Evaluation of suitability of the wastewater stream as a nutrient source for algal biofilm growth (2) Evaluation of the ability of the technology to meet the required wastewater treatment goals (3) Determination of optimum growth conditions for biomass productivity and/or nutrient uptake (4) Determination and optimization of system processes to favor the production of the desired bioproducts.

3.2 Biomass Productivity and Nutrient Removal Capacity

Bench- and pilot-scale studies have shown that animal (i.e., dairy and swine) and municipal wastewaters can support algal biofilm growth with biomass productivity values ranging from 0.5 to 31 $gm^{-2}d^{-1}$ (Berner et al. 2014; Kesaano and Sims 2014). Similarly, wastewater treatment has also been demonstrated. The main nutrient removal mechanisms are attributed to assimilation of N and P into algal biomass (Kebede-Westhead et al. 2006; Su et al. 2011; Posadas et al. 2013), in addition to ammonia volatilization and phosphorus precipitation resulting from a medium pH increase due to microalgae photosynthesis (Wei et al. 2008). Mulbry et al. (2008) reported that about 70–90 % of the influent N and P was recovered in algal biomass with nutrient loading rates below 1 g TN m^{-2} d^{-1} and 0.15 g TP m^{-2} d^{-1}, respectively, but the recovery rates decreased to 50–80 % at higher loading rates. Different nutrient loading rates have been tested in wastewater-based algal biofilms from 0.11 to 4.53 g N m^{-2} d^{-1} and 0.01 to 0.58 g P m^{-2} d^{-1} for influent N and P, respectively (Wilkie and Mulbry 2002; Kebede-westhead et al. 2003). Biomass productivity, nutrient removal rates, and nutrient (N and P) content of algal biomass generally increase with increasing nutrient loading rates. However, the linear relationship ceases when the algal biofilms reach their maximum nutrient uptake capacity (Boelee et al. 2011). Thick biofilms experience substrate transport limitations in addition to light attenuation, which eventually leads to sloughing (Flora et al. 1993). It is important to note that the nutrient removal capacity of algal biofilms is highly variable and depends on the culture composition, nutrient concentrations, hydraulic retention time, amount of biomass, biofilm growth phase, and nutrient effluent criteria considered. For instance, Boelee et al. (2011) showed that the internal biomass N:P ratios decreased with an increase in loading rates from 23:1 to 11:1 probably due to luxury uptake of P. In addition, the algal biofilm system they studied only met the target effluent values of 2.2 mg L^{-1} N and 0.15 mg L^{-1} P when the loading rates were at most 1 g N $m^{-2}d^{-1}$ and 0.13 g P $m^{-2}d^{-1}$, respectively. Kesaano et al. (2015) also showed that the highest nutrient removal capacity in algal biofilms was observed during the exponential growth phase. Overall, algal biofilms account for approximately 30–100 % nutrient removal efficiencies, with reported nutrient removal rates of 0.07–14.1 g N $m^{-2}d^{-1}$ and 0.013–2.1 g P $m^{-2}d^{-1}$ (Kebede-westhead et al. 2003; De Godos et al. 2009; Christenson and Sims 2012; Zamalloa et al. 2013).

Accordingly, the challenge is not whether algae can grow and subsequently take up nutrients from wastewater, but if the productivity and nutrient removal capacity are sufficient to meet the needs of the biomass end use and/or wastewater treatment goals. There is also a need for more directed research on the fundamental biological and physiological processes (i.e., growth factors, species interactions, mass transport mechanisms, etc.) occurring within wastewater-based algal biofilm communities in order to improve the modeling and predictability, feasibility (in terms of system performance and reliability), sustainability, and scale-up of these systems.

3.3 Industrial Wastewaters

Phycoremediation of industrial wastewaters is a promising field of application for algal biofilm studies. The use of algae for the treatment of industrial wastewaters has the advantages of removal of metals, degradation of organic pollutants, utilization of CO_2 as a carbon source, and the potential for bioproducts generation (Al-rajhia et al. 2012; Vijayakumar 2012). In particular, the ability to generate revenue through bioproduct production as a result of waste stream remediation is of specific value to industry. The coupling of phycoremediation with valuable bioproduct production within a biorefinery framework may increase the economic viability of many proposed industrial waste remediation efforts.

Due to the varied environments provided by industrial effluents, algal strain selections are of key importance. Factors to consider when selecting a unialgal or mixed culture system are geographical location, temperature, light, pH, nutrients, bioproducts of interest, and the potential impacts of culture contamination (Markou and Georgakakis 2011). The natural resiliency of biofilms lends itself to the

Fig. 3.3 Produced water effluent after primary hydrocarbon separations (*left*). Rotating algal biofilm reactors (RABRs) operating in produced water for the production of high-value pigments (*center* and *right*) (Courtesy of the Sustainable Waste-to-Bioproducts Engineering Center (SWBEC), Utah State University)

Table 3.1 Remediation potential of single-strain and mixed culture phototrophic biofilms

Wastewater	Organisms	Target contaminant	Remediation potential	References
Synthetic acid mine drainage	*Ulothrix* sp.	Heavy metals	20–50 %	Orandi et al. (2012)
Synthetic acid mine drainage	*Klebsormidium* sp.	Heavy metals	35–50 %	Orandi and Lewis (2013)
Synthetic sewage	*Scenedesmus obliquus*	Cobalt	94.50 %	Travieso et al. (2002)
Electroplating effluent	*Chlorella sorokiniana*	Nickel(II)	43.1 mg/g biomass	Akhtar et al. (2004)
Modified BG-11	*Phormidium granulatum*	Cadmium(II)	98–99 %	Kumar et al. (2012)
Nutrient medium spiked with diesel oil	*Oscillatoria*, *Phormidium*, *Chroococcus*, and *Burkholderia*	Total petroleum hydrocarbons	94–99 %	Chavan and Mukherji (2010)
Seawater medium of varying salinities	Mixed culture cyanobacterial mat	Petroleum model compounds	<30 %	Abed et al. (2006)
Saline ASN III medium	*Oscillatoria salina*, *Plectonema terebrans*, and *Aphanocapsa* sp.	Crude oil	45–55 %	Raghukumar et al. (2001)

often extreme and unstable nature of many industrial wastewaters. Often, high levels of organic chemicals, total dissolved solids, and heavy metals are present. Cyanobacteria genera, in particular *Oscillatoria*, *Phormidium*, and *Lyngbya*, are often reported in industrial effluents and are especially well suited to flourish in extreme environments (Vijayakumar 2012) (Fig. 3.3).

High concentrations of metals may be found in many industrial wastewaters such as acid mine drainage, oil and natural gas-produced water, and metallurgical wastewaters. The bioremediation of metals by algal biofilms is a potentially low-cost and high-efficiency recovery process (Table 3.1). Algae remediate metal-contaminated wastewaters by uptake, precipitation, and biosorption mechanisms. Immobilized algal biofilms have been shown to remove higher levels of metal contaminants than suspended culture in large part due to increased biosorption (Singh et al. 1989; Vijayakumar 2012). Important factors to consider for algal metal biosorption are pH, temperature, competitive inhibition, and metals recovery. Elevated pH within algal biofilms and their associated exopolymeric matrices increases metal biosorption, as well as precipitation, due to a decreased mass transfer out of the biofilm (Liehr et al. 1994; Parker et al. 2000). However, the biosorption of metal ions in algal cultures decreases dramatically with increased concentrations of "light" metal ions such as Na^{+}, Ca^{2+}, and Mg^{2+} due to their competitive adsorption rates (Fortin et al 2007). Recovery of the metals from the resulting biomass has been achieved by dilute acid washes and by adsorption/desorption cycles of adapted algal strains, but more studies on the ultimate fate and bioproduct utility of the recovered metals and algal biomass residuals are needed (Orandi and Lewis 2013).

Organic chemical contamination, such as hydrocarbons and aromatic compounds, are often found in surface waters that have been exposed to oil and natural gas exploration. Produced water from the oil and natural gas industries is the largest waste stream that is generated by these hydrocarbon recovery activities. Algal biofilm growth and the degradation of organic chemicals in oil- and gas-produced water represent a large area of potential for phototrophic biofilm studies. Organic chemical degradation facilitated by algae has been largely attributed to, and observed in, cyanobacteria-microbial biofilms (Table 3.1) (Edwards and Daniel 1992; Roeselers et al. 2008). This degradation is often initiated by associated bacteria and enhanced through oxic/anoxic diurnal shifts created within the algal biofilm. Shifts in oxygen profile allow for multiple aerobic and anaerobic microbial communities to utilize the organic breakdown products

(Cohen 2002). The supplementation of nitrogen has been shown to enhance the breakdown of hydrocarbons (Coffin et al. 1997). Therefore, heterocyst- and non-heterocyst-forming nitrogen-fixing cyanobacteria may enhance biofilm communities by providing a source of bioavailable nitrogen (Roeselers et al. 2008). This nitrogen-fixing capability may be particularly important in industrial wastewaters where there is a nitrogen limitation and/or significant ammonia volatilization over time. The localized area effect within a biofilm offers an advantage to cyanobacterial biofilms over suspended culture, where the degradation-enhancing benefits of cyanobacteria co-culture with bacteria are generally dispersed at a faster rate.

4 Conclusions

There is an inherent variability in algal biofilm-based systems due to the system designs and operation, growth conditions, and biofilm communities, and as such standardization of parameters across studies is important for universal implementation of these systems. Growing predefined or constructed consortia in set conditions will probably yield more reproducible results, which is desirable for large-scale commercial operations. Due to the widely varying chemical compositions of industrial effluents, characterization, with respect to algal culture, is of great importance to future studies. Additionally, the co-recovery of valuable bioproducts from the remediation process flow will incorporate the economic and sustainability benefits from a biorefinery operation. At field scale, long-term in situ operational studies are needed to evaluate the seasonal impacts of environmental and community fluctuations.

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Biofuel Production Along with Remediation of Sewage Water Through Algae

4

T.V. Ramachandra, Durga Madhab Mahapatra, Sudarshan P. Bhat, and N.V. Joshi

Highlights

- The study reveals that man-made lake systems, Bellandur and Varthur, Bangalore, performed well under higher organic load with a COD removal efficiency of 70 %, TN removal efficiency of 73 % and TP removal efficiency of 22 %.
- Facultative pond-based systems at Mysore were very effective in suspended solid (SS) removal of up to 93 % and BOD removal of up to 82 %.
- The extended aeration-based Sewage Treatment Plant (STP) systems, Bangalore West, were good in terms of SS removal of up to 88 %, COD removal of up to 74 % and BOD removal of up to 63 % but were highly ineffective in nutrient removal.
- The existing large lake systems and the facultative pond systems can be designed for a better photosynthetic yield resulting in higher algal biomass which would not only polish the wastewater but at the same time will act as substrate for lipid/biofuel generation by a novel algal trap mechanism.
- The treatment efficiency analysis showed that the facultative pond-based systems situated at Mysore were the most effective options for the urban wastewater systems compared to the lake-based systems as well as the energy-intensive mechanical systems with their nutrient-integrated treatment efficiency of more than 68 % and the final effluents with an adorable effluent quality for disposal for irrigation.

T.V. Ramachandra (✉)
Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

Centre for Sustainable Technologies [CST], Indian Institute of Science, Bangalore 560012, India

Centre for Infrastructure, Sustainable Transport and Urban Planning [CiSTUP], Indian Institute of Science, Bangalore 560012, India
e-mail: cestvr@ces.iisc.ernet.in; energy@ces.iisc.ernet.in; http://ces.iisc.ernet.in/energy

D.M. Mahapatra
Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

Centre for Sustainable Technologies [CST], Indian Institute of Science, Bangalore 560012, India

S.P. Bhat • N.V. Joshi
Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

1 Introduction

Liquid waste generated in the domestic, commercial, industrial and agricultural sectors comprises organic and inorganic constituents. Wastewater mostly consists of water (~99 %) and solids (~1 %). Numerous treatment processes are being adopted to treat wastewaters depending on the nature, type and extent of contamination. The treatment of wastewater is essential to minimise the contamination of land, water and soil. Treated wastewater can then be reused for various applications that help in reducing freshwater usage. Treatment of wastewater requires an analysis of wastewater characteristics, which helps in finding appropriate technologies pertaining to the region. The quantum of domestic sewage generation has multiplied manifolds due to the rampant development, increased industrialisation and rapid urbanisation. It is estimated that water demand is about 42×10^6 MLD (million litres per day) (National Commission for Integrated Water Resource Development 2010) (NCIWRD 1999) (Fig. 4.1), and about 33.6×10^6 MLD domestic wastewater is generated in India. The urban pockets are the potential generator of wastewaters, i.e. 23,000 MLD, and out of which 5000 MLD is being treated. This necessitates cost-effective sustainable treatment options.

Urbanisation in India since the last century shows an increase in urban population, the number of urban centres

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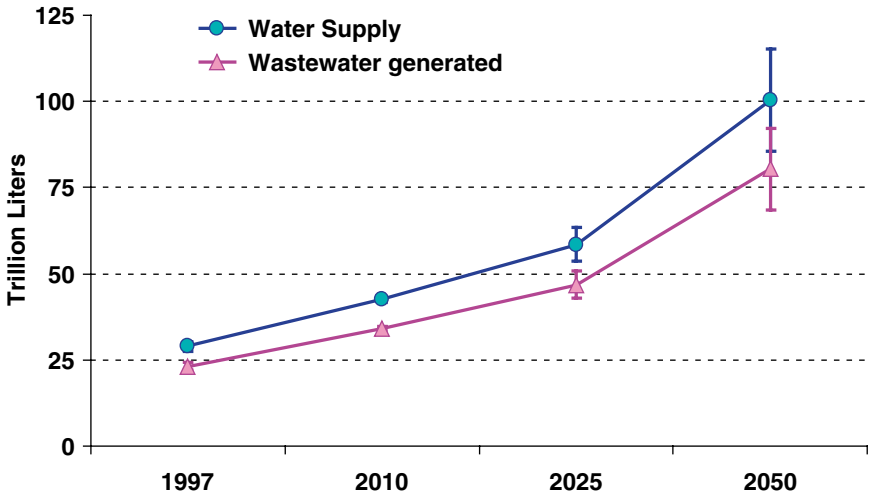

Fig. 4.1 Water supply (NCIWRD 1999) and wastewater generated in India

and a rapid rise after 1951. The number of urban dwellers has risen to 285 million from 25.8 million persons in 1901, highlighting an 11-fold increase in urban population over the period 1901–2001 (CPCB 2011). Due to increased population, substantial volume of domestic wastewater is being disposed into surface water bodies resulting in water pollution and is a consequent threat to the aquatic biota and human health. Indiscriminate disposal of wastewater has contaminated the groundwater and soil adding to the existing miseries of humankind.

The population of India is expected to stabilise at ~1.7 billion people by 2050 (NCIWRD 1999). As per the census of 2001, the urban population is 285 million, and keeping in view of the population projection for the year 2051, it is likely to be of the magnitude of 1093 million. Based on these, wastewater generation in 2051 would be about 132 billion litres per day (CPCB 2011). Hence, there is an urgent need for a proper, feasible, cost-effective (Onkal and Demir 2006) and less energy-intensive wastewater treatment approach to suit the tropical climatic scenario while attaining maximum wastewater treatment in urban centres. However, the associated costs need to be assessed before planning of the treatment plant projects (Asano 1991; Hernandez-Sancho et al. 2011).

A large number of algae grow copiously in wastewater capitalising on available organic carbon and inorganic nutrients (N and P) and also play vital function of remediation by removing N and P (Mahapatra et al. 2013a, b; Mahapatra and Ramachandra 2013; Ramachandra et al. 2013). The use of algae in wastewater treatment has been highly effective with the conventional oxidation (stabilisation) ponds or the suspended algal pond systems such as high-rate algal ponds (Green et al. 1995; Hoffmann 1998; Munoz and Guieysse 2006). In algal systems, photosynthetic algae with O_2 generation help in aeration avoiding the requirement of energy and labour-intensive mechanical aeration. Oxygenation of ponds through algae also aids in bioremediation of organic and inorganic compounds by heterotrophic aerobic bacteria (Oswald et al. 1957). Furthermore, algae-based remediation does not generate additional pollutants and provides an opportunity for efficient recycling of nutrients, while it is an environmentally sound and sustainable option to manage wastewater.

Algae can grow at higher densities in highly concentrated wastewaters (Wang et al. 2009) to secondary treatment wastewaters often also used for tertiary polishing of wastewaters (Oswald et al. 1957). Algae transform wastewater C (organic/inorganic C) into algal biomass C. It has been reported that a substantial amount of this C is also found as lipids in certain wastewater algae (Mahapatra et al. 2013a; Mahapatra and Ramachandra 2013; Ramachandra et al. 2013; Wang et al. 2009). Lipid synthesis in wastewater algae provides additional benefits of algal biofuel development coupled with nutrient removal and wastewater remediation and ensures maximum resource utilisation (Mahapatra et al. 2014). Earlier growth studies by culturing algae in wastewaters have revealed high biomass productivities with reasonable lipid yield (Mahapatra et al. 2013a, 2014; Mahapatra and Ramachandra 2013). Such investigations have also shown high potential of wastewater algae (Mahapatra et al. 2013a) and algal consortia (Mahapatra et al. 2014) for removing C, N and P (Oswald et al. 1957; Wang et al. 2009). This highlights the scope of wastewater-grown algae for biofuel production and as potential alternate energy sources. Experimental studies involving wastewater-grown algae will help in addressing the driving factors to maximise biomass production and subsequent efficient harvesting for optimal lipid extraction.

Algal biomass as a potential biofuel feedstock paves suitable path in exploring potential options to meet the energy shortfalls (Mahapatra et al. 2013a). The dwindling stock of fossil fuels coupled with escalating oil prices and growing concerns towards greenhouse gases due to global warming and consequent changes in the climate has necessitated exploration for viable energy alternatives (Ramachandra et al. 2009). Challenges in algae-based biofuel production are consistent supply of nutrients, harvesting of algae and effective lipid extraction techniques (Shen et al. 2009). The fuel production involves a series of unit processes as algal species selection, mass cultivation, biomass harvesting, biomass concentration, lipid extraction and refining. This entails an understanding of algal downstream processing and process optimisation for its sustainable utilisation and commercial exploitation.

Studies on efficiency of the wastewater treatment plants based on water reuse and alternative source of water resources have used analytical benchmarking method of DEA (Data Envelopment Analysis) (Hernandez-Sancho and Ramon 2009). Provisions are to be made to improvise the pond systems for better treatment efficiency and to improve the effluent quality and hence safeguard drinking water resources. Therefore, detailed studies are required for understanding the mechanism of the pond-based systems to tackle the problem in best possible way which completely reduces the organic and nutrient load and at the same time kills pathogens for its use in agriculture and other activities.

Earlier several studies have investigated the treatment plant efficiencies in many cities of India as New Delhi (Jamwal and Mittal 2010), Indore (Sharma and Dubey 2011), Yamuna basin (Sato et al. 2007), Bangalore West (Kumar et al. 2010) and Mysore (Shakunthala et al. 2010) and in other countries as Algeria (Driche et al. 2008), Greece (Andreadakis et al. 2003), Spain (Colmenarejo et al. 2006), Iran (Sadeghpoor et al. 2009), Mexico (Alcocer et al. 1993), South Africa (Samie et al. 2009), Brazil (da Costa and Medri 2002), etc. These studies however do not address the socio-economic aspects of the treatment systems. The main focus of the present study is to assess lipid and bioremediation potential of algae, which involves:

1. Evaluation of treatment efficiency of the large man-made lake systems with facultative pond-based systems and mechanical STP (using extended aeration and/or activated sludge processes)
2. Scope of biofuel production from algae growing in wastewater-fed lakes and facultative ponds
3. Valuation of the wastewater system considering the capital, environmental and societal aspects
4. Formulating an alternative treatment option for urban cities
5. Ranking/grading the plant performance considering critical parameters based on efficiency criteria

2 Materials and Methods

2.1 Study Area

The city of Bangalore spans over an area of more than 741 km^2 and is one among India's fastest growing city. A current temporal analysis of wetlands, however, indicates a decline of 58 % in Greater Bangalore which can be attributed to intense urbanisation processes. This is evident from a 466 % increase in built-up area from 1973 to 2007 (Ramachandra and Kumar 2008). The undulating topography, featured by a series of valleys radiating from a ridge, forms three major watersheds, namely, the Hebbal Valley, Vrishabhavathi Valley and Koramangala and Challaghatta Valleys that form important drainage courses for the interconnected lake system which carries storm water beyond the city limits (Mahapatra et al. 2013b). Bangalore, being a part of peninsular India, had the tradition of harvesting water through surface water bodies to meet the domestic water requirements in a decentralised way. After independence, the source of water for domestic and industrial purposes in Bangalore is mainly from the Cauvery River and groundwater. Untreated wastewater is let into the storm water drains which progressively converge at the water bodies (Mahapatra et al. 2013b).

The city of Mysore spans over an area of more than 130 km^2 and is a very first urbanising region. It has five major catchment districts. The topography of the city divides into five prominent drainage basins. The city's wastewater is let into three wastewater treatment plants through gravity draws. The northern region is handled by a 30.0 MLD STP (powered by aerators). The south-western regions' wastewater flows to STP (67.65 MLD) which uses organic microbe-mix inocula (OS-1 and OS-2) for treatment before entering Dalavai kere. Further south is the STP of 60 MLD at HD Kote Road working with microbial solutions as inocula to treat the wastewater. All these plants consist of facultative aerated lagoons followed by sedimentation basins. During the study period the treatment pond basically was functioning as an algal pond. The different wastewater treatment plants, their installed capacities and total water supply in Bangalore and Mysore are provided in Tables 4.1 and 4.2, respectively.

Table 4.1 Capacities of sewage treatment plants at Bangalore and Mysore

Sl. no.	Location	Capacity in MLD	Treatment facility
	Bangalore – CWSS stages I, II and III		
1	Vrishabhavathi Valley	180	Secondary: trickling filters
2	K&C Valley I	163	Secondary: activated sludge process
3	Hebbal Valley	60	Secondary: activated sludge process
4	Madivala	4	Secondary: UASB + oxidation ponds + constructed wetlands
5	Kempambudhi[a]	1	Secondary: extended aeration
6	Yelahanka	10	Activated sludge process + filtration + chlorination (tertiary)
	Bangalore – CWSS stage IV, phase I		
7	Mylasandra	75	Secondary: extended aeration
8	Nagasandra	20	Secondary: extended aeration
9	Jakkur	10	Secondary: UASB + extended aeration
10	K.R. Puram	20	Secondary: UASB + extended aeration
11	Kadubeesanahalli	50	Secondary: extended aeration
12	K&C Valley II	30	Secondary: extended aeration
13	K&C Valley III	55	Secondary: CMAS
14	Raja canal	40	Secondary: extended aeration
	Total	**718**	
	Mysore – HWSS stages I, II and III		
1	Kesare Valley	30	Secondary: alternate extended aeration
2	Vidyaranyapuram	67.5	Secondary: pond-based fermentative bacteria (OS)
3	Rayankere	60	Secondary: pond-based microbe solution (Fermenta)
	Total	**157.5**	

[a]Under progress

Table 4.2 Total water supply to Bangalore and Mysore cities

Sl. no.	Projects	Supply capacity (MLD)
	Bangalore (CWSS)	
1	Arkavathy (TG Halli)	60
2	Cauvery stage I	135
3	Cauvery stage II	135
4	Cauvery stage III	300
5	Cauvery stage IV, phase I	270
6	Cauvery stage IV, phase II*	(270)
7	Cauvery stage IV, phase III*	(500)
		900 (1670)
	Mysore (HWSS)	
1	Hongally WSS (stage I & II),	30
2	Hongally WSS (stage III),	45
3	Belagola WSS	43.5
4	Melapur WSS	41.5
		160

*in progress

1. Man-made lake systems: Varthur and Bellandur Lakes (~500 MLD), Bangalore South
2. Mechanical treatment systems: aeration and activated sludge (75 MLD), Bangalore
3. Facultative pond-based systems: STP (67.5 MLD), Mysore

2.2 Field Sampling and Laboratory Analysis

Field samplings of influent (1), middle (2) and effluent (3) water samples were carried out between March and August 2011 (Figs. 4.2 and 4.3). The qualities of influents and effluents were assessed to determine the efficiency of the treatment plant.

On-site analysis was performed to record pH, water temperature, electrical conductivity (APHA method 205), oxidation-reduction potential (ORP), total dissolved solids (TDS), salinity, dissolved oxygen (DO), dissolved free carbon dioxide (free CO_2) and turbidity using the standard methods. One litre subsample was analysed according to standard methods (American Public Health Association) (Public Health Association) AWWA WEF 1995): total biochemical oxygen demand over 5 days (BOD_5) (APHA method 507), filtered BOD_5; chemical oxygen demand (COD) (APHA, 5220 C); suspended solids (SS) (APHA method 209c); SAR (APHA 1206); turbidity (Hach turbidimeter, APHA method 214a); ammoniacal nitrogen (APHA method 417a); total nitrogen (TN) (APHA method 420a, total nitrogen) and phosphates (APHA, 4500-P D). In addition, visual clarity or transparency of the lake/STP wastewater was measured with a Secchi disc. Algal species collected from the lake systems and facultative ponds (both

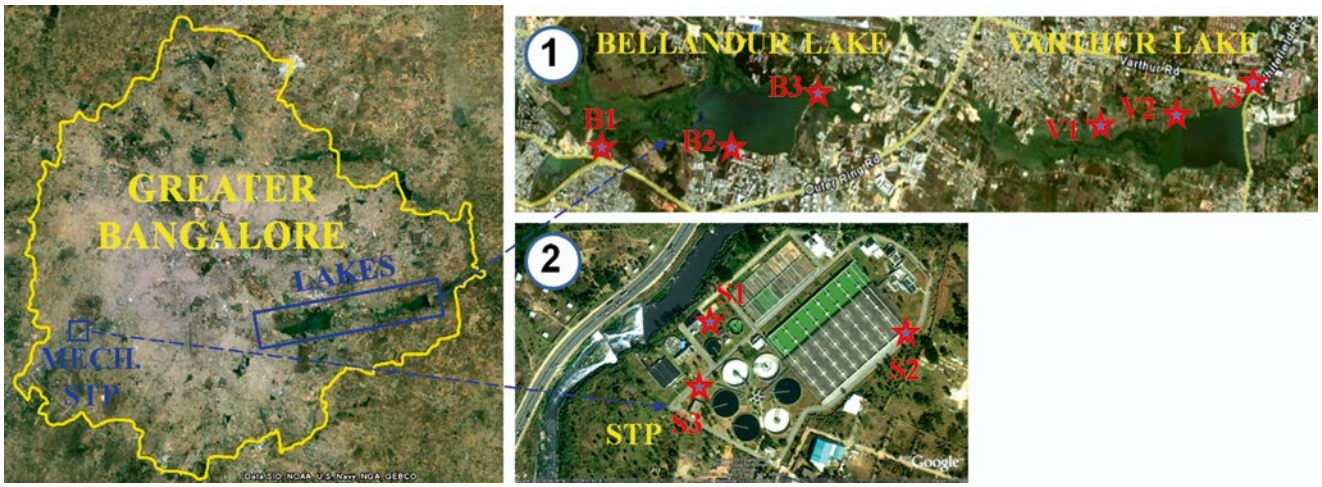

Fig. 4.2 Greater Bangalore: (*1*) lake systems and (*2*) sewage treatment plant

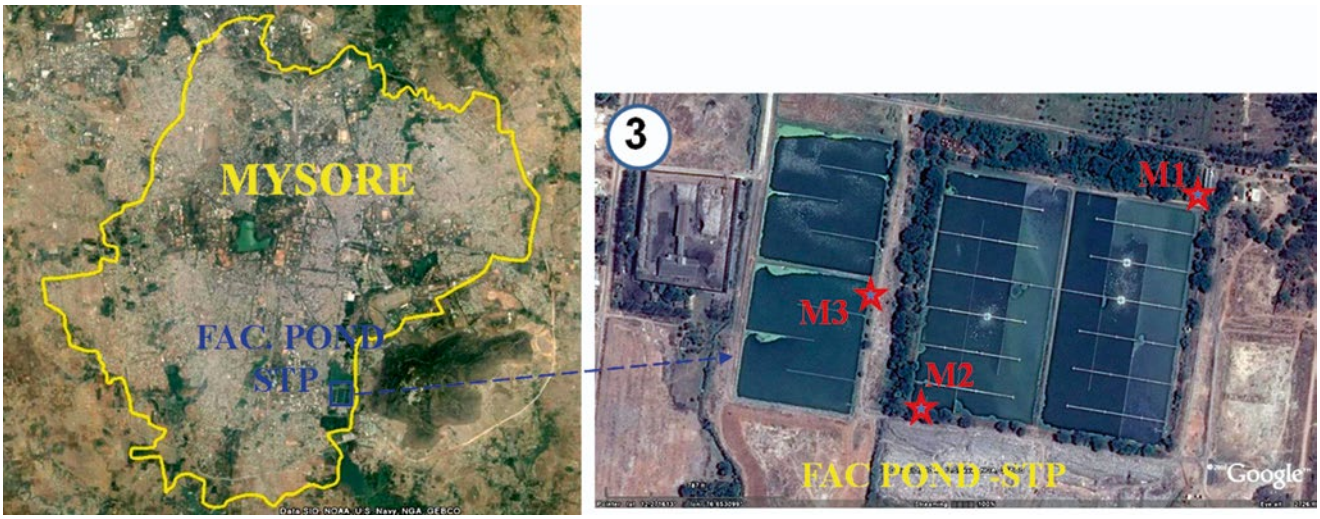

Fig. 4.3 Mysore: (*3*) facultative pond-based STP

unialgal and as algal consortia) were cultured and evaluated for their biofuel potential. Lipid quantification (gravimetry), extraction (cell disruption, solvent treatment, thin-layer chromatography and solvent separation) and FAME composition were conducted following earlier studies (Mahapatra et al. 2013a).

2.3 Process Description

2.3.1 Lake Systems

Lake systems were initially constructed for storage of water to meet the local drinking and other domestic purposes. But, with time, the water yield in the catchment has declined due to the unplanned urbanisation-driven rapid land use changes and also results in the generation of large quantum of wastewater. The series of interconnected lakes convey the city's wastewater. Consequently, these water bodies act as man-made lagoons performing primary, secondary and tertiary treatment due to the interaction of biotic and abiotic components with sufficient detention time (Mahapatra et al. 2011a, b). These water bodies are shallow, and the wind causes a great deal of turbulence aiding oxygen diffusion for adequate aeration and mixing. This process is further supplemented by rapid generation of dissolved oxygen by algal photosynthesis (Mahapatra et al. 2011b). Despite having a massive daily wastewater load of ~500 MLD, the prolific algal growth aided in the uptake of nutrients from these wastewaters further improvising the system's efficiency. Hydraulic retention time (HRT) for Bellandur Lake is ~7–8 d (Fig. 4.4) and Varthur Lake is ~5 d (Fig. 4.5), respectively.

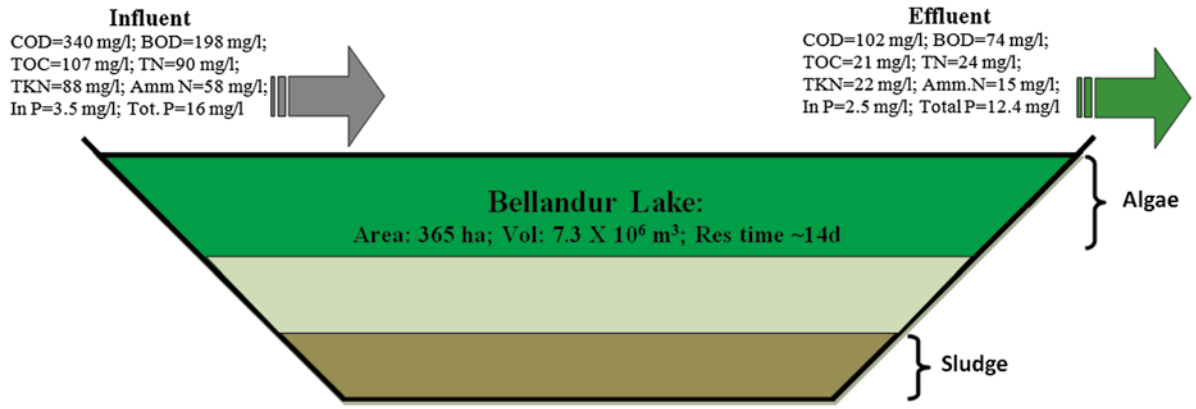

Fig. 4.4 Inflow and outflow process parameters at Bellandur Lake, Bangalore

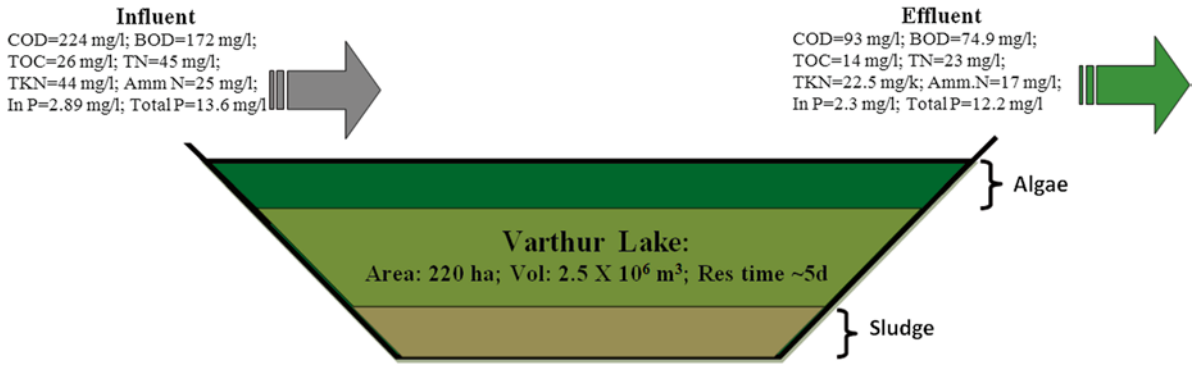

Fig. 4.5 Inflow and outflow process parameters at Varthur Lake, Bangalore

2.3.2 Facultative Ponds

The facultative treatment pond system consists of facultative and maturation ponds (Fig. 4.6). The organic matter is primarily removed in the initial anaerobic zone of the facultative pond. Subsequently algal growth takes place owing to availability of nutrients such as N and P. The high algal growth in the facultative ponds helps in suspended solid (SS) and carbon (C) removal. Maturation pond is meant for pathogen removal, nutrient balance and more importantly removal of suspended solids (stabilisation) (Mahapatra et al. 2013b) together with the removal of a little quantity of BOD. The HRT depending upon feed fluctuations for the initial facultative ponds and maturation ponds is ~3–9 d and ~2–6 d, respectively.

2.3.3 Mechanically Aerated Systems

The mechanically aerated systems comprise of a screen and a grit chamber installed before the aeration and activated sludge systems (Fig. 4.7). Raw wastewater passes through the screen and grit chamber to the aeration tanks (powered by surface aerators) and gets treated by activated sludge. These are continuously fed with recirculation sludge from the secondary clarifiers. The secondary clarifiers help in water detention as well as acts as a storage tank for the inoculum to be regularly supplied to the inflow wastewater. These extended aeration systems are designed with a depth of 4–6 m for a hydraulic retention time (HRT) of ~8 h.

2.4 Comparative Valuation of Treatment Systems: Economic, Environmental and Social Aspects

The total annual cost was calculated which essentially comprised of construction (infrastructure), operation and maintenance (O&M) and land costs (Sato et al. 2007). Infrastructure cost includes procurement of screen, grit chamber construction, wastewater treatment infrastructure, sludge treatment infrastructure, etc. at STPs. O&M costs include expenses on human resources (wages), energy requirement/power, repair and essential chemicals. Rs. 4.8/kWh was taken as the cost involved in power consumption. The wear and tear involved the repair costs for infrastructure/civil structures and mechanical and electrical equipments that are estimated annually as a certain factor/percentage multiplied by total construction cost. The annual repair cost for civil work was calculated at 0.4 % and 0.15 % of the capital cost for aeration with activated sludge processes (ASP) and pond processes, respectively. Likewise, the annual repair cost for mechanical and electrical equipment was calculated at 3 % of the total

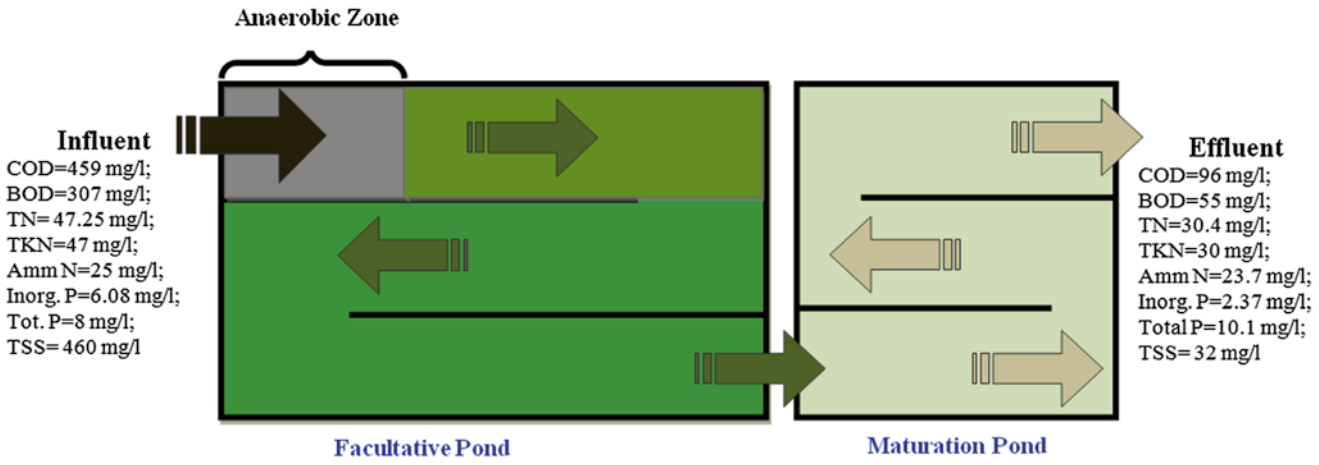

Fig. 4.6 Inflow and outflow process parameters at facultative pond, Mysore

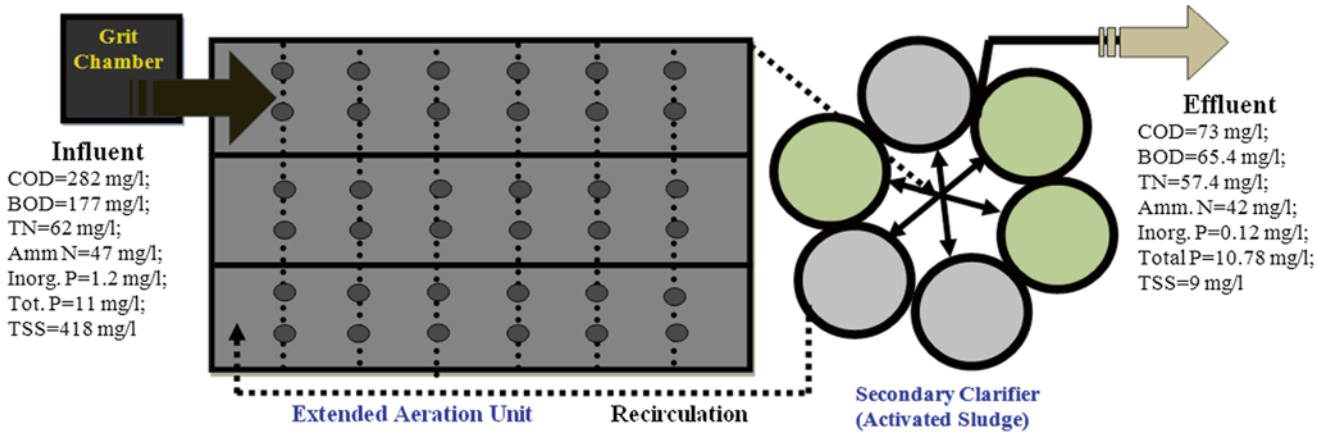

Fig. 4.7 Inflow and outflow process parameters at aeration and activated sludge STP, Bangalore

construction cost. In addition, it was assumed that mechanical and electrical equipment requires replacement every 6 years. This replacement cost was included in the repair cost. The service life of STP was estimated at 30 years, which served as the period for life cycle cost (JICA 2005). The total annual cost was calculated by Eq. (4.1):

$$AC_{TOTAL} = RF_{CAPITAL} \quad IC_{TOTAL} + OMC \tag{4.1}$$

where AC_{TOTAL} is the total annual cost, $RF_{CAPITAL}$ the capital recovery factor, IC_{TOTAL} the initial cost (e.g. for construction, land) and *OMC* the operation and maintenance cost (e.g. human resource, power/energy, repair, chemicals).

3 Results and Discussions

3.1 Water Allocation and Wastewater Generation

Bangalore city receives water of about 900 MLD from the Cauvery and about 600 MLD from groundwater. Out of this, wastewaters account to ~1200 MLD. The existing sewage treatment plants at Bangalore (under CWSS I–IV) handles only half of the wastewaters generated (718 MLD) with a modest treatment level, and the rest of the wastewater generated is directed to several lakes and streams. Many of these plants are running at half the installed plant capacity with partial treatment. Such circumstances and associated reasons make it imperative to find a less energy-intensive, cost-effective and sustainable wastewater treatment system for Bangalore to handle the city's wastewaters and treat to acceptable levels. The existing lake systems are playing a major role in treatment of these wastewaters up to satisfactory levels by virtue of its high detention time with the interplay of biotic and abiotic factors (Mahapatra et al. 2011a, b, c). The study of the functional aspects of the interactions between biotic and abiotic elements (Mahapatra et al. 2011a, b, c) in the lake systems has provided the basic understanding to design and formulate an optimal open algal pond treatment system which works on its own without much cost and maintenance to treat huge quantities of wastewaters.

In the case of Mysore, the water supply accounts to 160 MLD, and about 95 MLD is drawn from groundwater sources which sums up to 255 MLD. Assuming 80 % of the original

water supply being wastewaters, the total wastewater generation in Mysore city hovers to ~204 MLD. Wastewater treatment plants in the city have a net capacity of treating 157.5 MLD indicating a shortfall for sewage treatment for another 50 MLD. The treatment plants were insufficiently running to its intended installed capacity resulting in voluminous untreated and partially treated water that finally joins the rivers Cauvery 12 km and Kapila 40 km downstream and is thus a matter of grave concern for the future water requirement and its security.

3.2 Raw Sewage Characteristics

Table 4.3 lists the raw wastewater characteristics and features and is comparable to domestic wastewater. Higher biodegradability of the wastewater is indicated by the BOD_5/COD ratio of 0.60. A meagre 50 MLD is treated up to secondary levels through aeration and activated sludge STP at Bangalore (installed for 75 MLD) compared to the Bellandur and Varthur lake systems that receive an enormous volume of >500 MLD treating the wastewater up to secondary levels by virtue of its detention time and algal nutrient uptake activity. The sewage treatment farm with facultative pond system at Mysore has been designed to treat 67.5 MLD, but it works at less than half of its installed capacity (~30 MLD) during the study period.

Table 4.3 Physico-chemical characterisation of raw sewage

Parameters	Mean	± Standard deviation
pH	7.73	0.20
Temperature (°C)	25.70	2.68
Elec. conductivity (µS/cm)	1013.80	277.70
Total dissolved solids (mg/l)	774.33	156.02
Total suspended solids (mg/l)	328.00	56.00
Turbidity (NTU)	229.23	42.84
Dissolved oxygen (mg/l)	0.22	0.35
Free CO_2 (mg/l)	48.59	19.48
COD (mg/l)	248.50	52.48
BOD (mg/l)	152.58	31.79
Nitrates (mg/l)	0.63	0.50
Ammonia (mg/l)	72.00	16.00
TKN (mg/l)	88.00	8.00
Phosphates (mg/l)	2.34	0.76
Total phosphates (mg/l)	10.20	4.60
Alkalinity (mg/l)	380.00	83.90
Total hardness (mg/l)	296.00	108.69
Calcium (mg/l)	72.00	12.00
Magnesium (mg/l)	28.00	6.60
Chloride (mg/l)	147.16	146.53
Sodium (mg/l)	254.02	420.04
Potassium (mg/l)	26.75	29.01
ORP (mV)	−121.33	85.91

Fermentative bacteria (organic solutions) are fed to this plant as inoculum which helps in COD/BOD, TSS and odour removal. Considering the population of both the cities, the average per capita water usage is considered as 120 l/d. The quantity of BOD produced was estimated at 18.2 g/d/person, and the amount of TSS was estimated at 39.36 g/d/person.

3.3 Biofuel Prospects

The algae abundant in facultative ponds and lakes showed total lipid contents of ~25–28 % (w/w). The morphological and cell surface studies (through electron microscopy) of euglenoids from these facultative ponds showed the presence of nano-lipid channels (pores) across the stria pattern that possibly help in cellular lipid secretions periodically under stress conditions (Mahapatra et al. 2013c). The Raman spectroscopic analysis with a confocal attachment of algal lipids in vivo revealed clusters of lipid scattered across the cell cytoplasm that were associated with pigments as chlorophyll and carotenoids (Mahapatra et al. 2013d). The unialgal species showed high unsaturated fatty acids (~52 %) compared to the algal consortia grown in batch mode that showed ~24 % unsaturated fatty acids. The order of the dominant fatty acids in the unialgal species is palmitic acid C16:0 (42 %) > linoleic acid C18:2 (22 %) > linolenic acid C18:3 (23 %) > stearic acid C18:0 (4 %) (Table 4.4) (Mahapatra et al. 2013a). The unialgal species showed higher polyunsaturated fatty acids (~47 %) compared to monounsaturated ones (~4 %).

The algal consortia tested in laboratory mostly comprising of euglenoids and members of Chlorophyceae showed ~76 % of saturated fatty acids compared to unialgal species. In contrast to unialgal species, monounsaturated fatty acids were high (~14 %) compared to polyunsaturated ones. The order of dominant FAME is given by palmitic acid C16:0 (42 %) > stearic acid C18:0 (~26 %) > oleic acid C18:1 (~11 %) > linoleic acid C18:2 (~5 %) (Table 4.4). In both unialgal and consortia of algae, the percentage of C16–C18 (desirable fatty acids from a biodiesel perspective) is >90 %. The FAME gas chromatograms of unialgal species and wastewater algal consortia cultured in laboratory (Mahapatra et al. 2014) reactors are elucidated in Fig. 4.8. Such algae have been also tested for its growth, nutrient removal and lipid production in continuous culture systems that yielded high biomass density close to 1 g/l with good lipid productivities ~50 mg/l/d with high P stocks (2–3 %) in algal cells (Mahapatra et al. 2013e) and thus can create new avenues in decentralised wastewater treatment and energy generation. During such continuous operations these mixed algal species form flocs, which float on the surface of the bioreactor (in the final stages) enabling easier harvesting and concentrating algae for biofuel production.

Table 4.4 FAME composition of algal lipids Mahapatra et al. (2013a, 2014)

No.	FAME (chemical name)	Chemical formula	RT	% FAME	
				Unialgal	Consortia
1	Butanedioic acid, dimethyl ester	C6:0	7.49	–	2.76
2	Dodecanoic acid, methyl ester	C12:0	19.023	0.04	–
3	Methyl tetradecanoate	C14:0	24.92	1.40	0.84
4	Tetradecanoic acid, 12-methyl ester	C14:0–12 CH_3	26.91	–	1.61
5	Pentadecanoic acid, methyl ester	C15:0	28.529	0.11	–
6	Methyl 4,7,10,13-hexadecatetraenoate	C16:4(4,7,10,13)	29.99	–	1.90
7	7,10-Hexadecadienoic acid, methyl ester	C16:2(7,10)	30.27	–	1.14
8	7,10,13-Hexadectrienoic acid, methyl ester	C16:3(7,10,13)	30.47	–	1.67
9	7-Hexadecenoic acid, methyl ester	C16:1(7)	30.66	–	1.38
10	9-Hexadecenoic acid, methyl ester	C16:1 (9)	31.038	3.36	–
11	Hexadecanoic acid, methyl ester	C16:0	31.61	42.05	42.30
12	Heptadecanoic acid, methyl ester	C17:0	34.42	0.11	0.95
13	8,11,14-Eicosatrienoic acid, methyl ester	C18:3(8,11,14)	35.92	–	0.05
14	Methyl octadecatetraenoate	C18:4(6,9,12,15)	35.94	–	0.30
15	6,9,12-Octadecatrienoic acid, methyl ester	C18:3(6, 9,12)	36.352	1.47	–
16	9,12-Octadecadienoic acid, methyl ester	C18:2(9,12)	36.49	22.22	5.27
17	9-Octadecadienoic acid, (Z)-methyl ester	C18:1(9)	36.74	–	10.87
18	11-Octadecadienoic acid, methyl ester	C18:1(11)	36.84	1.09	1.42
19	9,12,15-Octadecatrienoic acid, methyl ester	C18:3(9,12,15)	37.131	22.98	–
20	Octadecanoic acid, methyl ester	C18:0	37.63	3.68	25.69
21	Eicosanoic acid, methyl ester	C20:0	43.08	0.18	0.42
22	Docosanoic acid, methyl ester	C22:0	48.39	–	0.38
23	Tetracosanoic acid, methyl ester	C24:0	51.69	1.31	0.36
24	Hexadecanoic acid, methyl ester	C26:0	53.80	–	0.38
25	Octacosanoic acid, methyl ester	C28:0	55.52	–	0.32
	Saturated FA			48.89	76.00
	Unsaturated FA			51.11	24.00
	Monounsaturated FA			4.45	13.67
	Polyunsaturated FA			46.66	10.33
	Unsaturated to saturated FA ratio			1.05	0.32
	C16-C18 FA			96.95	91.04
	Total lipid content (%)			24.60	28.50

More than 1200 MLD of domestic wastewater is generated in Bangalore city (Mahapatra et al. 2011a). There is a scope of resource recovery from the nutrient-laden waters in the city (Chanakya and Sharatchandra 2008; Chanakya et al. 2013; Ramachandra and Mahapatra 2012). The algae-rich water bodies (urban algal ponds) help in 70–80 % nutrient and ~90 % C removal from influent wastewaters (Mahapatra et al. 2011a). These algae could be harvested for use as biofuels. Algae proliferate due to nutrient enrichment in the water bodies and subsequently die after the growth cycle. Apparently the organic matter, dead algal matter and other debris in the lower strata of these water bodies decompose and critically reduce dissolved oxygen (DO) rendering the system anaerobic favouring GHG emissions.

The present algal biomass productivity in the wastewater-fed algal reactor systems tested in the laboratory was ~0.1 g/l/d. It is reported that species like *Euglena* can potentially produce 6.52 tonnes of crude lipid per hectare per annum (Ramachandra et al. 2013). If algal ponds are used for treating the entire wastewater generated in Bangalore, then an average ~120 tonnes of algal biomass can be generated every day. If only 50 % of the biomass is harvested, with an average lipid content of ~25 % (as per present experiment), then daily 15 tonnes of crude lipid could be produced. As per earlier experiments 20–30 % of the crude lipids are TAG; therefore, ~3.75 tonnes/day of biodiesel can be generated that yields ~1125 tonnes of biodiesel/annum. Earlier studies have shown a reasonably high calorific value of the dried wastewater algae (heat value ~18 MJ/kg) showing the potential of whole cell algae for energy generation without cell disruption and expensive solvent treatments (Mahapatra et al. 2013f). After lipid extraction, the spent biomass that is left

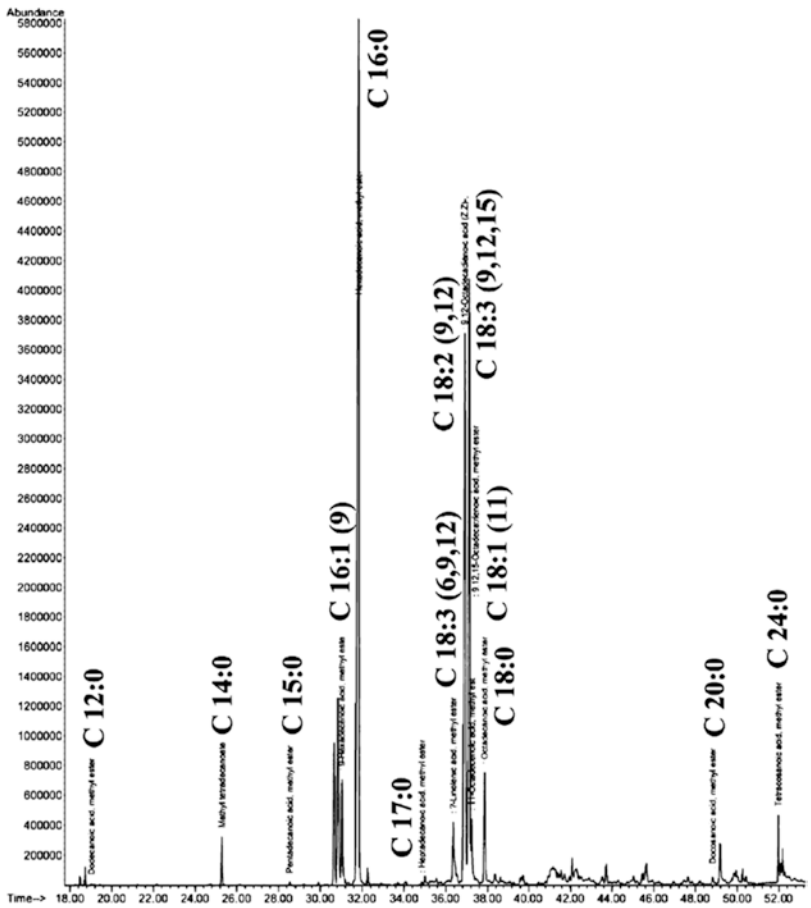

Fig. 4.8 Gas chromatogram of FAME mix from *Euglena* sp. (Mahapatra et al. 2013a)

out mainly comprising of carbohydrates and proteins can be reutilised for energy generation via fermentation yielding bioethanol (heat value ~30 MJ/kg) or pyrolysis producing liquid crude (heat value >30 MJ/kg). In the due course the sludge produced in these algal bioreactors could be used for biogas generation (heat value ~21 MJ/m^3), and the slurry left behind after bio-methanation can be used as potential manure (Chanakya et al. 2012).

3.4 Treatment Plant Efficiency

The treatment of wastewater has been assessed through conventional water quality parameters: COD, BOD, TSS, TDS, P and N. The differences at inlet and at outlets reveal the efficiencies of the respective treatment. The aeration-based mechanical treatment systems showed high removal of COD (74 %) and BOD (63 %; Fig. 4.9) but have low removal efficiency of total nitrogen (TN) (~8 %; Fig. 4.10) and P (~2 %; Fig. 4.11). TSS removal is about ~88 % (Fig. 4.12). These results are tabulated in Table 4.5.

Facultative pond-based treatment in Mysore has higher removal efficiency of BOD (82 %; Fig. 4.9) and TSS (93 %; Fig. 4.12) compared to N (36 %; Fig. 4.10) and almost no removal of P (Fig. 4.11). In the case of pond-based systems, the removal efficiencies are dependent on its working/operations, i.e. aerobically or facultatively (Table 4.5). As per earlier reports, the aerobic ponds have detention times of ~3–10 days and facultative ponds, 5–30 days (Reed et al. 1995). Longer retention in facultative ponds aids in the higher removal of N and pathogens (Water Environment Federation/American Society of Civil Engineers (WEF/ASCE) 1992; Mara 1997; Oakley 2005).

Earlier studies have reported high removal efficiency of BOD (90–95 %), TSS (90–95 %) and faecal coliforms (92–99.99 %) and lower removal of TP (10–20 %) and TN (15–25 %) in the mechanical treatment systems. The stabilisation/facultative pond-based treatment has comparably high removal efficiency of TSS (90–95 %) and faecal coliforms (90–99.90 %) but has medium to high removal efficiency of BOD (75–95 %) and low to medium removal efficiency of P (10–50 %) and TN (10–60 %) (Gilbert 1976; Pettygrove and Asano 1984; Palme et al. 2005; Hannah et al. 1986; Reed 1991; Reed et al. 1995; United Nations Environment Programme 1997; US 2002; Metcalf and Eddy 2003) as shown in Table 4.6.

The large lake-based system showed a moderate removal efficiency of COD (70 %; Fig. 4.9) and BOD (62 %; Fig. 4.9)

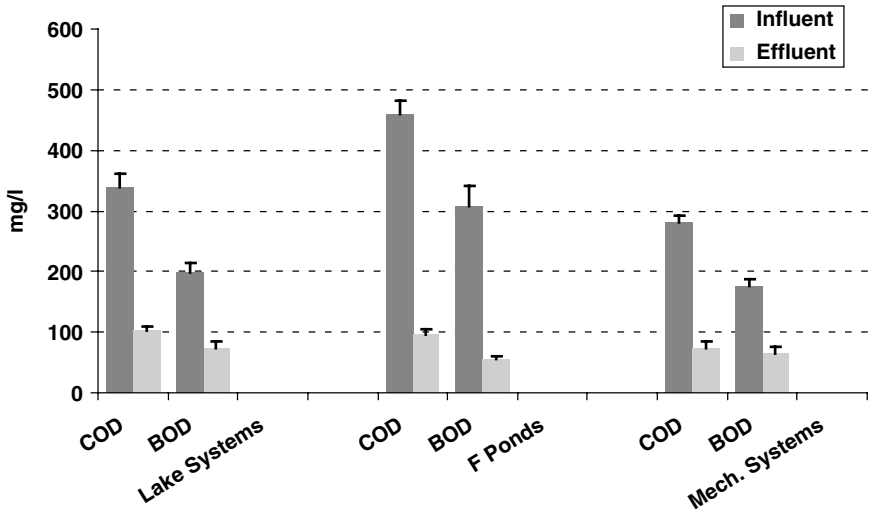

Fig. 4.9 COD and BOD removal in the three systems

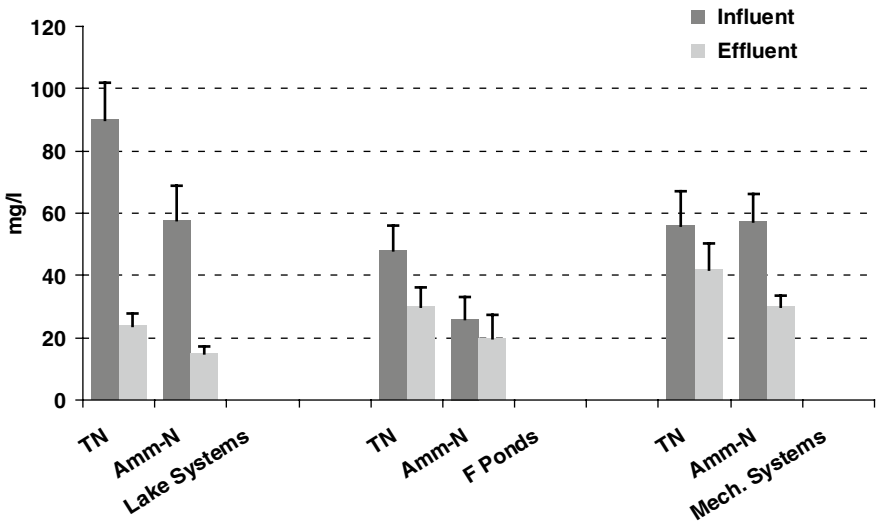

Fig. 4.10 TN and ammonia N removal in the three systems

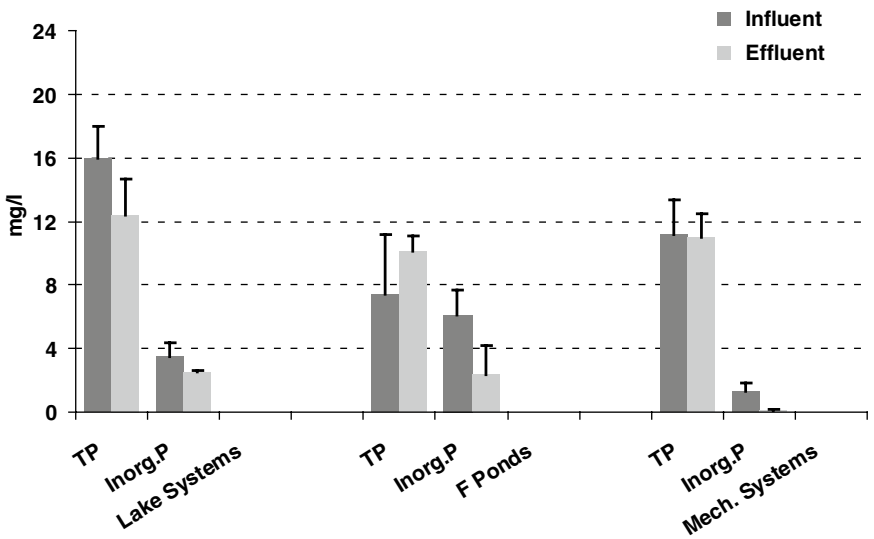

Fig. 4.11 TP and inorganic P removal in the three systems

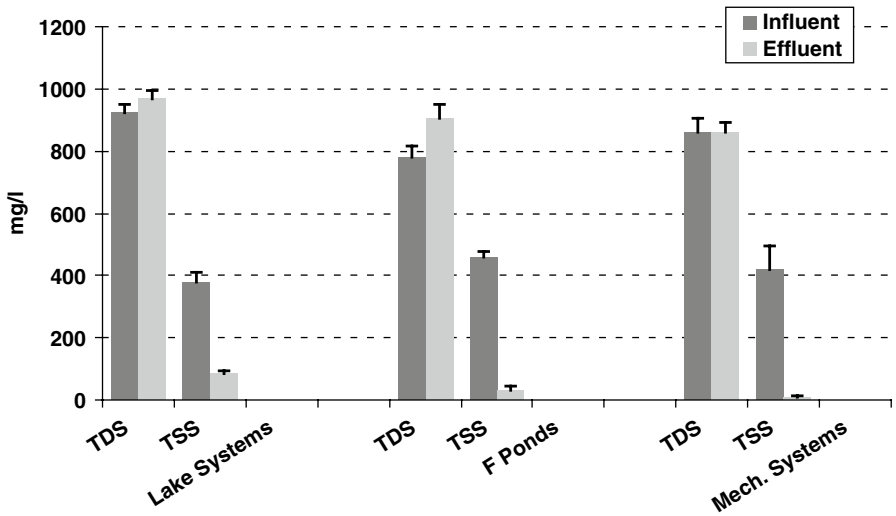

Fig. 4.12 TDS and TSS removal in the three systems

Table 4.5 A comparative analysis of the removal efficiencies (treatment parameters)

	Lake-based systems						Facultative pond			Mechanical aeration		
	Bellandur			Varthur			Mysore			Bangalore		
Parameters	Infl.	Effl.	%Rem	Infl.	Effl.	%Rem	Infl.	Effl.	%Rem	Infl.	Effl.	%Rem
COD (mg/l)	340	102	70	224	93	58.5	458.7	96	79.07	282	73	74
BOD (mg/l)	198	74	62	172	74.9	56.4	307	55.1	82.02	177	65.4	63
TSS (mg/l)	380	83.6	78	288	79	72.5	460	32	93.4	418	9	98
Turbidity (NTU)	386	71.2	81.5	325	65	80	326	16	95	329	6.58	98
TN (mg/l)	90	24	73	45	23	48	47.25	30.4	35.7	62	57.4	8
Ammonia N (mg/l)	58	15	74.1	25	17	32	25.67	20.99	18.2	42	29.8	29
Nitrates (mg/l)	0.72	0.88	–22	0.64	0.62	3	0.202	0.015	92.5	1.05	0.24	77
Inorganic P (mg/l)	3.5	2.5	28	2.89	2.3	20.4	6.08	2.37	61	1.28	0.126	90
Total P(mg/l)	16	12.4	22.5	13.6	12.2	10.2	7.36	10.12	–37.5	11.2	10.99	2

with a comparably high removal of N (73 %; Fig. 4.10) and TSS (78 %; Fig. 4.12). The characteristics of the effluent normally indicate treatment efficacy and also the nature of discharge options for potential reuse.

3.5 Valuation of Sewage Treatment Systems

3.5.1 Economic Evaluation

Figure 4.13 compares initial investment costs of the existing treatment systems in Indian cities. These analyses are based on the cost required to treat million litres per day (MLD). The analysis showed higher initial and O&M costs for mechanical systems with ~100 lakhs/MLD and ~6.8 lakhs/MLD/annum compared to pond-based systems with ~40 lakhs/MLD and ~1 lakhs/MLD/annum, respectively. The mechanical treatment systems are either suspended growth processes or attached growth processes. The suspended growth processes mainly involve two techniques as extended aeration and activated sludge. The former operates at lower organic loading and high cell retention time. However, the activated sludge process is conducive for small populations as a large bioreactor volume is required to attain high cell retention. The operating and process costs of both the techniques are influenced by the SS removal capacity and the age of the treatment unit. In addition to this in the case of activated sludge technique, the costs are related to the removal efficacy of organics.

The attached growth processes are pronounced for small populations. During the bacterial bed processes, the O&M costs are determined by the age of the treatment unit alone. However, the other variants of the attached systems as biodisc and peat bed techniques (Hernandez-Sancho et al. 2011) require material replacement at very frequent intervals, so the plant age becomes insignificant, and the SS removal efficiency decides the O&M costs of the treatment unit.

The final stages of the treatment involve various techniques for tertiary treatment as membrane bioreactors, ultrafiltration, microfiltration, ferric chloride-polyelectrolyte

Table 4.6 Comparative analysis of the STP treatment techniques with nutrient removal efficiencies

No.	Place (STPs)	Treatment approaches	Effective approach	% Removal efficiency	References
1	Ixtapan de la Sal, Mexico	[High-rate clarifier + anaerobic pond + facultative pond + maturation pond]; [anaerobic pond + facultative pond (2) + maturation pond]	[High-rate clarifier + anaerobic pond + facultative pond + maturation pond]	84 % BOD	Alcocer et al. (1993)
				70 % COD	
				45 % TS	
				55 % SS	
				43 % TDS	
				97 % F. coli	
2	Yamuna river basin, India (15 STPs)	UASB and facultative pond units	Facultative ponds	67 % COD	Sato et al. (2007)
				70 % BOD	
				−14 % $N\text{-}NH_3$	
				36 % TSS	
				8 % F. coli	
3	Mpumalanga, South Africa (14 STPs)	Ponds; activated sludge; [ponds + trickling filters]; [anaerobic digestion + trickling filters + maturation pond]; [activated sludge + ponds]; [activated sludge + ponds]; [tricking filter + activated sludge]; and [trickling filters + activated sludge + ponds]	[Activated sludge + ponds]	99 % F. coli	Samie et al. (2009)
4	Santa Catarina, Brazil (swine wastewater)	Stabilisation ponds	Aerobic facultative pond	83 % COD	da Costa and Medri (2002)
				85 % $N\text{-}NH_3$	
				65 % $P\text{-}PO_4$	
5	Mysore, India (3 STPs)	[Alternate aeration + maturation pond]; [pond-based fermentative bacteria]; [facultative microbes and stabilisation ponds]	Facultative ponds	82 % COD	Shakunthala et al. (2010)
				89 % BOD	
				87 % SS	
				9 % TDS	
6	Bangalore, India (2 STPs)	Extended aeration; high-rate aeration with biofilters	Extended aeration	28 % TS	Kumar et al. (2010)
				99 % TSS	
				97 % BOD	
7	New Delhi, India (16 STPs)	Activated sludge, extended aeration, trickling filters, high-rate aeration, oxidation pond, BIOFORE (physical, chemical and biological removal treatment)	Extended aeration, oxidation pond and BIOFORE	99 % F. coli99% BOD	Jamwal and Mittal (2010)
				96 % COD	
				68%TKM	
				99 % turbidity	
8	(a) Varthur and Bellandur Lakes, Bangalore India	Man-made lakes: huge open lagoons [Activated sludge + extended aeration + clarifier] [Facultative pond + maturation pond]	Facultative pond-based systems	79 % COD	Present study
	(b) Activated | sludge (aeration) STP, Bangalore			82 % BOD	
	(c) Facultative pond, Mysore, India			93 % SS	
				36 % TN	
				18 % $N\text{-}NH_3$	
				95 % turbidity	
				99 % bacteria	

F Coli, faecal coliform

addition, reverse osmosis, etc. These unit processes help in achieving a better water quality at the end of the chains if the treatment cascades. In such cases the O&M costs are directly influenced by the organic content left in the system and nutrient (N and P) removal efficiencies. The man-made lakes are almost free treatment units with the minimum cost. Operation and maintenance (O&M) costs associated with wastewater treatment comprise manpower and energy/power and replacement and reinstallation purchase of equipments and chemicals. Figure 4.13a illustrates cost requirement for mechanical treatment, which is ~2.5 times higher than a facultative pond system and ~5 times higher than man-made lake systems, if these options are implemented for treatment purposes. The higher cost in mechanical systems is due to

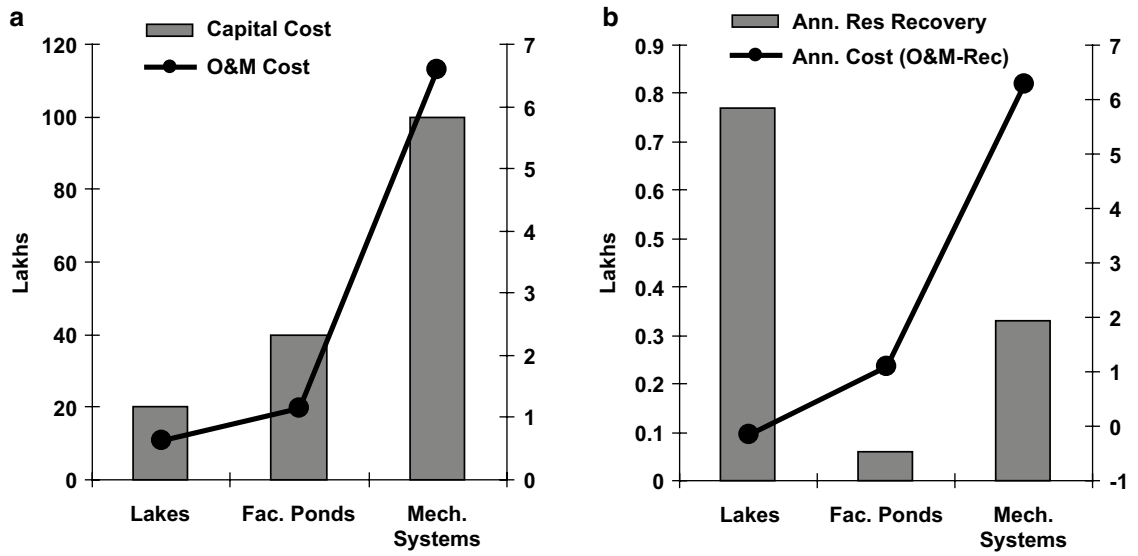

Fig. 4.13 (**a**) Comparative capital and O&M cost for different technologies and (**b**) comparative annual cost and annual resource recovery for different technologies

highly mechanised equipment with energy-intensive processes. In the present study massive algal biomass was observed in lakes and to an extent in certain parts of facultative ponds that had a lipid content ranging from 18 to 30 % (w/w) of the dry biomass. In such conditions the value-added algal biomass in turn can generate revenue by algal biofuel production. Considering the worth of algae from such non-mechanically aerated systems, an annual resource recovery of ~0.75 lakhs/annum can be derived from man-made lake systems compared to ~0.3 lakhs/annum in the case of mechanically aerated systems only when sludge biomethanation and recycled water selling are carried out. Thus, the annual cost (O&M resource recovery cost) is negative in the case of lake systems compared to higher annual costs of >6 lakhs in the case of mechanically aerated systems (Fig. 4.13b). The cost calculations are tabulated in Table 4.7.

The cost of community wastewater treatment (user cost) depends on the treatment process, its efficiency, population size served and the method adopted for the effluent discharge. The smaller population incurs more charges (user cost) than the larger population with a larger plant capacity. Therefore, the user costs can be too high to individuals in smaller communities, especially low-income dwellers. The pond-based systems are cost-effective as it can potentially reduce costs by at least one half (Helmer and Hespanbol 1997).

3.5.2 Environmental Evaluation

The energy consumptions are mostly due to the operation and maintenance costs during aeration and pumping of water and solids (Middlebrooks et al. 1981). Figure 4.14 depicts varied energy needs across different wastewater treatment technologies. Here, the activated sludge/aeration tank-based processes require more energy than either facultative ponds or lake-based systems. Higher carbon footprint is associated with high energy use. For a population of 1000 people, the activated sludge system may generate ~1400 tonnes of CO_2 for operation and 50 tonnes of CO_2 for maintenance over a period of 15 years (Emmerson et al. 1995).

Among many viable approaches, algal pond-based systems help in treating wastewater while generating energy. The type of material selection for the treatment technology has its own embodied energy. The conventional mechanical treatment systems comprise of concrete infrastructures with an embodied energy of 18.4–1580 MJ/M litres (Horvath and Hendrickson 1988). The energy analysis (Fig. 4.14) showed that the mechanically aerated systems require much larger energy (~0.72–6.36 million units/MLD) compared to facultative ponds (~0.36–1.68 million units/MLD) and a very low energy of ~0.02–0.24 million units/MLD is required for lake systems accounted for energy cost (weed removal, sludge clearance, etc.) and incurred for electricity and maintenance.

3.5.3 Social Evaluation

Public participation is an important criterion while selecting a suitable wastewater treatment system for a particular community. People's participation and their opinion on treatment and disposal options would help in the further refinement. In India most of the urban dwellers are unaware of the type of wastewater treatment happening around them. At the same time they pay charges to the urban bodies for sanitation services. One of the essential criteria for assessing the role of the public is the assessment of level of knowledge/awareness

Table 4.7 Valuation of treatment (economies and net annual costs) technologies

Evaluation approaches	Unit	Existing lake systems	Facultative pond systems	Mechanical systems
Economic				
Construction costs (land + infrastructure costs)	Rs in lakhs/MLD	20	40	100
Operation and maintenance	Rs in lakhs/MLD	0.62	1.16	6.6
User cost	Rs/month	–	18	30
Annual resource recovery	Rs in lakhs/year	0.77	0.06	0.33
Net annual cost = O&M resource recovery	Rs in lakhs/year	−0.15	1.1	6.27
Environmental				
Average energy use	kWh/MLD (kWh/m^3)	0.05×10^6	1.7×10^6	5.98×10^6
Chemical oxygen demand (COD)	% Removal	70	79	74
Biochemical oxygen demand (BOD)	% Removal	62	82	63
Total suspended solids (TSS)	% Removal	78	93	88
Turbidity (NTU)	% Removal	80	95	98
Nitrogen (TN)	% Removal	73	36	8
Phosphorus (TP)	% Removal	22	–	2
Social				
Public participation	Qualitative measure	Yes	No	No
Community size served	Population/MLD	8000	13000	11428
Aesthetics	Measured level of nuisance from odour	Moderate	Moderate	High
Human resources to operate plant	Staff/MLD	–	0.2	0.32
Level of education/awareness	Operational requirements	Average	Good	Higher
Availability of open space	Hectare/MLD	1	0.6	0.08

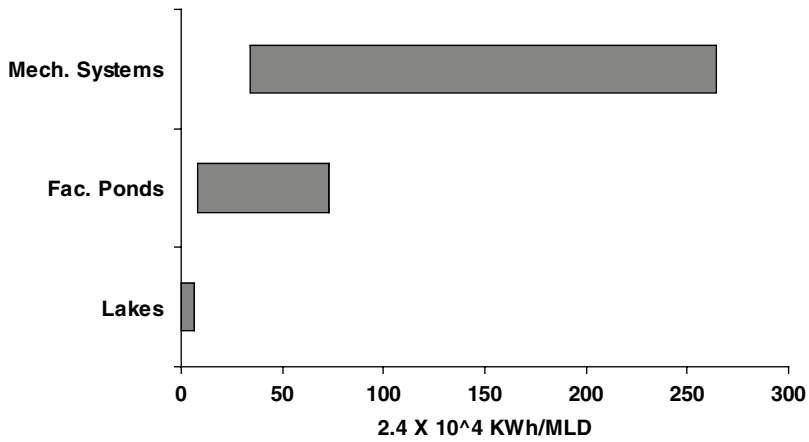

Fig. 4.14 Total energy requirements in different wastewater treatment plants

among the users (Palme et al. 2005). Today affordability and the appropriateness of the technology are considered critical leading to the adoption of cost-effective appropriate technology over more mechanised technology (Sperling 1996).

A larger plant capacity is indicative of a large population. Mechanical and pond-based systems can meet the treatment requirements for a large population than other treatment processes. In this context the mechanically driven systems are opted over pond-based systems in urban areas in India where there is low land/open space availability. In this context for assessment of sustainability of any treatment systems, suitable ways of evaluation of such systems are a prerequisite. This involves computing appropriate mass balances, assessment of nutrient fluxes from a systems perspective and cause-consequence evaluation. This helps in proper estimation of nutrients in various subsystems in the treatment process that helps in evading the accumulation/deficiency of nutrients.

The gaseous by-products during the wastewater containment and treatment may generate foul odour. This results in hampering the aesthetics, raises societal concerns and seeks regulatory measures. Forced aerated systems have a higher foul odour generation potential compared to pond-based systems due to unhandled solids/particulates. In mechanically

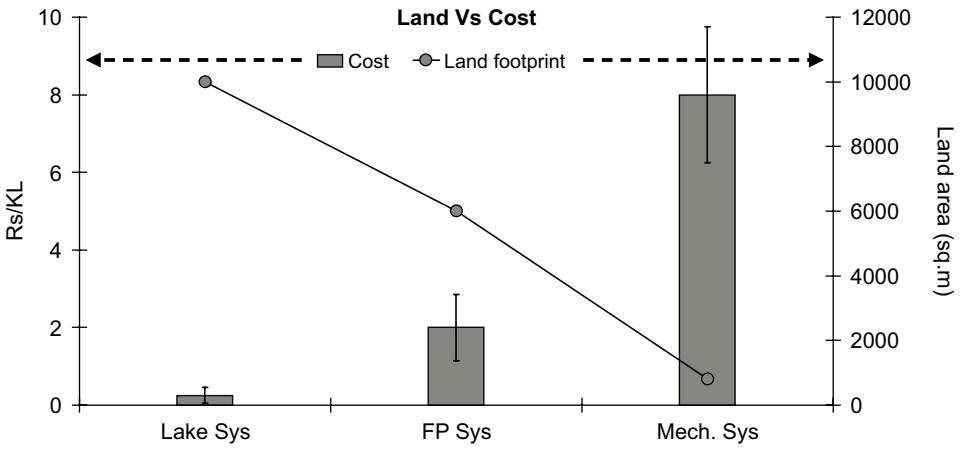

Fig. 4.15 Land vs. cost trade-off for wastewater treatment systems

aerated systems, the aeration and activated sludge systems at Bangalore, the foul odour problems emanate from wastewater containment in pipes, storage basins, pumping units and manholes in different locations. However, the foul odour from treatment units can be due to higher total maximum daily load (TMDL) or due to accumulation of surface scum and also from sludge deposits. Sludge accumulation from facultative pond-based systems may also contribute to the problem. According to WEF (1992) the unit processes in treatment plants as primary clarifiers and trickling filters have a higher odour potential compared to aeration which has a moderate odour potential, and finally the lagoon-/pond-based systems and secondary clarifiers have a low/moderated odour potential. In the present study the lake systems were found to have a small odour compared to the other technologies attributed to a higher organic load and release of H_2S.

Human resource requirement depends upon the plant capacity. According to WEF (1992), an average of one member (staff) is needed to manage the treatment plant (1 MLD). Thus, smaller pond-based plants require small work force, contrary to mechanically driven systems that need more staff.

Awareness and the level of environmental aspects is a crucial social factor for the treatment practice survival processes. The level of sophistication/complexity of a wastewater treatment system often decides the type of operator skill needed for the plant operation based on their educational skills. In the present study the STP based on more mechanisation as in the case of the extended aeration-based treatment process involved more technically sound labourers, supervisors and well-qualified plant engineers unlike the pond-based system where there is not much requirement of a technical staff. Earlier studies show that factors like lack of proper systems understanding in process control and operation leads to limitation in the performance in treatment plants (Water Environment Federation/American Society of Civil Engineers (WEF/ASCE) 1992).

Open space availability is yet another important evaluation criterion where in the mechanical systems having a short hydraulic retention time (HRT) of 3–8 h (Metcalf and Eddy 2003) is indicative of a much smaller land requirement compared to the facultative pond-based systems that require more land area due to a longer detention time. The minimum land area requirement for energy-intensive mechanical systems (extended aeration/ASP), facultative pond systems and partial mix aerated pond systems varies from 0.1acre/MLD and 12–42 acre/MLD to 7–12 acre/MLD (Metcalf and Eddy 2003). The land requirement for the current study showed 0.08 Ha/MLD, 0.6 Ha/MLD and >1 Ha/MLD for mechanical systems, facultative pond-based systems and lake-based systems, respectively (Table 4.7). The land to total cost trade-off of the various wastewater treatment systems currently studied is illustrated in Fig. 4.15.

3.6 Sustainability of the Treatment Systems

Here the sustainability of the existing wastewater treatment systems has been studied considering the economic, environmental and social aspects.

The conventional mechanical systems are capital and energy intensive apart from higher running costs leading to high user cost. Furthermore, such systems have high foul odour generation affecting the aesthetics of the surrounding region. These systems contribute less to the economy of a community by employing the less number of staff per plant capacity, than other treatment systems. Such energy-intensive processes being control systems driven require less human resources and meagrely contribute to the revenue/economy of the society when compared to other treatment systems as ponds. Despite these setbacks in achieving sustainability, the mechanical systems of wastewater treatment are efficient in BOD, TSS and pathogen removal.

The facultative pond-based processes are economical while providing a large community open space and effi-

ciently tap nutrients as N and P through bioremediation. Aeration of the pond systems usually adds additional costs in terms of infrastructure and energy (O&M cost) compared to non-aerated pond systems (US 2002). Foul odour generation is seasonal (especially in summer) in ponds. However, the studied facultative pond systems did not pose any type of odour. In terms of cost (infrastructure and O&M) and energy usage, the pond systems are beneficial with high solid, nutrient and pathogen removal providing maximum stabilisation to the treated effluent that makes them appropriate for treatment at decentralised/community levels.

The design of the existing pond systems can be further improvised by increasing the number of ponds and specifically constructing an anaerobic pond that provides better C removal and solid settling. The BOD in the effluent of the pond systems is mainly the algal BOD. Therefore, a suitable algal trap mechanism (dark algal settling chamber) is envisioned. This design/alternative treatment option would function by integrating the wastewater treatment algal pond systems with the algal capture mechanism by providing a novel settling chamber (unpublished design).

Although these pond systems require large area than mechanised systems, they can contribute more to the economy of the plant by generating revenue by algal biomass capture and subsequent lipid production. Of all three systems, the facultative pond-based treatment systems have the least overall impact (Table 4.7) and also the integrated nutrient treatment efficiency (Table 4.8). Advantages of facultative pond-based systems are lower infrastructure and O&M cost and low energy footprint and results in a small user cost with high BOD, SS, N and pathogen removal and low foul odour generation. The land-intensive pond systems garner a large open space for societal interaction and provide better employment, which adds revenues to the local economy. Finally, the algal biomass growing in this type of wastewater is useful as feedstock for biofuel thus fostering sustainability. The present study highlights algae-based lake systems and facultative pond-based systems to be a more sustainable choice, considering economic, societal and environmental issues.

3.7 Nutrient-Integrated Treatment Efficiency for the Various Sewage Treatment Systems

The investigation on the physical, chemical and biological parameters reveals efficiencies considering the influent wastewater characteristics, detention time and performance of micro-biota in bioremediation. This suggests the need to devise a cumulative efficiency indicator/index that determines the systems efficiency of the treatment unit which aids as an important decision-making tool for further downstream treatment and management of the wastewater.

Colmenarejo et al. (2006) reported an efficiency indicator in terms of treatment parameters (TSS, COD, BOD5 and NH_3-N) to evaluate the efficacy of treatment units. In the present study, new nutrient-integrated treatment efficiency is suggested by considering the mean of TSS, BOD, TN and TP efficiencies in removal. Ideally the efficiency of such systems in the tropical climate should be closer to 90 %:

$$\mathrm{NITE} = 1/4[\mathrm{E_{TSS}} + \mathrm{E_{BOD5}} + \mathrm{E_N} + \mathrm{E_P}]$$

where NITE is the nutrient-integrated treatment efficiency in (%), E_{TSS} is efficiency of turbidity removal (%), E_{BOD5} is efficiency of BOD5 removal (%), E_N is efficiency of N removal (%) and E_P is efficiency of P removal (%).

The above results reveal a very low nutrient-integrated efficiency of the effluent indicating an immediate requirement of an additional detention unit which would help the improvement of the NITE values.

4 Conclusion

The present study reveals that man-made lake systems as Bellandur and Varthur Lakes in Bangalore appreciably treated the influent wastewater having higher organic load. The treatment resulted in 70 %, 73 % and 22 % removal of COD, TN and TP, respectively. However, the facultative pond-based systems in Mysore were very effective in removal of suspended solid (SS) (93 %) and BOD (82 %). The mechanically aerated (extended aeration) STP in

Table 4.8 Nutrient-integrated treatment efficiencies

Treatment unit	Area (ha)	Capacity/flow rate (MLD)	Nutrient-integrated treatment efficiency (NITE) (%)	Desired NITE (%)	Driving factor
Bellandur Lake	365	595	58.75	90	TN
Varthur Lake	220	600	43.17	90	TN
Facultative ponds	25	67.5	62	90	BOD
Aeration and activated sludge STP	7.5	75	42.75	90	TSS

Bangalore was superior in removal of SS (88 %), COD (74 %) and BOD (63 %) but were highly ineffective in nutrient removal.

The wastewater algal biomass present in ponds and lakes showed promising lipid contents, and the FAME analysis revealed high C16:0 (>40 %) followed by C18 fatty acids that further provides scope for algal biofuel generation to meet the regional energy demand. Such biofuel generation requires efficient methods of harvesting algae periodically. This necessitates an efficient algal capture mechanism to capture algal biomass from the final treatment plant effluent that helps in removing algal BOD and solids.

The economic evaluation of treatment processes assessed through the capital investment, annual O&M costs, COD removal cost and land needs reveals that the mechanical systems require five times more capital and O&M costs than facultative ponds. Evaluation of treatment systems in terms of capital investment, human resources, chemical usage, wear and tear repair, electricity needs and land requirement showed that lake-based systems followed by facultative pond-based system are economically a better alternative than mechanically aerated technologies. Finally, it was found that large algal pond-based systems could economically be a potential option for the country considering all factors that include expenses and treatment efficiency.

The existing large lake systems and the facultative pond systems can be innovated for a better photosynthetic yield resulting in higher algal biomass which would not only polish the wastewater but at the same time will act as substrate for lipid/biofuel generation by specific algal trap mechanism.

The treatment efficiency analysis for the period of study showed facultative pond-based systems to be the most effective options for the urban wastewater systems compared to the lake-based systems as well the energy-intensive mechanical systems with a nutrient-integrated treatment efficiency (NITE) of >68 % with the treated water that can be reused for irrigation and domestic purposes.

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The Role of Anaerobic Digestion in Algal Biorefineries: Clean Energy Production, Organic Waste Treatment, and Nutrient Loop Closure

5

J.L. Ramos-Suárez, N. Carreras Arroyo, and C. González-Fernández

1 Introduction

Algae are aquatic organisms which obtain energy via photosynthesis. Algae capture CO_2 and transform it into organic carbon. Algae comprises of two major groups – macroalgae and microalgae.

Microalgae are a diverse group of unicellular organisms with more than 30,000 known species. Although microalgae, in their strictest sense, are eukaryotes, this chapter will also consider cyanobacteria (blue-green algae), which are prokaryotes with photosynthetic capacity. In spite of the great number of species that belong to this group of living forms, only a handful are currently of commercial significance (Bruton et al. 2009).

Microalgae are attracting great interest in recent years due to their high photosynthetic efficiency, which can be as high as 5 % (Acién et al. 2012a). As a consequence, they grow faster than terrestrial plants and have a high potential for bioenergy production (Brennan and Owende 2010). Moreover, microalgae show other advantages compared to traditional energy crops such as their high CO_2 consumption during their growth (Chisti 2007), their ability to grow on marginal lands, and use of marine or wastewater (Park et al. 2011). This biomass can be used for biofuel production or extraction of a wide range of value-added products. Pigments, ω-3 fatty acids, feed, food, fertilizers, and natural food colorants can be obtained from microalgal biomass (Spolaore et al. 2006; Romero García et al. 2012). The economical revenue obtained from these value-added compounds makes biofuel production from algae even more attractive. However, the current market is not yet open to high value-added products obtained from waste streams, and thus, as of today, the only recovery from this biomass can be achieved by its conversion into an energy form.

In this context, this book chapter is devoted to the production of biogas coupled with the use of waste effluents for microalgae biomass growth. Considering all available technologies producing biofuels, anaerobic digestion along with thermal liquefaction is the only conversion route that uses the whole organic content of microalgae to produce energy. Furthermore, biogas generation seems to be the least complex since anaerobic digestion avoids energy-intensive steps such as biomass drying and extraction. Therefore, AD has a higher energy efficiency compared to the other options (Sialve et al. 2009). Biogas can be produced as the main product from microalgae (direct anaerobic digestion of the whole biomass) or can be a coproduct of an industry culturing microalgae for different purposes (organic waste treatment in biorefineries) (Ramos-Suárez and Carreras 2014). Additionally, there is a synergy between anaerobic digestion and microalgae growth: (1) biogas contains a high percentage of CH_4 and CO_2, and if it is combusted in CHP units, CH_4 is converted to CO_2; (2) the digestate produced after anaerobic digestion is a liquid medium where most of the nutrients of the organic substrate are mineralized. Therefore, the two main products of anaerobic digestion (CO_2 and nutrients) could serve as sources of growth enhancer for microalgae culture (Sialve et al. 2009; Uggetti et al. 2014).

2 The Anaerobic Digestion Process

Anaerobic digestion is a suitable technology for the treatment of almost all types of organic residues generated in agro-industrial processes. This technology has been shown to be environmentally friendly and cost competitive with

J.L. Ramos-Suárez (✉)
Procycla SL, Pau Casals, 18 4-4, 08240 Manresa, Spain
e-mail: jlramos@procycla.com

N.C. Arroyo
Environment Department, Ciemat, Avenida Complutense, 40, Edif. 20, 28040 Madrid, Spain

C. González-Fernández
Biotechnological Processes for Energy Production Unit – IMDEA Energy, Avda. Ramón de la Sagra, 3, 28935 Móstoles, Madrid, Spain

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more than 9232.7 ktoe of primary energy produced from decentralized biogas plants only in Europe during 2012–2013 (EurObserv'ER 2014).

The anaerobic digestion or biomethanation is a biological process whereby organic matter is degraded into a number of gaseous products, known as biogas, and a by-product known as digestate. This bioprocess is conducted in the absence of oxygen where degradation of organic matter is performed through a complex series of biochemical reactions that are carried out by different groups of anaerobic microorganisms.

2.1 Anaerobic Process Stages

Biochemical and microbiological studies conducted so far divide the anaerobic decomposition process into four phases or stages: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis. Any of these four stages can be the limiting step in terms of the overall reaction rate. Hydrolysis is usually the limiting step when dealing with complex substrates, as it is the case for some microalgal strains (Mussgnug et al. 2010). Figure 5.1 shows a diagram of the various steps involved in the anaerobic digestion process, the microorganisms involved, and intermediate products generated. The numbers indicate the bacterial population responsible for the process.

2.1.1 Hydrolysis

Out of the four stages, hydrolysis is the initial step in the anaerobic degradation of complex organic substrates. Anaerobic microorganisms can only use soluble organic matter that can pass through the cell wall, and thus, hydrolysis of organic matter is a must. The organic material mainly comprises of three basic types of macromolecules: carbohydrates, proteins, and lipids. During hydrolysis, the bacteria transform the complex organic substrates into simple soluble compounds, i.e., proteins, carbohydrates, and fats are converted to amino acids, monosaccharides, and fatty acids, respectively.

The hydrolysis of these complex molecules is carried out through the action of extracellular enzymes produced by hydrolytic microorganisms.

The rate of hydrolytic degradation of lignocellulose materials composed mainly of lignin, cellulose, and hemicellulose is so slow that it is often the limiting step of the AD process. This is because lignin is highly resistant to degradation by the anaerobic microorganisms and also affects the biodegradability of the cellulose, hemicellulose, and other carbohydrates. It is noteworthy to mention that in contrast to lignocellulosic material, microalgal biomass does not contain lignin, and therefore, their hydrolysis and overall methane production is favored.

2.1.2 Acidogenic Phase

During acidogenesis, the fermentation of soluble organic molecules takes place via facultative bacteria. The end product of this reaction includes alcohol, hydrogen, carbon dioxide, and several fatty acids like acetic acid, formic acid, propionic acid, valeric acid, lactic acid, etc. Some of the end products of this reaction like acetic acid, formic acid, and hydrogen can directly be used by the methanogenic bacteria.

The formation of one or another acid depends on the concentration of H_2 produced during the digestion. When the H_2 concentration in the gas produced is very low (5–50 ppm), acetic acid is preferably formed. When the H_2 concentration increases, acetic acid decreases, and the fraction of long-chain acids (e.g., propionic, butyric, etc.) increases.

In this phase, also alcohols are produced. The kinetics of the process is relatively fast; the acid-producing bacteria are fast growing with minimum doubling times of 30 min.

2.1.3 Acetogenic Phase

In the third stage, known as acetogenesis, the other products of the acidogenic phase, namely, propionic acid, butyric acid, and alcohols, are transformed by acetogenic bacteria in hydrogen, carbon dioxide, and acetic acid. The small organic molecules, especially VFA (volatile fatty acids), are converted into acetic acid. The bacteria involved in this process are facultative, live in close collaboration with methanogenic bacteria, and can only survive in symbiosis with the genre that consumes hydrogen, since they are inhibited by high hydrogen concentrations (Anderson et al. 2003). These bacteria have slower growth rates than acidogenic bacteria, with minimum doubling times from 1.5 to 4 days.

2.1.4 Methanogenic Phase

This phase constitutes the final stage in which compounds such as acetic acid, hydrogen, and carbon dioxide are transformed into CH_4 and CO_2. Bacteria involved constitute a single group composed of several species of different shapes and cell structures. There are two main types of strictly anaerobic microorganisms, which degrade acetic acid (acetoclastic methanogenic bacteria) and those that consume hydrogen (hydrogenotrophic methanogens). The main route for methane formation is the first one, with about 70 % of the methane produced.

Acetoclastic methanogenic bacteria produce methane from acetate. They have a slow growth (minimum doubling time of 2–3 days) and are not affected by the concentration of hydrogen in the biogas. The hydrogen-consuming methanogenic bacteria produce methane from hydrogen and CO_2. This reaction has a dual function in the anaerobic digestion process; on the one hand, methane is produced and, on the other, gaseous hydrogen is removed.

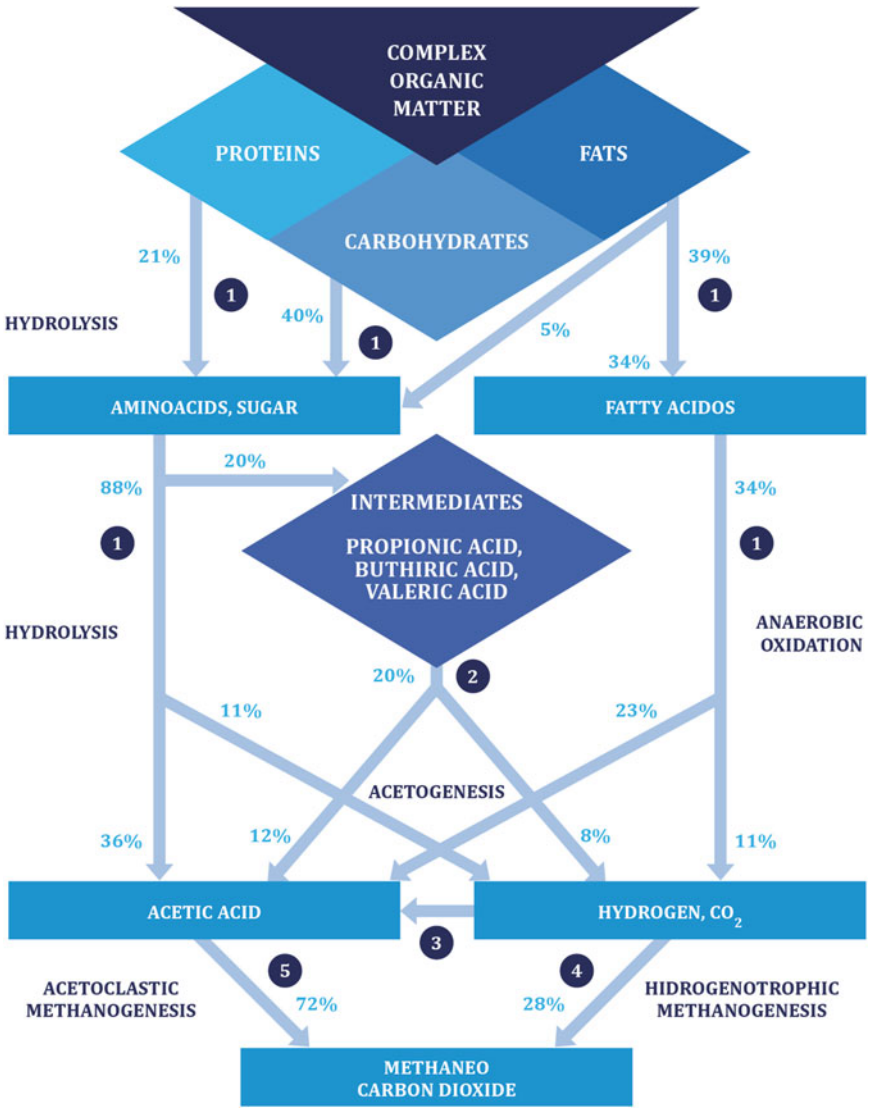

Fig. 5.1 Degradation steps of anaerobic digestion process and microorganisms involved (*1* fermentative bacteria, *2* hydrogen-producing acetogenic bacteria, *3* homoacetogenic bacteria, *4* hydrogenotrophic methanogenic bacteria, *5* acetoclastic methanogenic bacteria) (*Source*: adapted from Gujer and Zehnder (1983))

With high ammonia concentrations, acetate-utilizing methanogens have limited activity in methanogenesis (Hansen et al. 1998). Symptoms of limitation of acetoclastic methanogens have also been observed in anaerobic digestion of microalgae due to high ammonia concentrations (Ramos-Suárez et al. 2014b).

2.1.5 Formation of Hydrogen Sulfide

In addition to the bacteria described above, there is a group called sulfate-reducing bacteria (SRB), which are particularly important if sulfates are also present (Espinosa-Chávez et al. 2007). SRB are able to reduce sulfate to sulfide. This reaction is crucial in anaerobic digestion, since SRB can compete with methanogens and decrease the production of methane. Moreover, SRB are also able to reduce the sulfates using the hydrogen produced by the acid-forming bacteria. In this case, the hydrogen cannot be used by the methanogenic bacteria. Sulfur content of microalgae cells varies normally between 1.5 and 1.6 μg mg^{-1} dry weight, whereas the concentration range of sulfur (in the form of salts) in the growth media is usually in the range of grams per liter (Grobelaar 2004). Remains of growth media will probably be introduced in the digester together with microalgal biomass, increasing sulfur content and, therefore, hydrogen sulfide production. Moreover, chemicals with sulfur could be used in some extraction process, which could remain in spent biomass under digestion (Romero García et al. 2012). It is therefore important to monitor the presence of sulfates during the process, since besides affecting the methanogenesis, hydrogen sulfide is corrosive and can affect several processes and structures (Hidalgo and García 2001). In microalgal biorefineries, if biogas is supplied as carbon source, hydrogen sulfide can be inhibitory for the growth of microalgae (Kao et al. 2012a, b).

2.2 Process Parameters

Anaerobic digestion is affected by various parameters which influence the kinetics of the different reactions and the production of biogas. Those parameters affecting the digestion process include: control parameters (nutrients, temperature, pH, redox potential) and operational parameters (agitation, hydraulic retention time, organic load). Moreover, many of these parameters are used to monitor the digestion course in industrial plants.

2.2.1 pH and Alkalinity

The different groups of bacteria involved in the process have optimum activity levels around neutral pH:

- Fermentative bacteria: 7.2–7.4
- Acetogenic bacteria: 6.0–6.2
- Methanogenic bacteria: 6.5–7.5

Generally, the pH of the digester should not be lower than 6.0 or higher than 8.3 (Bazara et al. 2003). If the pH of the medium is below 6.5, the activity of methanogenic acetoclastic bacteria diminishes, while at pH below 4.5, the activity ceases completely (Lema and Méndez 1997).

Organic overloading can mediate instability of the digestion process due to organic acid accumulation. A high concentration of organic acids decreases the pH, decreases methane production, and can cause reactor souring or reactor failure (Rittmann and McCarty 2001).

Alkalinity is defined as the capacity to neutralize acids (Rittmann and McCarty 2001). Bicarbonate alkalinity of at least 500–900 mg/l $CaCO_3$ is required for a pH greater than 6.5 (Rowse 2011). In the anaerobic digestion of microalgae besides the alkalinity caused by the carbonate-bicarbonate equilibrium, ammonia produced from protein degradation plays an important role increasing the buffer capacity.

2.2.2 Redox Potential

Methanogenic bacteria are strict anaerobes; therefore, their tolerance to changes in the redox potential is lower than other microorganisms involved in the digestion process. In pure cultures, methanogens require a redox potential of at least −350 mV to ensure the strongly reduced environment that these bacteria need for optimal activity (Anderson et al. 2003).

2.2.3 Temperature

Methanogenic microorganisms are extremely sensitive to temperature changes. There are three temperature ranges: (1) psychrophilic (5–20 °C), (2) mesophilic (25–45 °C, with the optimum of 30–37 °C; above 40 °C can cause denaturation of the enzymes), and (3) thermophilic (45–65 °C, being the optimum 55–60 °C) (Anderson et al. 2003). At the industrial level, it is common to find mesophilic (35–40 °C) and thermophilic digesters (55–60 °C). Stability decreases with increasing temperature, as a consequence of higher accumulation of VFA, greater toxicity of ammonium, an increased sensitivity to temperature changes, and foam and odor problems (Parkin and Owen 1986).

Specific methane production rates are 50–100 % higher for thermophilic anaerobic digestion than for mesophilic anaerobic digestion (Rittmann and McCarty 2001). In the anaerobic degradation of microalgae, the use of higher temperatures sometimes leads to higher methane yields (Golueke et al. 1957). Other studies showed a decrease in the methane yield (Samson and LeDuy 1986). Whereas Golueke et al. (1957) suggested a higher rate of degradation of algal cells due to the increased temperature, the latter study pointed to an increase of ammonia sensitivity with increasing temperatures due to a shift to the unionized form of ammonium.

2.2.4 Nutrients

The main nutrients required for growth of microorganisms are carbon, nitrogen, and phosphorus. Also, a series of mineral elements such as sulfur, potassium, sodium, calcium, magnesium, and iron should be present at trace levels. Carbon is the main power source for bacteria and the main component of biogas. This carbon comes mainly from carbohydrates contained in the biomass degraded.

On the other hand, nitrogen is a major source for the synthesis of proteins. Nitrogen deficiency disables bacteria to metabolize the carbon, which would lead to a reduced efficacy in the degradation. Conversely, if there is an excess of nitrogen, its accumulation in the form of NH_3 (ammonia) is toxic to anaerobic microorganisms.

Therefore, the C/N ratio of the substrate is a key indicator of digestibility and potential methane yield of the biomass (substrate) to be degraded. This value is specific to each substrate, and in the case of microalgae, it depends on the species and the cultivation process (Sialve et al. 2009). Optimal C/N ratio for anaerobic digestion is between 10 and 30, and it depends on operational conditions, substrate composition, and microorganism acclimation to a particular substrate (Chen et al. 2008; Pagés Díaz et al. 2011). Microalgae, due to their high protein content, show normally a low C/N ratio which could be detrimental for the anaerobic digestion process (Sialve et al. 2009).

2.2.5 Inhibitors

The anaerobic digestion process can be inhibited by the presence of toxics in the system which affects the development

of bacterial activity. Furthermore, the threshold toxicity concentration of specific compounds (ammonium, sulfide, volatile fatty acids, etc.) depends also on other parameters such as temperature or pH. Out of the anaerobic microorganisms, methanogenic bacteria are generally the most sensitive although generally all groups of microorganisms involved in the process are affected.

2.2.6 Mixing

Mixing aims at facilitating mass transfer processes and preventing dead zones and insufficient contact between organic matter and microorganisms. Mixing increases the kinetics of the anaerobic digestion process by accelerating the biological conversion. Additionally, mixing allows uniform heating of the reactor (Tchobanoglous et al. 2003).

Mixing can be provided mechanically through conventional impellers rotating immersed within the digester at low speed, pneumatical recirculation by injecting biogas via spargers at the bottom of the digester (Tchobanoglous et al. 2003), or by recycling the effluent at the bottom of the digester.

2.2.7 Retention Time

The retention time defines the time that the substrate is in contact with the active biomass within the digester. In digesters without biomass retention mechanisms (e.g., CSTR), the retention time is equivalent to the hydraulic retention time (HRT). HRT is defined as the average amount of time one reactor volume of actively digesting sludge stays within the reactor. HRT can be calculated with the following equation:

$$HRT = \frac{V}{Q} \tag{5.1}$$

where:

HRT = hydraulic retention time (d)
V = volume of reactor (m^3)
Q = influent flow rate (m^3/d)

Anaerobic reactors should be designed with sufficient retention time to allow an effective volatile solid conversion to biogas and the development of methanogenic populations (Vesilind 1998; Parkin and Owen 1986). In this context, there are digesters (e.g., fluidized bed reactors, membrane reactors) where the solid retention time (SRT) is increased by means of biomass retention mechanisms which separate the liquid and the solid streams. These digesters are normally used for the treatment of high volumes of wastewater with low solid content. Although not applied yet, these types of digesters could be used for degrading highly diluted microalgal biomass.

2.2.8 Organic Loading Rate (OLR)

The organic loading rate (OLR) is defined as the mass of volatile solids added each day per reactor volume (Vesilind 1998) or the amount of BOD or COD applied to the reactor volume per day (Tchobanoglous et al. 2003). Organic loading rate is related to the hydraulic retention time by the following equation:

$$OLR = \frac{Q \cdot CVS}{V_{\text{digester}}} = \frac{CVS}{HRT} \tag{5.2}$$

where:

OLR = organic loading rate (kg VS m^{-3} d^{-1})
Q = volumetric flow rate (m^3 d^{-1})
CVS = concentration of volatile solids in the substrate (kg VS m^{-3})
V_{digester} = reactor volume (m^3)
HRT = hydraulic retention time (days)

OLR thus depends on the waste composition and the retention time. It is one of the parameters commonly used to characterize the treating capacity of anaerobic digesters.

According to Rittmann and McCarty (2001), the recommended rate of organic loading for high-rate anaerobic digestion is 1.6–4.8 kg VSS m^{-3} d^{-1}, and the recommended organic loading rate for low-rate anaerobic digestion (digestion with no heat and no mixing) is 0.5–1.6 kg VSS m^{-3} d^{-1}. Vesilind (1998) recommended that the peak organic loading rate for high-rate anaerobic digestion should be 1.9–2.5 kgVS m^{-3} d^{-1}. These values can vary for different substrates and digesters. If the loading rate is too high for the system conditions, methanogenesis can become inhibited by organic overloading. Therefore, organic loading rate should be set in a conservative way.

2.3 Biogas Recovery and Use

Biogas is mainly composed of methane and carbon dioxide. The energetic value of biogas (between 20 and 25 MJ m^{-3}) is determined by the concentration of methane in the biogas (Werner et al. 1989). Besides methane and carbon dioxide, biogas contains other minor constituents such as water vapor (H_2O), hydrogen sulfide (H_2S), ammonia (NH_3), hydrogen (H_2), and nitrogen (N_2). Additionally, some traces of volatile organic compounds (e.g., siloxanes, mercaptans, terpenes) can be present in biogas produced in landfills but are rarely produced in agro-industrial applications (Rasi 2009). Biogas composition affects the possibilities for its use, since the methane concentration determines the LHV of the fuel. Moreover,

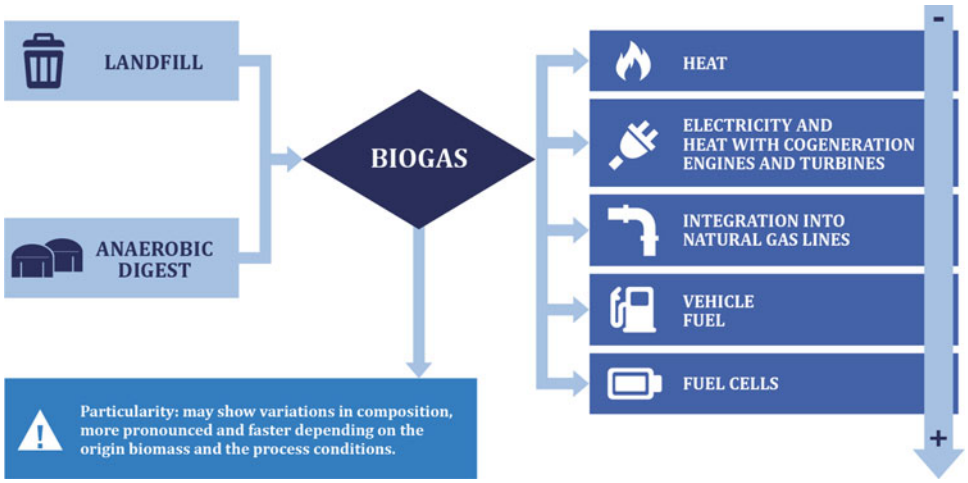

Fig. 5.2 Current applications of biogas produced in industrial plants and required degree of refinement

high concentrations of some of these trace components may impede the use of biogas for certain energetic purposes.

Biogas can be used practically for the same energy applications developed for natural gas. Nowadays, the applications of greatest interest (see Fig. 5.2) are (1) heat by direct combustion, (2) power generation in engines, (3) power generation in engines with heat recovery (cogeneration), (4) integration in natural gas grid, and (5) fuel for vehicles. Nevertheless, the most common ones are direct combustion for heat and power generation with cogeneration engines. However, there is a growing interest in other alternatives such as its application as motor fuel and its integration into the natural gas grid (AEBIOM 2009).

Therefore, depending on the application in which biogas is used, a different degree of cleaning is required. Biogas purification systems are based on different techniques (chemical, physical, biological) and are normally designed for the removal of a single component. In agro-industrial biogas plants, the minor constituents that require removal include water vapor, carbon dioxide, and hydrogen sulfide, depending on their concentration and the intended application. Commercial techniques for hydrogen sulfide removal include iron sponges, activated carbon, micro-aeration (inside digester), water scrubbing, chemical absorption, biological filters, and membranes. Whereas for CO_2 removal, the following techniques are available in the market: physical and chemical absorption, water scrubbing, pressure swing adsorption (PSA), vacuum swing adsorption (VSA), membrane separation, and cryogenic separation.

Microalgae cultures can be used for biogas purification by fixing CO_2 and sulfur in their biomass. Although this option is still under study and no commercial systems based on this technology exists, biogas purification with microalgae could show some benefits compared to the other techniques, such as no use of chemicals, production of a valuable product (microalgal biomass) or additional energy (if biomass is digested), and simultaneous removal of CO_2, H_2S, and other compounds. This option will be further discussed in this chapter.

2.4 The Digestate

The anaerobic digestion process produces a by-product commonly known as digestate. It is a mixture of stabilized influent and microbial biomass. For a certain substrate, the type of digester and the operation parameters determine the properties of the digestate. As already mentioned, during the anaerobic process, part of the organic matter is converted into methane; therefore, the organic content of the digestate is lower than in the influent. The reduction of the C/N is beneficial when the end product is intended for agricultural purposes.

The agricultural valorization of digestates focuses mainly on two aspects: the direct use of the digestate as fertilizer and solid–liquid separation and further usage of the solid fraction for the preparation of high value-added fertilizers through composting and the use the liquid fraction as a liquid fertilizer.

The fertilizer value of the digestate depends mainly on the nutrient concentration of the degraded substrate. During the digestion process, organic carbon is converted to methane and carbon dioxide, whereas most of the nutrients that were associated to organic molecules are mineralized and remain in the digestate. For instance, organic nitrogen is converted to ammonia nitrogen, whereas total nitrogen remains virtually unchanged (some ammonia will be found in the gas phase). Therefore, the digestate exhibits a great fertilizer value and could be used for the growth of terrestrial crops and also for microalgae cultures, as it will be further discussed in this chapter.

3 Biogas Production from Microalgae

3.1 Introduction

The initial studies on anaerobic digestion of microalgae are from the end of the 1950s (Golueke et al. 1957; Golueke and Oswald 1959). In their very first work published, Golueke et al. (1957) observed the difficulties that the anaerobic degradation of microalgal biomass (*Scenedesmus* and *Chlorella*) could entail, both in mesophilic and thermophilic range. Authors inferred three possible reasons: (1) low C/N ratio, (2) ability of algae to survive in the digester, and (3) resistance of algae cell wall to bacterial attack.

Gunnison and Alexander (1975a) worked to understand the resistance of microalgae to bacterial attack under natural conditions. The main conclusion of their study was that the composition of microalgal cell wall is a decisive factor for this resistance. Afterwards, the same authors analyzed the constituents of the cell wall of some microalgal species that showed an effective resistance to bacterial degradation (Gunnison and Alexander 1975b). In many species (e.g., *Staurastrum* sp., *Pediastrum duplex*, and *Fischerella muscicola*), complex carbohydrates supported high resistance to bacterial attack and decomposition. Considering these results, research conducted in the recent years has pursued different goals:

- Coupling anaerobic digestion to microalgal growth units for clean energy production
- Application of pretreatments to break microalgal cell walls in order to enhance biodegradation and consequently biogas production potential
- Co-digestion of microalgal biomass with high C/N substrates in order to balance nutrients and to enhance microbial activity
- Anaerobic digestion of microalgal residues produced after the extraction of high-value products, as waste treatment process in a biorefinery

3.2 Microalgae as Energy Crop

The literature on the direct use of microalgal biomass for biogas production is to some extent scarce. However, in the recent years, a new approach is emerging: the use of microalgae grown in wastewater for direct biogas production. In this case, the energy savings generated by replacing the traditional wastewater treatment processes by microalgae-based treatments make the cultivation process profitable, and the use of mixed algal-bacterial populations for biogas production adds to even higher energy gains. There are important R&D projects trying to implement this concept at medium and industrial scale with encouraging results to date (e.g., All-Gas project, led by Aqualia: http://www.all-gas.eu/).

Different microalgae species have been assessed for methane production. Microalgae assessed in the different studies include chlorophytes or green microalgae (*Chlorella* sp., *Scenedesmus* sp. *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Monoraphidium* sp.), cyanobacteriae (*Arthrospira platensis*, *Oscillatoria* sp., *Spirulina maxima*), euglenoids (*Euglena graciliis*), and haptophytes (*Isochrysis galbana*) (Golueke et al. 1957; Golueke and Oswald 1959; Samson and LeDuy 1986; Varel et al. 1988; Mussgnug et al. 2010; Ras et al. 2011; Hernández et al. 2013; Prajapati et al.; 2014a; Tran et al. 2014; Mottet et al. 2014; Santos et al. 2014). The results are reported to be influenced by operational parameters and experimental conditions of each study (e.g., batch or continuous tests, temperature, hydraulic retention time, organic loading rate), but they provide an insight into intra- and interspecies variability in methane potential of microalgae (see Fig. 5.3 for a comparative evaluation of some of these results).

Mesophilic methane yield of the different species ranged from 139.7 ± 0.9 L_{CH4} $kgVS^{-1}$ for *Scenedesmus* sp. (Tran et al. 2014; Ramos-Suárez et al. 2014a) to 387 L_{CH4} $kgVS^{-1}$ for *Chlamydomonas reinhardtii* (Mussgnug et al. 2010) or 446.8 L_{CH4} $kgVS^{-1}$ for an algal sludge mainly composed of *Scenedesmus*, *Chlorella*, *Euglena*, and *Oscillatoria* (Golueke and Oswald 1959), the latter at thermophilic digestion (45 °C).

From all these studies, the main conclusion that can be drawn is that biodegradability, and therefore methane potential production, is species and strain specific (Mussgnug et al. 2010; Prajapati et al. 2014a). Moreover, within the same species, this potential could change depending on the culture growing method, or more specifically, on the nutrients supplied to the culture during its growth which determine its biochemical composition and, therefore, its methane production potential (Hernández et al. 2013).

It has been concluded, that biodegradability is mainly conditioned by the presence or absence of a cell wall composed of complex polymers, hardly biodegradable, that impede the degradation of intracellular organic molecules by microorganisms (Mussgnug et al. 2010).

Another drawback for an optimum anaerobic degradation of microalgae is the high content of nitrogen and low C/N ratio of this biomass. The major fraction of microalgae cultured without nutrient limitation is protein (Rebolloso Fuentes et al. 2000), which could be up to 60 % (Mahdy et al. 2014a). During anaerobic digestion, proteins are degraded and ammonium is produced. If ammonium concentration reaches important levels, microorganisms could be inhibited reducing or even stopping biogas production

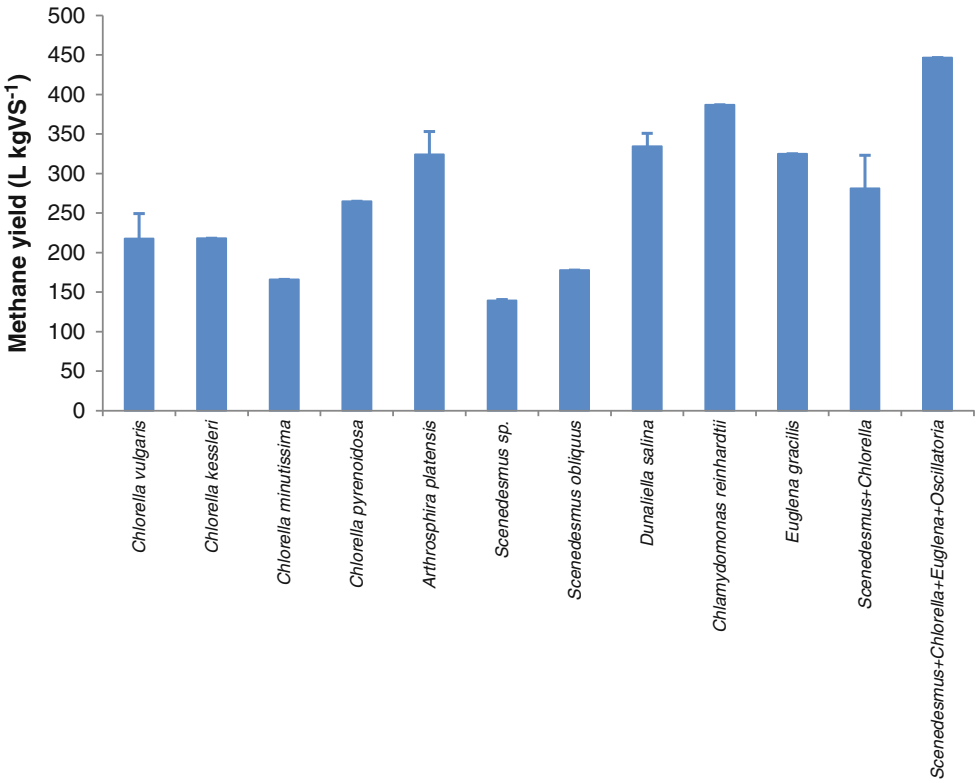

Fig. 5.3 Comparative evaluation of methane potential of different microalgae species

(Samson and LeDuy 1986). In literature, a wide range of ammonia inhibition thresholds are shown (from 1.7 to 14 g L^{-1} being the necessary concentration to cause a 50 % reduction in methane production). The inhibiting concentration could change with differences in substrates, inocula, environmental conditions (temperature and pH), and acclimation period (Chen et al. 2008).

Additionally, for marine species, salinity could also play an important role, as high salinity levels can diminish anaerobic digestion performance. Mottet et al. (2014) showed that salinity could be counteracted by means of adapted or acclimated inoculum, which could withstand high salinity levels yielding similar methane as low-salinity-level microalgae.

3.2.1 Enhancement of Methane Yields

3.2.1.1 Pretreatments for Microalgal Biomass

Algae consist of complex organic matter which implies a difficult enzymatic hydrolysis and hence limits the efficiency of anaerobic digestion. The limited hydrolysis is due to the biomass features such as biochemical composition and cell wall characteristics of the different microalgae strains. One of the approaches followed for enhancing methane production is the pretreatment of microalgae biomass. This approach enhances the hydrolysis rate of microalgae during anaerobic digestion by facilitating cell wall disruption. In this sense, organic matter contained in microalgal biomass becomes readily available for anaerobic microorganisms, and therefore, methane production and productivity increase. Pretreatments to open up the cell wall structure have been widely studied in activated sludge and lignocellulosic biomass; however, there is not enough information regarding the effect of those pretreatments on microalgae. Even though microalgae does not contain lignin which renders this substrate easier to degrade than lignocellulosic substrates, microalgal biomass contains some other compounds, such as algaenans and sporopollenin, which confer the cell wall a high resistance to bacterial attack (Burczyk and Dworzanski 1988).

3.2.1.1.1 Pretreatment Features: Organic Matter Solubilization and Structural Changes

The pretreatments applied to microalgal biomass prior to anaerobic digestion results in different changes in biomass structure and organic matter solubilization. By these means, microalgae biomass suffers changes which ultimately lead to methane production enhancement.

Traditionally, the organic matter solubilized upon pretreatment of activated sludge has been directly correlated to methane yield enhancement (Bougrier et al. 2005). Opposite to activated sludge, a parameter that can be used as an indicator of methane yield enhancement after pretreatment is lacking. As mentioned above, pretreatments employed for activated sludge are being adapted for microalgae biomass, and therefore, the first indicator followed was soluble COD increase after pretreatments. Nevertheless, this attempt has shown that microalgae biomass behaves differently than

other biomasses upon pretreatments and no direct linkage exists (González-Fernández et al. 2012a; Alzate et al. 2012). In the constant search of finding a key indicator, proteins and carbohydrates have been also studied. These two macromolecules have been pointed out as potential indicators since microalgae biomass is mainly composed of these components (González-Fernández et al. 2010; Mendez et al. 2014a). In this sense, these macromolecules are main components, but what is indeed causing the low hydrolysis of these substrates in anaerobic digestion is the composition of the cell walls. It is important to understand the chemical composition of the targeted microalgal cell wall. *C. vulgaris* is one of the most commonly studied microalgae, and as reported in historical literature, it has a cellulose-based cell wall (Loos and Meindl 1984), but the latest studies are showing that this may not be correct (Gerken et al. 2013; Kim et al. 2014). By taking a closer look into carbohydrates, Mendez et al. (2014a) were able to identify a close relationship between carbohydrate solubilization and methane production enhancement after thermal pretreatment. Nevertheless, when trying to reproduce this trend in different microalgae strains, the results indicated that the overall fraction of carbohydrates was not a proper indicator (Mendez et al. 2014b). It can be thus concluded that the carbohydrate fraction itself cannot be used for this purpose, but study on the different carbohydrates that constitute the microalgae cell wall deserves further investigation. More specifically, uronic acids, neutral sugars, and amino sugars have been identified in the microalgae cell wall (Cheng et al. 2015). Studies have always claimed that this microalga possesses a carbohydrate-based cell wall mainly composed of cellulose and hemicellulose (González-Fernández et al. 2012b). Nevertheless, lately, few investigations have pointed out that this might not be true. For instance, Kim et al. (2014) used cellulases to hydrolyze *C. vulgaris* cell wall and the results were not as expected. Their investigation revealed that cellulase and amylase did not have any effect on cell wall disruption. To verify which enzyme would be more efficient for cell wall degradation, they tested pectinase, cellulase, amylase, xylanase, β-glucosidase, chitinase, lysozyme, and sulfatase. Their results confirmed that only pectinase had a significant effect on the degradation of polysaccharides from the cell wall of *C. vulgaris*. Similarly, another recent publication reached the same conclusion. It seems likely that *C. vulgaris* does not have a cellulose rigid cell wall, but rather uronic acids and amino sugars are conferring this microalgae its hardness (Gerken et al. 2013). At this point, it seems of relevant importance to characterize the carbohydrate fractions released upon pretreatments in order to gain insights on the effect that this disruption method has on the microalgae cell wall.

Proteins have been studied to a minor extent than carbohydrates. One of the main reasons for this limited information on proteins is related to the fact that proteins are weaker macromolecules than carbohydrates, and thus, upon pretreatments, proteins are quite often converted into other polymers and therefore not quantified in the soluble phase (Mendez et al. 2013). The low solubilization of proteins has been attributed to the occurrence of Maillard reaction. In this context, the available reducing sugars and amino acids reacted leading to the formation of complex molecules. Maillard reaction course is strongly affected by factors such as temperature, heating duration, water content, pH, and amino acid to sugar ratio. In this study, the low solubilization of proteins recorded was attributed to this type of reaction taking place when proteins and carbohydrates are soluble at high temperatures. As proteins react with reducing sugars, the amount of carbohydrates and proteins solubilized was indeed higher than the determined value. Nevertheless, the polymerization of the solubilized macromolecules reduced their solubility.

Microalgal cell walls have been reported to contain 10–30 % protein (Burczyk et al. 1999; Blumreisinger et al. 1983). The presence of proteins in the cell wall cannot be neglected since this polymer may also be responsible of the low anaerobic digestibility of microalgae. This macromolecular fraction has been pointed out as the polymer limiting the anaerobic digestion of other biomasses such as activated and primary sludge (Mottet et al. 2010; Miron et al. 2000). Lately, studies focusing on enzymatic hydrolysis of microalgae cell wall have elucidated the relevance of cell wall proteins for biogas production purposes. In this context, Mahdy et al. (2014a) tested two enzymatic cocktails, namely, carbohydrase and protease, which resulted in high hydrolysis efficiencies rendering almost all the particulate carbohydrates and proteins available in soluble phase. When subjecting carbohydrase and protease hydrolyzed biomass to anaerobic digestion, methane production was greatly enhanced for biomass pretreated with proteases. The importance of proteins for biogas production has been evidenced in chlorophyta, namely, *Chlorella* sp. and *Scenedesmus* sp. (Mahdy et al. 2015). Proteins frequently reported on microalgae cell wall conferring rigidity belong to the glycoprotein family (Voigt et al. 2014). Glycoproteins themselves are not difficult to digest but their linkage can be of great importance. For instance, *Chlamydomonas reinhardtii* exhibiting glycoproteins is a substrate easy to digest (Mahdy et al. 2014a), while *Scenedesmus* is probably one of the most difficult microalgae to degrade. In this latter one, glycoproteins are cross-linked via carbohydrate side chains and not via transglutaminase-dependent reactions or peroxidase-catalyzed isodityrosine formation as it happens in C. *reinhardtii* (Voigt et al. 2014).

The other change that is observed during microalgae pretreatments is the formation of aggregates upon cell wall disruption. Particle size distribution shifts to higher particle diameters when applying temperatures greater than 80 °C or when subjecting the microalgae biomass to ultrasound

pretreatment at high energy levels (Ometto et al. 2014; González-Fernández et al. 2012c). The reason for this increased diameter has been attributed to the release of intracellular material. Some other evidences of cell wall disruption during pretreatments have been provided microscopically. Change in structural integrity or breakdown of microalgal cells are not detected under normal microscopic observation. However, cell wall disruption has been proved by using dyes, such as Sytox green (González-Fernández et al. 2012a), or through transmission electron microscopy (Passos and Ferrer 2014). These two methods are useful to fully ascertain that organic matter released during the different pretreatment is intracellular and not due to the exopolymers attached to the cell wall. In this context, Sytox green dyes the cell when the wall is disrupted; on the other hand, if only extracellular organic matter is released, microalgal cells would not be stained. Transmission electron microscopy has been also confirmed as a helpful tool to identify structural changes occurring upon microalgae cell wall pretreatments. During thermal hydrolysis, the cell wall are expanded and partially disaggregated inside the cell boundaries, while cell turgidity is less evident after enzymatic hydrolysis (Ometto et al. 2014). Authors attributed this less distorted cell structure after enzymatic hydrolysis to the degradation of specific cell wall components. FT-IR spectra of raw and pretreated microalgae biomass has also been used widely for obtaining information of chemical and structural changes in different biomasses (Monlau et al. 2012; Salehian et al. 2013). When applied to microalgal biomass, the intensity of peaks related to the bond C–O–C of polysaccharides diminishes at increasing temperatures, and also changes in the fingerprint regions of amide band (proteins) were registered during thermal pretreatment (Mendez et al. 2014a). Therefore, infrared spectra can support qualitative changes of the biomass upon pretreatment but not quantitative because the changes in peak intensity are too low to determine structural changes.

As it can be seen, the need of finding an indicator to elucidate whether the cell wall was damaged or not during biomass pretreatment has been successfully achieved during this last decade of research. Nevertheless, no clear indicator that can be related to methane production enhancement after biomass pretreatment has been provided. Most likely, the reason for this is the different cell wall composition and matrix linkage of the microalgae studied. However, the last decade of intensive research on microalgae biomass has shown a wide range of efficient pretreatments for biogas production. Most of the pretreatments studied recently are done through thermal, physical, or chemical means.

3.2.1.1.2 Thermal Pretreatments

Thermal pretreatments affect weak hydrogen bonds when mild temperatures (below 100 °C) are applied while complex carbohydrates solubilizes when higher temperatures are employed (González-Fernández et al. 2012a; Garrote et al. 1999). Mild temperatures of 50 °C when applied to microalgal biomass resulted in organic matter solubilization (Mahdy et al. 2014b). Carbohydrate solubilization recorded for *Chlorella* sp. was 15 and 32 % for *Scenedesmus* sp. Despite the organic matter release, methane yield of these biomasses did not increase significantly (Mahdy et al. 2014b; Passos et al. 2013). The almost negligible increase in methane yield was attributed to the fact that the organic matter solubilization was probably mediated by exopolymer released during the pretreatment rather than by cell wall breakage. At higher temperatures of around 90 °C, *Scenedesmus* biomass is disrupted after 30 min, and methane production is doubled (González-Fernández et al. 2012a). At this point, it should be stressed out that *Scenedesmus* is probably one of the most difficult microalgae to digest (Mussgnug et al. 2010). Similarly, out of the three mild temperatures tested on *Nannochloropsis oculata*, at 30, 60, and 90 °C for 4 h, an enhancement in methane yield of 41 % was recorded at 90 °C compared to the raw biomass (Marsolek et al. 2014). This was due to the organic matter solubilization registered after thermal treatment (40 %). In the same range, 32 % methane yield enhancement was registered after the thermal treatment at low temperature (60 °C for approximately 4 h) of a microalgal mixture of *Pediastrum* sp. and *Micractinium* sp. where 11 % organic matter was solubilized after the pretreatment (Kinnunen et al. 2014). Interestingly, these authors also reported the effect of freeze-thawing the biomass. This pretreatment resulted in 18 % organic matter solubilization but double methane yield. It can thus be confirmed that organic matter solubilization is a too general parameter, and the chemical composition of the different organic matter solubilized is crucial for methane production. This fact has also been confirmed by other authors. The different pretreatments and different pretreatment conditions differently affect microalgal biomass disruption, and thus, different disruption mechanisms lead to different chemical compositions of organic matter that ultimately determine the methane yield achievable by these pretreated substrates (González-Fernández et al. 2012a; Ometto et al. 2014). Overall, it may be hypothesized that this range of temperatures (around 100 °C) would be enough to open up all microalgae cell walls and make their organic material available for anaerobic microorganisms.

When evaluating higher temperatures, carbohydrate solubilization prevails over proteins (Mendez et al. 2013). This investigation dealt with *Chlorella vulgaris* subjected to 120 °C for 20 min, and 40 min resulted in 4- and 4.5-fold carbohydrate content in the soluble phase, respectively. Even though the differences attained were low, this had a major effect on the methane yield achieved. While biomass subjected to 120 °C for 20 min increased methane yield by 30 %, the biomass heated for 40 min doubled the methane

production. Similarly, Cho et al. (2013b) reported a methane yield enhancement of 20 % together with 30 % organic matter solubilization when pretreating a microalgae mixture (70 % *Chlorella* sp. and 30 % *Scenedesmus* sp.) at 120 °C for 30 min with regard to 336 L_{CH4} $kgVS^{-1}$ achieved by the raw biomass. These enhancements decreased to 14 % and 4 % when the mixture was pretreated at 80 and 50 °C, respectively. Nevertheless, as pointed out before, these enhancements are strain specific due to the differences in their cell wall and biochemical composition. In this manner, applying the same pretreatment to *Scenedesmus* sp. biomass supported an enhancement of 21–27 % (Mendez et al. 2014b). Even though carbohydrate profile for *Scenedesmus* biomass showed solubilization pattern similar to *Chlorella* biomass, the methane yield enhancement was lower for *Scenedesmus* than the observed value for *Chlorella* biomass. These results could be attributed to the high strength of *Scenedesmus* cell wall. In the case of saline biomass, thermal pretreatment at 120 °C for 2 h has been tested on *Nannochloropsis salina* (Schwede et al. 2013a). This investigation did not follow the organic matter solubilization, but authors observed a twofold increase in methane yield. The same enhancement was observed in semicontinuously operated reactors; however, the absolute methane yield values were diminished by half. In this sense, pretreated biomass digested in batch assay provided methane yield of 570 L $kgVS^{-1}$, while the digestion in continuous mode mediated 270 L $kgVS^{-1}$. The reason for such a decrease was attributed to the high ammonium and salt concentration in the feedstock which ultimately led to volatile fatty acid accumulation.

Moving upwards to higher temperatures in the range of 140–180 °C (applied for 10 min) increased the carbohydrate content in the soluble phase by four- to sixfold, while protein solubilization was enhanced by one- to twofold (Mendez et al. 2014a). Concomitantly with the enhanced carbohydrates solubilization, methane yield was improved by 1.4–1.6-fold in comparison to that of the raw *Chlorella* biomass. Ometto et al. (2014) also studied high temperatures in the range of 120–165 °C. Their results showed an organic matter solubilization of around 40 % when applying temperature at 165 °C for *Scenedesmus obliquus* and *Chlorella sorokiniana*. Despite the fact that the organic matter solubilization was similar, the methane yield in the case of *Scenedesmus obliquus* was enhanced by 200 % and for *Chlorella sorokiniana* by 100 % in comparison to the raw biomass (88 and 118 L_{CH4} $kgVS^{-1}$, respectively). Within the high temperature range, high-pressure thermal hydrolysis (170 °C at 800 KPa) as pretreatment for *Scenedesmus* hydrolysis has been also tested (Keymer et al. 2013). These conditions of temperature and pressure were maintained for 30 min and resulted in 11-fold organic matter solubilization and 81 % increase in methane yield over that of raw algae (150 L_{CH4} $kgVS^{-1}$).

3.2.1.1.3 Chemical Pretreatments

Chemical pretreatments have been studied to a lesser extent due to the need to readjust the pH changes prior to feeding into the reactor and associated chemical cost. The positive effect of acid catalysts in thermal pretreatment on other substrates, such as lignocellulose, has been confirmed. Thermo-acid pretreatment clearly affects the cell wall by solubilizing polymers, thus favoring anaerobic microbial degradation. This pretreatment, for instance, has been proven particularly efficient in solubilizing microalgae carbohydrates (Mendez et al. 2013). Treating *Chlorella vulgaris* at 120 °C for 40 min at pH 2 increased carbohydrates in the soluble phase by sevenfold, while the acid addition alone provided only 2.3-fold increase. Proteins were also solubilized; however, their conversion into other complex molecules made the quantification impossible. With regard to methane yield of this thermo-acid pretreatment, the higher solubilization of carbohydrates did not support an enhanced methane yield compared to only the thermally treated biomass. Thermally pretreated biomass yielded 267.7 L_{CH4} kg COD^{-1}, while when this pretreatment was combined with pH reduction, methane yield reached 228.6 L_{CH4} $kgCOD^{-1}$. Chemical supplementation combined with this temperature hindered the methane production, probably mediated by unidentified side product released during the pretreatment.

In case of lignocellulosic biomass, alkali addition disrupts the lignin structure and breaks the linkage between lignin and other cell wall carbohydrates. This feature does not affect microalgal biomass since these substrates are lignin-free. Nevertheless, alkali pretreatment enlarges the surface area of cellulose by biomass swelling and reduces cellulose crystallinity by cleavage of carbohydrate glycosidic bonds (Hsu 1996). Alkali pretreatment at pH 10 and temperature of 120 °C led to an increase of proteins in the soluble phase by 1.7- and 1.9-fold when pretreating *Chlorella vulgaris* biomass for 20 and 40 min, respectively (Mendez et al. 2013). Under this alkali scenario, carbohydrates were solubilized in a similar fashion like the thermal pretreatment which did not involve chemicals. Thermo-alkali pretreatment reported slightly higher values on methane yield than that observed for thermo-acidic pretreatment. Methane yield was reportedly enhanced by 1.73-fold when a combination of 120 °C for 40 min and pH 10 was used as pretreatment, while for the raw biomass, it was 139 L_{CH4} $kgCOD^{-1}$ only.

Carbohydrate solubilization is also reported when pretreatment involves lower temperatures (50 °C), in addition to alkali (Mahdy et al. 2014b). The solubilization of polymer is strain specific. For *C. vulgaris*, it ranged from 1 to 18 %, while for *Scenedesmus* sp., it was 15–44 %. Once again, even though the organic matter solubilization was higher for *Scenedesmus*, methane yield enhancement was higher for *C. vulgaris*. The reason for that was attributed to the nature of carbohydrates solubilized. This study showed low methane

production enhancement under this conditions (approximately 15 % when biomass was pretreated with 5 % w/w NaOH). This low enhancement was ascribed to the fact that the organic matter solubilization was probably mediated by exopolymers released during the pretreatment rather than those from within the cell. When alkali pretreatment was performed over a mixture of microalgae (70 % *Chlorella* sp. and 30 % *Scenedesmus* sp.) without heating, only 5 % organic matter solubilization was observed at pH 9 and 11, while this value increased up to 21 % at pH 13 (Cho et al. 2013b). Nevertheless, pH 11 and 13 negatively affected methane production and inhibited the process. Subjecting the biomass to pH 9 provided only a slight enhancement. It can be thus concluded that alkali pretreatment of microalgal biomass is not a promising method.

Overall, acidic pretreatments increased the carbohydrates released into the medium while under alkaline conditions; proteins were solubilized to a greater extent. The drawback of using concentrated chemicals includes material corrosion and formation of by-products that could result in inhibition of digestion (Monlau et al. 2014).

3.2.1.1.4 Physical Pretreatment

Physical pretreatment involves the reduction of particle size and increase in surface/volume ratio available for hydrolysis. Within this category, microwave and ultrasound are the most commonly used methodologies. Microwave pretreatment involves boiling of water using microwave radiation. This process resulted in cell hydrolysis and structural changes in proteins (Park et al. 2010). Microwave pretreatment (900 W for 3 min having specific energy of 70,000 kJ kg VS^{-1}) has been tested on a mixture of microalgae, and the results on methane yield were not significant (Passos et al. 2014a). Minor increase in methane yield was attributed to the possible cell wall damage, but since lysis did not occur, any significant increase in yield was precluded. However, it remains to be seen if this pretreatment has strain specificity. For application of ultrasound to microalgal biomass, literature is more extensive. As a matter of fact, a detailed review on the effect of this pretreatment on different biomasses, including microalgae, is available (González-Fernández et al. 2014). Ultrasound consists of elastic waves with frequency range between 20 kHz and 1 GHz. Bubbles are formed in the liquid and filled with the liquid's vapor and dissolved gases. Above a critical value of local pressure, the bubbles implode violently, producing powerful hydromechanical shear forces in the liquid medium surrounding them. On micro scale, the cavitation process produces temperature of around 5000 °C and pressure of 50 MPa for microseconds (Suslick 1990). Ultrasound pretreatment has also been proven as an effective pretreatment for two of the most robust microalgae, *Chlorella vulgaris* and *Scenedesmus obliquus*. When subjecting *Scenedesmus obliquus* to different levels of ultrasound energy, the results showed methane production at a twofold higher level on lower ultrasound levels (35.5 MJ kg^{-1} TS^{-1}) and a fourfold level when applying higher energy levels (76.5–130 MJ $kgTS^{-1}$) (González-Fernández et al. 2012b). The highest energy supplied (100–130 MJ $kgTS^{-1}$) almost doubled the methane production for the untreated *S. obliquus* (51 L_{CH4} $kgVS^{-1}$). This enhancement is similar to that observed for thermal pretreatment of this biomass, and therefore, due to lower energy requirements of thermal application, this latter one might be a preferable pretreatment (González-Fernández et al. 2012a). Ometto et al. (2014) compared the effect of ultrasound on three photosynthetic microorganisms, namely, *C. vulgaris, S. obliquus*, and *A. maxima*. This investigation showed that the microalgae *C. vulgaris* provided the highest methane yield enhancement (44 % in comparison to the raw biomass which attained 169 L_{CH4} $kgVS^{-1}$) when the biomass was subjected to 35 MJ $kgTS^{-1}$. In the case of *A. maxima*, 82 % organic matter solubilization and 33 % methane yield enhancement were observed, regardless of the increase in power output applied (from 0.35 up to 35 MJ $kgTS^{-1}$). This was due to the fact that *A. maxima* has a weaker cell wall compared to other microalgae, and therefore, even the lowest energy level applied provided the very high solubilization and methane yield achievable out of this biomass. On the other hand, when applying a specific energy of 32 MJ $kgTS^{-1}$ to a microalgal mixture composed of *Monoraphidium* sp., *Stigeoclonium* sp., and the diatoms *Nitzschia* sp. and *Amphora* sp., the methane yield enhancement was 11 % (raw biomass exhibited 148 L_{CH4} $kgVS^{-1}$) (Passos et al. 2014b). This low enhancement was attributed to the presence of diatoms which have a silica-based cell wall which is only slightly degradable. Methane yield increased by 33 % after subjecting this biomass mixture to 67.2 MJ $kgTS^{-1}$; nevertheless, the authors have also pointed out that the preliminary energy assessment indicated that the energy input was higher than the extra energy produced. The general conclusion that can be pointed out of the ultrasound pretreatment studies confirms that the thermal pretreatments can be more effective than ultrasound in enhancing microalgae digestibility (González-Fernández et al. 2012b; Cho et al. 2013b; Ometto et al. 2014).

3.2.1.1.5 Enzymatic Hydrolysis

For enzymatic hydrolysis, the correct choice of enzymes is crucial for a successful pretreatment. So far, very little work has been done on the effect of enzymatic hydrolysis of microalgae for methane production purposes (Miao et al. 2013; Ciudad et al. 2014). Cell wall enzymatic pretreatment may not be useful for biofuel production only but also for value-added products that can be extracted from this biomass. It is of high importance to understand the chemical composition of the targeted microalgae cell wall. Enzymatic pretreatment precludes the formation of inhibiting by-

products. The overall process cost of enzymatic hydrolysis may be lower than thermochemical hydrolysis as it avoids corrosion of the container and requires mild temperature. Likewise, enzymes can be produced naturally by a wide range of bacteria and fungi.

As mentioned above, the cell wall composition is one of the key parameters. *Chlorella vulgaris* is probably one of the most studied microalgae, but the composition of its cell wall is still not clear. Some studies have claimed that this microalga possesses a carbohydrate-based cell wall mainly composed of cellulose and hemicellulose. Nevertheless, lately, few investigations have pointed out that this might not be true (Kim et al. 2014). It seems likely that *C. vulgaris* does not have a rigid cell wall because of cellulose, but uronic acids and amino sugars are conferring this microalgal cell wall its hardness (Gerken et al. 2013). Therefore, enzymatic hydrolysis may be extremely useful to gain insights on cell wall composition.

The addition of carbohydrase and protease mediated high carbohydrate and proteins solubilization (86–96 %) when applied on *Chlamydomonas reinhardtii* and *Chlorella vulgaris* (Mahdy et al. 2014a). Out of these two biocatalytic cocktails, protease addition was more beneficial for biogas production. In the case of *C. reinhardtii*, protease addition increased methane production by 1.17-fold in comparison to the raw biomass (263 L_{CH4} $kgCOD^{-1}$). This enhancement was low due to the inherent high biodegradability of this biomass. On the other hand, hydrolyzed *C. vulgaris* mediated an enhancement of 51 % compared to the raw biomass (190 L_{CH4} $kgCOD^{-1}$). In order to optimize the protease dosage, results were tested by decreasing dosages. This attempt resulted in diminished hydrolysis efficiency concomitantly with decreased methane yield enhancement (Mahdy et al. 2014c). Thus, the optimum protease dosage was set at 0.585 AU g DW^{-1}. This dose was tested at increasing biomass loads to elucidate whether the viscosity of the broth was affecting the hydrolysis. The results indicated that this dose can be employed up to 65 g TS L^{-1} without markedly affecting the hydrolysis efficiency or the methane yield enhancement. At this point, it should be stressed out that proteases are released under biomass storage. Using some other biocatalyst cocktails, Ometto et al. (2014) tested a mixture of endoglucanase and cellulase and a mixture of esterase and protease in two microalgae and one cyanobacterium. Their results showed that the first mixture was better to hydrolyze *Scenedesmus obliquus* and increased methane yield by 5.6-fold, while the enhancement in the case of *Chlorella vulgaris* was decreased by 3.15-fold. The second mixture of enzymes provided an enhancement in methane yield of 3.9- and 3.2-fold for *S. obliquus* and *C. vulgaris*, respectively. In the case of the cyanobacterium *Arthrospira maxima*, no significant differences were attained between the two cocktails; thereby, both of them provided a biogas yield increase of approximately eightfold.

Only one study is available in the recent literature concerning the use of noncommercial enzymes for hydrolyzing microalgae biomass. In this context, marine bacteria with cellulolytic capacity (mainly exhibiting endoglucanase activity) were tested on *Nannochloropsis gaditana* (Muñoz et al. 2014). After 25 days of digestion, methane yield was enhanced by 2.5-fold compared to the raw biomass (109 L_{CH4} $kgVSS^{-1}$). For commercial cocktails which are mostly active at 50 °C, these authors also pointed out that the activities of these enzymes were maximum at 30 °C. Thereby, it can be inferred that energy costs can be decreased due to moderate requirement of temperature. Once identified, the most appropriate enzyme (or a cocktail) which is able to disrupt a broad range of microalgae strain may be produced in situ.

3.2.1.2 Co-digestion

Due to the high nitrogen content, microalgal biomass is characterized by a low C/N ratio. Co-digestion aims to increase the C/N ratio of the substrate introduced into the digester in order to balance nutrients. Different substrates rich in carbon could be combined with microalgal biomass thereby increasing the C/N ratio up to levels close to the optimum for anaerobic digestion, which oscillates between 10 and 30 (Pagés Díaz et al. 2011). The reason for such wide optimum C/N ratio is that it depends on several factors such as the chemical composition of the digested substrate, temperature of the process, and adaptation of the microorganisms to high levels of potentially inhibitory compounds, such as nitrogen (Chen et al. 2008).

Algae could potentially be integrated in a wastewater treatment plant and combine the benefits of nutrient removal, energy production, and CO_2 sequestration. Recently, a study was conducted to evaluate the potential of microalgae biomass as co-substrate for anaerobic digestion of primary and secondary sludge, thereby increasing the methane production and improving the energy balance of the whole process (Mahdy et al. 2014d).

Co-digestion with different substrates has been tested with different microalgae species such as *Spirulina maxima* (Samson and LeDuy 1983), *Arthrospira platensis* (El-Mashad 2013), *Scenedesmus* sp. (Yen and Brune 2007; González-Fernández et al. 2011; Ramos-Suárez et al. 2014a), *Chlorella* sp. (Yen and Brune 2007; Ehimen et al. 2009, 2011; González-Fernández et al. 2011; Wang et al. 2013; Park et al. 2013), *Nannochloropsis salina* (Park and Li 2012; Schwede et al. 2013b), *Microcystis* sp. (Zhong et al. 2012, 2013; Zhao and Ruan 2013), *Isochrysis galbana*, and *Selenastrum capricornutum* (Caporgno et al. 2015). In turn, co-substrates employed are diverse, mostly rich in carbon (sewage sludge, peat hydrolyzate, paper residues, glycerin, waste fat and oils, kitchen wastes, corn straw, switch grass, and prickley pear) and in once case a substrate rich in nitrogen: swine manure.

Most of these studies showed a positive effect when co-digesting microalgal biomass. Yen and Brune (2007) observed an increase in methane production and an improvement in the kinetics of degradation when co-digesting *Scenedesmus* and *Chlorella* with paper residues. Besides the beneficial effects of the balance of nutrients and alkalinity, they found an increase in the activity of cellulase when paper residues were added to the digesters together with microalgal sludge. The cellulase activity registered mediated a better degradation of *Scenedesmus* and *Chlorella* cell wall, and thus, the anaerobic biodegradability of these substrates was improved. Methane yield of microalgal sludge digested alone (C/N= 6.7) was 143.2 L_{CH4} $kgVS^{-1}$, whereas a mixture composed of 40 % algal sludge and 60 % paper residues in VS basis (C/N= 22.6) increased methane yield by 124 %. On the other hand, Wang et al. (2013) suggested that the increase in the biodegradability of *Chlorella* when it was co-digested with activated sludge was consequence of the high quantity and diversity of microorganisms in the sludge that aided in the hydrolysis of microalgal cell wall.

However, not all the studies have shown positive results. González-Fernández et al. (2011) co-digested *Chlorella vulgaris* and *Scenedesmus obliquus* with swine manure expecting microalgae to act as a carbon source to improve the digestion of swine manure. Although the cell walls of both species are rich in carbon, their complexity impeded the degradation and the methane production decreased. A decrease in methane production was also observed when *Spirulina platensis* was co-digested with switch grass (El-Mashad 2013). According to the author, the high lignin content of switch grass was the cause of this reduction even though C/N ratio was higher.

Ammonia produced by nitrogen degradation increased the buffer capacity of the digestion system. Therefore, microalgae could be used as co-substrate of easily degradable energy crops, increasing the organic loading rates (OLR) achievable. Schwede et al. (2013b) observed that the addition of *Nannochloropsis salina* to maize silage in a continuous digestion process facilitated the increase in organic loading rates to higher levels than in the monodigestion of maize silage. Similarly, Ramos-Suárez et al. (2014a) reached OLR as high as 5.33 gVS L^{-1} d^{-1} (HRT of 15 days) in the co-digestion of *Scenedesmus* sp. and *O. maxima* with a methane yield of 307.8 L $kgVS^{-1}$.

Considering the different studies concerning co-digestion of microalgal biomass, it seems that the addition of carbon-rich substrates enhanced the digestion process and an increase in the methane production along with an improvement in the kinetics of degradation. However, each co-substrate needs to be studied separately, since the increase in the C/N ratio cannot be used as a sole indicator of the process performance (Table 5.1).

Table 5.1 Compilation of different co-digestion studies, specie or strain evaluated, its C/N ratio and methane yield; type of assay (B = Batch, C = Continuous); temperature; co-substrate added; %VS of microalgae in the mixture; C/N ratio of the mixture; methane yield in co-digestion

Microalgae	Monodigestion (L_{CH4} $kgVS^{-1}$)	Assay	T (°C)	HRT	Co-substrate	$\%VS_{algae}$	$C/N_{mixture}$	Co-digestion (L_{CH4} $kgVS^{-1}$)	References
S. maxima (C/N = 4.2)	160–190	C	35	20	Peat hydrolyzate	90.4	4.5	200	Samson and LeDuy (1983)
						65	6.3	280	
						48	8	220	
					Sewage sludge	90.7	4.4	310	
						67.3	5.3	280	
						50.6	6.2	360	
					Sulfite liquor	90.7	4.7	250	
						66.1	6.9	50	
						50.6	9.6	30	
Scenedesmus + *Chlorella* (C/N = 6.7)	143.2	C	35	10	Waste paper	75	11.8	242	Yen and Brune (2007)
						50	18	292.5	
						25	36.4	79.3	
						66.7	13.3	274.3	
						40	22.6	321.4	
						33.3	27.2	142.7	
Chlorella (LE-1-butanol) (C/N = 5.6)	267.5	B	37	–	Glycerol	96.5[a]	–	286[a,b]	Ehimen et al. (2009)
Chlorella (LE-acid catalysis) (C/N = 5.6)	222						–	230[a,b]	
C. vulgaris + *S. obliquus* (C/N = 8.3)	128.9[c]	B	35	40	Swine manure	85.4[c]	–	143[c]	González-Fernández et al. (2011)
						50[c]	–	219.9[c]	
						14.6[c]	–	238[c]	

(continued)

Table 5.1 (continued)

Microalgae	Monodigestion (L_{CH4} $kgVS^{-1}$)	Assay	T (°C)	HRT	Co-substrate	$\%VS_{algae}$	$C/N_{mixture}$	Co-digestion (L_{CH4} $kgVS^{-1}$)	References
Nannochloropsis salina (LE) (C/N = 4.4)	130	C	37	40	Grease and oil	33	–	440[b]	Park and Li (2012)
				40		50	–	400[b]	
				40		67	–	360[b]	
				27		33	–	100[b]	
				27		50	–	540	
				27		67	–	260[b]	
				20		33	–	0	
				20		50	–	50	
				20		67	–	50	
				13		33	–	0	
				13		50	–	0	
				13		67	–	0	
Microcystis sp. (C/N = 6.0)	201	B	35	30	Corn straw	–	16	240[b]	Zhong et al. (2012)
						65	20	325	
						50	25	275[b]	
Microcystis sp. (C/N = 6.0)	160	C	35	10	Corn straw	80	15	200[b]	Zhong et al. (2013)
						65	20	234	
						50	25	190[b]	
S. platensis (C/N = 5.3)	355	B	35	40	Switch grass	13	25.5	142.6	El-Mashad (2013)
						17	21.6	160[b]	
						33	13.4	198	
S. platensis (C/N = 5.3)	358.3	B	50	40		13	25.5	198	
						17	21.6	210[b]	
						33	13.4	235.9	
Chlorella sp. (–)	124.2	B	37	45	Activated sludge	4	–	298.6[d]	Wang et al. (2013)
						11	–	272.3[d]	
						41	–	295.8[d]	
N. salina (C/N = 6.5)	210	B	40	36	Corn silage	66.7	9.1	305[b]	Schwede et al. (2013b)
						33.3	14.4	377[b]	
						14.3	21.2	410[b]	
					Corn-cob-mix	25	17.6	380[b]	
C. vulgaris (–)	366	B	35	25	Sewage sludge	50	50	420	Park et al. (2013)
Scenedesmus sp. (C/N = 6.0)	140.3	B	37	40	*Opuntia maxima*	75	7.3	141.6	Ramos-Suárez et al. (2014a)
						50	9.7	154.5	
						25	15.6	233.6	
Isochrysis galbana (C/N = 7.1)	439[e]	B	33	35	Sewage sludge	75	–	440[b,e]	Caporgno et al. (2015)
						50	–	460[b,e]	
						25	–	413[e]	
	261[e]		50	20		75	–	310[b,e]	
						50	–	420[b,e]	
						25	–	510[b,e]	
Selenastrum capricornutum (C/N = 9.2)	271[e]		33	35		75	–	394[b,e]	
						50	–	392[b,e]	
						25	–	330[b,e]	
	185[e]		50	20		75	–	245[b,e]	
						50	–	350[e]	
						25	–	452[b,e]	

[a]Expressed in TS basis (%TS; L_{CH4} $kgTS^{-1}$)
[b]Value estimated on the graphs shown by authors
[c]Expressed in COD basis (%COD; L_{CH4} $kgCOD^{-1}$)
[d]Calculated
[e]Expressed in biogas (L_{biogas} $kgVS^{-1}$); *LE* Lipid extracted biomass

3.3 Anaerobic Digestion in Microalgae Biorefineries

Definitely, there is a great potential in microalgae for biofuel production, but the truth is that nowadays microalgal biofuels are far from being economically viable. In this regard, the upper limit value of biomass production cost has been agreed at 0.5 US$ kg^{-1} (Acién et al. 2014), although Chisti (2012) suggested a production cost of 0.25 US$ kg^{-1} for algal fuels to be competitive with petroleum-derived fuel.

Production cost estimates vary widely from study to study due to the lack of industrial plants working at full capacity and a defined technology (Acién et al. 2014). Chisti (2007) estimated production costs of 2.95 US$ kg^{-1} and 3.80 US$ kg^{-1}, respectively, for PBRs and raceways, assuming free carbon dioxide and a facility with an annual biomass production of 100 tons. Acién et al. (2012b) estimated production cost of 69 € kg^{-1} for a 3 m^3 tubular PBR facility producing *Scenedesmus almeriensis*. Authors indicated that by a simplification of the production system and due to economics of scale, increasing annual biomass production up to 200 tons $year^{-1}$ (1570 m^3 of PBRs) could reduce production costs to 12.6 € kg^{-1}, still far away from the upper limit mentioned above. Other authors (Norsker et al. 2011) estimated production costs of microalgae in closed tubular PBRs, flat panels, and raceways to be 4.15, 4.95, and 5.96 € kg^{-1}, respectively, and stated that optimizing production conditions could reduce production costs to 0.68 € kg^{-1}.

In any case, microalgae production costs need to be reduced to meet requirements of the energy market (Acién et al. 2012b). Microalgae industry is expected to keep growing in the near term for the nutraceutical, pharmaceutical, cosmetic, food, and feed industries, whereas fertilizers, bioremediation, and chemical demands are also future applications of interest. If several compounds and products are obtained from microalgae at the same time, the economics of production would improve substantially. It has to be taken into account that all these products have higher market prices than biofuels, and therefore, the production of the latter can be expected to be as a marginal case at the end of a production line. Whatever the compound/s to be extracted or product/s to be produced from microalgae, it is expected that in the large scale this activity would produce great amount of organic residues that would require appropriate treatment (Ramos-Suárez and Carreras 2014).

Anaerobic digestion can be used in a biorefinery concept, as the appropriate process with huge synergistic possibilities for energy generation with microalgae culture. The coupling of anaerobic digestion to the extraction of proteins from microalgae could improve the economics of the process by the generation of renewable energy, the recycling of the digestate as growth medium (Uggetti et al. 2014), and the use of raw biogas or combusted biogas as carbon source (Douškova et al. 2010).

3.3.1 Organic Waste Treatment and Clean Energy Production

The first goal of anaerobic digestion in any biorefinery is the treatment of the organic waste and the production of clean energy in form of biogas, and in the case of microalgal biorefineries, this is not different. In this regard, different options have been assessed up to now, although available literature is pretty scarce.

As already mentioned in this chapter, the major fraction of microalgae grown without nutrient limitation is protein (Rebolloso Fuentes et al. 2000). Therefore, if the goal of a biorefinery is to use most of the generated biomass, protein should be leveraged properly. After the extraction of proteins, the residual biomass could be converted into biogas. Furthermore, the digestion process is improved due to the disruption of the cell wall prior to protein extraction and the increase in the C/N ratio (Ramos-Suárez and Carreras 2014). In their study with *Scenedesmus* biomass digested in CSTR, Ramos-Suárez et al. (2014b) observed an increase in the biodegradability and biogas yield in amino acid-extracted residual biomass similar to that observed after thermal and thermochemical pretreatments by other authors. Biogas yield of *Scenedesmus* residual biomass increased up to 409 L_{biogas} $kgVS^{-1}$ (71 $\%CH_4$) with a digester efficiency of 1084 L_{CH4} $m^{-3}_{digester}$ d^{-1} at and OLR of 3.85 gVS L^{-1} d^{-1} and 20 days of HRT.

Several authors have pointed anaerobic digestion as a convenient supplemental process to biodiesel production from microalgae (Sialve et al. 2009; Chisti 2007), since the produced biogas could serve the electrical and thermal energy necessary to run the biodiesel production process. In this regard, different studies have assessed the combination of lipid extraction from microalgae and subsequent anaerobic digestion of lipid-extracted residual biomass.

Two studies assessed methane potential of *Chlorella* biomass both in batch and continuous mode and after different lipid extraction methods (Ehimen et al. 2009, 2011). A reduction in methane potential was observed when lipid-extracted biomass was digested, although kinetics of the process was improved. The decrease in the methane production was a consequence of the extraction of the lipid fraction, which yields higher methane than proteins and carbohydrates (Sialve et al. 2009). It is important to note the solvent used during the extraction method since it can remain the residual biomass and affect the microorganisms. In fact, it has been demonstrated that the extraction of lipids with a chloroform-methanol mixture in the conventional lipid extraction process can inhibit the process (Ehimen et al. 2009). Contrarily to the results observed by Ehimen et al. (2009), other studies have shown that the lipid

extraction could benefit a subsequent digestion of the residues compared to raw biomass. An increase in methane yield for *Scenedesmus* and *Nannochloropsis gaditana* lipid-extracted biomass has been observed. For *Scenedesmus* biomass, increases of 33.3 and 51.3 % have been observed after lipid extraction (Keymer et al. 2013; Ramos-Suárez and Carreras 2014), whereas for *Nannochloropsis gaditana*, a slight increase of 10 % was observed (Alzate et al. 2014). Results obtained suggest that the increase or decrease of methane potential is dictated by the specie under digestion. In species with resistant cell walls, such as *Scenedesmus*, the rupture of the cell wall is enough to produce a significant increase in the methane production compared to that obtained from raw biomass, even though lipids are extracted from the biomass.

Although major part of the research on this field is focused on the use of lipid- or protein-extracted residual biomass, the ideal biorefinery would use all precious components in a multistep extraction before introducing residues in an anaerobic digester. Lipids and amino acids could be extracted in sequential processes; afterward, residues could be used for biogas production. Moreover, sugars or other high-value products depending on the species could also be extracted. However, the sequential application of different extraction processes is difficult due to the intensive processes used. Normally, the extraction of certain component causes the loss or degradation of the other components, preventing further use of generated residual biomass. Researchers in the University of Almería (Spain) are working currently in the development of multipurpose extraction process (see Fig. 5.4), minimizing residues but using them for anaerobic digestion as a final step to produce biogas and digestate which could be used as fertilizer (Fernández Sevilla 2014).

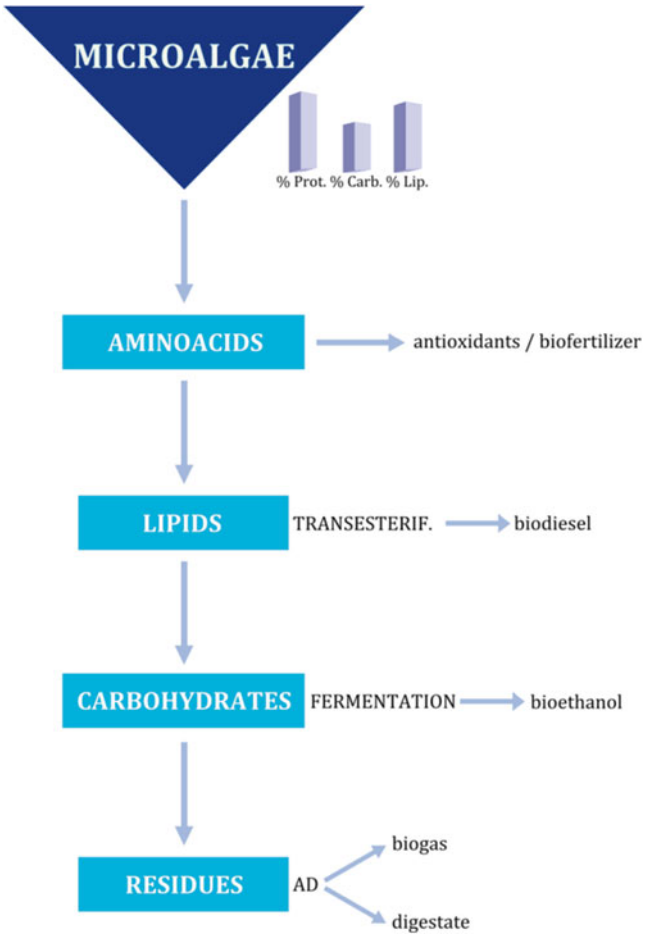

Fig. 5.4 Example of a biorefinery scheme with final anaerobic digestion of spent biomass

3.3.2 Closing the Nutrient Loop

As already said, the two main products of anaerobic digestion are biogas and the digestate. Biogas is formed mainly by methane and carbon dioxide, although it has typically other minor components such as water vapor, hydrogen sulfide, ammonia, hydrogen, and nitrogen. On the other hand, the digestate is an aqueous sludge where almost all nutrients remain in a mineralized form, therefore being used as biofertilizer and/or soil amendment. Both products could be used for microalgae culture, the first as carbon source for their autotrophic growth and the second as growth medium, supplying the necessary mineral nutrients for microalgae growth. Possibilities for their use are described in this section.

3.3.2.1 Biogas Upgrading

There are two possibilities for biogas to be used by microalgae (see Fig. 5.5): (1) biogas upgrading by microalgae to produce methane-enriched biogas and subsequent use of methane-enriched biogas in different applications which can provide additional CO_2 for microalgae culture and (2) supplying the flue gases produced after the combustion of biogas in traditional CHP engines or boilers to microalgae cultures.

Based on these two basic combinations, different additional processes could be included. For instance, in Fig. 5.5a, instead of using the upgraded biogas in CHP units or boilers, biogas could be further upgraded to be used as vehicle fuels, in fuel cells, or to be injected in natural gas grids. Additionally, in both schemes, cultivated microalgae could be introduced in the anaerobic digester as co-substrate to increase biogas production. The use of flue gases as carbon source in microalgae cultures has been extensively studied and will not be covered in this chapter, where we will focus on biogas upgrading by microalgae.

The need of biogas purification depends on the application in which it is being used, as shown in Fig. 5.2. Hydrogen sulfide and carbon dioxide removal technologies are widely applied in biogas plants all over the world, with different existing options based on chemical, physical, or biological methods.

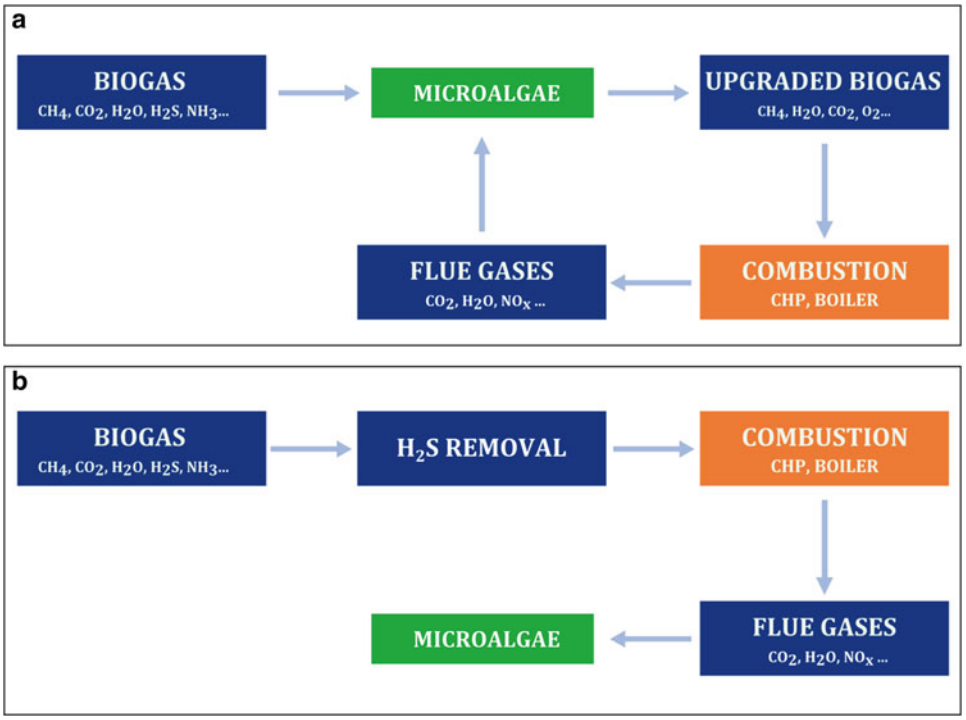

Fig. 5.5 Biogas and microalgae combination schemes: (**a**) with biogas upgrading by microalgae and (**b**) without biogas upgrading but flue gases utilization by microalgae

If microalgae are used for biogas upgrading, the technology needs to be competitive with the existing technologies in the market. Table 5.2 shows a comparison between the main technologies used nowadays for biogas purification. Microalgae show potential in biogas purification at farm scale although capital costs are high compared to other low-cost technologies such as iron sponge, activated carbon, iron chloride addition to substrate, and microareation. Moreover, it is the only technology that has the potential for simultaneous removal of H_2S and CO_2.

The main benefits of microalgal purification over the other technologies are:

- Effective absorption of CO_2 in the form of biomass.
- Generation of microalgal biomass which can be further marketed.
- Microalgal biomass could be used as co-substrate increasing biogas production.
- No residues are produced and no chemicals are used.
- The digestate could be also used as growth medium, removing nitrogen and phosphorous

Microalgal biogas purification is a new technology, poorly developed, that even has some uncertainties that will be important to determine the feasibility of the process. Besides the factors influencing microalgal growth in any conventional culture system (light, mineral nutrients, temperature, pH, mass transfer capacity), if biogas is used as carbon source, some additional factors come into play.

Biogas composition influences purification efficiency according to three factors: (1) CH_4 concentration, (2) CO_2 concentration, and (3) H_2S concentration. Another important point that limits the efficiency of the system is the oxygen concentration in the upgraded biogas.

3.3.2.1.1 Methane Concentration

Methane concentration in raw biogas could reach levels of up to 70 % in some cases. In several studies where biogas has been upgraded by microalgae, the growth rate of these microorganisms has been negatively affected due to high CH_4 concentrations (Kao et al. 2012a; Yan et al. 2014; Douškova et al. 2010). Although there was an evident decrease in the growth rate, in all cases, it was a slight decrease that would not suppose an impediment to the use of biogas as carbon source.

Despite this limitation, methane concentration in purified biogas has been above 90 % in most cases (Kao et al. 2012a; Yan et al. 2014; Travieso et al. 1993; Yan and Zheng 2013; Converti et al. 2009).

3.3.2.1.2 Carbon Dioxide Concentration

In studies where biogas has been used as carbon source of microalgae, no inhibition has been observed with CO_2 concentrations up to 55 % (Yan et al. 2014). However, in other studies performed with flue gases, inhibition of microalgal growth has been observed with CO_2 concentrations above 5 % (Chiu et al. 2009).

In any case, an excessive addition of CO_2 to microalgal cultures could cause the acidification of the growth medium,

Table 5.2 Comparative study of different biogas purification technologies according to the removed contaminant, applicability to farm scale biogas plants, capital costs, O&M costs, ease of operation of the system, regeneration ability of the used adsorbent (if applicable), purified biogas with less than 250 ppm H2S, and environmental impact

Comp.	Technology	Applicable to farm scale	Capital cost	O&M costs	Ease of operation	Regener.	H_2S (<250 ppm)	Env. impact
H_2S	Iron sponge	Yes	Low	Medium	Medium	Partial	Yes	High
	Activated carbon	Yes	Low	Medium	Medium	No	Yes	High
	Chemical absorp.	Neutral	High	High	Difficult	Partial	Yes	Medium
	Biofilters	Neutral	High	Medium	Medium	Partial	Yes	Low
	Iron chloride add.	Yes	Low	Low	Ease	n.a.	No	Medium
	Microareation	Yes	Low	Low	Difficult	n.a.	Yes [a]	Low
CO_2/H_2S	Amine scrubbing	No	High	High	Difficult	Yes	Yes [a]	High
	Water scrubbing	Neutral	Medium	Medium	Medium	Yes	Yes [a]	High
	Org. phys. scrubb.	Neutral	Medium	Medium	Medium	Yes	Yes [a]	High
	Membranes	Neutral	High	High	Difficult	n.a.	Yes [a]	Medium
CO_2	PSA	Neutral	Medium	High	Difficult	Yes	Yes [a]	Medium
	Cryogenic	No	High	High	Difficult	n.a.	Yes	Medium
CO_2+H_2S	Microalgae	Yes	High	Medium	Difficult	n.a.	Yes	Low

Source: adapted from McKinsey (2003); Deublein and Steinhauser (2008)
n.a. not applicable
[a]possible need to install an additional H_2S removal step

a decrease in microalgae growth rate, and the subsequent reduction of purification efficiency (Acién et al. 2012a; Sumardiono et al. 2014; Yan et al. 2014). Therefore, addition of CO_2, and consequently of biogas, must always be controlled as a function of pH, matching CO_2 addition to the CO_2 consumption by microalgae.

Several studies have explored the possibility of injecting biogas and air intermittently, observing an increase in microalgae growth rate (Sumardiono et al. 2014; Kao et al. 2012b). This kind of systems would entail at list two duplicate systems working in parallel to achieve a constant-in-time purification of biogas, avoiding at the same time the dilution of purified biogas with air. Also, it has to be taken into account that there are microalgae species more resistant to high concentrations of CO_2, as it is the case of a mutant strain of *Chlorella* sp. (Kao et al. 2012b).

3.3.2.1.3 Hydrogen Sulfide Concentration

Hydrogen sulfide is often present in biogas in concentrations up to 1 % (10,000 ppm). This component is probably the one which brings more uncertainties to the efficiency of the microalgal biogas upgrading system. Moreover, there exists little information about what happens with this component during biogas supplying to microalgal cultures. Some authors have hypothesized about what is the mechanism of removal of H_2S from biogas. Travieso et al. (1993) suggested that this removal would be caused mainly by absorption of hydrogen sulfide into the aqueous growth medium; once absorbed, sulfur would be consumed by microalgae cells for the production of amino acids but at a slower rate than the absorption rate (Travieso et al. 1993). Therefore, at first, we might consider that biogas cleaning by microalgae removes both CO_2 and H_2S, as has been shown in some studies (Travieso et al. 1993; Mann et al. 2009; Douškova et al. 2010). However, other studies showed that the presence of H_2S in the system is inhibitory to the growth of microalgae (Kao et al. 2012a). Consequently, in other studies, H_2S is removed before employing biogas as carbon source for microalgae (Yan and Zheng 2013). It is probably an excessive accumulation of H_2S in growth medium which caused inhibition of microalgal growth in some cases.

Although this point may not seem important, economic viability compared to other methods of biogas upgrading could change dramatically if there is a need to install an additional system to remove H_2S, as often happens in the conventional systems shown in Table 5.2.

3.3.2.1.4 Oxygen Concentration in Upgraded Biogas

In any system where microalgae grow, oxygen will be produced due to photosynthetic activity. This oxygen should be removed from the growth medium to impede photosynthesis inhibition due to oxygen saturation (Acién et al. 2013). Therefore, one of the technical challenges is how to remove this oxygen from the growth medium avoiding at the same time the accumulation of oxygen in the purified biogas. The maximum oxygen concentration would be determined by the application in which the biogas is to be used and the flammability risks of the gas mixture.

Normally, the minimum oxygen concentration (MOC) below which the combustion of a gas mixture is not possible oscillates between 5 and 15 %. For the case of methane, the MOC is 12 %. However, this relation changes with pressure

and temperature. The higher the pressure and the temperature, the lower the MOC, i.e., gas mixture is more explosive. This is a very important factor which has to be taken into account due to safety reasons.

3.3.2.2 Digestate Recycling as Growth Medium

The digestate is an aqueous medium which is composed of stabilized organic matter (active biomass and hardly biodegradable organic matter) and mineralized nutrients. Digestate is an easy product to handle and can successfully substitute mineral fertilizers in agricultural applications (Lukehurst et al. 2010). Anaerobic digestion is a closed system where the only inputs are the substrate and energy. Therefore, the fertilizer value of the digestate depends on the substrate composition, i.e., the nutrients that are supplied to a digester via the feedstock are the same as those in the digestate. However, nutrients in the feedstock will change in form during the anaerobic digestion process due to biochemical reactions that take place inside the digester, enhancing their availability to crops (Lukehurst et al. 2010).

The use of digestate as growth medium could improve economics and environmental impact of microalgae production by reducing water needs and fertilizer costs. For the specific case of biofuel production from microalgae, the availability of these two inputs is crucial for its viability (Uggetti et al. 2014).

Use of digestate as a growth medium for microalgae culture implies to consider some important points. First of all, it is necessary to remove the solid fraction of the digestate. The removal of the solids from the digestate will improve light availability and ease final separation of higher-quality microalgal biomass. Digestate liquid fraction is characterized by high ammonium content (Lukehurst et al. 2010) and high turbidity (Noike et al. 2004), factors that could influence microalgal growth and reduce the suitability of digestate as growth medium (Uggetti et al. 2014). According to Collos and Harrison (2014), who studied the effect of different ammonium concentrations on several species of microalgae, there are significant differences between classes of unicellular algae. According to this study, chlorophytes are the most tolerant class to high ammonium, whereas dinoflagellates are the least tolerant. Furthermore, ammonia toxicity is associated with NH_3 at $pH > 9$, whereas at $pH < 8$, toxicity is likely to be associated with the ammonium ion. The effects of ammonium on microalgae are evident on long-term growth rates (days), but also on short-term physiological processes such as uptake rates, photosynthetic rates, and enzyme activities (minutes to hours).

Moreover, the use of digestate as fertilizer is normally regulated in order to protect animal and human health as well as the quality of the crops (Lukehurst et al. 2010). Therefore, the use of digestate as growth medium for microalgae culture could limit the ultimate fate of the produced biomass.

In literature, relatively very few recent studies have investigated on the use of digestate as growth medium for microalgae culture (Cho et al. 2013a; Uggetti et al. 2014; Ficara et al. 2014; Prajapati et al. 2014b; Fouilland et al. 2014; Franchino et al. 2012; Sheets et al. 2014; Marcilhac et al. 2014). From most of these studies, it can be concluded that the ammonium concentration in digestate negatively affects microalgae growth, and therefore, it should be diluted before being used (Cho et al. 2013a; Uggetti et al. 2014; Prajapati et al. 2014b; Fouilland et al. 2014; Franchino et al. 2012; Sheets et al. 2014). This statement applies to several microalgae strains: *Chlorella*, *Scenedesmus*, *Chroococcus*, *Botryococcus*, *Nannochloris*, *Dunaliella*, *Lyngbya aestuarii*, *Neochloris* and *Nannochloropsis*. The impact of ammonia varied from strain to strain, as already concluded by Collos and Harrison (2014). Conversely, Ficara et al. (2014) observed no severe inhibition when using undiluted digestate to grow an algal suspension made of a mixture of *Scenedesmus* and *Chlorella*, although a decrease in nitrogen removal efficiency was evident. Dilution could be done with any available water, but preferably, wastewaters with low ammonium content should be used in order to reduce environmental impact.

On the other hand, there is no consensus about the effect of turbidity and color of the digestate on microalgal growth. Some authors pointed out that biomass concentration limits light availability, and therefore microalgal growth rate, to a greater extent than turbidity (Uggetti et al. 2014). Contrarily, other studies suggested that digestate color, even when diluted up to 30 %, hindered light availability and microalgal growth (Prajapati et al. 2014b; Marcilhac et al. 2014).

It should be noted again that digestate composition and color depend on the substrate being fed into the digester and on the process that takes place in each digester; therefore, the successful use of different digestates as growth medium will vary from case to case and also with a high dependence on microalgae strain (Marcilhac et al. 2014). This can be seen in Table 5.3, where the optimal dilutions found by some of the studies conducted are shown.

Table 5.3 Optimal digestate concentration in growth medium for different microalgae

Microalgae	Digestate conc. (% v/v)	Feedstock (anaerobic digester)	References
N. salina	7	Municipal wastewater	Sheets et al. (2014)
C. vulgaris	10	Cattle slurry and raw cheese whey	Franchino et al. (2012)
Scenedesmus spp.	20	Microalgae (*Scenedesmus* spp.)	Fouilland et al. (2014)
Chroococcus sp.	30	Microalgae (*Chroococcus* sp.)	Prajapati et al. (2014b)

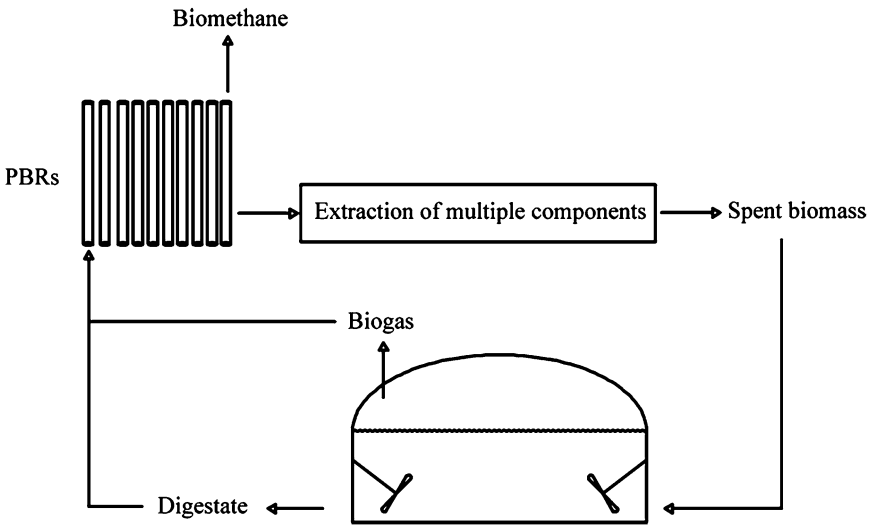

Fig. 5.6 Simplified scheme of a biorefinery with integration of anaerobic digestion

The use of digestate as growth medium will reduce, or even eliminate, the use of additional nutrients. The level of self-sufficiency of nutrients will depend on the composition and availability of the digestate. For instance, Ramos-Suárez et al. (2014b) prospected a reduction of 30 % in nitrogen needs if digestate from amino acid-extracted microalgae degradation was recycled as growth medium in a closed biorefinery concept. In this specific case, the extraction of amino acids reduced nitrogen content of digested biomass and, therefore, nitrogen content in the digestate. In this kind of closed microalgae biorefineries, for different classes of spent biomass, savings in fertilizer could be higher.

4 Conclusion and Future Prospects

The integration of different technologies in a biorefinery aims at maximizing benefits while reducing the environmental impact. Considering what we have shown in this chapter, the future of algae biorefineries would include the extraction of several components from microalgae and simultaneous reduction of the waste biomass. Waste biomass would be treated by anaerobic digestion thus reducing the pollutant load while producing energy and recycling nutrients for microalgae culture. A simplified scheme of the prospected biorefinery is shown in Fig. 5.6.

According to authors, current bottlenecks for the development of this concept of biorefinery include:

- The extraction of different components from biomass in a sequential process
- Effective biogas upgrading by microalgae
- Use of the digestate as growth medium without dilution

If the nutrient loop is closed, profitable processes will be achieved. Consequently, biofuels and high-value products would be obtained at the same time from microalgal biomass, reducing environmental impact and increasing profits.

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Algae-Based Biohydrogen: Current Status of Bioprocess Routes, Economical Assessment, and Major Bottlenecks

6

Richa Kothari, Arya Pandey, Virendra Kumar, and V.V. Tyagi

1 Introduction

Hydrogen is a most efficient fuel and has the highest energy density among known fuels (143 GJ/tonne) in terms of energy values as well as from an environmental point of view. It is a zero emission fuel which does not contain carbon, sulphur, or nitrogen and generates water only as a by-product on combustion. Recently it is being very efficiently used as a vehicle fuel in automobiles and also for electricity generation via fuel cells. Commercially, hydrogen is produced by using fossil fuels such as coal, methane, and other heavy hydrocarbons (Kothari et al. 2008). All these processes of hydrogen production are very expensive and not environmentally friendly. Recently, researchers have sought alternative methods for hydrogen production including photolysis of water and biological methods of hydrogen production (Nayak et al. 2014). Biologically produced hydrogen by using microorganisms such as bacteria and algae by photosynthetic and fermentative routes (Monlau et al. 2013; Julia et al. 2014; Kothari et al. 2011; Venkata et al. 2007; Levin et al. 2004) provides a sustainable approach for society. Biological processes can scale up biohydrogen production by using various microorganisms and making it potentially competitive with chemical processes including thermal gasification, pyrolysis, and reforming among others. Biohydrogen production via a biological route is beneficial because it is neutral regarding CO_2 emission and free from other greenhouse gases such as carbon monoxide and hydrogen sulphide and it does not require any kind of treatment before use in the fuel cell to generate electricity. Yield of biohydrogen production depends on operating cost whereas its rate depends upon its installation cost or reactor cost.

Biophotolysis (direct biophotolysis and indirect biophotolysis), photofermentation, and dark fermentation (Venkata et al. 2009) are the emergent bioprocess routes for the production of biohydrogen. Among these, algae-based bioprocess production routes are projecting more scope in the R&D sector with commercialization. Indeed, algae present several advantages compared to terrestrial plants in virtue of: (1) algae have a higher growth rate than plants and they are more capable in CO_2 fixation; (2) they can be grown easily in water and wastewater (Venkata et al. 2012); (3) they are rich in carbohydrates and have a lack of lignin (Nayak and Das 2013). Besides these, algae is a third-generation biofuel produced from macroalgae, and microalgae are more advantageous than second-generation biofuel produced from nonedible crops because they do not require fertile land for their growth and they have the potential to provide jobs for skilled and unskilled members of society.

There is very modest information available in the literature regarding the journey of lab-scale to large-scale commercial production of biohydrogen with algae. Hence, the present chapter aims to make available considerable research and developmental progress with major bottlenecks through bioprocess routes for algal-biomass–based biohydrogen production with emphasis on the major factors involved.

2 Bioprocess Routes for Biohydrogen Production by Algae

Algae have wide potential for bioenergy generation by their metabolic activity as well as their anaerobic fermentation due to their rapid growth and rich carbohydrate contents. Biohydrogen production through biological process is significant and economically viable by algae because it is less expensive, has an easily available feedstock, and can use

R. Kothari (✉) • A. Pandey • V. Kumar
Bioenergy and Wastewater Treatment Laboratory, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India
e-mail: kotharicha21@gmail.com

V.V. Tyagi
School of Energy Management, Shree Mata Vaishno Devi University, Katra, Jammu & Kashmir 182320, India

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waste material as a substrate for growth (Venkata et al. 2007). In this section, we mainly focus on biophotolysis (BP) routes and dark fermentation (DF) routes for biohydrogen using algal biomass.

2.1 Direct and Indirect Biophotolysis

2.1.1 Direct Biophotolysis

Direct biophotolysis is the process responsible for algal photosynthetic activities; solar energy is directly converted to hydrogen in the reaction routes of photosynthesis (Eq. (6.1)). This natural process is part of its attraction among scientists because it converts available substrate water to oxygen and hydrogen.

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \quad (6.1)$$

This process works at a partial pressure of near one atmosphere of O_2. On the other hand, oxygen sensitivity to the hydrogenase enzyme reaction always creates a hindrance in the process (Frigon and Guiot 2010). Monlau et al. (2013) reported hydrogen production rates on the order of 0.07 mmol/h per litre in their experimental study with direct biophotolysis.

2.1.2 Indirect Biophotolysis

Algal-based biohydrogen production with indirect BP is completed in two stages: the first involves the synthesis of carbohydrate by using a photosynthetic process, and the second stage covers the degradation of stored carbohydrates in anaerobic condition (Azapagic and Stichnothe. 2011). Stage 1 and stage 2 are reported as photofermentation and dark fermentation with light and without light, respectively (Tommasi et al. 2012). Cyanobacteria also have the unique feature of using ambient CO_2 as a carbon source and solar light as an energy source (Eq. (6.2)). The cells take up CO_2 first to produce cellular substances, which are subsequently used for hydrogen production (Eq. (6.3)). The overall mechanism of hydrogen production in cyanobacteria can be represented by the following reactions:

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (6.2)$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \quad (6.3)$$

Both algae and cyanobacteria have the capacity to produce biohydrogen but algae are better than cyanobacteria because they require high-energy intensive enzymes and ATP requires nitrogenase for biohydrogen production but in cyanobacteria production of biohydrogen and oxygen both take place at separate times and places known as indirect biophotolysis (Tommasi et al. 2012). Algae produce biohydrogen by a water-splitting process to form hydrogen, but the rate of biohydrogen production is not as high as the CO_2 reduction. In this process oxygen is also produced, which inhibits the production of biohydrogen, because hydrogenase is highly sensitive to oxygen. Therefore, research work is being done in this field to discover the key component that reduces the production of oxygen out of which sulphur deprivation is best and potassium deficiency has also been found as a biological switch that reduces oxygen production. Here, oxygen is not a problem but solar conversion efficiency is low (Julia et al. 2014). Table 6.1 shows the result obtained after review of the existing literature based on algal and cyanobacteria biomass available for biohydrogen production by the bioprocess route of direct and indirect biophotolysis.

2.1.3 Factors Affecting Biophotolysis (BP)

Factors affecting the process are numerous but only a few important ones are discussed here in the subsections.

2.1.3.1 Immobilization

Microalgae cultivated in the form of immobilized cells would have versatile applications because their CO_2 capturing rate is high to convert them into organic compounds. The rate of biohydrogen production via immobilized cells is higher than free cells (Brouers and Hall 1986).

2.1.3.2 pH

Biohydrogen production is directly related to pH (Table 6.2). Hydrogenases and nitrogenase are the biohydrogen-producing enzymes, sensitive to pH because at low pH (less than 5) it reduces the enzymatic activity and also the biohy-

Table 6.1 Algae and cyanobacteria biomass for producing biohydrogen cited in the literature

Broad classification	Name of algae	Reference
Green algae	*Chlamydomonas reinhardtii*	Julia et al. (2014)
	Chlorella sorokiniana	Chader et al. (2009)
	Chlorella vulgaris	Rashid et al. (2011)
	Chlorella fusca	Das and Veziroglu (2008)
	Scenedesmus obliquus	
	Chlorococcum littorale	
	Platymonas subcordiformis	
Cyanobacteria (indirect biophotolysis)	*Oscillatoria*	Pinto et al. (2002)
	Calothrix	
	Gloeocapsa	

Table 6.2 Algal biohydrogen production with different substrates at optimal pH

Name of sp.	Substrate used	Optimal pH	References
Chlorella vulgaris	Malt extract	8.0 to 9.0	Rashid et al. (2011)
C. renhardtii	glucose	6.9	Kosourov et al. (2002)

drogen production rate. The pH value is also varied for freshwater algae and marine water because the requirement of the pH value for freshwater algae is different to marine water algae because marine algae require low nitrate uptake. The main factor responsible for the change in pH is nitrate uptake along with the fixation of carbon.

2.1.3.3 Carbon Source

The carbon source is one of the important factors for the cultivation of microalgae. During the process of photosynthesis these microalgae use carbon and store it in the form of starch and glycogen but the storage of this starch and glycogen is limited as a result of which biohydrogen production is also limited, thereby requiring an exogenic source of carbon (organic carbon such as glucose, fructose, malt extract, etc. in wastewater). The role of the carbon source in the cultivation of microalgae is not well understood but some research work has been done on the effect of the carbon source on microalgae in anaerobic conditions.

A cyanobacteria and green algae *Microsystis aeruginosa*, *Chlorella vulgaris*, respectively, were used on substrates including malt extract, glucose, and sucrose, and maximum biohydrogen production was on the malt extract: 1300 ml/l. *Anabaena* species strain CH_3 was cultivated by using fructose, galactose, sucrose, and glucose as a feeding material and it was found that the most suitable substrate for biohydrogen production was fructose and sucrose that produced 0.0016 mol and 0.001 mol of biohydrogen production, respectively (Table 6.3).

2.1.3.4 Light

The most suitable light frequency that provides energy for algal growth is in the 400 to 700 nm wavelength. In a temperate climate algal biomass production is much lower than in a tropical climate because of variation in solar radiation. Sutherland et al. (2013) have investigated that in summer algae biomass production increased about 250 % because of the presence of three times more solar radiation in summer than in winter.

Table 6.3 Yield of algal-based biohydrogen with different substrates as carbon source

Microbial sp.	Substrate used	Biohydrogen Production Rate	Reference
Microsystis aeruginosa (cyanobacteria)	Malt extract	1300 ml/L	Song et al. (2011)
Chlorella vulgaris (green algae)	Malt extract	1300 ml/L	Song et al. (2011)
Anabaena sp. Strain CH3	Fructose	0.0016 mol/200 ppm	Chen et al. (2008)
	Sucrose	0.001 mol/200 ppm	

2.2 Dark Fermentation (DF)

Dark fermentation is a simple process manifested by anaerobic bacteria with the capacity to produce biohydrogen by using organic acid and waste material as a substrate. This process mainly involves two pathways: acetate and butyrate. There are two common pathways in the production of hydrogen by dark H_2 fermentation (Kothari et al. 2012): one producing acetate and the second butyrate. Theoretically, the hydrolytic fermentation of 1 mol of glucose yields 4 and 2 mol of H_2 through acetate and butyrate pathways, respectively (Angenent et al. 2004):

$$1.\ C_6H_{12}O_6 + 2H_2O = 4H_2 + 2CO_2 + 2CH_3COOH \quad (6.4)$$

(Hydrogen fermentation to acetate pathways)

$$2.\ C_6H_{12}O_6 = 2H_2 + CH_3CH_2CH_2COOH + 2CO_2 \quad (6.5)$$

(Hydrogen fermentation to butyrate pathways)

2.2.1 Factors Affecting DF

2.2.1.1 Substrate

Microalgae and cyanobacteria have recently been more emphasised for bioenergy production. The algal biomass is rich in carbohydrates (starch/glycogen/cellulose) and does not contain lignin as does other biomass. Thus, it is easier to obtain monosaccharides from algal biomasses than other lignocellulose material. Some species of cyanobacteria such as *Anabaena* sp., *Synechocystis* PCC6803, *Synechococus*, and *Spirulina* sp. can accumulate contents up to 20–30 % of dry weight (Cao et al. 2010). However, some cyanobacteria store carbohydrate in the cytoplasm and in their cell walls in the form of polysaccharides. These sugars need to be converted to monomers by the application of some pretreatment; chemical (acids and alkaline) and enzymatic hydrolysis are common pretreatment methods.

2.2.1.2 Inoculums

The anaerobic fermentation of algal biomass is mostly done by an anaerobic consortium taken from wastewater treatment (Table 6.4). There are various types of pure strains also used, such as species of *Clostridium* and *Enterobacter*. A mixed fermentative culture is more common for biohydrogen production as it is simple to operate and does not require sterile conditions as do pure strains. The mixed culture inoculums are mostly taken from soil and anaerobic sludge of wastewater treatment plants. These inoculums are mainly characterised by the bacteria belonging to the genus *Clostridia* and *Bacillus*.

Table 6.4 Effects of inoculum on biohydrogen production from various algal biomass in dark fermentation process

Feedstock	Carbohydrate contents (% of dried biomass)	Bacteria/inoculums	H_2 yield	References
Chlorella vulgaris	57.0	*Clostridium butyricum*	85.3 ml/g-TVS	Liu et al. (2012)
Chlamydomonas reinhardtii	11.8	*Clostridium butyricum* + *Rhodobacter sphaeroides*	128.3 ml/g-TVS	Kim et al. (2006)
Arthrospira platensis	44.4	Mixed culture	354.7 ml/g-TVS	Cheng et al. (2012)
C. Pyrenoidosa sp.	NA	Anaerobic digested sludge,	6.1 ml/g-TS	Sun et al. (2011)
Chlamydomonas Reinhardtii	NA	*C. butyricum* NCIB 9576	40 ml/g-TS	Kim et al. (2006)
Nannochloropsis sp.	NA	*Enterobacter aerogens* ATCC13048	48 ml/g-TS	Nobre et al. (2013)

2.2.1.3 Temperature

The process of hydrogen production is highly affected by temperature changes as a small increase or decrease in temperature might alter the substrate utilization process, hydrogen yield, or formation of liquid products as well as microbial community of the system (d'Ippolito et al. 2010; Hafez et al. 2012). Most of the studies of biohydrogen production are done under mesophilic conditions as they are preferable from economic and technical points of view to thermophilic bacteria and they exhibit high yield under stable conditions (Zhang et al. 2003; Munro et al. 2009). However, the mesophilic biohydrogen production process also favours the growth of nonhydrogen-producing microbes.

2.2.1.4 pH

pH has a profound effect on the fermentative hydrogen production process due to its major role in determination of the acidic and alkaline condition of the system, in the limitation of the growth of bacteria, and regulation of solvent production. Solvent generated at the end of fermentation decreases the pH by acid accumulation. The optimum pH for hydrogen production is found between 5.5 and 6.5 avoiding the solvantogenic phase (Khanal et al. 2004).

2.3 Factor Affecting Both BP and DF Bioprocess Routes

2.3.1 Reactors

There are various types of bioreactors used for algal biomass production for production of biohydrogen in particular. Details of some important bioreactors, different in structural designs (Fig. 6.1) are as follows.

2.3.1.1 Tubular Airlift and Bubble Column

This reactor having vertical transparent tubes made up of glass or polyethylene to get adequate light penetration and CO_2 supply is allowed through bobbing. As we know, fabrication of a vertical tubular bioreactor is cheap but it is not versatile. It does not provide high culture volume and efficient gas transfer because a bioreactor should possess a high area–volume ratio and due to lack of these things its photosynthetic efficiency also decreases (Martnez-Jeronimo and Espinosa-Chavez 1994). Another drawback is that it has a large angle size in comparison to sunlight therefore most of the sunlight would be reflected back, making it a disadvantage in terms of biomass productivity.

2.3.1.2 Helical Tubular Bioreactor

This bioreactor possesses a flexible tubular pipe with a coiled framework along with a heat exchanger and gas exchange tower. Due to its coiled conical shape structure it receives maximum solar radiation for algal growth. In HTR the area-to-volume ratio is high therefore it is possible to gate 6.6 % photosynthetic efficiency and have volumetric productivity of 0.9 g L^{-1} d^{-1} (Tredici and Rodolfi 2004).

2.3.1.3 Flat Plate Bioreactor

Such type of reactor is fabricated mainly to achieve maximum solar radiation therefore it is made by using narrow panels to achieve the maximum area-to-volume ratio. The main advantage of such a reactor is that it possesses an open unit of gas transfer which is also important due to restriction in oxygen buildup, which plays an inhibitory role in biohydrogen production; the main drawback is that its open unit may increase the chances of contamination. It is also beneficial due to its high productivity and uniform light distribution. This system can also be oriented towards the sunlight to achieve maximum radiation. A flat plate solar bioreactor has

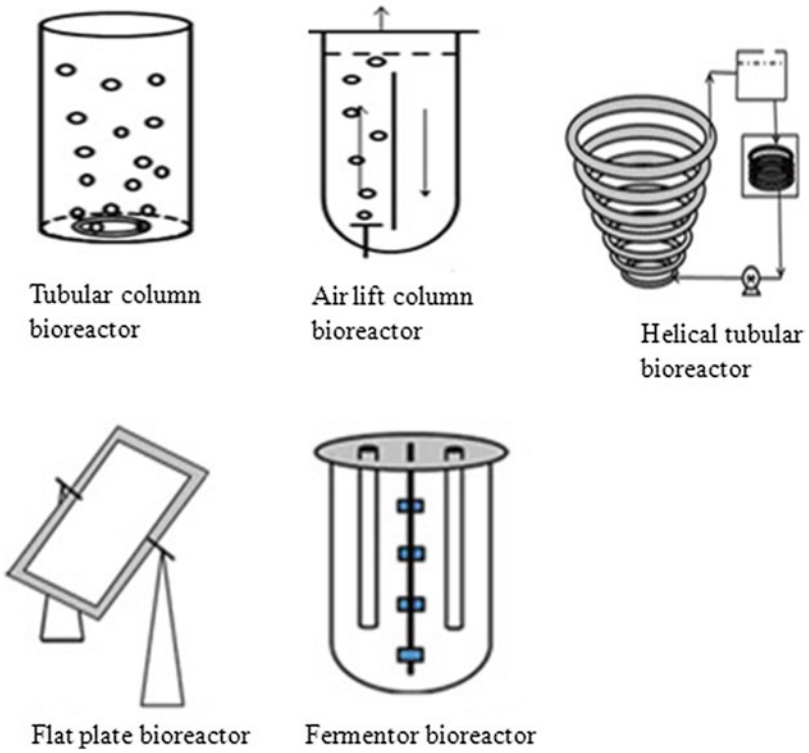

Fig. 6.1 Different types of photobioreactors (Dasgupta et al. 2010)

been made to produce 10 ml hydrogen L^{-1} h^{-1} with 6.5 L capacity and at 30° temperature (Eroglu et al. 2008).

2.3.1.4 Fermentor Type of Bioreactor

The main advantage of this bioreactor is control of parameters such as sunlight, flow rate, and mixing but the main drawback is that it does not do well in receiving solar radiation. It is not applicable at the industrial level (Pohl et al. 1988)

Commercial algal production is low worldwide. Probably 6000 t/year in terms of dry biomass are produced autotrophically in the presence of sunlight and CO_2. Today at the global level, there is no adequate and meaningful amount of algal-based biohydrogen being produced. Biohydrogen production using algal biomass is significant in terms of negative emission of carbon also because when bioenergy sources used atmospheric carbon for its growth at the same time it also removed atmospheric carbon, therefore it is also positive in the sense of carbon sequestration.

3 Economic Stresses on Bioprocess Routes

Economic feasibility of any system depends on the various parameters such as (1) algal biomass required; design of reactor; capital costs; operating costs including power, labour, and water; and general supplies for resulting biomass and energy balance outputs. In the case of biohydrogen production through algal biomass, capital costs were estimated based on vendor quotes, and prior literature studies on standard energy estimates (Tapie and Bernard 1988). Similarly, other factors responsible for economic stress may be fixed operation cost (labour, maintenance, insurance, tests), indirect capital cost, internal rate of return, plant's life duration, and so on. In spite of this, algal biomass production rate with the type of culture/strain is also an important factor in observing the economic status of any bioprocess route. After an extensive literature survey on concerned bioprocess routes for the last 10 years, various researchers discuss the pros and cons in respect of economic inputs, and are listed in Table 6.5.

It has been reported that efficiency of biohydrogen production by using solar energy is very low, that is, less than 1.5 %. Research work has been done to enhance the efficiency by applying some nutrient stress such as sulphur and potassium to suppress the production of oxygen, yet it increases only 10 %. In the photosynthesis process water is used as the substrate thus the operating cost is very low and it requires only its maintenance cost but production of biohydrogen is very low, therefore the reactor should be large and the installation cost is very high. Zaborsky (1999) has reported that a reactor having 10 % light conversion efficiency would cost about \$50/m^2 for a single stage but will cost \$100/m^2 for a two-stage system; he has also suggested

Table 6.5 Economic stresses and feasibilities of algae-based bioprocess routes for biohydrogen as per research accomplished in the last 10 years

S. No.	Description/Highlights	References
1.	Hybrid fermentation that incorporates hydrogen and methenation production both, provides an economically promising and applicable bioprocess route for alternative energy resources.	Arni et al. (2010)
2.	Researchers' work based on urban wastewater treatment in combination with biohydrogen production using microalgae provides the possibility of biofuel with nutrient removal and improves the economic profitability of the whole system for bioenergy prospects.	Batista et al. (2015)
3.	Wastewater treatment and bioenergy generation via algae is an integrated suitable approach, but for the production of economically viable and sustainable algae-based biofuel research work is needed to be done from cultivation of algae to conversion of biomass into energy.	Abbas et al. (2015)
4.	Technoeconomic assessment and life-cycle assessment are the most important tools through which one can easily understand the current status of algae and technologies related to the production of biofuel/biohydrogen, potential and conversion efficiency, and major R&D challenges required in the field of algae technologies.	Jason and Ryan (2015)
5.	For efficient algae culture and biomass harvesting, cost-effective technologies are needed.	Hallenbeck and Benemann (2002)
6.	The authors concluded that at present, biohydrogen productions via biological process routes are not economically viable in comparison to other fuel alternatives. Various technological and engineering challenges have to be solved preceding economic barriers. Economic analyses stated that major R&D challenges are concerned with development of cost-effective photobioreactors and improvement in photosynthetic efficiency.	Show et al. (2012)
7.	The bioprocess route for biohydrogen production requires less energy in comparison to chemical and electrochemical processes. It may be possible to produce biohydrogen in places where biomass is easily available in the form of waste and transport would be possible at low cost as well as low energy expenditure.	Olga and Pavel (2012)
8.	In the case of biological hydrogen production major challenges are low yield of biohydrogen and its production rate, making it not economically viable; these gaps can be bridged by the use of suitable algae species, with the improvement in bioreactor design and improvement in genetic and molecular engineering technologies.	Show et al. (2012)
9.	Researchers provide an inverse relationship between the bioprocess route and economic viability for sustainable energy production, that is, biohydrogen production through dark fermentation is economically viable but has a low yield. Photofermentation is efficient but not economically viable.	Song et al. (2011)
10.	Algal-based biofuel costs about €50 per litre which is far from an economical point of view.	Ahrens and Sander (2010)
11.	Economic possibilities with biofuel, an important energy source, reduces the dependency on fossil fuel and shows economical vulnerability for new era.	Demirbas and Ayhan (2009)
12.	By using activated sludge for biohydrogen production, a significant amount of biomass may be produced that may compete economically over fossil fuel and provide a better energy supply in the twenty-first century.	Ren et al. (2007)
13.	It had been predicted that biohydrogen production via indirect biophotolysis would have capital cost 2.4 $/gj/year	Resnick (2004)
14.	Compared with photobiological hydrogen production, fermentative hydrogen is three times more in per unit cost of energy generation and conversion efficiency in both cases is the same, almost 10 %.	Nath and Das (2003)

that for this process the cost of a tubular bioreactor would be $\$50/m^2$ and the project cost of biohydrogen production would be \$15/gj. It has also been reported (Amos 2008) that a pond type of bioreactor having an area 110,000 m^2 using unicellular green algae would have a reactor cost of $\$10/m^2$. It has also been reported that biohydrogen production by using cyanobacteria would incur a cost of $\$25/m^2$ (Block and Melody 1992).

Hence, economic stress for these discussed bioprocess routes (direct and indirect biophotolysis and dark fermentation) can be overcome through (1) biological and engineering improvement opportunities; (2) for significant cost reduction potential in capital and operating cost, research should be more focused on two parameters, lipid content and algal growth; and (3) optimizing nutrient stress conditions and CO_2 requirements to reduce the capital cost by utilizing suitable wastewater (urban and industrial) as a substrate.

4 Major Bottlenecks in Bioprocess Routes

There are certain shortcomings associated with algae-based bioprocess routes of biohydrogen production, which are obstacles and affect biohydrogen production, therefore these conditions should try to be minimized.

4.1 R&D: In Growing Stage

4.1.1 Suitable Substrate: Demand in Search

Waste materials from the ecosystems that are suitable as a substrate for biohydrogen production are also a challenge because the complex nature of organic compounds sometimes adversely affects biodegradability. Simple sugars such as glucose, maltose, lactose, and sucrose can be easily degraded and suitable for biohydrogen production. Agricultural and food industry waste are highly rich in starch, cellulose, and also in terms of carbohydrate. It is easy to produce biohydrogen from waste containing starch or carbohydrate because it can easily hydrolyse to glucose and maltose to form organic acid and then biohydrogen gas whereas using agricultural waste containing cellulose and hemicelluloses always possesses the problem of pretreatment. First, it has to go through the process of delignification because lignin content and the efficiency of hydrolysis are inversely proportional to each other. There are some industrial wastes which, like dairy, tannery, olive mill, and brewery wastewater are potential applicants for biohydrogen but the main challenge in using these wastes is that they require pretreatment to remove undesirable substances, then convert to organic acid, and then biohydrogen production. In a wastewater treatment plant, a huge amount of waste sludge is generated which is also rich in carbohydrate and protein content so this sludge can also be used as raw material for biohydrogen production; however, it also has toxic substances and complex organic compounds which cannot be easily degraded due to their complex nature. Therefore they also require pretreatment which is cost effective and not economically viable thus the use of wastewater as a raw material for algal biomass is also challenging when producing biohydrogen (Kothari et al. 2010, 2012; Bhaskar et al. 2008).

4.1.2 Optimization of Parameters: Challenge from Lab Scale to Pilot Scale

At lab scale there are various parameters which play an important role in biohydrogen production such as pH, temperature, nutrient ratio, and substrate (Krupp and Widmann 2009) among others, and these parameters can be maintained easily at lab scale as a result of which biomass productivity would enhanced. When doing this at industrial scale it is quite difficult to maintain these parameters and the cost factor is also prominent and cannot be ignored. There are various factors which affect the production of biohydrogen when high-level large-scale cultivation of algae requires additional fertilizers such as phosphorus and nitrogen and these fertilizers from the dry algal biomass cannot be ignored as they may have an adverse impact on biohydrogen production; therefore some technologies should be developed for nutrient recycling (Ferreira et al. 2013). The use of excess fertilizers can also cause nutrient pollution or eutrophication as a result of which the structure and function of the ecosystem of concern may change. By the process of leaching, if these nutrients leach to a nearby water body they could have an adverse effect on aquatic flora and fauna. Under controlled conditions, algae cultivation requires inputs of fossil fuel in the form of electricity and drying algae to form dry biomass natural gas is also required. Algae are also temperature sensitive, therefore maintenance of temperature also requires use of fossil fuel so we have to develop such a technology and system designed to minimize the use of energy and enhance biomass productivity (Slade and Bauen 2013). It is important to know that algae also produce some toxic substances including polypeptide ammonia and polysaccharide. At the end of the process by-products are sometimes used as manure so these toxins can have an adverse impact in the food chain of the ecosystem, therefore care should be taken in the selection of algae species.

4.2 Road to Commercialization

Biohydrogen production as a third-generation fuel is very new. Most of the work is being performed on a lab scale by using different micro- and macroalgae and bacteria but its industrial application is not as high as it should be. Although it has been reported that for the growth of algae, pure culture medium was being used, the scenario has now changed and there is a shift from pure culture medium to food and industrial waste as a substrate, which is easily and cheaply available and a renewable source for energy generation. In biohydrogen production, rate and yield are two important parameters that should always be in consideration. Scientific research efforts have focused on microalgae that are already commercially significant with the greatest prospects for highly efficient energy production coming from species such as *Chlorella*, *Spirulina*, *Dunaliella*, and *Haematococcus* (Bruton et al. 2009). These algae are already used in commercial nonfuel operations, where they are used to make a variety of high-value products for use in human and animal nutrition, aquaculture, and cosmetics (Spolaore et al. 2006).

4.2.1 Reactors

One major challenge in biohydrogen production is reactor design at the commercial level because it is has a direct relation to algal biomass production. There are various

types of bioreactors but designing a suitable bioreactor with relation to its efficiency is a tedious task. The most important parameters when designing a bioreactor are light penetration, mixing, and flow, which depend upon area-to-volume ratio. In order to get a high area and volume ratio several bioreactors of various shape and size have been designed which have given successful responses. The flat-plate bioreactor, tubular bioreactor, and fermentor type of bioreactor are designed to get high light penetration and based on the principle of high area-to-volume ratio for proper mixing, light penetration, and flow rate (Owende and Brennan 2010; Yeow et al. 2011).

Cultivation of algae at the commercial level is not feasible although it is technologically feasible at the lab-scale level. Commercialization of algal cultivation for biohydrogen production is too far from being realised (Richmond 1987). For the successful commercialization of algae-based biohydrogen production it is always a big obstacle to discover the best and most suitable fast-growing algae strain with high photosynthetic efficiency and high oil content. For commercialization of algae as a fuel two important things are that there should be an easy algae culture harvesting system and use of a photobioreactor should be economically viable (Davis et al. 2011). Supporting the infrastructure, maintenance, and operational costs for algae culture and biohydrogen production for its commercialization is very important. Today freshwater demand has increased and it is also required for agricultural crops therefore the freshwater requirement for algal growth would add pressure in areas where water is scarce. Algae cultivation for biohydrogen production in an open pond system is not suitable because the adjustment of parameters for optimum growth is not easy task. It is more suitable in a closed type of bioreactor but here we cannot enhance the production rate of biohydrogen. A life-cycle assessment report has shown that algae cultivation in an open pond system for biohydrogen production is not environmentally suitable in comparison to normal crop plants (Clarens et al. 2010) (Table 6.6).

Table 6.6 Status of major technologies and gaps http://www.intech.unu.edu/events/workshops/hfc05/chopra_ppt.pdf

Technology	International status	National status
Coal gasification	Commercially available	Efforts underway to set up pilot plant
Biological rout for hydrogen production	In precommercial stage	Demonstration plant set up
Metal hydrides for hydrogen storage	Hydrides with 1.5–2.0 wt% storage capacity for ambient conditions developed	Hydrides with 2.42 wt% storage capacity for ambient condition developed

5 Environmental Benefits of Biohydrogen Economy

For sustainable economic development in the world a biohydrogen economy with energy and environmental aspects provides a clean solution (Kothari et al. 2010, 2012; Panwar et al. 2012). These solutions are reviewed in the available literature and given in highlights below:

- Waste material generated by the combustion of hydrogen is water.
- It helps in the eradication of greenhouse gases.
- Elimination of fossil fuel pollution.
- Elimination of economy dependency.
- Biohydrogen production routes are commonly done at ambient temperature and pressure, therefore less energy is used in bioprocess routes.
- This is an ecofriendly method of bioenergy production and use of a renewable source of energy makes it significant because it is inexhaustible.

6 Conclusions

Technologies related to algae cultivation and bioprocess routes for biohydrogen production are commercially viable and give us a positive source of energy for our society. It is an integrated approach through which one can produce biohydrogen as energy, and at the same time it can also be used for wastewater treatment. Use of algal-based biohydrogen as an energy source is more significant than a conventional source of energy because it does not produce any kind of greenhouse gases and by the combustion of biohydrogen it produces only water vapour which is not harmful to our environment. Hence the economic analysis of biohydrogen production by algae shows that it is a most feasible feedstock for future energy production and in addition to a lack of lignin content and being rich in carbohydrate content make algae a promising feedstock for future energy production.

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Bio-oil and Biodiesel as Biofuels Derived from Microalgal Oil and Their Characterization by Using Instrumental Techniques

7

Dipesh Kumar, Bhaskar Singh, Kuldeep Bauddh, and John Korstad

1 Introduction

Microalgal oil has shown an immense potential in the production of biofuels. The biofuels that are derived from microalgal oil primarily include bio-oil and biodiesel. Bio-oil is derived from the pyrolysis of biomass which could be further processed to obtain a variety of chemical products. Bio-oil has physicochemical characteristics different from biodiesel. This is attributed to difference in their chemical characteristics. Bio-oil possesses high acidity, high viscosity, poor heating value, and poor stability. The comparatively low quality of bio-oil as compared to biodiesel is attributed to the high oxygen content in the former (30–55 wt%) as compared to around 11 % in the latter. To enhance the applicability and fuel characteristics of bio-oil, deoxygenation is done. The methods through which the amount of oxygen could be reduced are chemical (viz., hydroprocessing, cracking) and physical (viz., char removal, hot vapor filtration, liquid filtration, solvent addition) (Xiong et al. 2011). Wang et al. (2009) reported that bio-oil could be upgraded via catalytic hydrogenation, catalytic cracking, or steam reforming. It is reported that more than 400 compounds are present in bio-oil derived from fast pyrolysis of biomass. The presence of several compounds also results in a wide range of boiling points of bio-oil components. Bio-oil is said to be thermosensitive and undergoes various reactions, viz., decomposition, polymerization, and oxygenation. The bio-oil obtained from fast pyrolysis has been categorized into three fractions: light, middle, and heavy. The constituent in the light fraction comprises mostly of water with strong acidity, poor stability, and good fluidity. The middle fraction has less water content and moderate mobility. The heavy fraction is the one having high viscosity, absence of volatile matter, black solid like appearance and a comparatively higher heating value (Wang et al. 2009). The water content in the bio-oil has been reported to be around 15–35 wt%. The products of the fast pyrolysis include organic acids (viz., formic and acetic) which impart it a low pH value (2–4) and make bio-oil corrosive. The removal of water from bio-oil has been a challenging task due to its miscibility with hydrophilic thermolysis products from cellulose and hemicellulose. Phase separation of water also results to substantial loss in polar carbon compounds (small aldehydes, ketones, hydroxyaldehydes, few anhydrous sugars, and other compounds) (Zhang et al. 2010). Apart from this, compounds such as acids, esters, phenols, and lignin-derived oligomers are also formed due to complexity of the reactions involved in pyrolysis of biomass (Capunitan and Capareda 2013). The heating value, water content, and storage stability issues of bio-oil warrant for its upgradation (Zheng and Wei 2011). The properties of bio-oil can be improved by either physical or chemical means. The techniques for upgradation include filtration (for ash removal), solvent addition (for homogenization and reducing the viscosity of oil), emulsification of bio-oil with mineral diesel for its utilization as transport fuel or engine fuel, and other methods (Capunitan and Capareda 2013). For the upgradation of the fuel, Zheng and Wei (2011) reported that reduced pressure distillation could be done to obtain distilled bio-oil from fast pyrolysis. The method resulted to yield of 61 % with water phase of 29 % and residual content of 10 %. The oxygen content of the bio-oil got reduced to 9.2 %. The comparatively lower oxygen content has been reported to enhance the heating value of distilled bio-oil to 34.2 MJ/kg (around twice of that obtained via fast pyrolysis).

D. Kumar • B. Singh (✉) • K. Bauddh
Center for Environmental Sciences, Central University of Jharkhand, Brambe, Ranchi 835205, Jharkhand, India
e-mail: bhaskar.singh@cuj.ac.in

J. Korstad
Department of Biology and Chemistry, Oral Roberts University, 7777 South Lewis, Avenue, Tulsa, OK 74171, USA

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2 Bio-oil and Biodiesel: Characteristics and Components

As the fuel quality of bio-oil is inferior as compared to biodiesel, they have utility in different engines. The heating value of bio-oil has been reported to be one-third of that of diesel (No. 2) due to the presence of water and oxygen. The cetane number of pyrolytic bio-oil has also been reported to be low (5.6) (Ikura et al. 2003). While bio-oil could be used in boilers and turbines that are designed to burn heavy oil for generation of electricity, biodiesel is usually used as fuel in compression ignition (CI) engines to run transport vehicles and can be used either in neat form or blended form (because of its good solubility with mineral diesel). Bio-oil is considered to be immiscible with the hydrocarbons present in the biodiesel. Solubilization of bio-oil with mineral diesel could be achieved by addition of surfactants as emulsifiers (Zheng and Wei 2011). Bio-oil produced by fast pyrolysis is highly viscous, possesses high acidity, and has low ignitability due to high structural water content. To overcome these problems, the pyrolyzed bio-oil is emulsified. The cost of the emulsification is governed by the type of surfactant used (Ikura et al. 2003).

Bio-oil is also named as "pyrolysis oil," and the technology is termed as flash pyrolysis technology. Flash pyrolysis has been used to convert the solid biomass (wood waste, aquatic plant biomass, municipal solid waste, and agricultural and industrial residues) to potential energy resource. The composition of bio-oil varies with the type of biomass subjected to pyrolysis (due to the differences in biomass composition). The varying ratio of the major constituents in biomass, i.e., cellulose, hemicellulose, and lignin, has an impact on the quality of the bio-oil. Pyrolysis conditions also have a strong impact on the formation of end products (Tessarolo et al. 2014). Catalysts are used to deoxygenate the bio-oil and to simultaneously derive useful products from lignin. Pyrolysis is an old practice in which biomass is thermally decomposed in the absence of oxygen to produce solid, liquid, and gaseous products (Capunitan and Capareda 2013). Filtration technique has a potential to remove ash from bio-oil. Capunitan and Capareda (2013) suggested the fractional distillation of the bio-oil derived from corn stover to facilitate the separation of components. The heavy fraction that constituted around 53 % showed improved properties and composition that could be either upgraded further or blended with other liquid fuels. Continuous hydrotreatment of pyrolysis bio-oil with Pd/C as catalyst has been reported in a packed bed catalytic reactor at a temperature ranging 175–300 °C and pressure 50–150 bar for its upgradation (Chaiwat et al. 2013). For an effective fast pyrolysis aimed at improving the yield of bio-oil, it is suggested that the heating rate should be high, carefully controlled temperature (at about 500 °C), short vapor residence times (usually below 1 s), rapid removal of char from the reaction environment, and rapid cooling of the pyrolysis vapors (Alvarez et al. 2014). Bio-oil has got application as fuel (as blend with mineral diesel), in the synthesis of value-added products, as feedstock in production of hydrocarbons, and for obtaining hydrogen (via steam reforming). The abundantly available lignocellulosic biomass based bio-oil can be transported to refinery units for large-scale production of fuels with commercial value, viz., hydrogen. Each of the products have numerous applications at industrial level. The char could be utilized as fuel to provide heat to the process of pyrolysis. Alternatively, char could also be carbonized to obtain activated carbon that is considered as a very good adsorbent.

Biodiesel could be obtained from the microalgal oil by conversion of triglycerides present in it to fatty acid alkyl ester via transesterification. Apart from biodiesel, long-chain hydrocarbons could also be derived from microalgal oil (Vidyashankar et al. 2015). Silva et al. (2015) reported that the abundance of marine microalgae makes it a potential raw material for production of biodiesel. Biodiesel is considered to be clean, renewable, and biodegradable. However, the polar nature of biodiesel renders it to solubilize the metals from the container in which it is stored. There will be potential danger of release of toxic metals in the environment on the combustion of biodiesel. The biodegradable nature of biodiesel makes the fuel corrosive to the engine parts. This in turn may cause the biodiesel to go off-specification. Hence, a thorough characterization of biodiesel becomes important for its usage as a transport fuel. Hence, the characterization of biodiesel becomes important to ensure the suitability of fuel for usage in transport sector.

It is reported that the lipid profile of the majority of microalgal species is similar to that of the plant-derived vegetable oils and is suitable for the production of biodiesel. Microalgae have the capability to synthesize and accumulate neutral lipids that are considered to be most suitable for the production of biodiesel (Swarnalatha et al. 2015). Among the lipid profile present in the microalgal oil, the saturated and monosaturated fatty acids are preferred (Sepúlveda et al. 2015). This could be due to the fact that they provide a better compromise in between oxidation stability and cold flow properties (e.g., cloud point, cold filter plugging point) of biodiesel.

As per the European specifications (EN 14214), biodiesel should posses a minimum 96.5 % of fatty acid alkyl ester to be considered for use as a transport fuel (Sharma et al. 2011). The other specifications (viz., acid value, viscosity, flash point, cetane number, etc.) are mentioned in American Society for Testing and Materials (ASTM D6751) and should be fulfilled. Nations also have their own specifications for

biodiesel. Specification for biodiesel in India comes under IS 15607. The specification for biodiesel in Germany is DIN V 51606 (Sharma et al. 2008).

3 Instruments for Characterization of Lipid in Microalgal Oil

Instrument offers an important mode for the characterization of lipid present in microalgal oil, biodiesel and bio-oil derived from microalgal biomass.

3.1 Nile Red Fluorescence Method

Nile red, a lipophilic dye, has been reported to be used to assess the relative quantity of lipid in strains of algae, yeast, fungi, and mammalian cells. The live cell is incubated in the dye, often along with a solvent, and fluorescence is recorded using a spectrophotometer. Fluorescence occurs when the dye penetrate the cell structure and diffuse into lipid droplets and fluoresces in the nonpolar environment. The fluorescence technique is reported to be rapid and can be integrated with the measurement of optical density. Hence, this allows to track the growth and lipid levels through the different stages of growth of microalgae (Higgins et al. 2014). Fluorescence technique serves as a fast-screening method for the characterization of lipids derived from a microscopic species. Poli et al. (2014) have reported quantification of neutral lipid from the yeast cell based on fluorescence. The technique has been reported to be more efficient than the conventional technique of gravimetric analysis as lesser amount of organic solvent is used. The excitation and emission wavelength for total lipid (neutral and polar) have been reported to depend on the individual organism as well as composition and content of intracellular lipids. The characteristic wavelength of excitation and emission range from 470 to 547 nm and 540 to 628 nm, respectively. The sample preparation in fluorescence method requires mixing the cell suspension with isopropanol in the solution of potassium chloride phosphate buffered saline. The limitation of Nile red fluorescence has also been reported. It has been reported that in few microorganisms, Nile red may not penetrate intracellular component of the microorganism to form the Nile red-lipid complex. Hence, separate method is required for such organisms.

3.2 PAM Fluorometry

White et al. (2011) have reported pulse amplitude modulated (PAM) fluorometry to measure the physiological stress in the microalgae and synthesis of cellular neutral lipids. It is reported that the fluorescence technique is a tool that could be utilized to examine energy metabolism in photosynthetic cells and interactions between carbon and nutrient assimilation in microalgae. The light energy is absorbed by the chlorophyll to do photochemical work or is reemitted as heat. The energy used to do photochemical work is inversely related to the amount of fluorescence emission from chlorophyll *a*. PAM fluorometry has been reported to be a common, noninvasive, and rapid mode to measure chlorophyll fluorescence and photosynthetic performance. PAM fluorometry has earlier been used to study physiological stress, viz., temperature, salinity, nutrient, and irradiance. The dual-channel PAM fluorometer detects the variability in photosynthesis activity in photosyntheses I and II. The "maximum quantum efficiency" of PSII (Fv/Fm) is reported to be constant in the nonstressed culture and decreases when the culture is stressed. PAM fluorometry can be used to determine the stress induced by the environmental factor in the microalgae. The stress induced by iron depletion was observed to be minimal. Using the PAM fluorometer gave the extent of neutral lipid synthesis in freshwater microalgae, *Chlorella* sp. The physiological stress induced by nutrient limit and complete nutrient deprivation was accessed by decrease in rETR, Fv/Fm, and E_k values. Insausti et al. (2013) have reported estimation of various parameters (FAME, cetane number, heat of combustion, and color) in biodiesel and diesel blend using synchronous fluorescence. Szabo et al. (2014) have reported multiwavelength chlorophyll fluorescence analyses for examining the photosynthetic efficiency of *Nannochloropsis oculata* that was grown under high and low irradiance of light. The type of lipid accumulation in the algal cell was found to be influenced by the type of irradiance. The high irradiance of light resulted in high accumulation of saturated fatty acids, whereas low irradiance led to high accumulation of polyunsaturated fatty acids.

3.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a very important instrumental technique for qualitative and quantitative analyses of fatty acids and its derivatives. It is a rapid analytical tool offering authentic and reproducible results. Isotopes having a nonzero spin are NMR active. ^{1}H and ^{13}C remain the most widely used isotopes, having two different spin states (α and β). Naturally, the orientation of the nuclei does not follow a pattern and their orientation remains random. However, in the presence of an applied external magnetic field, the orientation of the nuclei is not random in space, and they remain either aligned with or against the applied magnetic field. As it is energetically more stable (lower energy "α state"), most of the nuclei align with the magnetic field. The energy gap

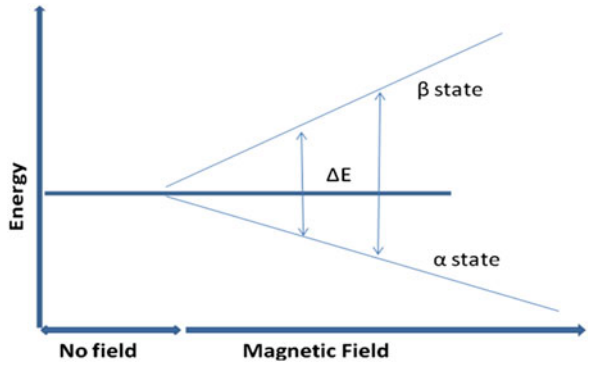

Fig. 7.1 Nuclei spin state splitting in the presence of an external magnetic field

between the two spin states is minimal and increases with the strength of the applied magnetic field (Fig. 7.1). This energy gap corresponds to the energy of radio waves.

In the presence of an applied field, nuclei tend to behave as a tiny bar magnet and generate its own magnetic field, which opposes the external magnetic field. Different nuclei of even the same isotope present in different chemical environment experience different values of net effective magnetic field as they generate local magnetic field of variable strength (B*net* = B*applied*-B*local*). When irradiated with radio waves, NMR active nuclei absorb energy corresponding to energy gap between the spin states, and this promotes nuclei to higher energy state. As being in higher energy state is energetically unstable, the nuclei tend to jump back to lower energy state simultaneously giving off radio waves that it had absorbed. Emitted radio waves have variable frequencies, and each frequency serves as a fingerprint for a particular chemical species present in a given chemical environment. Typical components of an NMR instrument are shown in Fig. 7.2.

Since different NMR spectrometers have different magnetic field strength, all signals are recorded relative to a standard, TMS {tetramethylsilane: (Si $(CH_3)_4$)}, and the scale used is called the delta (δ) scale, and values are reported in ppm. One major advantage offered by NMR spectroscopy is its ability to analyze intact and bulk algal biomass and thus avoids lengthy and inefficient oil extraction procedures. The methylene peak signal intensity is generally used as an indicator of lipid presence (generally TAGs). Table 7.1 shows characteristic chemical shift values (^{1}H NMR) for different types of lipid. Time-domain (TD) NMR is an alternative to classical NMR (^{1}H, ^{13}C, ^{31}P NMR, etc.) which is based on the difference in relaxation times of hydrogen nuclei present in the different phases of the sample analyzed (Todt et al. 2001). TD-NMR is cheaper as it works under low magnetic field (uses permanent magnet). ^{31}P NMR in combination with ^{1}H or ^{13}C can help differentiate between neutral triacylglycerol which is best suited lipid type for biofuel production and

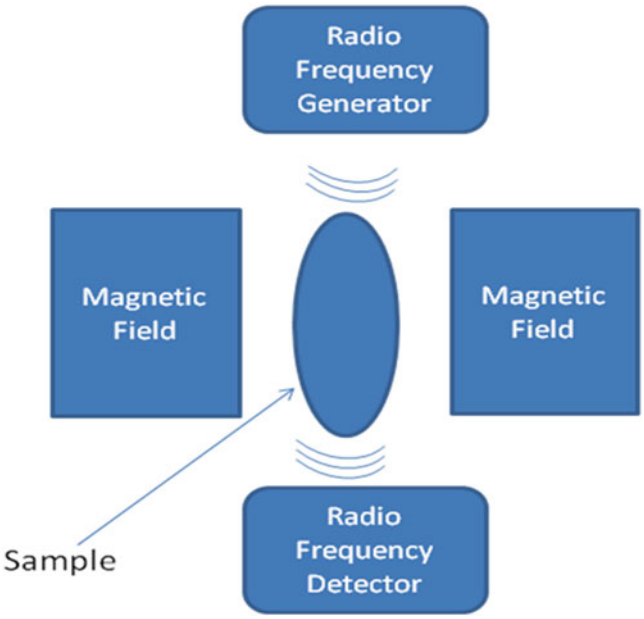

Fig. 7.2 Components of NMR spectrometer

Table 7.1 Typical chemical shift (δ) values for different lipid classes

Lipid class	^{1}H NMR (δ:ppm)
Triacylglycerol (TAG)	4.34
Total fatty acid	2.35
Phospholipids and glycolipids	4.53–4.38

Nuzzo et al. (2013)

polar phospholipids constituting a major fraction of cell membrane and is considered unsuitable for biofuel production.

Gao et al. (2008) used TD-NMR to rapidly quantify the lipid content in *Chlorella protothecoides* and obtained better correlation for TD-NMR(R^2 = 0.9973) than Nile red-based staining method (R^2 = 0.9067) when compared to traditional gravimetric analysis based on solvent extraction procedure. Gelbard et al. (1995) used ^{1}H NMR to determine the yield of biodiesel produced by transesterification of rapeseed oil with methanol. Yield determination was based on disappearance of signal from proton located adjacent to the methylene group in triacylglycerol and appearance of signal due to the proton in the alcohol moiety of the methyl ester. Dimmig et al. (1999) used ^{13}C NMR to study turnover and transesterification kinetics of oil derived from rapeseed with methanol and reported that acylglycerol formation from triacylglycerol (slowest) as the rate determining step. Points on unsaturation (double bonds) on individual fatty acid molecules can be used to assess the oxidation of biodiesel on prolonged storage in the presence of air, and upon oxidation changes in NMR, spectra peaks can be identified and assessed easily (Knothe 2006). There are several advantages and limitations associated with NMR spectroscopy, as shown in Table 7.2.

Table 7.2 Advantages and disadvantages of NMR

Advantages of NMR	Disadvantages of NMR
1. Ability to analyze intact algae (Beal et al. 2010)	1. Expensive instrument
2. Structural details can be obtained	2. Relatively new technique
3. Non-destructive analysis	3. Use of ^{13}C NMR requires large sample volume, because of its lower natural abundance (Davey et al. 2012)
4. Lower maintenance cost	4. Although more accurate, compared to TD and ^{1}H NMR, ^{13}C NMR is time taking (Beal et al. 2010)
5. Lower analysis time	5. TD-NMR provides limited qualitative results (Beal et al. 2010)
6. Easy sample preparation	
7. Ability to distinguish between polar and neutral lipids when combined with ^{31}P NMR	
8. Allows for continuous monitoring of oil content in growing cells	
9. Automated technique	
10. Require very small sample volumes	
11. Ability to measure TAG content across all oleaginous microalgae species producing triacylglycerides	
12. TD-NMR is an extremely fast technique (Gao et al. 2008)	
13. High reproducibility	
14. Can be used as an online technique for continuous lipid analysis, under different stages and cultivation conditions, as live cells can be analyzed directly	
15. Can be used to study oxidation of lipids and biodiesel (Knothe 2006)	

3.4 Gas Chromatography–Mass Spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is a powerful hyphenated analytical technique which combines the separation power of gas chromatography with analytical power of mass spectrometry. The combination of GC-MS overcomes the shortcomings and limitations of either technique individually and provides a powerful analytical technique. Typical components of a hyphenated GC-MS system are shown in Fig. 7.4.

3.4.1 Gas Chromatography (GC)

Gas chromatography is the most widely used analytical tool for fatty acid profile analysis. Like any other chromatographic technique, GC involves a mobile phase and a stationary phase. In GC, the mobile phase is a nonreactive gas (also known as carrier gas) and a stationary (which is usually a liquid coated onto a solid surface) phase. Before the sample enters the stationary phase which is kept in a thermostatic oven in the form of a coiled column, it is heated so as to vaporize all the sample components. The sample is heated in the injection port which is situated upstream of the stationary phase, at a temperature which is about 50 °C higher than the average boiling point of the sample components. The vaporized mixture is then carried along the stationary phase by a carrier gas. The various components of the sample have different affinity for the stationary phase at a given temperature and therefore elute out of the column at different rates. Temperature programming is often required for sample components having wide range of boiling points. Thus, different

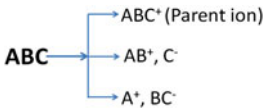

Fig. 7.3 Pattern of ionization and fragmentation

sample components spend different amount of time (retention time) in the GC system before the come out of the column one by one. Based on the retention time values and peak integration values of individual sample components, qualitative and quantitative details can be obtained.

One major limitation of GC is its inability to analyze nonvolatile (within the temperature range of the system) sample components. Fatty acids and triacylglycerols (TAGs) are relatively less volatile and cannot be analyzed directly by GC. These compounds therefore require derivatization into some other compound having higher volatility before they can be analyzed by GC. Fatty acids and TAGs are usually converted into their respective esters prior to their analysis by GC. GC separation can be time-consuming if temperature gradient is low but can provide high resolution result. Higher temperature gradient on the other hand can be fast but provides poor resolution.

3.4.2 Mass Spectrometry (MS)

Mass spectrometry requires samples of high purity, which is provided by GC. After being separated by GC, the individual sample components enter into mass spectrometer one by one. First of all the individual sample components are ionized and fragmented into parent and daughter ions by high energy electrons (Fig. 7.3).

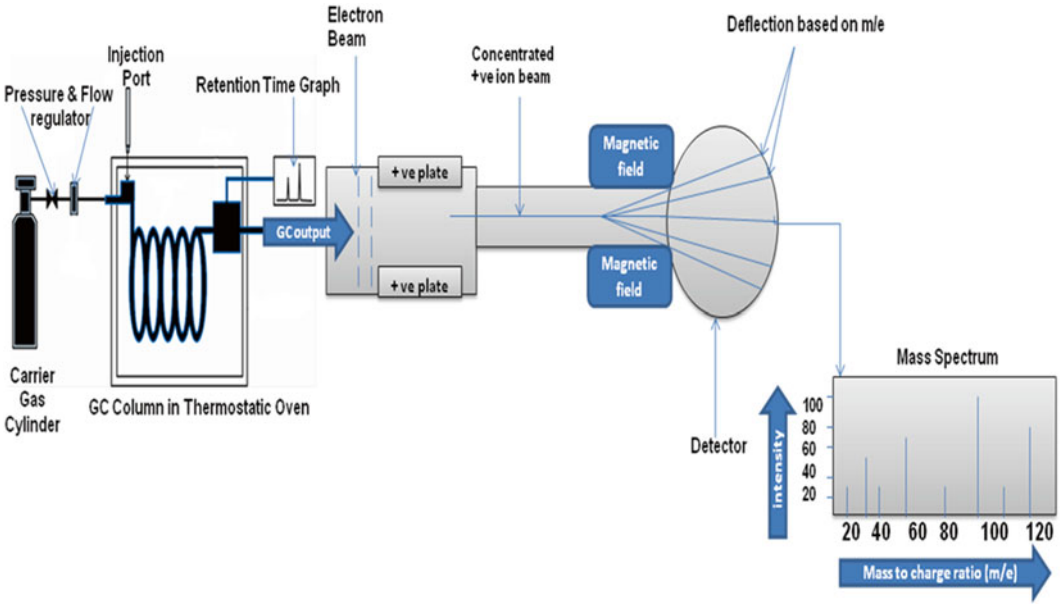

Fig. 7.4 Schematic diagram of GC-MS

These ions are accelerated toward the detector by creating an electrical potential. These ions are then deflected by a magnetic field from their path depending on their mass-to-charge ratio, in which the ions having lower mass-to-charge ratio are deflected more than ions having higher mass-to-charge ratio. By varying the strength of the magnetic field, all the ions can be brought to the detector (Fig. 7.4). The detector which consists of a metal box donates electrons to the positively charged ions thereby neutralizing the ions and creates electron deficit in the metal. The electrons shuffle along to fill the gap and thus create current which is amplified and recorded. The largest peak (base peak) in mass spectrum denotes ions having highest number and is given a numerical value of 100, while intensities of all other ions are expressed relative to the base peak. The parent ion represents the mass of the parent molecule from which all other ions are derived. The fragmentation process of molecules follows a definite pattern which depends on bond strength and stability, and thus relative abundance of ions helps elucidate structure of the molecules. Mass spectrometry is a destructive analytical technique. Methyl esters coming out of a GC column are ionized and fragmented by using high-energy electrons, and based on the pattern of fragmentation and m/e of individual fragments, the parent molecule can be identified. GC remains the most extensively used analytical technique for qualitative and quantitative analysis of fatty acid methyl esters derived from lipids. Suitability of vegetable oil as biodiesel feedstock is greatly dependent on the constituent fatty acid profile. Identification of individual fatty acids is dependent of library database or use of internal standards. Although GC is the most widely used analytical technique for analyzing biodiesel, there can be ambiguities related to individual compound identification due to problems such as signal overlapping and baseline drift (Knothe 2001). GC coupled with MS can potentially eliminate any ambiguity related to the nature of material coming out of a GC column as mass spectra for individual compounds is inherently unique (Knothe 2001).

Qin et al. (2012) used GC-MS for studying the characteristic fatty acid profile of deacidified *Pistacia chinensis* oil. Barman et al. (2012) carried out an algal oil screening study for determining suitability as biodiesel feedstock based on algal oil derived from 21 algal taxa using GC-MS dependent fatty acid profiling. Omotoso et al. (2011) used GC-MS in a comparative suitability analysis of *Jatropha* oil and palm oil as biodiesel feedstock based on individual fatty acid profile and yield under identical transesterification condition.

Gas chromatography and gas chromatography coupled with mass spectrometry have been widely used in the characterization and identification of the fatty acid alkyl ester and its amount in biodiesel. The common method for assessing the level of unsaturation in biodiesel is by determining the iodine value. An alternative and direct method for the determination of unsaturation has been suggested by Fernandes et al. (2014) using a technique called "easy ambient sonic-spray ionization mass spectrometry" (EASI-MS). The easy ambient sonic-spray ionization mass spectrometry has been reported to be direct, simple, and rapid, along with high specificity and sensitivity in determination of the iodine value of biodiesel. The technique has been reported to be effective in quantitative analysis of unsaturated fatty acids in biodiesel obtained from various feedstocks (soybean, canola, and Jatropha). As per the European standard (EN 14214), biodiesel must have atleast 96.5% of fatty acid methyl esters

Table 7.3 Advantages and disadvantages of GC-MS

Advantages of GC-MS	Disadvantages of GC-MS
1. Most widely used analytical techniques for fatty acid profile analysis	1. Oil extraction is required
2. Hyphenated technique of separation and analysis	2. Derivatization is required (usually to ester) Lin et al. (2012)
3. High resolution	3. Longer analysis time
4. Wide range of column types (depending on polarity) and detectors available allow high selectivity depending on the sample composition	4. Destructive analysis
	5. High speed only at the expense of quality signal-to-noise ratio and resolution
5. Minor components can be quantified with higher accuracy (Knothe 2001)	6. Only volatile and thermally stable compounds can be analyzed
	7. Factors such as signal overlapping and baseline drift can affect accuracy (Knothe 2001)

(FAME) content. The calibration curve plotted for the FAME was reported to show good linearity (correlation coefficient > 0.99) and spike recovery (ranging from 76 to 127 %). The relative standard deviation was reported to range from 5.1 to 17.7 %. Fernandes et al. (2014) report the method to be fast with a high accuracy. Table 7.3 lists various advantages and limitations of a GC-MS system.

3.5 Fourier Transform Infrared Spectrometer (FTIR)

An infrared spectrometer subjects the sample under analysis to infrared radiation having sufficient energy to cause bonds in the molecule to vibrate, and each type of functional group has a characteristic frequency at which its constituent bonds undergo vibration. Fourier transform infrared (FTIR) is a special type of infrared spectrometry in which a polychromatic infrared radiation is used instead of using a monochromatic radiation, and thus all wavelengths are detected and measured at the same time. An infrared spectrum serves as a fingerprint of a sample in which absorption peaks correspond to the frequency of radiation responsible for bond vibrations. Interferometer is the major component of an FTIR spectroscope. The interferometer divides the infrared radiation from the source into two beams and creates an optical path difference (OPD) between the beams. The beams are later recombined to produce repetitive interference signals which are measured by a detector as a function of OPD. As it passes through the sample, the interference signal obtains spectral information of the sample components. The interference signal is recorded in the form of an interferogram. The interferogram is subsequently decoded by a mathematical operation called Fourier transform. Figure 7.5 represents FTIR instrument schematically.

Different types of lipids absorb infrared radiation at different wavelengths, and their simultaneous detection is facilitated by FTIR spectrometer. FTIR is a very fast analytical technique and completes a wide spectra analysis within a matter of seconds. Intact algal cell can also be analyzed directly and thus facilitates continuous monitoring of cell

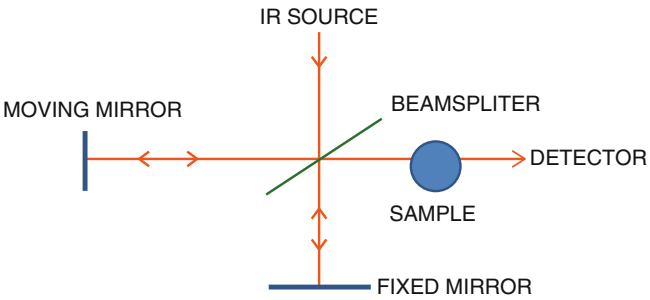

Fig. 7.5 Schematic diagram of FTIR

composition and effects of metabolic control of cellular composition (Dean et al. 2010). Nitrogen starvation is known to enhance lipid accumulation in microalgae, and this has been confirmed by several researchers based on several analytical techniques including FTIR.

Dean et al. (2010) studied *C. reinhardtii* and *S. subspicatus* under variable availability of nitrogenous fertilizers and reported high absorption at 1740 cm^{-1} characteristic of lipids for algae grown under nitrogen-starved conditions. Giordano et al. (2001) based on his carbon allocation pattern analysis on intact *Chaetoceros muelleri* in response to optimized nitrogen availability reported diversion of carbon from other biomolecules (carbohydrate, protein) and chlorophyll toward lipids, producing IR spectra with enhanced absorption at 1740 cm^{-1}. According to Ivanoiu et al. (2011), the presence of broadband signal between 2500 and 3300 cm^{-1} can be used as an indication of presence of free fatty acid and moisture in algal oil. The methyl peak ($O\text{-}CH_3$) at 1436 cm^{-1} reflects the methyl esters of all types and can be used to monitor conversion of triglycerides into biodiesel via transesterification (Bergougnou et al. 2009). The advantages and disadvantages of FTIR are presented in Table 7.4.

4 Conclusions

The biofuels, viz., bio-oil and biodiesel, find application as fuel in transport sector. The two biofuels should adhere to the national/international specifications for their usage. The

Table 7.4 Advantages and disadvantages of FTIR

Advantages of FTIR	Disadvantages of FTIR
1. Intact algal cells can be analyzed (Dean et al. 2010)	1. Only IR active molecules can be analyzed
2. Nondestructive analysis	2. Solvents must be transparent in the spectral region of interest
3. Relatively lower scanning time	
4. Higher signal-to-noise ratio	3. Requires freeze-dried algal samples (otherwise oil extraction is required)
5. Relatively inexpensive	
6. Wide spectra analysis	4. Requires exogenous lipid standards.
7. Easy maintenance	5. Possibly requires preparation of separate calibration curves for varying algae species
8. Derivatization is not required	
9. Tolerant to a limited level of variation in the samples (Han et al. 2011)	
10. Very little sample preparation requirement (Han et al. 2011)	

characterization of the biofuels involves advanced analytical techniques that include Nile red fluorescence method, PAM fluorometry, NMR, GC-MS, and FTIR as the major instrumental techniques available for qualitative and quantitative analyses of algal oil. Each of these methods has their own advantages and limitations. Therefore, the selection of a particular method is subjective and depends on different factors, viz., cost, time, sample preparation, and accuracy and precision desired, among others. Among the various techniques available, NMR offers characteristic chemical shift values for individual components along with its structural details in relatively short time span. It can also differentiate between neutral and polar lipids. NMR has emerged as a powerful technique in the characterization of the biofuels, biodiesel. It takes a very short time to quantify the amount of fatty acid alkyl ester present in the biodiesel. Another similar technique, i.e., TD-NMR, is rapid but offers limited qualitative details. Hyphenated technique of GC-MS offers high resolution and signal-to-noise ratio with a wide range of column and detector types to choose from depending on the requirement, but its sample preparation procedure is complicated and time taking as the sample requires derivatization. Another useful technique, FTIR uses a polychromatic IR radiation and thus facilitates simultaneous detection of different functional groups present within a matter of seconds. It can also be used to directly analyze freeze-dried algal biomass.

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Remediation of Dyes from Aquatic Ecosystems by Biosorption Method Using Algae

8

Poulomi Chakravarty, Kuldeep Bauddh, and Manoj Kumar

1 Introduction

1.1 Aquatic Pollution

Water, the elixir of life, is subjected to contamination and pollution in the hands of man. The aquatic ecosystem around the globe is facing a major threat by anthropogenic activities. Although 70 % of the Earth's crust is covered by water, only less than 1 % of that is fresh water and is used by all for consumption as well as nonconsumptive purposes. Industries around the world consume a major share of fresh water for processing, washing and cooling of industrial products. Apart from the direct usage of water in the production of materials, the industries also foul the water bodies by discharging effluents in them. The toxic and hazardous effluents released from industries create havoc in the aquatic ecosystem and also affect organisms on terrestrial ecosystems when water is consumed directly or used for irrigation. The dye industry poses a major threat to the aquatic ecosystem as the colours from the dyes not only reduce the aesthetic value of the water but also increase toxicity in the water body which can hamper the normal growth of aquatic organisms as well as terrestrial organisms when it reaches the higher trophic levels. The water usage in textile industry alone is about 9 trillion gallons annually around the world (Blacksmith Institute Annual Report 2012). An estimate by World Bank suggests that the textile industries contribute around 17–20 % of the total industrial water pollution globally (Blacksmith Institute Annual Report 2012). The world population will be about 9.3 billion in the year 2060 (UN Report 2011). The ever-increasing population will create stress on the existing resources, and fresh water will be amongst the scarcest natural resources.

P. Chakravarty • K. Bauddh • M. Kumar (✉)
Centre for Environmental Sciences, Central University of Jharkhand, Ranchi 835205, Jharkhand, India
e-mail: msinhabit@gmail.com

1.2 Dyes

Unfortunate are those men who cannot see the beauty of the Earth in its vibrant hues. This statement has perfect truth as we cannot imagine this world without colours. A substance having an affinity towards the substrate on which it is applied is described as a dye. The dyes are of a certain colour as they absorb visible rays of the electromagnetic spectrum at a particular wavelength (Pereira and Alves 2012). Natural dyes are known to be used by man since 3500 BC according to the historical records (Kant 2012). Organic colours were also used by Egyptians 4000 years back in the wraps for the mummies in the form of blue dye, indigo (Gordon and Gregorn 1983). The first synthetic dye was accidentally discovered by W.H. Perkins in the year 1856 which paved the way for modern day dye industry. More than one lakh commercial dyes are present today, and over 700,000 tonnes of dyes are produced annually (McMullan et al. 2001; Pearce et al. 2003).

Dyes are used in textile, paper, leather, pharmaceuticals, cosmetics, agricultural, wood staining, food and many other industries. Natural dyes were used earlier which had a drawback of low colour fastness. The natural dyes are less bright in colour also the dyes fade away with time. Therefore, the use of synthetic dyes has increased at present times which are polluting in nature. The natural dyes can also harm the environment as substances known as mordants have to be used with them to fix or bind the colour on fibres. Chromium is an example of such mordant which is a potentially toxic heavy metal for all living organisms (Kant 2012).

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1.2.1 Types of Dyes

The dye molecules comprise of two major components the chromophore group and the auxochrome group. Chromophores are electron acceptors, and they are responsible for the light absorption in molecules of the dye. Auxochromes are the electron donors, and they are the colour enhancers in the dye molecules (Gomes 2001; Pereira and Alves 2012). The dyes can be classified on the basis of application and on the basis of chemical structure. The anionic dyes are of three types: reactive, direct and acid dyes (Fu and Viraraghavan 2001). Reactive dyes are used in textile industries, and these dyes show a fast colour even after washing and exposure to light. Direct dyes are used for colouring cellulose fibres without the application of mordants. Acid dyes are mostly used in colouring of protein fibres; azo dyes come under the category of acid dyes. Basic dyes fall in the category of cationic dyes; these dyes are bright in colour but are not fast to light and water. These dyes are usually used in textile industries for silk fibres. Disperse dyes are nonionic in nature, mostly used in synthetic fibres in paper or textile. Disperse dyes are insoluble in water; therefore, their colour is fast and bright (Fu and Viraraghavan 2001).

1.2.2 Toxicity of Dyes

Synthetic dyes are complex compounds in nature, manufactured in a way to increase the longevity of the coloured product. Dyes are xenobiotic in nature and thus resist degradation by traditional wastewater treatments and reach the aquatic ecosystem (Crini 2006). The undesirability of the presence of dyes in water increases because of the toxic and carcinogenic nature of majority of dyes and the breakdown products released by them, for example, benzidine, naphthalene and other aromatic compounds (Alves de Lima et al. 2007; Tsuboy et al. 2007; Suteu et al. 2009a, b; Zaharia et al. 2009; Oplatowska et al. 2011; Li et al. 2012; Punzi et al. 2015). The usage of water in the dye industry is also very high where almost 90–94 % of water is used in processing and the rest of the water is used for cooling purposes in case of textile industries. The amount of textile dyes lost during dying process itself is 10–25 %, and 2–25 % of dyes are directly released as effluents into water bodies (Zaharia and Suteu 2012). According to the annual report of Blacksmith Institute of 2012, the dye industry contributes around 400,000 DALYs (disability-adjusted life years) out of the total 17,147,600 DALYs which is the total burden of disease in the 49 countries which were assessed. The DALY is a measurement of life expectancy adjusted according to several health hazards caused due to pollution and other factors. The first basic problem with the effluents released from dye industry is that it gives the water an unnatural hue which ruins the aesthetic value of that water body. Figure 8.1 depicts the impacts of effluents discharged by dye industries on the aquatic and terrestrial ecosystem. The coloured dyes on reaching the water body obstruct solar radiation penetration and thus consequently reduce the photosynthesis rate of phytoplanktons and other macrophytes present in water. The pH and salinity of the water are affected by the presence of dye effluents as the dye effluents are high in pH and salinity; thus, these factors in turn disturb the maintenance of the equilibrium in the water bodies (Chia and Musa 2014).

The homeostasis of aquatic organisms is adversely affected by toxicity and water quality degradation by dye effluents. The effluents from dye industry are organic in nature; they tend to lower the dissolved oxygen level of water bodies which affects the overall ecological balance of the water body (Ratna and Padhi 2012). The lowering of DO leads to increase in biological oxygen demand (BOD) and chemical oxygen demand (COD) which leads to death of aquatic organisms and increase in anaerobic bacteria (Khopkar 2004). When the water polluted by effluent is used for irrigation, it leads to clogging of pores in the soil, hardening of soil particles and prevention of root penetration in soil which further results in reduction in soil productivity (Kant 2012). Effluents discharged from industries usually do not stay fixed at the point of release. Explanation about the impact of dyes on agricultural crops is given in Fig. 8.2. The effluents if discharged into water bodies may enter the terrestrial ecosystem in form of irrigation water. The effluents from the dye industries are found to have an inhibitory effect on the germination of seeds (Nirmalarani and Janardhanan 1988). The effluents may also bring about alterations in the biological and chemical status of water and soil which affects the overall development and productivity of plants. When the concentration of solids present in the effluent is higher, it reduces the DO level and restricts the development of seedlings (Saxena et al. 1986). The chlorophyll content in leaves of such plants is also lowered because of the presence of dissolved solids (Gadallah 1996). These toxic effluents may also lower the tolerance of the plants towards abiotic and biotic stresses.

Several of the dyes are found to be mutagenic and carcinogenic in nature specially the azo dyes and the anthraquinone dyes (Rochat et al. 1978; Kornbrust and Barfknecht 1985; IARC 1987; Moghaddam et al. 2004; Puvaneswari et al. 2006; Mathur and Bhatnagar 2007; Jayaraj et al. 2011; Mayson and Suad 2012; Kousha et al. 2012; Chia and Musa 2014). A detailed explanation on the dyes and their toxic impact on health of organisms are given in Table 8.1. Organically bound chlorine is found in 40 % of the dyes found globally which is found to be a carcinogen (Kant 2012).

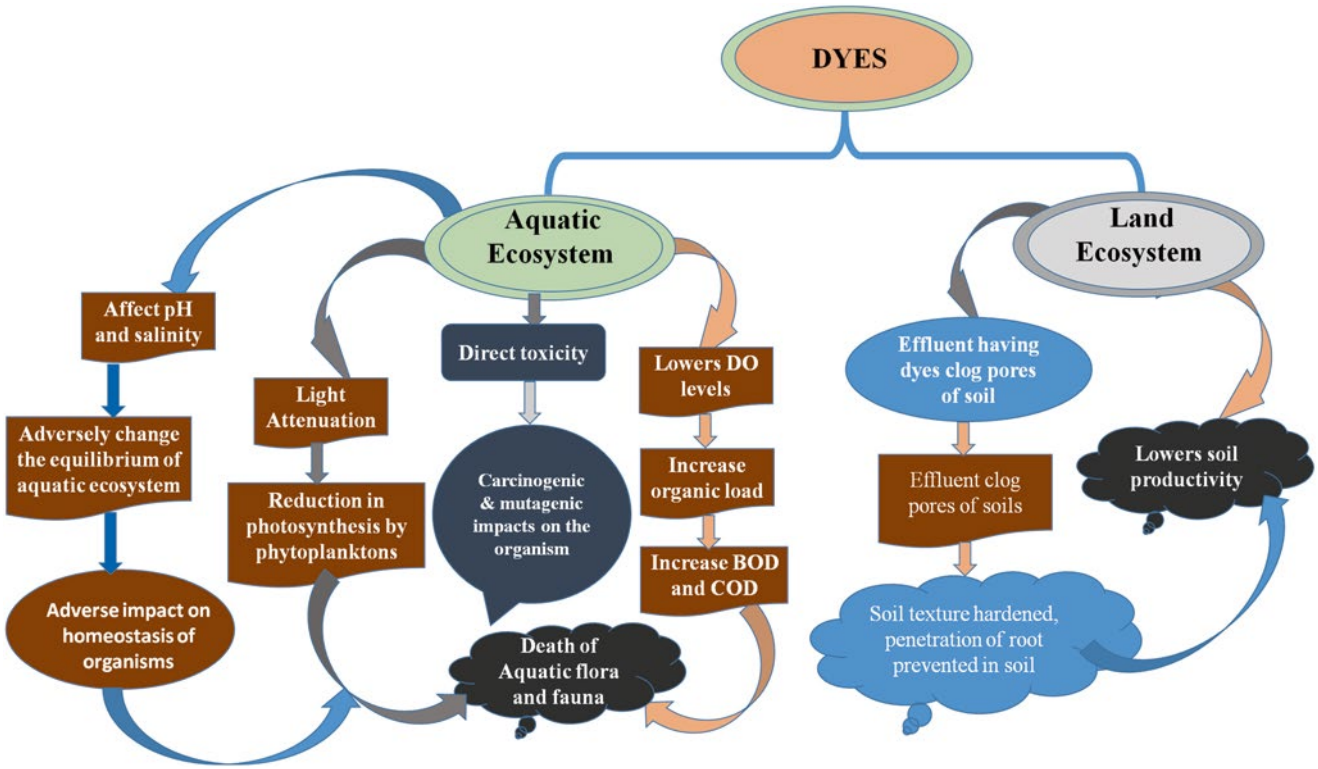

Fig. 8.1 Effects of dyes on aquatic and land ecosystems (Khopkar 2004; Kant 2012; Ratna and Padhi 2012; Chia and Musa 2014; deLuna et al. 2014)

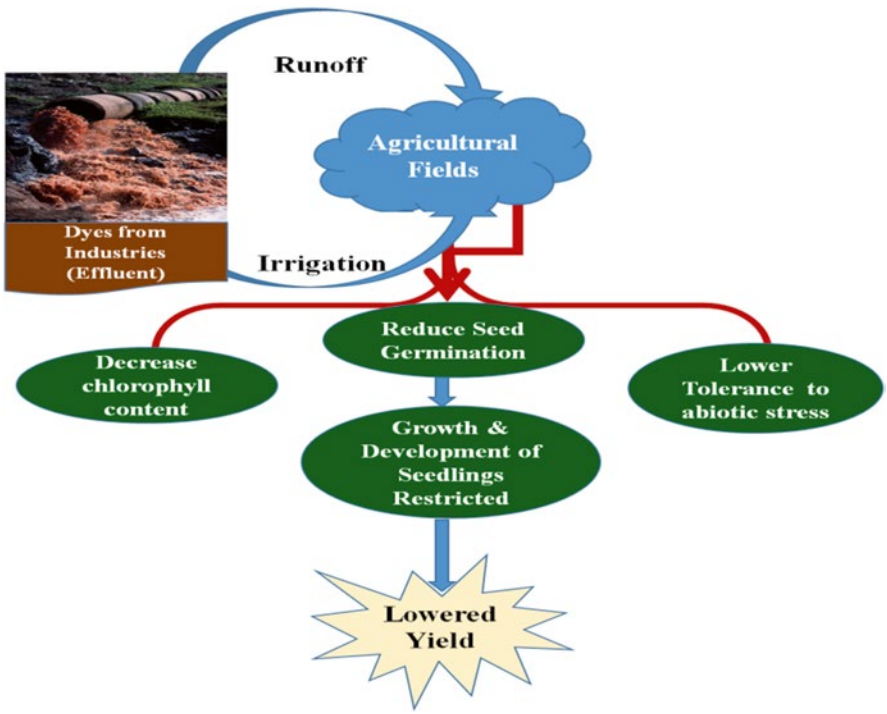

Fig. 8.2 Effects of dyes on growth and yield of agricultural crops (Nirmalarani and Janardhanan 1988; Saxena et al. 1986; Gadallah 1996; Image source: Google images)

Table 8.1 Effects of dyes on human health

Dye	Class	Application	Toxicity impact	Reference
Azo dyes (general)	Azo	Textile industries	Bladder cancer in workers handling dyes	Puvaneswari et al. (2006)
Rhodamine B	Xanthene	Textile and food industry, used as water tracer	Irritation to skin, eyes, respiratory tract Carcinogenic, reproductive and development toxicity, neurotoxicity	Rochat et al. (1978), Kornbrust and Barfknecht (1985), Jain et al. (2007) and IARC (1987)
Acid black	Diazo	Paints, inks, plastics, leather	Irritation to eyes, skin and respiratory system	Daneshvar et al. (2012)
Acid orange ll	Azo	Textile, paper	Carcinogenic	Kousha et al. (2012)
Acid green (3)	Triphenylmethane	Textile	Animal carcinogens, tumour growth observed in fish species	Hazrat and Shah (2008)
Methylene blue	Phenothiazine	Textile	Vomiting, shock, increased heart rate, tissue necrosis in humans, Heinz body formation	Hameed et al. (2007)
Malachite Green	Triphenylmethane	Textile, biological stain	Carcinogenic and mutagenic	Moghaddam et al. (2004)
Congo Red	Diazo	Textile, paper and pulp industry, biological stain	Carcinogenic and mutagenic	Jayaraj et al. (2011), Mayson and Suad (2012)
Indigo dye	Azo	Textiles, paper, leather, plastic, food, drug, cosmetics	Mutagenic effect on bacterium *Salmonella typhimurium*	Mathur and Bhatnagar (2007)
			Acute toxicity effect seen on *S. quadricauda*	Chia and Musa (2014)

2 Removal of Dyes from Aquatic Ecosystem

Presence of dyes in the water bodies is a nuisance at present times due to toxic and polluting nature of dyes. Removal of colour is a must from all effluent as it ruins the aesthetic appearance of water bodies and reduces light penetration. The laws are stringent regarding organic content in effluents; therefore, it is necessary to remove dyes from the effluents before their release into the water bodies (Crini 2006). Since the popularisation of synthetic dyes in the modern world, several techniques have been employed for removal of dyes. Very few methods have been successful considering the holistic view (Ghoreishi and Haghighi 2003).

2.1 Traditional Methods

The technologies for removal of dyes can be categorised into physical, chemical and biological techniques as described in Fig. 8.3 (Robinson et al. 2001). Physical treatment methods include membrane filtration techniques such as reverse osmosis, electrodialysis, nanofiltration, adsorption processes, etc. Membrane filtration techniques are not economically feasible because of low life span of the membranes which requires periodic replacement (Crini 2006). Adsorption is considered as one of the most feasible and effective techniques for removal of dyes (Dabrowski 2001; Crini 2006). Adsorption by activated carbon is referred to as one of the most efficient dye removal techniques of current by US EPA (Derbyshire et al. 2001). The activated carbon adsorption method is not very cost-effective, nonselective and considered ineffective for vat and disperse dyes (Babel and Kurniawan 2003). Chemical treatment methods for removal of dyes from wastewaters include coagulation and flocculation followed by filtration, precipitation using Fe(II)/$Ca(OH)_2$ followed by flocculation, electrokinetic coagulation, oxidation using ozone and irradiation. These methods are expensive and contain toxic sludge which poses a problem for disposal, and excess chemical usage may add to the burden of existing pollution problem (Crini 2006). Biological treatment processes are the most cost-effective alternatives for treatment of dye effluents in comparison to traditional physical and chemical methods. Microbial degradation, fungal decolourization, biosorption by microbes and macro-organisms and bioremediation techniques fall under the category of biological treatment processes. Bacteria, algae, fungi and yeasts are known to accumulate or degrade effluents (Banat et al. 1996; Fu and Viraraghavan 2001; McMullan et al. 2001; Crini 2006). Biological treatment processes also have some constraints in terms of application and technology. The disadvantages of biological treatment processes are as follows: they require a larger area (Bhattarcharyya and Sharma 2003); complete colour elimination is not possible (Robinson et al. 2001); and some complex compounds being xenobiotic in nature cannot be completely degraded (Ravi Kumar et al. 1998). Nonconventional methods are now being developed to combat the problem of pollution resulting from dyes.

2.2 Nonconventional Methods of Dye Removal

2.2.1 Adsorption

Adsorption techniques can be considered as one of the most environmental friendly technologies for the successful elimination of dyes from the wastewater. The use of activated carbon made from waste materials from agriculture, industries

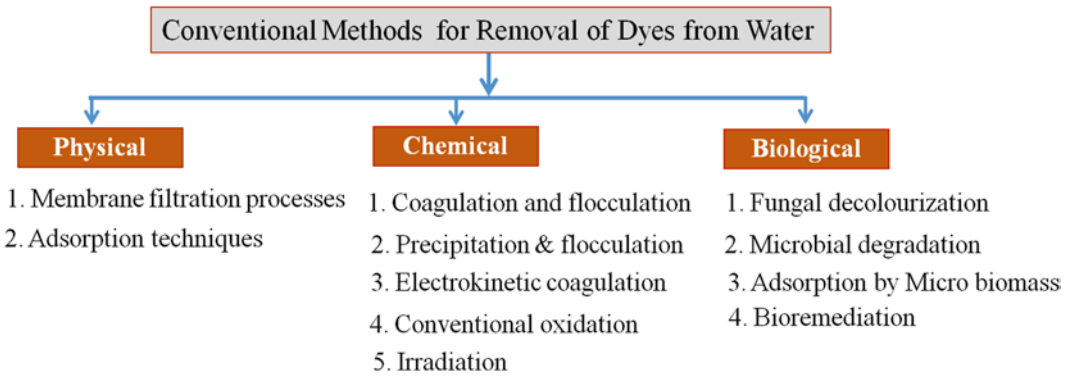

Fig. 8.3 Methods for removal of dyes from water (Source: Crini 2006)

Table 8.2 Adsorption efficiency of some conventional and nonconventional adsorbents

Adsorbent	Dye	Class	Adsorption capacity (mg g^{-1})	Reference
Chitosan	Reactive blue 2	Anthraquinone	2498	Chiou et al. (2004)
Activated carbon Taipei Chemical corp. (Taiwan)	Acid yellow	Azo	1179	Chern and Wu (2011)
Chemviron Carbon (UK)	Remazol yellow	Anthraquinone	1111	Al-Degs et al. (2000)
Pinewood	Acid blue 264	Anthraquinone	1176	Tseng et al. (2003)
Biomass	Reactive black 5	Azo	588.2	Aksu and Tezer (2000)
Tree fern	Basic red 13	Methine	408	Ho et al. (2005) and Gupta et al. (2003)
Peat	Basic violet 14	Triarylmethane	400	Sun and Xang (2003)
Activated sludge	Reactive yellow 2	Azo	333.3	Aksu (2001)
Clay	Basic blue 9	Triarylmethane	256.41	Gulnaz et al. (2004)
Yeasts	Remazol blue	Anthraquinone	173.1	Aksu and Donmez (2003)
Zeolite	Reactive red 239	Diazo	111.1	Ozdemir et al. (2004)

and municipal solid wastes is becoming popular nowadays as they are cost-effective; also it makes use of the materials which are polluting in nature and thus are beneficial in all respects. Natural materials such as clays, zeolites and silicates also are used as nonconventional adsorbents (Crini 2006). Table 8.2 shows the adsorption capacities of different conventional and nonconventional adsorbents (Cardoso et al. 2011). Chitosan is observed to have the maximum adsorption capacity followed by AC Taipei Chemical Corp (Chiou et al. 2004; Chern and Wu 2011).

2.2.2 Biosorption

The use of biomass for the accumulation of pollutants from effluents or other aqueous solutions can be termed as biosorption. The biosorbents are effective in removal of dyes due to their complex nature and chelating properties (Crini 2006). According to Aksu and Tezer (2005), biosorption consists of several processes independent of metabolism such as chemical and physical adsorption, chelation, microprecipitation, complexation and ion exchange; these take place within the cell wall. The process of biosorption includes two phases: one is a solid phase involving biosorbent that is the biological material and the second one is the liquid phase consisting of a solvent usually water which has a dissolved adsorbate (dyes) that needs to be sorbed (Ramachandra et al. 2005). Biosorption is a lucrative technique for dye removal because of its cost-effectiveness, high efficiency and selectivity and abundance of biosorbents found in nature or present in waste materials which can be effectively used (Aksu and Tezer 2005).

2.2.3 Phycoremediation

Algae have the potential to become suitable biosorbent because of their abundance in nature, very fast growth in a simple medium and cost-effectiveness (Aksu and Tezer 2005; Rawat et al. 2011; Olguín and Sánchez-Galván 2012; Prajapati et al. 2013; Malla et al. 2015). Phycoremediation can be defined as the use of microalgae and macroalage to transform contaminants with potential hazards present in water or soil into nonhazardous materials. The use of algae is being popularised nowadays for treatment of wastewater instead of using conventional bacteria. Algae are considered as a better option for the treatment processes because some of the pollutants have nutritional value for the algae; thus, it is efficiently taken up (Rawat et al. 2011; Olguín and Sánchez-Galván 2012; Singh et al. 2012; Prajapati et al. 2013; Sode et al. 2013; Laffont-Schwob et al. 2015). Heavy metals such as iron and chromium are essential for the photosynthetic process and metabolism of the algae; thus, these

heavy metals are effectively removed from the environment by them. Algae, which live in such conditions where there is a lack of these metals, develop special mechanisms to collect the essential nutrients. This feature of algae can be successfully used to treat effluent laden with heavy metals, toxic materials and dyes (Kumar et al. 2007; Gomes and Asaeda 2009; Olguín and Sánchez-Galván 2012; Kumar et al. 2015; Machida and Horizoe 2015). One of the advantages of using algae over bacteria is that algae do not require oxygen to remove pollutants instead they release oxygen and gather carbon dioxide. The additional advantage of carbon sequestration can be obtained from the process of detoxification (Dwivedi 2012). Treatment of coloured water by algae is proving to be highly beneficial because of several factors such as abundance nature of algae, cost-effectiveness, efficiency, no competition with other aquatic species, etc. (Chisti 2007; Kumar et al. 2014). In some instances where nonliving algal biomass is used for biosorption, the de-oiled algal biomass is used for biosorption of dyes. This provides double benefit as biofuel is obtained by algae and same biomass is used for waste water treatment. *Microspora* sp. (ATCC PTA-12197) was first de-oiled, and then the biomass was used for biosorption of methylene blue dye by Maurya et al. (2014).

Algae have been found to cleanse toxic effluents and wastewater by the processes of bioaccumulation, biosorption and biodegradation. The biosorption capability of macroalgae and microalgae can be attributed to the porous cell walls which allow movement of ions and molecules in aqueous solutions (Sheng 2004; Wang and Chen 2009; Fakhry 2013). The functional groups such as carboxyl, hydroxyl, phosphate, amino and other charged groups that are present on the cell surface of algae are responsible for binding of dye molecules and separation of pollutants from water (Srinivasan and Viraraghavan 2010; Çelekli and Geyik 2011; Çelekli and Bozkurt 2011; Fang et al. 2011). The extracellular biopolymers comprise mostly of alginate in Phaeophyta which are permeable to small ions (Vieira and Volesky 2000). Agar, porphyran and carregeenan which are sulphated galactans are found in Rhodophyta (Davis et al. 2003). These extracellular biopolymers which have long-chain structure are responsible for the adsorption of dyes and heavy metals (Fakhry 2013). The adsorption of contaminants or heavy metals in live microalgae is carried out in two steps: first step takes place rapidly where the adsorption is onto the cell surface and the second step is absorption which is lengthy and takes place within the cell and is dependent on cell metabolism (Dwivedi 2012). The macroscopic structure and rigidity in shape makes some macroalgae such as *Azolla filiculoides* very efficient in biosorption column applications (Padmesh et al. 2005). Some algae are capable to convert complex toxic compounds and dyes into simple compounds like *Chlorella vulgaris* which is capable of removing about 63–69 % of colour from water in mono-azo dye tectilon yellow 2G by breaking it up into aniline (Acuner and Dilek 2004). The use of immobilised algae for removal of colour is being studied with positive results. In a study by Chu et al. (2009), it was found that alginate-immobilised *Chlorella vulgaris* is capable of removing more colour than the suspension culture.

3 Biosorption Studies and Adsorption Kinetics

3.1 Biosorption by Macroalgae

Macroalgae have been reported to eliminate dyes successfully from aqueous solution in laboratory experiments (Khataee and Dehghan 2011; Khataee et al. 2010, 2011; Daneshvar et al. 2012; Kousha et al. 2012; Salima et al. 2013). Acid black 1 which is a diazo dye was efficiently removed from the solution using macroalgae *S. glaucescens* and *S. marginatum* where adsorption by *S. marginatum* and by *S. glaucescens* was found to be 30.9 mg g^{-1} (Table 8.3.) and 27.0 mg g^{-1}, respectively (Daneshvar et al. 2012). Kousha et al. (2012) found that C_3H_9N-treated *S. marginatum* was capable of 71.05 mg g^{-1} biosorption of acid orange dye, whereas untreated *S. marginatum* removed 35.62 mg g^{-1} of same dye. *Padina pavonica* removed 11.72 mg g^{-1} of acid-fast yellow dye (Fakhry 2013). Jayaraj et al. (2011) found that *Valoria bryopsis* could remove 10.5 mg g^{-1} of Congo red dye. *Chara aspera* has been found to remove 60–81 % methylene blue dye with pH ranging from 2.40 to 11–16. Basic blue 3 was removed by *Chara aspera* with an efficiency of 17.36–27.33 % at pH of 2.17–9.43 (Low et al. 1994).

3.2 Biosorption by Microalgae

Acutodesmus obliquus efficiently removed 44.24 mg g^{-1} of acid red 66 dye (Sarwa et al. 2014). *Microspora* sp. (ATCC PTA-12197) was capable of removing 86 % of methylene blue dye (Maurya et al. 2014). Aksu and Tezer (2005) found that *Chlorella vulgaris* effectively removed remazol black B, remazol red R and remazol golden yellow with accumulation rate of 368.8 mg g^{-1}, 181.9 mg g^{-1} and 52.8 mg g^{-1}, respectively (Table 8.4).

The treatment process where algae are used is highly pH dependent. The pH of the solution influences the dye chemistry in water as well as the dye binding sites at the cell surface. When the pH is low, the biomass has a net positive charge; it is assumed that in this case, amines or imidazoles present in the biomass will be protonated when pH has acidic values. The lower the pH, the higher the adsorption can be caused due to negative dye anions and positive cell surface. When the pH increases, the sites with positive charge decrease and sites with negative charge increase causing electrostatic repulsion between dyes and cell surface; thus, the adsorption capacity decreases (Crist et al. 1981; Gardea-

Table 8.3 Biosorption efficiency of macroalgae for different dyes

Algae	Dye	Class	Concentration of dye (mg L^{-1})	Rate of accumulation (mg g^{-1})/efficiency	pH	Biosorbent dosage (g L^{-1})	Time (h)	Adsorption isotherms	Reference
S. glaucescens	Acid black 1	Diazo	10–50	27.0	2	1–9	1.5	Temkin and Freundlich	Daneshvar et al. (2012)
S. marginatum	Acid black 1	Diazo	10–50	30.9	2	1–9	1.5	Temkin and Freundlich	Daneshvar et al. (2012)
S. marginatum	Acid orange	Azo	30–90	35.62	2	1	1	Langmuir	Kousha et al. (2012)
C_3H_9N-treated *S. marginatum*	Acid orange	Azo	30–90	71.05	2	1	1	Langmuir	Kousha et al. (2012)
CH_3OH treated *S. marginatum*	Acid orange	Azo	30–90	29.08	2	1	1	Langmuir	Kousha et al. (2012)
HCHO treated *S. Marginatum*	Acid orange	Azo	30–90	34.06	2	1	1	Langmuir	Kousha et al. (2012)
HCHO/HCOOH treated *S. marginatum*	Acid orange	Azo	30–90	14.95	2	1	1	Langmuir	Kousha et al. (2012)
Padina pavonica	Acid-fast yellow	Azo	5–160	11.72		2	1.5	Pseudo second-order model	Fakhry (2013)
Valoria bryopsis	Congo red	Diazo	5–25	10.5	5	50–250 mg		Langmuir, Freundlich, DKR adsorption isotherm	Jayaraj (2011)
Chara aspera	Methylene blue	Basic	100	60.0–81.25 %	2.40–11.16	0.05–0.5	2	Langmuir	Low et al. (1994)
Chara aspera	Basic blue 3	Triaryl–methane	100	17.36–27.33 %	2.17–9.43	0.25–1	2	Langmuir	Low et al. (1994)

Table 8.4 Biosorption efficiency of microalgae for different dyes

Algae	Dye	Class	Concentration of dye (mg L^{-1})	Rate of accumulation (mg g^{-1})/efficiency	pH	Biosorbent dosage (g L^{-1})	Time (h)	Adsorption isotherms	Reference
Acutodesmus obliquus	Acid red 66	Azo	10–50	44.24 (max)	2	0.1	1	Langmuir	Sarwa et al. (2014)
Microspora sp. (ATCC PTA-12197)	Methylene blue	Basic	50	86 %	7	10		Pseudo second-order model	Maurya et al. (2014)
Chlorella vulgaris	Remazol black B	Azo	20–800	368.8	2	1	–	Freundlich, Redlich-Peterson, Kolbe-Worigan	Z.Aksu and Tezer (2005)
C. vulgaris	Remazol red R	Azo	20–800	181.9	2	1	–	Langmuir	Z.Aksu and Tezer (2005)
C. vulgaris	Remazol golden yellow	Azo	10–200	52.8	2	1	–	Langmuir	Z.Aksu and Tezer (2005)

Torresdey et al. 1990; Aksu 1998; Aksu and Tezer 2005; Tam et al. 2002; Tien 2002). Most of the studies indicated that the optimum pH of 2 was most suitable for biosorption of dyes from the aqueous solution.

The dye removal efficiency of macro- and microalgae is calculated by the following formula:

$$\text{Removal efficiency } (\%) = (C_0 - C_f) * 100 / C_0 \tag{8.1}$$

where C_o is the initial dye concentration and C_f is the equilibrium dye concentration in the solution (mg/l)

$$q_e = V(C_0 - C_f) / m \tag{8.2}$$

where V is the volume of the solution, M is the mass of biosorbent (g), and q_e is the dye biosorption.

3.3 Adsorption Isotherms

Adsorption models are used to investigate and study the adsorption mechanisms and surface properties of the biosorbents. The maximum adsorption capacity of any adsorbent is determined by the adsorption equilibrium measurements. Langmuir, Freundlich, Redlich-Peterson and Temkin isotherms are commonly used. Freundlich isotherm was developed in 1906 by Freundlich which described the heterogeneous surface equilibrium, and monolayer capacity is not assumed in this case (Lin and Juang 2009). The surface is considered as homogeneous in Langmuir isotherm where all adsorption sites exhibit equal affinity for solute particles (Langmuir 1918). Redlich-Peterson isotherm combined the elements from Langmuir and Freundlich isotherms (Redlich and Peterson 1959). The initial dye concentration also determines the adsorption capacity of biosorbent; when the initial concentration is high, the adsorption increases as more driving force is provided to overcome resistance from mass transfer between the solid and aqueous phases (Aksu and Tezer 2005). Similarly, the amount of biosorbent dosage when increased increases the rate of adsorption.

4 Conclusions

Algal biosorption can prove to be a boon for the people who are suffering from the adverse impacts of dyes present in the effluents releasing from different industries like textile, paper and pulp, tanning and pharmaceuticals. The algal biomass can have multipurpose use in extraction of oil, waste water treatment and carbon fixation. Marine and freshwater algae have both been made use of to detoxify the dye from wastewater. Algae being cosmopolitan and cost-effective can be used at even community level for the removal of dyes contamination. Some technical interventions like application of genetic engineering can be engaged which may further enhance the rate of phycoremediation of dyes with accelerated amount of lipid content.

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Bioremediation and Decolourisation of Biomethanated Distillery Spent Wash

9

Sushil Kumar Shukla and Pradeep Kumar Mishra

1 Introduction

With the advent of industrialisation, modern society was blessed with leisure life, but it has also suffered due to rapid pollution being created by a number of these industries, which is currently creating havoc. With increased pollution load, various remedial measures are being planned and executed by the planner, researchers and development agencies. Water, being one of the most important commodities for the survival of humankind, faces a number of problems, threatening it both in quantitative and qualitative terms. Rapid urbanisation, industrialisation and changing consumption pattern have pushed readily available fresh water resources to extinction levels. The reckless disposal of industrial effluents in surface water has converted nearly all the major rivers into wastewater stream. These developments have resulted in severe water crisis in majority of countries worldwide.

Even in India, large volumes of wastewater is being generated, and its safe disposal is posing a great challenge to the researchers and environmentalists. As per UNESCO report, India was under water-rich category up to the 1950s, but currently it has been ranked under water-stressed category. It invites immediate attention towards the conservation of water resources, its harvesting, treatment and recycling to help the nation to come out of this emergent scenario.

To fill the demand and supply gap of alcohols, distillery industry in India is growing at fast rate. These industries can be ranked in the top bracket of most polluting industries as they generate large quantity of wastewater (12–14 L/L alcohol produced) containing excessive COD and BOD. In India currently, approximately 3.0×10^9 liters of alcohol is being produced and it generates 5×10^{10} liter equivalent of wastewater each year (Uppal 2004). The most famous conventional secondary treatment technique for this wastewater is the aerobic treatment using suspended culture. These reactors need larger space and installation cost; with increasing quantities of wastewater, larger space and more monetary investment are required, that is, the biggest roadblock for this technology (Fumi et al. 1995). As distilleries are using molasses as a raw material to produce alcohol, we can find a large number of integrated sugar mills and distilleries in India. Bagasse and pressmud are other two prominent wastes being generated from sugar industries. Bagasse is generally used as the raw material for paper manufacturing or as a fuel for boilers, whereas pressmud has no notable use (Nandy et al. 2002). Recently, pressmud was found to have an interesting application in energy generation; a few of the entrepreneurs are using pressmud for making briquette. The main concern from the environmental point of view is large effluents being generated from molasses-based distilleries known as spent wash (SW). As per the average estimate, molasses-based distillery generates nearly 12–15 L of spent wash per litre of alcohol produced (Beltran et al. 2001). Spent wash having low pH, high temperature (70–80 °C), dark-brown colour, high ash content and high percentage of dissolved organic and inorganic matter is very difficult to dispose as it is very hazardous to surface water (Beltran et al. 1999; Yeoh 1997). As per Nandy et al. (2002), the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the spent wash typically range between 35,000–50,000 and 100,000–150,000 mg/L, respectively. However, it is not easy to predict the exact characteristics and volume of wastewater being generated from distilleries as it heavily depends on the raw material being used and processing technology. Other prominent discharges contributing to the quantity of wastewater being generated from distilleries include washing water used to clean the fermenters, cooling water and boiler water (Pant and Adholeya 2007; Satyawali and Balakrishnan 2007). The intensity of

S.K. Shukla (✉)
Centre for Environmental Sciences, Central University of Jharkhand, Ranchi 835205, India
e-mail: shuklask2000@gmail.com; sushil.shukla@cuj.ac.in

P.K. Mishra
Department of Chemical Engineering & Technology, IIT (BHU) Varanasi, Varanasi 221005, Uttar Pradesh, India
e-mail: pkmishra.che@iitbhu.ac.in

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potential pollution being created by this distillery wastewater based on BOD can very well be understood by the population equivalent of 1.25 billion for India. It implies that this organic pollution load is approximately seven times more than that of total organic load of municipal and domestic discharges based on the total population of India.

There is a minimum standard to be followed as promulgated by the Central Pollution Control Board (CPCB) as per the regulations of Environment Protection Act, 1986, known as minimal national standards (MINAS) for discharge of distillery wastewater. According to MINAS, only distillery wastewater having BOD <30 mg L^{-1} and COD <250 mg L^{-1} can be disposed into inland surface water. Similarly, prior to disposal on land, this wastewater should have BOD <100 mg L^{-1}, pH of 5.5–9.0 and Total Suspended Solids (TSS) <100 mg L^{-1}. Effort should also be made to minimise colour to the maximum extent (CPCB 2003).

1.1 Wastewater Generation and Characteristics

As per the CPCB, distilleries fall under the category of most polluting industries in India, ranking among the top 20 most polluting industries. The wastewater being generated at various stages in a typical distillery and their typical composition can be seen from Table 9.1 to Table 9.2. Major contributors are spent wash, fermenter cleaning and cooling steps.

The wastewater being generated by molasses-based distilleries have dark-brown colour. This colour can primarily be attributed to a dark-brown pigment, melanoidin. The presence of phenolic compounds and caramel is also responsible for this (Kalavathi et al. 2001). Melanoidins are nitrogen-containing natural heterogeneous polymers produced by nonenzymatic browning reaction known as the Maillard reaction. This is a chemical reaction which takes place between the carbonyl groups of reducing sugars and the amino groups of amino acids, peptides or proteins. Temperature at around 50 °C and pH of 4–7 are favourable for melanoidin production through the Maillard reaction, and these are responsible for the the characteristic dark brown colour of spent wash of distilleries (Rivero-Pérez et al. 2002).

As per Martins and Van-Boekel (2004), spent wash contains 2 % melanoidins, which has an empirical formula of $C_{17}H_{26-27}O_{10}N$. The conjugated carbon–carbon double bonds –C = C – in melanoidins are responsible for the brown colour (Kim et al. 1997). The molecular weight of this group lies in the range 5000–40,000. As melanoidins contain recalcitrant compounds, it is very difficult to remove its colour through conventional treatment technique. The colour intensity even increases during anaerobic digestion, due to re-polymerisation (Satyawali and Balakrishnan 2007). Melanoidins are also having antimicrobial properties which are toxic to a number of microorganisms involved in wastewater treatment processes (Sirianuntapiboon et al. 2004).

1.2 Profile of Lords Distillery Ltd., Nandganj, Ghazipur, UP, India

The samples were collected for the study from Lords Distillery Ltd., Nandganj Ghazipur, UP, India. The installed capacity of Lords Distillery Ltd. for manufacturing rectified spirit (RS) is 14,850 KL (94.5 % alcohol) per annum. The molasses is converted into alcohol by fermentation using yeast through a batch fermentation process. The yeast culture used for fermentation converts the sugar present in molasses into alcohol. Alcohol is extracted from the fermenter wash by distillation. The final products are rectified spirit, country liquor and Indian-made foreign liquor (IMFL). The capacity utilisation of the plant in the study period has been almost 41.9 %. The first stage of Effluent Treatment Plant (ETP) is an anaerobic biological digestion. The second stage comprises of a primary clarifier, an anaerobic digester with extended aeration system using diffused aeration mechanism and a secondary clarifier. The third stage comprises of an aerobic digester consisting of a hybrid aerobic biological reactor wherein microorganisms supported by activated carbon particles are kept suspended within the reactor by means of air diffusion and a tertiary clarifier. In addition to the above treatment process, the company has developed a botanical treatment plant using the root zone process. Two lagoons of capacities 1435 and 890 m^3 have also been provided for storage of spent wash in case of emergencies.

Table 9.1 Wastewater generations in different distillery operations

Distillery operations	Average wastewater generation (KLD/distillery)	Specific wastewater KL wastewater/KL alcohol
Spent wash (from distillation)	511.4	11.9
Fermenter cleaning	108.2	2.5
Fermenter cooling	307.7	7.2
Condenser cooling	34.2	0.8
Floor wash	47.6	1.1
Bottling plant	126.9	3.0
Others*	33.3	0.8

Source: The Energy and Resources Institute (CPCB 2003)
* Wastewater generated through other distillary operations

Table 9.2 Typical characteristics of various wastewater streams in distilleries

Parameter	Spent wash	Fermenter cooling	Fermenter cleaning	Condenser cooling	Fermenter wash	Bottling plant
Colour	Dark brown	Colourless	Colourless	Colourless	Faint	Colourless
pH	4.0–4.5	6.25	5.0–5.5	6.8–7.8	6.0	7.45
Alkalinity (mg L^{-1})	3500	300	Nil	_	40	80
Total solids (mg L^{-1})	1,00,000	1000–1300	1000–1500	700–900	550	400
Suspended solids (mg L^{-1})	10,000	220	400–600	180–200	300	100
BOD (mg L^{-1})	45,000–60,000	100–110	500–6000	70–80	15	5
COD (mg L^{-1})	80,000–1,20,000	500–1000	1200–1600	200–300	25	15

Source: TERI (2003)

Table 9.3 Characteristics of distillery spent wash and anaerobically digested distillery effluent (BDE) of Lords Distillery Ltd. Nandganj, Ghazipur

Parameters	Distillery spent wash	Anaerobically biodigested distillery effluent (ABDE)
pH	4.5–5.4	7.5–8
BOD5	55,000–65,000	5000–8000
COD	110,000–130,000	40,000–52,000
Total solid (TS)	130,000–160,000	70,000–75,000
Total volatile solid (TVS)	60,000–75,000	48,000–60,000
Total dissolved solids (TDS)	90,000–120,000	35,000–50,000
Chlorides	6000–8500	5000–5500
Phenols	8000–10,000	7000–8000
Sulphate	7500–9000	3000–5000
Phosphate	2500–2700	1500–1700
Total nitrogen	5000–7000	4000–4200

Unit of all the parameters is mg L^{-1} except pH

Effluent from the ETP is diluted, if required, and subsequently discharged onto the neighbouring agricultural land. The characteristics of spent wash and anaerobically biodigested distillery effluent (ABDE) of Lords Distillery Ltd. are given in Table 9.3, along with the detailed flow sheet of effluent treatment plant used in Lords Distillery Ltd., Nandganj, shown in Fig. 9.1.

1.3 Challenges and R&D Focus Areas in Distillery Effluent Treatment

Anaerobic treatment yielding methane is a key secondary treatment being applied to the treatment of the spent wash, and these processes result in the generation of energy in addition to considerable reduction of pollution load. Majority of ETPs in distilleries are using aerobic digestion of post-biomethanated effluent, but it does not give the desired yield of methane in spite of considerable energy consumption in aeration. The two-stage aerobic digestion currently being used reduces COD considerably but it is nearly ineffective in colour removal. Decolourisation of distillery effluent is a challenge to R&D effort, and it also needs complete knowledge of colour-causing compounds and its characteristics prior to designing any such process. Numerous efforts to test the efficacy of conventional physical, chemical and biological treatment for this purpose have been reported with little success (Satyawali and Balakrishnan 2007; Pant and Adholeya 2007). The main disadvantage associated with physicochemical processes such as adsorption, flocculation, oxidation and coagulation is sludge handling in addition to the economy of the process (Rajor et al. 2002). Adsorption still attracts attention, but focus should be on the selection of any prominent solid waste being generated by industries as it will potentially reduce the process cost. To reach to zero discharge goals, the industries are currently forced to use highly expensive technologies like reverse osmosis (RO). Although the superiority of biodegradation processes for the treatment of distillery effluent cannot be questioned due to the large amount of wastewater being generated by these industries, efforts are certainly required for an integrated approach to deal with the problem and minimise the wastage of freshwater being currently used for dilution purposes.

1.4 Treatment Processes Employed in Distilleries

The removal of BOD, COD, Suspended Solid (SS), nutrients (nitrate, phosphate and potash), colourants, pathogens and

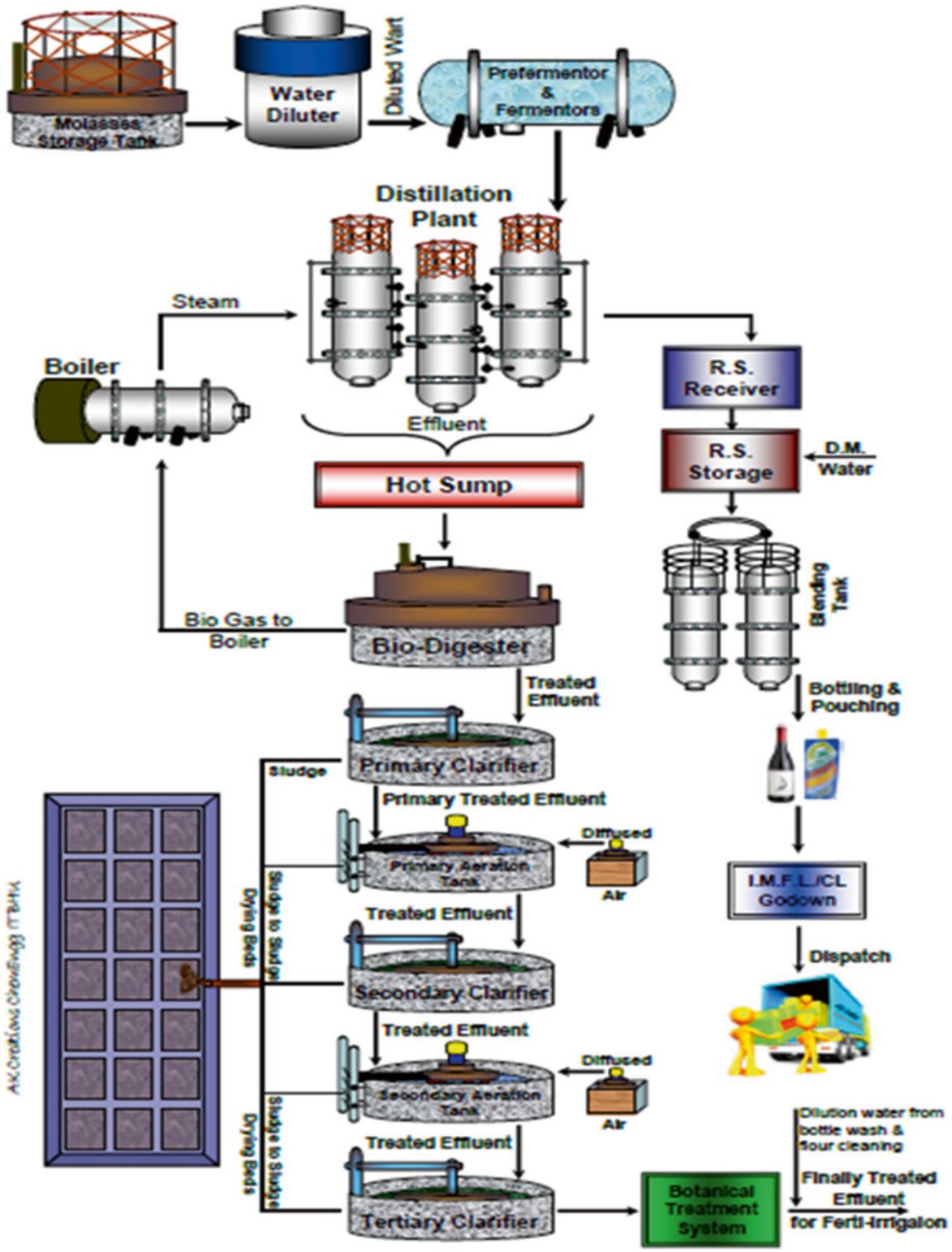

Fig. 9.1 Effluent treatment plant used in Lords Distillery Ltd., Nandganj

toxicity is required to make wastewater fit for disposal into the environment. The organics present in water primarily consume dissolved oxygen (DO) present in water to facilitate degradation, resulting in depletion of DO level in any surface stream leading to death of aquatic animals. The suspended particulate matters are primarily removed through

sedimentation or filtration, and when required, some chemical aids can also be used for this purpose. The excessive release of nutrients leads to eutrophication resulting in the growth of unwanted plants such as algae and aquatic macrophytes. The excessive nitrate concentration above 45 g m^{-3} in drinking water has been reported to lead to blue baby syndrome (Lincolin and Earle 1990).

Certain investigations have revealed the positive impact of the spent wash on certain crops that is why till the 1970s it was disposed on the land. Due to its high nitrogen, phosphorus and organic content even in Brazil, *vinasse* produced during the fermentation of sugarcane juice is being used as fertiliser. As per Rodríguez (2000), this can be used as fertiliser under controlled conditions. The main drawback associated with land disposal of the spent wash is its strong colour and odour in addition to some toxic effects. In addition to this, it requires a large area preferably in the vicinity of distilleries, and in order to avoid its transport into the surface or groundwater, the disposal site should lie in low and medium rainfall area (Sheehan and Greenfield 1980). Groundwater contamination is the most potent danger in this practice (Jain et al. 2002). Animal feed and potash production through evaporation and incineration, respectively, are other two important options validated by researchers (Sheehan and Greenfield 1980; Wilkie et al. 2000).

The physical, chemical and biological treatments either in isolation or in combination can be used to make wastewater safe for disposal. Suspended pollutants are mostly removed through physical treatment, whereas colloids and dissolved contaminants can be removed through chemical treatments (Stumm and Morgan 1962).

A critical review of existing literature in the field of distillery wastewater treatment reveals that still there is a scope of improvement and various microbes and algae should be examined for their efficacy. In addition to this, attempt should also be made to investigate the hybrid processes suitable for this treatment scheme.

1.4.1 Biological Treatment Processes

In recent years, a number of studies have focused on microorganisms, which are able to biodegrade and bioasorb colouring compounds present in wastewater. With increasing consciousness about pollution control, biological decolourisation is receiving prime attention for effluent treatment. With economic constraints on pollution control process, affordable and effective methods have become a necessity. Since spent wash is a high-strength wastewater, it is generally subjected to anaerobic digestion in huge methane reactors to convert organics into methane, which is used as a fuel. The ABDE is, then, treated aerobically in activated sludge treatment plants to further reduce BOD/COD and used for composting pressmud, a by-product of sugar mills. The colour of spent wash persists even after bio-methanation. A number of microorganisms have been evaluated in recent years to either biodegrade or bio-sorb the colour-causing components; still a lot more has to be done to make these processes economically viable.

1.4.1.1 Anaerobic Biodegradation

Anaerobic biodegradation is certainly the most important biological treatment as far as spent wash is concerned and it is capable of converting substantial portion of COD (more than 50 %) to biogas (Wilkie et al. 2000). The fuel generated can be used for electricity generation. This step reduces COD by 90 % and BOD by 80–90 %, and 85–90 % of biochemical energy is recovered as fuel (Wolmarans and de Villiers 2002). There are various types of reactors used in anaerobic treatment such as downflow fixed film reactor, granular bed anaerobic baffled reactor (GRABR), upflow anaerobic sludge blanket (UASB) reactor, anaerobic contact filter, etc. Out of these reactors, upflow anaerobic sludge blanket (UASB) reactors are commonly used reactors in distilleries (Bories and Ranyal 1988; Akunna and Clark 2000; Harada et al. 1996; Vijayaraghavan and Ramanujam 2000).

Toxicity, pH, temperature and nutrient concentrations are the most important factors in determining the success of biological treatments. To get the influent at required condition before biological reactors, the spent wash is heavily diluted to maintain its pH and toxicity within permissible range. As per Asthana et al. (2001), the effluent of anaerobic digester does not meet surface discharge standards due to excessive organic loading of spent wash. Effluents obtained after anaerobic digestion are called anaerobically biodigested distillery effluent (ABDE), which is characterised by dark-brown colour with high organic load. Therefore, aerobic treatment is considered essential and effective for anaerobically treated final effluent before discharging (Kim et al. 1997).

1.4.1.2 Aerobic Biodegradation

Anaerobically biodigested distillery effluent (ABDE) is treated aerobically in a two-step process with aerator in activated sludge-type (using mixed or pure culture) reactors or other advanced bioreactors. The following sections discuss the utilisation and prospects of pure cultures including bacterial, fungal, algal strain and mixed consortium in aerobic biodegradation of distillery effluent. Several workers have been using pure aerobic bacterial cultures for the treatment of distillery effluent in their studies (Mohana et al. 2007; Chavan et al. 2006; Dahiya et al. 2001a, b; Kumar and Chandra 2006).

Nowadays, various fungal spp. (viz. basidiomycetes and ascomycetes) are utilised to decolourise the characteristic dark brownish colour caused by natural and synthetic melanoidin in distillery wastewater. Fungal treatment is more effective than bacterial treatment due to the presence of pallets in fungi which provide a large surface area for the absorption in treatment of melanoidin and also reduces BOD/COD to a large extent. Beside this, filamentous fungi are least

affected by the variation in process parameters such as nutrients (C, H, N and trace metals), pH, temperature and aeration. Fungal spp. have been found to a potential agent for the treatment of distillery wastewater (Shukla et al. 2010; Pant and Adholeya 2009; Shayegan et al. 2004; Rajor et al. 2002).

The application of microalgae in wastewater treatment is an attractive and useful option when compared to other biotreatments because it synthesises carbohydrates by photosynthesis utilising CO_2 which is produced during aerobic degradation of organic compound by fungi and bacteria and also removes nitrogen and phosphorus from the environment (De la Noüe and De Pauw 1988; Goldman 1979; Shelef et al. 1980; Soeder et al. 1978; De Pauw and Van Vaerenbergh 1983; Oswald and Gotaas 1957).

The microalgal genera such as *Chlorella*, *Ankistrodesmus*, *Scenedesmus*, *Euglena*, *Chlamydomonas*, *Oscillatoria*, *Micractinium* and *Golenkinia*, belonging to various classes like Chlorophyta, Cyanophyta, Bacillariophyta and Euglenophyta, are frequently used in waste oxidation ponds and lagoons (Palmer 1969, 1974). Various algal spp. such as *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium* are also able to grow in adverse condition (Ramachandra 1993).

Various researchers reported the efficiency of algal spp. in different industries such as sewage treatment, distilleries, tanneries, heavy metals, etc. (Shelef et al. 1980; Mohamed 1994; Ibraheem 1998; De Pauw and Van Vaerenbergh 1983; Zaid-Iso 1990; Ma et al. 1990; Phang 1990, 1991; Kaplan et al. 1988; Soeder et al. 1978; Gerhardt et al. 1991; Hammouda et al. 1995; Cai-XiaoHua et al. 1995).

1.4.2 Treatment of Distillery Effluent by Coagulation

Aerobic biological treatment is only effective at higher dilution because melanoidin retards the activity of conventional micro- and macroorganisms due to its recalcitrant nature. So, coagulation seems to be an effective alternative pretreatment process in biological aerobic treatment in order to avoid heavy use of water for dilution, making the process cost-effective and efficient. The coagulants or flocculants are cheaper, accessible and have been used extensively in wastewater treatment. Sufficient literature are available (Dilek and Gokcay 1994; Al-Malack et al. 1999; Sundin and Hartler 2000a, b; Dilek and Bose 2001; Georgiou et al. 2003; Dugal et al. 1976, Lathia and Joyce 1978; Joyse et al. 1979; Srivastava and Jalan 1994, Srivastava et al. 2005; Beulker and Jekel 1993; Stephenson and Duff 1993, 1996a, b; Garg 1996) on the use of coagulants for the treatment of wastewater specifically, being disposed from textile, pulp and paper and allied industries. However, various researchers have also been investigating the efficacy of coagulation for the treatment of distillery effluent (Singh and Dikshit 2010; Chaudhari et al. 2007; Mishra and Chaudhary 2007; Pandey et al. 2003; Migo et al. 1997; Ramachandra and Singh 1999; Olthof and Eckenfelder 1975; Hayase et al. 1984; Mandal et al. 2003).

The coagulation with alum (Kawamura 1987), $FeCl_3$ (Olthof and Eckenfelder 1975) and $MgSO_4$, (Serger 1977) physically removed the colour of spent wash, but the voluminous sludge generated posed disposal problems. In the case of $FeCl_3$, ferric ions further imparted colour. Ramachandra and Singh (1999) attempted decolourisation of anaerobically biodigested distillery effluent by precipitation with aluminium sulphate and bleaching powder.

1.5 Objective of Present Investigation

Several reports on treatment techniques are available in the field of distilleries. A combination of physicochemical and biological methods is required to obtain a stream which can be recycled and reused to attain zero discharge status. One of the major drawbacks dealing with biological treatments, particularly aerobic biodegradation, is requirement of higher degree of dilution. The anaerobic digestion of spent wash is undoubtedly attractive and viable as it renders considerable decrease in COD and BOD loads besides generating methane, which is also considered a potential source of energy. To overcome the problem faced by excessive dilution before aerobic digestion, coagulation is supposed to have tremendous potential in treating the anaerobically biodigested distillery effluent and making it viable for supplementary aerobic degradation. Appropriate polymeric flocculants may be used for coagulation to reduce the amount of coagulant required. The use of fungal spp. and microalgae in treatment of distillery wastewater has been regarded as a cost-effective approach in removing refractory organic compounds along with nutrient elimination by maintaining aerobic conditions.

In light of the above discussion, the present investigation has been planned to study the treatability of anaerobically biodigested distillery effluent (ABDE) purchased from Lords Distillery Ltd., Nandganj, Ghazipur, UP, India. An integrated treatment approach combining coagulation-aerobic fungal degradation has been tested.

2 Materials and Methods

2.1 Wastewater

The sample (distillery spent wash and ABDE) for the decolourisation study was taken from cane molasses-based Lords Distillery Ltd., Nandganj, Ghazipur, UP, India, and kept in

deep freezer at low temperature (3–4 ± 2 °C). The physico-chemical analysis of samples was carried out in the laboratory based on the Standard Methods for the Examination of Water and Wastewater, APHA (1985, 1989), as shown in Table 9.4.

2.2 Coagulant Treatment

Anaerobically biodigested distillery effluent was pretreated with coagulants such as aluminium chloride ($AlCl_3$), ferric chloride ($FeCl_3.6H_2O$) and potash alum ($K_2SO_4.Al_2(SO_4)_3.24H_2O$) which were LR grade (commercial), procured from M/S Merck Specialities Pvt. Ltd., Mumbai, and optimized dose and temperature for maximum decolourisation were decided by a jar test (VELP Scientifica, Model JLT 6, France).

2.3 Microorganism and Inoculums

Pure culture of *Aspergillus niger* ATCC No. 26550 and NCIM No. 684 was procured from National Chemical Laboratory (NCL), Pune, and maintained on Potato Dextrose Agar (PDA) under incubattion at 30 °C (Miranda et al. 1997). The maintenance and growth medium were the same and showed maximum activity in 72–96 h.

2.3.1 Flask Cultures

The aerobic treatment of coagulated ABDE was carried out in 250 mL Erlenmeyer shake flasks with 100 mL sample in nutrient broth. Various process parameters such as nutrient concentration, temperature of the solution, pH, inoculum volume and shaking speed were optimized for maximum decolourisation of ABDE. Samples were collected at the end of every 24 h for observing maximum decolourisation at various operating conditions.

Table 9.4 Composition of anaerobically biodigested distillery effluent (ABDE) obtained from Lords Distillery Ltd., Nandganj, Ghazipur, UP

Parameter	ABDE
COD	42500.0
BOD	6200.0
Colour	Blackish brown
pH	7.6
Ca	175.9
K	930.0
Total N	3900.0
SO_4	3200
Fe	18.0
PO_4	1650

Note: All values are in mg/L except colour and pH

2.4 Decolourisation Assay

During aerobic reaction, the sample collected after every 24 h interval was centrifuged at 10,000 rpm for 10 min prior to optical density measurement of the supernatant with Systronics double beam spectrophotometer (2202) at 475 nm. Decolourisation of effluent was measured in percentage as given below:

$$\%\,\text{decolourisation} = [(\text{initial OD} - \text{final OD}) / \text{initial OD}]\,100$$

3 Results and Discussion

Batch studies were performed in 250 ml Erlenmeyer flask using *Aspergillus niger* inoculums for biodegradation of anaerobically biodigested distillery effluent (ABDE) procured from Lords Distillery Ltd., Nandganj, Ghazipur. The primary investigation revealed the requirement of excessive dilution resulting in additional wastage of water. The maximum percentage of decolourisation obtained from *Aspergillus niger* was 63.5 % at excessive dilution of 100 %. Depending upon its cost-effectiveness and degradation ability, potash alum was selected as the best coagulant among the three as shown in Table 9.5. The cost-effectiveness of aerobic treatment was enhanced by coagulation as a pretreatment step. Coagulation reduced the dilution of ABDE up to 90 % in aerobic treatment. The typical characteristics of ABDE before and after treatment by coagulants are shown in Table 9.6 and it was observed that the maximum colour and COD reduction was achieved by treatment with potash alum, i.e. 92.45 % and 78.5 %, respectively, among the three coagulants. The organic content and dissolved solids were precipitated due to coagulation and flocculation processes, resulting in removal of colour and COD reduction.

3.1 Biodegradation of Coagulated ABDE

In aerobic biodegradation, coagulated ABDE filtrate was used and various process parameters like nutrient concentration, pH, temperature, stirring speed and inoculation volume were optimized for maximum decolourisation and COD reduction by *Aspergillus niger*. This fungus was characterised by growth of small, compact and uniform pallets (2–6 mm diameter) in 72–96 h.

3.2 Benefits of Algae Wastewater Treatment Processes

Microalgae have several properties by which they can play an important role in the field of wastewater treatment partic-

Table 9.5 % colour, COD and pH reduction of ABDE at optimum doses of coagulants

Particulars	Optimum dose (g/L)	% Colour removal	% COD reduction	pH reduction of ABDE
Alum	80	92.45	78.5	4.8
Aluminium chloride	35	74.6	66.8	3.9
Ferric sulphate	30	67.0	59.4	4.2

Table 9.6 Characteristics of ABDE before and after coagulation

Parameters	ABDE	Treated ABDE with		
		Alum	$AlCl_3$	$FeCl_3$
COD	42500.0	9450.0	14110.0	17425.0
BOD	6200.0	1705.0	2790.0	3410.0
Colour	Blackish brown	Light yellow	Light brown	Greenish brown
pH	7.6	4.8	3.9	4.2
Ca	175.9	38.8	27.28	58.6
K	930.0	860.0	86.0	820.0
Total N	3900.0	1390	2118.0	2245.0
SO_4	3200	1232.0	1650.0	1834.0
Fe	18.0	67.86	8.92	1035.7
PO_4	1650	1043.0	1254.0	1287

Note: All values are in mg/L except colour and pH

ularly for organic-rich effluents. Distillery industries employ various treatment techniques to treat effluent, but the most effective technique is anaerobic digestion followed by two-stage aerobic treatment by activated sludge processes. Aerobic treatment requires an aerator which maintains oxygen concentration in reactor, triggering the degradation processes by aerobic microorganism present in activated sludge. This process requires huge amount of energy to maintain oxygen concentration. The effluent after aerobic treatment contains large amounts of organic load along with high nitrogen and phosphorus concentration which are produced as stable products in aerobic biodegradation process and the effluent can not be disposed off as such because of strict environmental legislation.

So, the future application of microalgae in aerobic biodegradation process along with fungi seems to be a viable cost-effective and ecofriendly technique when compared to conventional treatment processes as discussed below:

(a) Microalgae absorb CO_2 in the presence of sunlight and produces useful biomass along with oxygen by the process of photosynthesis (Greeno et al. 1996). These properties of microalgae reduces the cost of conventional treatment in which mechanical aerator is required to maintain oxygen concentration in the reactor. It has been estimated that 1 Kw of electricity is required to maintain oxygen concentration in the reactor which is sufficient to remove approximately 1 kg f BOD from the reactor. The production of 1 Kw of electricity is achieved by burning of fossil fuel which emits almost 1 kg of CO_2 in the atmosphere. So the conventional aerator system is neither cost-effective nor ecofriendly (Oswald 1988a, b).

(b) Sludge management is an important step in conventional treatment process. Nowadays, lots of new techniques are being employed in industries for the treatment and reuse of sludge. By this activity, industries are trying to reduce the cost of treatment and the pressure on natural resources. The application of microalgae also reduces the sludge problem because it utilises the by-product of aerobic degradation in photosynthesis and other metabolic activities and produced biomass can be used to produce valuable products for humankind like biofuel, edible products, raw materials for industries, etc.

(c) Greenhouse gas (GHG) emission is a major problem of conventional wastewater treatment. The algal-based wastewater treatment process emits very less amount of GHG in the atmosphere because most of the CO_2 and other important gases are utilised by algae in photosynthesis and metabolic activity.

(d) Microalgae have excellent carbon dioxide sequestration potential because it absorb huge amount of CO_2 and produces useful biomass. According to the National Renewable Energy Laboratory (NREL), under controlled environment, algae can produce up to 40 times more biodiesel when compared to terrestrial oil plant (Sheehan et al. 1998; Wald 1988). So, wastewater treatment with algae will be a cost-effective, ecofriendly and sustainable technique along with production of some useful by-products.

(e) Algal wastewater treatment also reduces the cost of disinfectant which is commonly used in conventional treat-

ment for harmful pathogens. In carbon-limited condition, microalgae can utilise carbonate as a carbon source and liberate hydroxide ions in the solution which increases the pH of the solution up to 9–10. At this pH most of the pathogens are killed, so the water obtained after this treatment is free of pathogens when compared to conventional treatment (Watanabe and Hall 1996; Zhu et al. 2008).

4 Conclusions

Nowadays, distilleries are considered as one of the most polluting and growth-oriented industries in the world. Distilleries consume huge amount of water in the manufacturing of alcohol and produce large amount of wastewater containing high organic load, acidic nature and are usually dark brown in color. This wastewater alters the physical, chemical and biological characteristics of receiving waterbody and soil if discharged directly into the environment without any treatment. Water is one of the most important natural resources for the survival of the living being on this planet Earth.

So, the development of effective treatment plan for distilleries is one of the challenges to environmental engineers. Taking this in consideration, developing effective treatment plan for distilleries, i.e. coagulation followed by mixed culture aerobic treatment (fungal and algal), seems to be a viable cost-effective and ecofriendly technique in future.

Keeping these in mind, a case study of the distillery effluent from Lords Distillery Ltd., Nandganj, Ghazipur, UP, India, has been discussed to evaluate an integrated approach combining coagulation + aerobic degradation (fungal) for a high removal of the contaminants from anaerobically biodigested distillery effluent (ABDE) and reduction of the cost of treatment.

This work on bioremediation and decolourisation of anaerobically biodigested distillery effluent (ABDE) shows that an incorporated planning approach, considering cost–benefit analysis of conventional treatment vs fungal treatment, is a more economical and viable option of treatment as compared to conventional treatment with following advantages :

(a) Reduces the cost of treatment by absorbing raw material of aerobic biodegradation and maintaines oxygen by photosynthesis even in extreme growth conditions (extreme pH, high salinity, etc.).
(b) It produces useful biomass which can act as raw material for industries and reduces the pressure on fossil fuel by producing biofuel.
(c) It acts as natural disinfectant and removes heavy metal from effluents.

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Genetic Engineering Tools for Enhancing Lipid Production in Microalgae

10

Sheena Kumari, Poonam Singh, Sanjay Kumar Gupta, and Santhosh Kumar

1 Introduction

Microalgae have been of considerable interest in the last decade as a possible resource for biofuel production, owing to its potential for high biomass generation rates compared to terrestrial plants (Radakovits et al. 2010). Many microalgal species are reported to produce substantial amount of storage lipids (triacylglycerols) under stress conditions, which could be effectively utilized for the production of biofuels. However, naturally growing microalgae might not be able to provide enough resources for the production of renewable biofuels so as to replace fossil transportation fuels (Li et al. 2008). To date, numerous research work have been performed to improve the lipid accumulation in microalgae which includes modifying the cultivation conditions such as nutrient stress, light and temperature.

Currently, several efforts are being made globally to increase the storage lipid accumulation in microalgae, by employing methods other than improving cultivation conditions. One potential approach to enhance lipid production is by employing genetic engineering (GE), based on the key enzymes involved in lipid biosynthesis. Many studies have been carried out in the recent past to understand the basic lipid metabolism of different microalgal strains. With the advent of advanced molecular techniques, the complete nuclear and chloroplast genome sequencing of many microalgal species is currently available. A basic knowledge of the key genes present in microbial genome would assist researchers in the manipulation of algal genomes more specifically and would form a strong platform for the future GE strategies in microalgae.

Microalgal GE for enhanced lipid production has mainly focused on manipulating the key genes associated with lipid synthesis through forward or reverse genetics (Radakovits et al. 2010; Hlavová et al. 2015). In this chapter, we focus briefly on the lipid and fatty acid biosynthesis pathways in microalgae and the common genetic engineering tools that have been employed to enhance lipid production. Some of the challenges affecting microalgal genetic engineering research have also been discussed in this chapter.

2 An Overview of Lipid Biosynthesis Pathways in Microalgae

Arabidopsis is considered as a model organism for understanding the enzymes involved in lipid synthesis of all photosynthetic organisms. Based on the sequence homology of genes involved in lipid metabolism of microalgae and Arabidopsis, it was generally assumed that microalgae have similar lipid metabolic pathway as that of higher plants. However, after the whole genome sequencing of *Chlamydomonas*, it was established that microalgae have simple lipid metabolic pathway compared to higher plants. Biosynthesis of fatty acids in microalgae occurs in the chloroplast and is regulated by an enzyme complex acyl carrier protein (ACP) fatty acid synthase (FAS) type 2 (Harwood and Guschina 2009). FAS enzyme is a polypeptide chain with multiple domains, each having distinct enzyme activity required for fatty acid biosynthesis. The first step in fatty acid biosynthesis is the formation of malonyl-CoA from acetyl CoA, catalysed by acetyl-CoA carboxylase (ACCase) (Blatti et al. 2013). In the chloroplast, photosynthesis provides an endogenous source of acetyl CoA, and more than one pathway may contribute in maintaining the acetyl CoA pool. ACCase is considered as the main enzyme that catalyses

S. Kumari (✉) • P. Singh • S.K. Gupta
Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa
e-mail: sheenas@dut.ac.za

S. Kumar
Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa

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the first step reaction in lipid biosynthesis. It has three major functional sites that promote the formation of malonyl-CoA, the main carbon donor for fatty acid biosynthesis. This is considered as a rate-limiting step in lipid synthesis which involves two stages: (a) the transfer of CO_2 (HCO_3^-) by biotin carboxylase portion of ACCase to a biotin prosthetic group that is attached to the E-amino group of lysine residue and (b) the transfer of activated CO_2 from biotin to acetyl CoA. Production of acetyl CoA and its conversion to fatty acid, however, depends on factors such as tissue type, light/dark condition, developmental stages and species (Thelen and Ohlrogge 2002).

Biosynthesis of fatty acid is a multistep reaction which involves condensation, reduction, dehydration and reduction. A malonyl group is first transferred from CoA to a protein cofactor acyl carrier protein (ACP) which is involved in the whole process until the formation of a 16- to18-carbon product, ready to be transferred either to the glycerolipids or exported from the plastid. The next step is a condensation reaction. At this stage, the malonyl group of malonyl-ACP undergoes a series of condensation reactions with acetyl CoA releasing CO_2, that helps to drive the reaction forward. At least three groups of condensing enzymes (commonly called 3-ketoacyl-ACP synthase) are involved in the synthesis of 18-carbon fatty acids (Hu et al. 2008). The initial condensation reaction leads to the formation of a four-carbon product, 3-ketoacyl-ACP, catalysed by 3-ketoacyl-ACP synthase III (KASIII). Other condensation enzymes are KASI which is thought to be involved in the production of 6- to 16-carbon chain products and KASII for the elongation of 16-carbon ACP to stearoyl-ACP. This reaction cycle is completed by a reduction reaction catalysed by enoyl-ACP reductase. Each cycle of these four reaction steps lengthens the fatty acid precursor chain by 2 carbons while still attached to ACP as a thioester (a process known as elongation) leading to the formation of saturated 16:0-ACP and 18:0-ACP. Unsaturated fatty acid is formed when a double bond is introduced by the enzyme stearoyl-ACP desaturase. This is followed by a reduction process where 3-ketoacyl-ACP is reduced at the carbonyl group by the enzyme 3-ketoacyl-ACP reductase and then dehydration by the hydroxyacyl-ACP dehydratase. Elongation of a fatty acid chain takes place in the plastid and continues until the acyl group is removed from ACP, either as a result of hydrolysis of acyl-ACP by acyl-ACP thioesterase, releasing free fatty acid, or by the transfer of fatty acid from ACP to glycerol-3-phosphate or to monoacylglycerol-3-phosphate by one of the two acyltransferases (Hu et al. 2008) (Fig. 10.1).

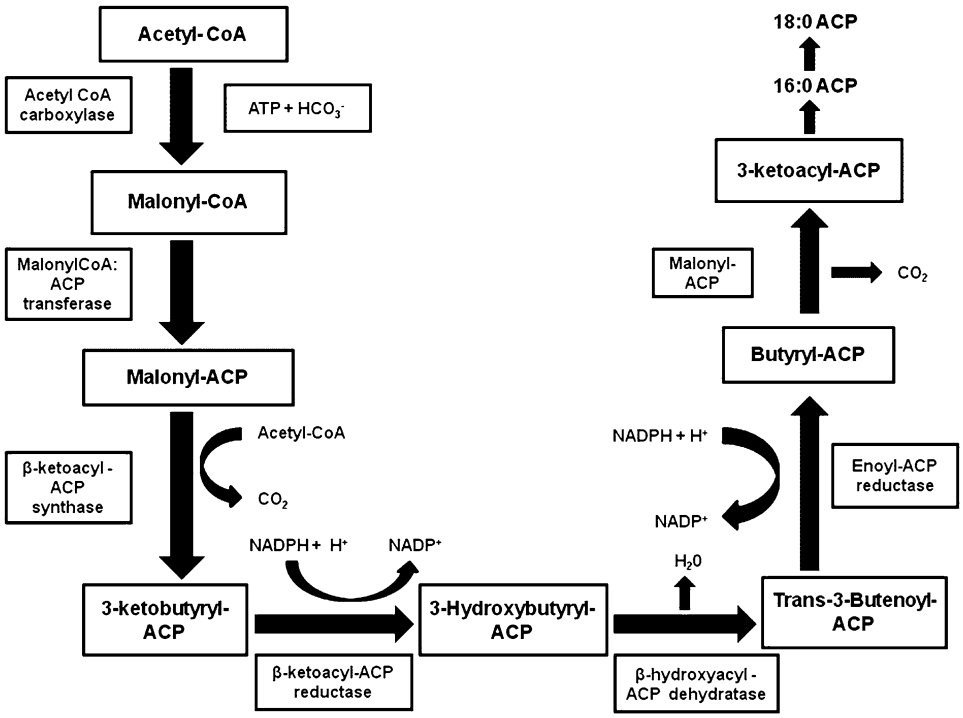

Fig. 10.1 Fatty acid biosynthesis pathway in microalgae

3 TAG Biosynthesis in Microalgae

The triacylglycerols (TAGs) are the storage form of metabolic energy in microalgae and various other organisms. Biosynthesis of TAGs starts with the two precursors, l-α-phosphoglycerol and acetyl CoA (Yu et al. 2011). Initially, the fatty acids synthesized in chloroplast are exported to cytosol where acylation reactions take place and the synthesized fatty acid is transferred to glycerol-3-phosphate to form phosphatidic acid (PA). This reaction is catalysed by the enzyme glycerol-3-phoshpate acyltransferase. In the next step, diacylglycerol (DAG) is produced by dephosphorylation of PA in the presence of phosphatidic acid phosphatase (Fig. 10.2). Synthesis of TAG is completed by the attachment of fatty acid to DAG in the presence of the enzyme diacylglycerol acyltransferase (Merchant et al. 2012). The acyltransferases involved in TAG synthesis may exhibit preference for specific acyl-CoA molecules which could be an important factor in determining the acyl composition of TAG. For example, the lyso-PC acyltransferase in *Nannochloropsis* cells prefers 18:1-CoA over 16:0-CoA (Roessler et al. 1994). Although lipid biosynthesis in algae has been assumed to share a similar pathway as that of higher plants, the annotation of chloroplast genes in microalgae has shown differences in the gene sequences. For example, gene annotation of *C. reinhardtii* has shown some differences in the type of gene families related to lipid biosynthetic pathways (Riekhof et al. 2005). Therefore, knowledge of the genes involved in the synthesis of fatty acids and TAGs specific to microalgae would allow for the improvement of GE technology for enhanced lipid production.

4 Genetic Engineering Approaches to Improve Lipid Synthesis in Microalgae

Various genetic engineering systems have been developed for strain improvement in different algal species. They are broadly classified into two main categories, viz. reverse and forward genetics. Reverse genetics involves the application of traditional random (chemical/physical) mutagenesis, whereas forward genetics involves identification of a particular gene of interest and manipulation of its expression level.

4.1 Forward Genetics Approach: Random and Insertional Mutagenesis

Chemical and physical mutagens are among the most widely used traditional methods for strain improvement. The most commonly used chemical mutagens are alkylating agents such as ethyl methanesulfonate (EMS) and N-methyl-N′-nitro-N-nitrosoguanidine (NTG) (Table 10.1). They were the first chemicals used for increasing the production of eicosapentaenoic acid (EPA) in *Nannochloropsis oculata* (Chaturvedi and Fujita 2006) and to increase the growth properties of Chlorella (Ong et al. 2010) through random mutagenesis.

Typical physical mutagens include different types of irradiation such as UV, gamma or heavy ion beams (Neupert et al. 2009; Hlavová et al. 2015). Among these, mutagenesis using UV light is simple and efficient, since it requires neither specialized equipment nor chemicals and can be performed by merely exposing the cells to germicidal UV lamps. This method has been used extensively in algal

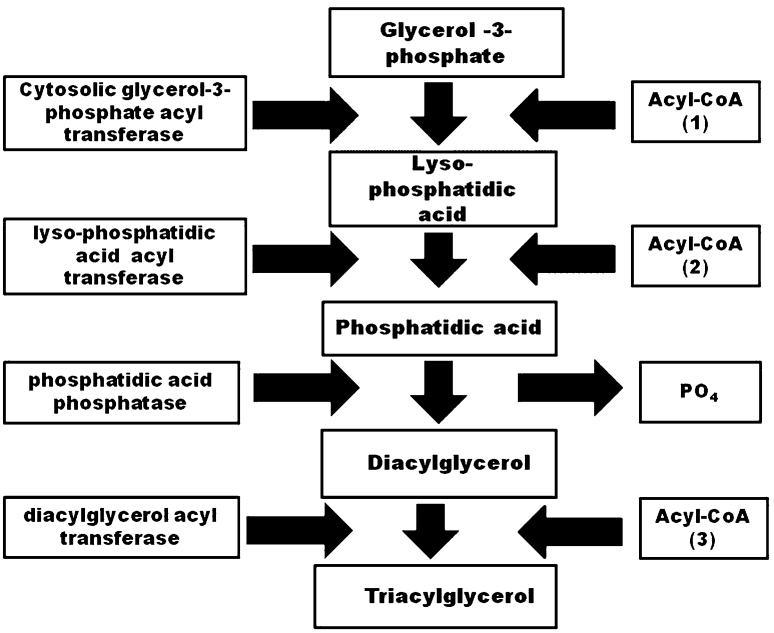

Fig. 10.2 Triacylglycerol biosynthesis pathway in microalgae

research to develop strains with specific features (Neupert et al. 2009) including enhanced oil production (Vigeolas et al. 2012; Jaeger et al. 2014). The applicability of gamma irradiation in microalgae was demonstrated by improved astaxanthin production (Najafi et al. 2011). However, gamma and heavy ion beam irradiation methods require specific equipment, hence not so widely employed in basic research. Though all mutagens have proven their merits in the production of mutants with desired phenotypes through forward genetics, there are some setbacks to this approach (Neupert et al. 2009; Hlavová et al. 2015). Though the initial step of mutagenesis appears simple as there is no need for prior knowledge on the genes of interest, the success of mutation lies in the proper selection of mutants with desired phenotypes (Fig. 10.3). Thousands of mutants may have to be screened to get a single desired phenotype which is one of the major bottlenecks in forward genetic approaches.

Insertional mutagenesis for gene inactivation is an alternative approach to obtain a mutant population for gene identification in various microalgal species (Dent et al. 2005). This can be classified both as forward and reverse genetics due to the nature of the approach. In this method, the mutation of DNA is achieved by insertion of one or two nucleotide bases into the coding region of the gene of interest, which in the loss of expression of that gene (Fang et al. 2006; González-Ballester et al. 2011; Hlavová et al. 2015). The main advantage of insertional mutagenesis over random mutagenesis is the ease of mutant isolation and identification of insertion points on the DNA fragment using specific markers (antibiotic resistant genes or amino acid synthesis gene or T-DNA) (Gonzalez-Ballester et al. 2005).

Table 10.1 Chemical and physical mutagens employed in reverse genetics and their mode of action

Mutagen	Mode of action	Mutation caused
EMS, NTG	Alkylation of DNA base particularly guanine	Point mutations
UV irradiation	Photochemical reaction leading to cyclobutane ring	Point mutations, deletions
Gamma irradiations	Ionization leading to double stranded break	Deletions
Heavy ion beams	Ionization leading to double stranded break	Chromosome breaks and exchanges

Adapted from Hlavová et al. (2015)

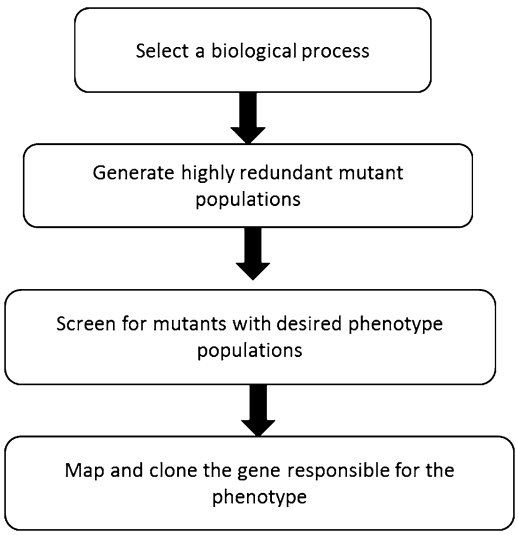

Fig. 10.3 Steps involved in forward genetic approaches (Adapted from Vuttipongchaikij 2012)

4.2 Reverse Genetic Approaches

In contrast to forward genetics, the reverse genetics start with the knowledge on the sequence of genes of interest and aims to alter its natural expression (Fig. 10.4). Overexpression, gene silencing, heterologous expression, homologous recombination, etc. are some of the common methods employed in reverse genetics (Fig. 10.5). A variety of transformation methods have been employed for the successful transformation of foreign DNA into microalgal cells, viz. use of glass beads or silicon carbide whiskers, electroporation, biolistic microparticle bombardment and *Agrobacterium tumefaciens*-mediated gene transfer (Radakovits et al. 2010). Nuclear transformation of microalgae generally results in the random insertion of genes into the host DNA. This may be suitable for transgene expression using random mutagenesis, however, not suitable to delete specific target genes. The screening of transgenic organisms is performed by the use of

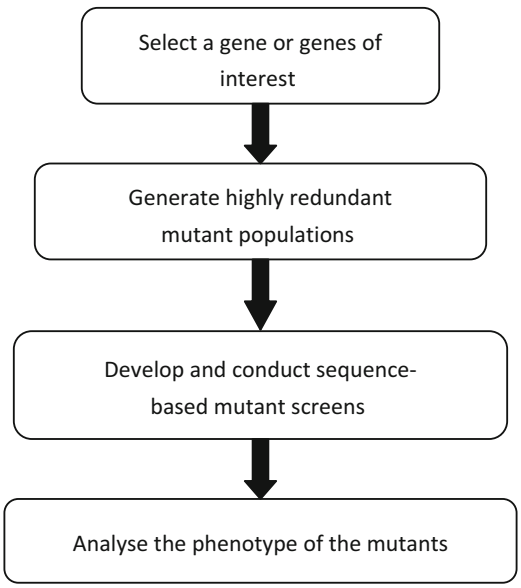

Fig. 10.4 Basic steps involved in reverse genetics (Adapted from Vuttipongchaikij 2012)

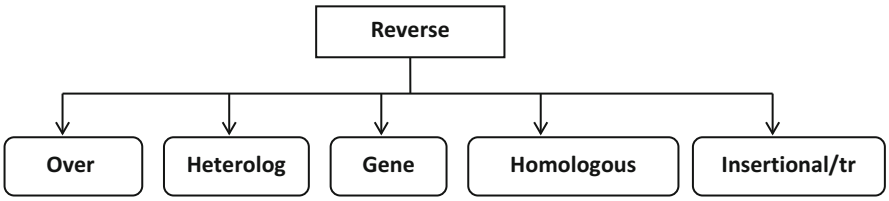

Fig. 10.5 Different reverse genetic approaches that are used for strain improvement in different species of microalgae

markers such as antibiotic resistant genes and fluorescent/biochemical markers (Radakovits et al. 2010). Knockdown of gene expression in *C. Reinhardtii* has been achieved using homologous recombination, however, with low efficiency (Radakovits et al. 2010). RNA silencing has also been successfully employed to knock down gene expression in *C. reinhardtii* and *P. tricornutum* (Radakovits et al. 2010; Kim et al. 2014). Recent improvements in gene silencing strategies include RNA interference (RNAi) or artificial RNA (amiRNA) constructs (Hlavová et al. 2015) or the creation of deletion mutants, either by homologous recombination (Fujiwara et al. 2013) or insertional mutagenesis (Zhang and Hu 2014).

The overexpression of genes is usually achieved by expressing the desired gene with a strong promoter (Gimpel et al. 2013; Hlavová et al. 2015). However, the major limitation of this method is the unpredicted mutation rates that could result due to the insertion of a piece of DNA into the genomic locus. This may sometimes cause additional phenotypic changes unrelated to the expression of the inserted construct. Recent developments in genetic engineering such as gene editing, however, have helped to overcome these limitations. Using these methods, it is possible to make precise changes in the target gene without affecting the expression of other loci. The methods of gene editing have been developed based on the combination of a number of factors which include DNA binding protein and zinc-finger nuclease (ZFN), transcription activator-like effectors (TALEs), gene deletion, gene replacement, introduction of a specific mutation in a gene, and creation of specific gene variants (Boch et al. 2009; Hartung and Schiemann 2014; Puchta and Fauser 2013; Yagi et al. 2014; Hlavová et al. 2015). However, thus far, there are only few reports on microalgae describing the use of ZFN-mediated gene editing (Sizova et al. 2013) and the use of TALEs (Daboussi et al. 2014).

4.3 Advancement in GE Approaches to Improve Lipid Synthesis in Microalgae

Traditionally, GE trails in microalgae have been carried out based on the genetic information on plant genomes. In the last 5 years, substantial progress has been made in microalgal genome research, which would facilitate a better application of GE approaches for strain improvement. Several microalgal nuclear and chloroplast genome projects have been completed thus far which includes: *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Cyanidioschyzon merolae*, *Ostreococcus lucimarinus*, *O. tauri*, *Micromonas pusilla*, *Nannochloropsis* spp., *Lobosphaera (Parietochloris) incisa*, *Trebouxiophyceae* sp., *Chlorella protothecoides*, *C. variabilis*, *C. vulgaris*, *Dunaliella salina*, *Volvox carteri* and *Botryococcus braunii* (Radakovits et al. 2010). The availability of full information on functionally important genes would enable researchers in enhancing production of high-value products from specific microalgal strains in the near future.

Successful genetic transformation has been reported in many species including green (*Chlorophyta*), red (*Rhodophyta*) and brown (*Phaeophyta*) algae; diatoms; euglenids; and dinoflagellates (Radakovits et al. 2010). Members of green algae that have been successfully transformed include *Chlorella reinhardtii*, *C. ellipsoidea*, *C. saccharophila*, *C. vulgaris*, *Haematococcus pluvialis*, *V. carteri*, *C. sorokiniana*, *C. kessleri*, *Ulva lactuca*, *Dunaliella viridis* and *D. Salina*. In most cases, transformation has resulted in stable gene expression. The studies have also indicated that the stability of expression can be improved through proper codon usage or by the use of strong endogenous promoters (Eichler-Stahlberg, et al. 2009; Radakovits et al. 2010). Moreover, the efficiency of transformation is reportedly species dependent and needs to be carefully optimized for each species. Several antibiotic resistance genes have also been optimized for specific microalgal transformant selection. This includes bleomycin, spectinomycin, streptomycin, paromomycin, nourseothricin, hygromycin and chloramphenicol (Radakovits et al. 2010).

Based on the nucleotide sequence knowledge of functional genes in microalgae, numerous trials have been carried out to investigate the feasibility of manipulating the genes of key enzymes relevant to lipid synthesis and to enhance lipid production of different species (Courchesne et al. 2009). Acetyl-CoA (ACCase) carboxylase, the enzyme that catalyses the first committing step of lipid biosynthesis pathway, is one of the commonly exploited enzymes. ACCase is responsible for regulating the rate of de novo fatty acid (FA) biosynthesis in plants and green algae. The modulation of carbon flux is started by the formation of malonyl-CoA through ACCase's activity, and this is the first rate-limiting step in the FA biosynthesis pathway (Davis et al. 2000). ACCase has been purified and characterized in several plants

which include maize (Nikolau and Hawke 1984), parsley (Egin-Bühler and Ebel 1983), spinach (Kannangara and Stumpf 1972) and wheat (Egin-Bühler et al. 1980) and also in microalgae (Livne and Sukenik 1990; Roessler 1990). Overexpression of this enzyme reportedly improved lipid accumulation in few species (Courchesne et al. 2009; Blatti et al. 2013; Liang and Jiang 2013). Davis et al. (2000) observed a sixfold increase in fatty acid synthesis rate while co-expressing *E. coli* ACCase (encoded by accA, accB, accC, accD) and thioesterases I (encoded by the tesA gene). These results confirmed that ACCase catalyzing the committing step was unquestionably the rate-limiting step for the fatty acid biosynthesis in *E. coli*. Overexpression of ACCase genes in *Brassica napus* improved lipid accumulation by 5 % (Thelen and Ohlrogge 2002). The microalgal ACCase was first isolated by Dunahay et al. (1996) from *Cyclotella cryptica* and successfully expressed in *Cyclotella cryptica* and *Navicula saprophila*. Though activity of this enzyme was increased in transgenic microalgal strains, there was no increase in lipid accumulation, which could be due to ACCase activity not being the rate-limiting step for lipid biosynthesis in these species (Dunahay et al. 1996).

Acyl-CoA:diacylglycerol acyltransferase (DGAT) is the most important enzyme in the Kennedy pathway (TAG biosynthesis) which converts diacylglycerol to triacylglycerols (Liang and Jiang 2013). The expression level of this gene has been checked in many plants such as *Brassica napus*, *Arabidopsis thalia*na and *Nicotiana tabacum*. Acyl-CoA:diacylglycerol acyltransferase gene from *Arabidopsis* was transformed into yeasts and tobacco, and DGAT activity as well as TAG accumulation was analysed. In transgenic yeasts, 200–600-fold increase in DGAT activity was observed, whereas TAG accumulation was only increased by three- to ninefold (Thelen and Ohlrogge 2002; Liang and Jiang 2013). Thus far only few studies have been reported for DGAT expression in microalgae. Wagner et al. (2010) have identified and characterized acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2) genes from the microalga, *Ostreococcus tauri.*

La Russa et al. (2012) overexpressed DGAT2 gene in the microalgae *Chlamydomonas reinhardtii* and observed an increase in (1.7–29.1 times) mRNA level as compared to wild strain. However, the increased level of mRNA expression did not result in elevated accumulation of TAG. This indicates the need for a comprehensive research in other microalgal strains to identify the role of DGAT and to develop strategies to enhance lipid accumulation (Yu et al. 2011). Other enzymes that may be targeted for enhanced fatty acid and TAG synthesis in microalgae are fatty acid synthetase (FAS), lysophosphatidate acyltransferase (LPAT), acetyl-CoA synthase (ACS), malic enzyme (ME) and ATP citrate lyase (ACL) (Liang and Jiang 2013). These enzymes are extensively studied in plants for improving lipid yields.

Apart from the above enzymes, overexpression of malic enzyme (ME), an enzyme that plays a major role in lipid biosynthesis by conversion of malate to pyruvate, is also reported to increase lipid biosynthesis in plants and microalgae (Zhang et al. 2007; Courchesne et al. 2009; Li et al. 2013; Xue et al. 2013). Recently, Xue et al. (2015) reported that an overexpression of ME gene (PtME) significantly enhanced the enzymatic activity of transgenic *Phaeodactylum tricornutum*. In their study, a 2.5-fold increase in total lipid content in the transgenic cells was observed with a similar growth rate to wild type. Further, an increase in the neutral lipid content to 31 % was also noticed under nitrogen-deprivation conditions on this transgenic *P. tricornutum*.

Multiple gene expression, i.e. overexpression of more than one key enzyme in the TAG pathway to enhance lipid biosynthesis, was also suggested by few researchers (Verwoert et al. 1995; Roesler et al. 1997; Li et al. 2013). Overexpression of the three functional genes, viz. acyl-CoA:glycerol-3-phosphate acyltransferase (GPAT), acyl-CoA:lysophosphatidic acyltransferase (LPAAT) and acyl-coA:diacylglycerol acyltransferase (DGAT), enhanced the quantity (up to twofold) of storage lipid in *C. minutissima* UTEX 2219 (Hsieh et al. 2012). Recently, Talebi et al. (2014) have successfully accomplished manipulation of carbon flux into fatty acid biosynthesis pathway in *Dunaliella salina*. This was achieved by transferring the pGH vector harbouring ME and AccD genes into the chloroplast genome of *D. Salina* using particle bombardment. The comparison of lipid and fatty acid profile of the transformed algal cell lines and control revealed a stable and overexpression of ME/AccD genes in the transformants, leading to a 12 % increase in total lipid content and substantial improvement in biodiesel properties.

Suppression of gene expression in the comparative pathways is another method to enhance lipid production in microalgae. Carbohydrate metabolism is the most important pathway to store and accumulate carbon as starch in many microalgae (Gonzalez-Fernandez and Ballesteros 2012; Ho et al. 2013). Inhibiting the carbohydrate metabolism may result in carbon flow towards lipid biosynthesis. A *Chlamydomonas* mutant has shown tenfold increase in the TAG accumulation after deactivation of ADP-glucose pyrophosphorylase, which catalyses the committed step in starch metabolism (Li et al. 2010). These findings indicated the probability of an increase in lipid production by redirecting the carbon from starch synthesis to lipid accumulation. However, knockdown of starch synthesis may result in decreased growth, thereby affecting biomass and lipid productivity.

Suppression of lipid catabolism is another potential strategy to be employed in microalgae to increase lipid accumulation. A mutant strain of *Thalassiosira pseudonana* has shown 3.5-fold higher lipid content after alteration in the

lipid catabolism by knocking down the regulation of multifunctional enzymes, lipase/phospholipase/acyltransferase (Trentacoste et al. 2013). The genes regulating these enzymes can be targeted for high lipid accumulation, without compromising growth. The quality of microalgal lipids however dictates the properties of subsequently produced biodiesel (Blatti et al. 2013). Therefore, a need exists to develop new strategies to improve the quality of microalgal lipids for the biodiesel to meet standard specifications.

The most suitable fatty acids for biodiesel production are saturated and monounsaturated fatty acids, preferably with a carbon length, 12:0, 14:0, 16:0, 16:1, 18:0 and 18:1 (Blatti et al. 2013). An enzyme, acyl-ACP thioesterase, regulates the fatty acid chain length by releasing the fatty acid chain from fatty acid synthase (Guo et al. 2014). Thioesterases from different organisms are specific for a particular chain length of fatty acids as per the metabolic needs of that organism. If the thioesterase genes from various organisms responsible for different chain lengths of fatty acids are transformed into microalgae, it could greatly improve the fatty acid composition desired for biodiesel synthesis. Two shorter chain length fatty acid acyl-ACP thioesterases from *Cinnamomum camphora* and *Umbellularia californica* were transformed to microalgae *Phaeodactylum tricornutum* (Radakovits et al. 2011). The fatty acid profile of transgenic *P. tricornutum* showed an increase in percentage composition of lauric (C12:0) and myristic (C14:0) acids (Xue et al. 2015). These molecular strategies for improving microalgal lipid quality hold immense significance for the successful production of microalgal biodiesel with the required specifications.

4.4 Bottlenecks in Microalgal GE Approaches and the Way Forward

GE approaches in microalgae have tremendous scope in enhancing lipid production; however, there are major limitations during scaleup of production. Though the commercial application of algal transgenics is beginning to be realistic due to the advancements in molecular techniques, there are challenges that are still needed to be overcome to use transgenic microalgae as a powerful tool for the production of commercial biomolecules (Cadoret et al. 2008). One of the major challenges in GE is the screening process, which is a critical parameter for both forward and reverse genetic approach (Hlavová et al. 2015). With the traditional methods, thousands of mutants may have to be screened to get the desired phenotype. However, various strategies have been proposed to improve the screening process which includes methods based on the survival of either mutant or wild type cells, under defined conditions. It is equally important to maintain similar screening conditions for all mutants in order to compare them directly, which is practically challenging (Hlavová et al. 2015).

Another key challenge is instability or loss of gene expression as the mutants are prone to reversion. The probability of reversion and stable expression, however, depends on many factors. It is reported that the large fragment of foreign DNA inserted into the genome by insertional mutagenesis is prone to reversion by gene silencing (Hlavová et al. 2015). This also could have a larger impact on the mutant strain, since silencing might spread to neighbouring regions of the genome. This could be partially overcome by growing the mutant strain constantly in the presence of antibiotics, if the antibiotic resistant genes were used as markers (Neupert et al. 2009; Hlavová et al. 2015). It is also recommended to limit subculturing by storing the mutant strain in liquid nitrogen to avoid mutant reversion. In the case of reverse genetics, the probability of gene reversion increases with the expression level, which could be partially avoided if an inducible promoter is used during the transcript construction (Iwai et al. 2014; Hlavová et al. 2015).

Possibility of low or no expression due to the absence of specific codons is another challenge that could affect the transgenic microalgae. It has been demonstrated that microalgal genomes contain very high GC content, for example, 71 % in *Monoraphidium* and 61 % in *Chlamydomonas* (Jarvis et al. 1992). This suggests a high variation in codon usage in microalgae as compared to other organisms. Therefore, codon optimization may be a prerequisite to improve the gene expression in different microalgal species. Codon optimization in *Chlamydomonas* showed a fivefold increase in its expression level (Fuhrmann et al. 1999; Hlavová et al. 2015). With an increase in the number of genome sequences being available for different microalgal species, researchers would be able to overcome this limitation in the near future.

Another major challenge is the suitability of transgenic microalgae for large-scale cultivation. Upon exposure to large-scale cultivation conditions (large-scale ponds), the strains experience situations that are more diverse from the controlled lab conditions, which subsequently affect its transgenic property. Consequently, productivity in outdoor cultivation never reaches that of the optimized laboratory conditions. Performances in large-scale ponds are sometimes also dependent on the mutant stability (Hlavová et al. 2015). Another major bottleneck in microalgal biotechnology is the cost involved in the whole transgenic process. Reverse genetics requires highly specialized equipment, and some processes are time consuming due to the numerous steps involved, from screening to large-scale cultivation, which would affect the cost immensely. Moreover, some countries do not support transgenics due to legislative policies against GMOs.

5 Conclusions

In microalgal research, considerable interest has been placed on bioprospecting, which is one of the key elements exercised for strain improvement. Various cultivation techniques are also used in practice for improving microalgal lipid biosynthesis but, however, face challenges during its scaleup. With the current pace in biotechnology advancements, genetic engineering in microalgae would be the most appropriate, efficient and cost-effective method for strain improvement. Both forward and reverse genetics have been employed in microalgal strain improvements. The forward genetics using chemical and physical mutagens is relatively simple; however, the screening process is extensive or exhaustive. In reverse genetics, specific knowledge on the target gene is imperative and initial optimization of each step is time consuming and costly. The genome sequencing of many microalgal species has been completed, and this would definitely form a strong foundation for improving the GE approaches for increased lipid production in the near future.

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Phycoremediation of Emerging Contaminants

11

Sanjay Kumar Gupta, Amritanshu Shriwastav, Sheena Kumari, Faiz Ahmad Ansari, Anushree Malik, and Faizal Bux

1 Introduction

1.1 Emerging Contaminants

Emerging contaminants (EC) or contaminants of emerging concerns (CEC) cover a broad spectrum of chemicals and compounds which are being recognized as potentially harmful in the last decade or so. These compounds may have been in application for a long period, but their environmental and health impacts are being observed and their hazardous nature identified recently; these might belong to a whole new class of compounds in application for their certain properties. The major concern over these emerging contaminants is the nonavailability of analytical techniques to identify them in the first place within the complex environmental matrices and to quantify them since their concentrations are very low (ranging from μg L^{-1} to ng L^{-1} or even lower). Because of the nonavailability of these protocols for either identifying or to quantify these compounds, regulatory limits are virtually nonexistent. This results in their uninhibited discharge into environment. With the advancement of analytical methods, such compounds are being increasingly identified and quantified. In addition, their environmental hazards are being recognized, thus raising a concern on their continued applications and initiating a search for better alternatives. The endocrine disruption potential is one of the major concerns of many of these products.

S.K. Gupta (✉)
Environmental Engineering, Department of Civil Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India
e-mail: sanjuenv@gmail.com

A. Shriwastav • S. Kumari • F.A. Ansari • F. Bux
Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa

A. Malik
Rural Development Technology, Indian Institute of Technology Delhi, 110016, New Delhi, India

1.1.1 Pharmaceutical and Personal Care Products (PPCP)

The continuous and unchecked use of pharmaceutical and personal care products (PPCP) over a long period has resulted in their presence in aquatic bodies all over the world (Li et al. 2013; Yang et al. 2013). These products include, but not limited to, following distinct classes of compounds (Ellis 2006): veterinary and human antibiotics (e.g., trimethoprim, erytromycine, lincomycin, sulfamethaxazole, chloramphenicol, amoxicillin, etc.), analgesics and anti-inflammatory drugs (e.g., ibuprofen, diclofenac, fenoprofen, acetaminophen, naproxen, acetylsalicylic acid, fluoxetine, ketoprofen, indometacine, paracetamol, etc.), psychiatric drugs (e.g., diazepam, carbamazepine, primidone, salbutamol, etc.), lipid regulators (e.g., clofibric acid, bezafibrate, fenofibric acid, etofibrate, gemfibrozil, etc), β-blockers (e.g., metoprolol, propranolol, timolol, sotalol, atenolol, etc), X-ray contrasts (e.g., iopromide, iopamidol, diatrizoate, etc.), steroids and hormones (e.g., estradiol, estrone, estriol, diethylstilbestrol, etc), fragrances (e.g., nitro, polycyclic, and macrocyclic musks; phthalates), sunscreen agents (benzophenone, methylbenzylidene camphor, etc.), insect repellents (e.g., N, N-diethyl toluamide), and antiseptics (e.g., triclosan, chlorophene, etc.). Recent advancements in analytical techniques, e.g., GC-MS, LC-MS, and LC-MS-MS, have lowered their detection limits to parts per trillion (ppt) levels (Oulton et al. 2010a), which has resulted in the increasing observations of these compounds or their metabolites in environmental matrices. The recent interest in these contaminants and their metabolites is due to their possible toxicological nature (including endocrine disruptive potential) to humans and other living organisms which is often complicated by their synergistic effects (Tyler et al. 1998). The sources of these products in aquatic bodies are mainly from wastewater treatment plant effluents and also due to leaching from landfills of domestic/medical garbage or sludge laden with these compounds (Kaplan 2013). Due to their persisting nature in environment, these compounds could affect many generations

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of organisms and produce exposure effects that may be correlated wrongly to other factors (Daughton and Ternes 1999; Kaplan 2013).

1.1.2 Pesticides

The use of pesticides in human civilization has a long history spanning centuries, where earlier pesticides were mainly of inorganic nature such as calcium arsenate and lead arsenate and some fluoroorganic compounds (Battaglin and Fairchild 2002). Since then, numerous new organic compounds have been developed for their application as pesticides. Many such pesticides, viz., DDT (dichlorodiphenyltrichloroethane), remain in the environmental matrices long after their targeted application, mainly due to their persistent nature. This results in their subsequent buildup and provided their toxicological profile for human health; over the years these pesticides have been identified as contaminants of concern. Although, with improved analytical methods and availability of their toxicological profile, many of these pesticides, for example, DDT and endosulfan, have been banned and their application discontinued; environmental matrices are still contaminated with their presence. A major concern is the carcinogenic nature and endocrine-disrupting potential of a majority of these compounds even at low concentrations (at ppb levels). As the information is garnered about these pesticides and their toxicological risks determined, new compounds presumably of lower risks are introduced as a replacement. However, with continued application of these new compounds, more information about their fate and hazardous characteristics are gathered, and new compounds are further searched. Such application of many pesticides through decades results in the buildup of a very complex mix of original compounds as well as their metabolites in environmental matrices and whose interactions are yet unknown and hence a matter of great concern.

1.1.3 Endocrine Disruptors

A normal hormonal function in a living organism is a prerequisite for good health. However, many natural and synthetic compounds mimic and possess similar chemical and biological properties like these hormones and thus disrupt/interfere with their normal functions or organisms. A wide variety of compounds has demonstrated their ability to interfere with the endocrine system and hence is collectively termed as endocrine disruptors or endocrine-disrupting compounds (EDC). These include pesticides (e.g., atrazine), surfactants, dioxins, polychlorinated biphenyls (PCBs), synthetic estrogens (e.g., 17β-ethinyl estradiol), natural estrogens (e.g., 17β-sitosterol, estrone), natural androgens (e.g., testosterone), phytosteroids (e.g., 17β-sitosterol), etc. (Richardson and Ternes 2005). The main complexity in streamlining the study and analysis of these compounds arises due to their highly variable affinities to hormonal receptors and different pathways. Due to these reasons, their endocrine potencies show a huge variability. Continued exposure of organisms to these EDCs may lead to drastic impacts on their health and the overall ecology of the system.

1.2 Major Challenges in Conventional Wastewater Treatment in Removal of Emerging Contaminants

Effluents from conventional wastewater treatment plants are recognized as a major source for these emerging contaminants into receiving bodies, mainly due to the limitations of these plants in achieving an effective removal (Battaglin and Fairchild 2002; Oulton et al. 2010b; Sedlak et al. 2000). This is in part due to the inherent limitations of the conventional treatment processes which are not designed and optimized for these emerging contaminants, for example, secondary treatment is optimally designed for BOD removal and in part due to the specific properties of these compounds which render it very difficult to be removed from water with conventional methods, such as high chemical stability, low biodegradability, low sorption coefficients, etc. Another major factor which complicates the removal of ECs is their highly variable response to a particular treatment process due to the sheer number and diversity of these compounds.

1.2.1 Low Levels and Limited Availability of Protocols for Identification and Quantification

The foremost challenge in the study of the fate of ECs in wastewater treatment plants is the unavailability of appropriate analytical protocols to identify and quantify such compounds and their metabolites at their low levels of presence (Battaglin and Fairchild 2002; Petrović et al. 2003). Without any identification and quantification, these products have not been an area of focus while developing the conventional processes. Hence, conventional treatments are not efficient in their removal because of the inherent design limitations.

1.2.2 Low Biodegradability

Most of these compounds have low biodegradability with conventional microbial populations found in treatment plants under natural conditions. This results in its inefficient removal during such treatments. For example, Ternes (1998) observed only 7 % removal of carbamazepine in municipal sewage treatment plants. Oulton et al. (2010a) also identified the conventional treatment plants to achieve removal efficiencies of all PPCPs not higher than 1-$\log_{10}$. A major reason for such poor biodegradability of these compounds in conventional treatments is the requirement of stable specific microbial culture for them. Since conventional activated sludge systems do not facilitate the enrichment of such

specific culture because of their suspended growth and low retention times, which may result in a wash out, the removal efficiencies are low. In comparison, processes such as trickling filters allow the development of a stable microbial culture which could be, with operation, enriched specifically for these endocrine disruptors and hence could perform better in removal of these compounds (Kasprzyk-Hordern et al. 2009).

1.2.3 High Water Solubility

Many of these compounds, mainly pharmaceuticals, demonstrate high solubility in water. For example, norfloxacin, tetracycline, and fluoxetine have water solubility of 1.78×10^5 mg L^{-1}, 5×10^4 mg L^{-1}, and 5×10^4 mg L^{-1}, respectively. Such high solubility also results in high variability in their levels in the influents. Petrie et al. (2014a) observed acetaminophen to appear at concentrations ranging from 6924 to 492,340 ng L^{-1} in influent wastewater. Such high variability in ECs concentrations complicates the optimization of a process design for their effective treatment.

1.2.4 Solid Phase Partitioning

Another important aspect of dealing with these compounds is its partitioning between solid and liquid phase depending on their sorption coefficients and subsequent hydrophobicity. Many organic pesticides and drugs (e.g., triclosan and triclocarban) are extremely hydrophobic in nature (log K_{ow} of 4.2–4.8) and thus are retained within the solid matrices (e.g., sludge etc.). For example, concentrations in excess of 1 mg kg^{-1} in biosolids have been reported for chemicals such as triclocarban, triclosan, bisphenol A, ciprofloxacin, ofloxacin, etc. (Petrie et al. 2014a). Disposal of such laden sludge is a major source of contamination for these compounds. In addition, the mobility of these compounds in the solid matrices, e.g., soil, is also dependent on many factors and widely varies among all compounds. For example, partitioning of charged endocrine disruptors is highly governed by electrostatic forces (Hyland et al. 2012). pH also plays a critical role in such partitioning behavior within the complex solid-water matrices (Petrie et al. 2014a).

1.2.5 Metabolites

Majority of these compounds are present with metabolites or undergo transformation into its metabolites during conventional treatment, both biological and physicochemical. Serious concerns about these metabolites have been raised recently, since these could reach concentrations highly in excess to their parent compounds and may also be biologically active in nature (Kasprzyk-Hordern et al. 2008; Petrie et al. 2014a). For example, Huerta-Fontela et al. (2010) observed carbamazepine to be present in influent wastewater at concentrations less than 1.5–113 ng L^{-1}, while concentrations of one of its metabolite carbamazepine epoxide ranged from 880 to 4026 ng L^{-1}. In addition, many of these metabolites are more toxic in nature than the parent compound and hence may pose a serious threat (Petrie et al. 2014a). The ability of these metabolites to again form the parent compound within the environmental matrices after treatment is also a concern, and limited information is available. A major limitation is that pathways for these metabolite formations are not yet identified and established for majority of the parent compounds.

1.3 Bioremediation

Bioremediation is a process where biologically mediated treatment of hazardous pollutants with naturally occurring organisms into compounds with less or no toxicity is achieved. The treatment occurs by the uptake of these compounds from environmental matrices by organisms and subsequent utilization for their growth or enzyme-mediated breakdown into other less hazardous compounds that are released into the environment.

1.3.1 Phytoremediation

Various plants have the ability to remove pollutants from environmental matrices and hence in the process provide treatment. Phytoremediation involves this ability of plants and their symbiotic microbes to effectively remove various pollutants from a contaminated site. This process has been successfully implemented worldwide to remediate heavy metals, pesticides, and other hazardous organic compounds from the environment (Ali et al. 2013; Malik 2004). Since it is a lost cost treatment option, phytoremediation is applicable at large contaminated sites where other treatments are not cost effective. Phytoremediation occurs by different distinct mechanisms such as phytoextraction, phytostabilization, and phytotransformation. The direct uptake of a pollutant from environment by plants is termed as phytoextraction. This process directly reduces the level of contaminants in the bulk surrounding medium. Uptake of heavy metals by plants occurs by phytoextraction (Ali et al. 2013). Another mechanism by which plants treat the contaminated sites is by stabilizing and containing the pollutants within the site. Plants do not uptake the pollutants but provide a microenvironment near their root zone, where with the help of symbiotic microbes, sequestering of the pollutants and sorption are supported. Such stabilization reduces the bioavailability of these pollutants and lowers their harmful effects in long term. Plants can also transform various compounds into less toxic metabolites with various enzymes by the process of biotransformation (Ali et al. 2013). However, phytoremediation also suffers from many limitations. Effective treatments of contaminated sites may need long duration depending on selected plant species, contaminants, and level of

contamination. Also, plants are able to treat low to mild levels of contaminants in sustainable manner, since high levels of pollutants are toxic for them too. Such phytoremediation also poses a very realistic threat of contaminating the whole food chain and requires proper management (Ali et al. 2013).

1.3.2 Phycoremediation

Pollution abatement from contaminated environmental matrices with the application of algae is termed as phycoremediation. Algae have historically been utilized for domestic wastewater treatment and have led to the development of specific processes (e.g., raceway ponds and photobioreactors). Their ability to uptake heavy metals has also lead significant research on their application for heavy metal removal (Chojnacka et al. 2005; Perales-Vela et al. 2006; Yu and Wang 2004) and other hazardous organic pollutants (Muñoz et al. 2006; Munoz and Guieysse 2006). In addition, algae have been demonstrated as a suitable sink for CO_2 (Jacob-Lopes et al. 2009). The ability of algae to grow on wastewaters in hitherto nonarable land with high productivity makes phycoremediation an attractive subset of bioremediation. The value-added algal biomass can potentially be utilized for extracting many useful products, for example, lipids, proteins, carbohydrates, pigments, etc. (Olguín 2012). The recent focus in phycoremediation is on following sustainable biorefinery approach, where wastewater treatment and removal of other pollutants is complimented with the value extraction from generated algal biomass (Prajapati et al. 2013b; Subhadra 2010).

2 Ecological Fate of Emerging Contaminants

Emerging contaminants are mainly released into the environment due to its unregulated applications. Such applications include domestic or medical discharges of various PPCPs and other compounds, agricultural applications or runoff of various pesticides, or inadequacy of conventional treatment in their effective removal from influent streams and thus discharge into receiving bodies. Once these contaminants enter environmental matrices, they undergo various processes which govern their ecological fate in the system.

Contaminants, as they reach aquatic bodies due to inefficient sewage collection/treatment or leaching from agricultural fields/domestic sources, pose a direct threat to living organisms and the ecology of these receiving bodies. Many of these dissolved contaminants (e.g., gemfibrozil, ibuprofen, ketoprofen, etc.) have shown evidences of undergoing photolysis and effective breakdown into harmless metabolites (Lin and Reinhard 2005). However, the ability of these compounds to undergo photolytic breakdown varies substantially due to their different structures. In addition, such photolytic degradation could also result in compounds of higher toxicity. However, the actual pathways of such degradation for majority of these compounds are still not available and pose a serious limitation in establishing their fate. Indirect photolysis of many such compounds also occurs due to the presence of free radicals in aquatic bodies (Ryan et al. 2011). Also, compounds which are resistant to such degradation persist in aquatic bodies and are more prone for uptake by living organisms and eventually undergo biodegradation or bioaccumulation. This uptake also reflects in the harmful effects on the health of these organisms and ultimately affects the ecology of the whole system.

Those compounds which are hydrophobic in nature get sorbed on various organic solids or sediments present within these matrices and are effectively removed from liquid phase. Such solid phase partitioning results in heavily laden solid mixture which is retained in the matrix where partial desorption may occur eventually, thus releasing these compounds into the aquatic phase. In addition, such solid–liquid phase partitioning is also governed by various environmental factors such as pH and temperature. The widely different characteristics of these compounds also determine the governing mechanism of such phase separation and their relative distributions. For example, charged and uncharged compounds experience different levels of electrostatic forces.

In addition to these major physicochemical processes occurring in the receiving bodies, emerging contaminants also undergo biological transformation during their original use or during their treatment within the treatment plants in both aerobic and anaerobic conditions. For example, Tiwari and Guha (2013b) studied the degradation of endosulfan in both aerobic and anaerobic conditions and established the degradation pathways. Degradation metabolites for acetaminophen and azithromycin have been observed in the effluent from a treatment plant (Gómez et al. 2010). Similarly, Tiwari and Guha (2013a) quantified various degradation metabolites of endosulfan and chlorpyrifos. However, the availability of information about degradation products and the governing pathway is very limited for majority of the emerging contaminants. This results partially due to the non-availability of analytical protocols for identification and quantification of these products and partially due to the complexity of the environmental matrices and highly variable formation mechanism for a single product from a parent compound. Since many of the intermediate metabolites are more toxic than parent compounds, the degradation actually worsens their impact on the environment. Hence, it is utmost important to focus on these metabolites along with parent compounds while performing their removal and degradation analysis within treatment plants or other environmental matrices.

3 Ecotoxicological Risks of Emerging Contaminants

The main reason behind increasing concerns over ECs is due to their ecotoxicological risks toward humans and other living organisms. Most of these compounds show acute or chronic toxicity depending on their concentrations in environmental matrices or the exposure duration. Main effect is their endocrine-disrupting potential where many of these compounds can mimic the hormonal functions within the body and disrupt/interfere with endocrine system. The toxicity of a particular compound is determined by acute toxicity test on a single organism, and such analyses have been performed for various parent compounds as well as their metabolites. Based on their EC_{50} values, compounds can be classified as harmful (EC_{50} as 10–100 mg L^{-1}), toxic (EC_{50} as 1–10 mg L^{-1}), or very toxic ($EC_{50} < 1$ mg L^{-1}). Petrie et al. (2014b) collated the information about EC_{50} values of different contaminants and analyzed their potential of being very toxic (e.g., erythromycin) to harmful (e.g., trimethoprim) for aquatic organism. However, such toxicity analysis is valid only for test species and is not universal. Other organisms can respond differently than the subject organism. Still, such EC_{50} values provide an indicative baseline for the toxic nature of these compounds. Farré et al. (2008) observed that the concentrations of emerging contaminants such as pharmaceutical products in environmental matrices are 2–3 orders of magnitude lower than their required levels for causing any acute toxicity. These compounds pose a more serious concern due to their potentials to cause chronic toxicity during prolonged exposure to living organisms even at low levels. Another factor which governs the toxicity profile of these compounds is their complex behavior in a mixture. Environmental matrices accommodate a mixture of various such compounds. The toxicological profile of these mixtures might be very complex and synergistic in nature. For example, the mixture of diclofenac, ibuprofen, naproxen, and aspirin showed increased toxicity than their individual effects, thus highlighting the synergistic behavior of these compounds in a mixture (Cleuvers 2004). In addition to the acute and chronic toxicity analysis of these compounds, Petrie et al. (2014b) also highlighted the importance of investigating the toxicological nature of different chiral enantiomers of these compounds which are used concurrently, since there might be a severe difference in their toxicity levels. To analyze and streamline the toxicity analysis of mixture of various such compounds, Eljarrat and Barceló (2003) proposed the concept of toxic equivalency factor to denote the overall toxicity of the whole mixture. However, the ecotoxicological analysis of these emerging compounds while incorporating their synergistic as well as enantiomeric effects remains to be streamlined and standardized for universal applicability and acceptance.

4 Phycoremediation of Emerging Contaminants

As per Chemical Abstracts Service Registry (CAS RN 1649503-59-2), presently, more than 91 million organic and inorganic substances are being formulated and in use, and more than 12,000 new formulations and compounds are being added daily (CAS 2012). All these chemicals and their metabolites are continuously being introduced, disposed, and dumped to various environmental matrices through industrial discharges, agricultural runoff, or inappropriate waste disposal practices and pose deleterious effects to the environment, all living beings, and ultimately to the human health (Daughton and Ternes 1999; Pavlostathis et al. 2003).

In recent years, the occurrence of traces of emerging contaminants such as pharmaceuticals and personal care products (PPCPs), endocrine-disrupting chemicals (EDCs), disinfection by-products (DBPs), persistent organic pollutants (POPs), pesticides, cyanotoxins, etc., in the natural and drinking waters has been reported widely. In aquatic systems, these chemicals get adsorbed and immobilized subjected to various transformations depending upon the biogeochemical processes and prevailing environmental factors. Such chemical contaminants remain available to the benthic microorganisms through the sediment water interface (Perelo 2010). The US EPA (2009) has listed 116 drinking water contaminants in Contaminant Candidate List 3 (CCL3), which have been detected in public water systems in the USA which are of serious concerns. The presence of such low levels of these contaminants in the environment may not stance lethal effects immediately, but in a long term it may pose catastrophic effects on aquatic organisms and human health.

It is now well established that biological remediation is eco-friendly, economically viable, and comparatively less expensive than chemical or physical treatment processes (Herbes and Schwall 1978). However, studies and reports on the phycoremediation of organic pollutants by microalgae and cyanobacteria lag far behind than that of bacterial and fungal biodegradation (Subashchandrabose et al. 2013).

As far as phycoremediation is concerned, microalgae and cyanobacteria have advantages over various species of bacteria and fungi as these species can grow autotrophically, heterotrophically, or mixotrophically in very harsh environmental conditions, i.e., low nutrient level, wide pH and temperature, etc. (Subashchandrabose et al. 2013). Various cyanobacteria and microalgae have been identified for their potential of wastewater treatment especially nutrient removal such as nitrogen and phosphorus and biomass production (Prajapati et al. 2013a; Shriwastav et al. 2014). However, literature on degradation of complex organic compounds such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pharmaceutical, and personal care products is limited.

Previous studies on microalgae-based wastewater treatments have demonstrated that the removal of organic contaminants (EDCs, PAHs, PCBs, pesticides, surfactants, etc.) takes place by virtue of various physicochemical processes which include both biotic transformation through microalgal accumulation, metabolization, and degradation and abiotic transformation through photodegradation, volatilization, sorption, and adsorption (Abargues et al. 2013; de-Bashan and Bashan 2010; Haritash and Kaushik 2009; Matamoros et al. 2015).

Recently, phycoremediation of 26 types of various emerging organic contaminants including pharmaceuticals and personal care products, pesticides, surfactants, anticorrosive agents, fire retardants, etc., in high-rate algal ponds (HRAPs) was reported by Matamoros et al. (2015). The findings of this study revealed 0–90 % removal, based on the nature of the chemical compound which is majorly achieved by photodegradation and biodegradation. However, hydraulic retention time (HRT) and seasonality affect the efficiencies of HRAPs.

Detoxification, degradation, and transformation of organic environmental contaminants by the use of microalgae/bacteria, microalgae/cyanobacteria, or bacteria/fungi consortia has been found to be more efficient and easy in comparison to the individual species (Subashchandrabose et al. 2013). As in microbial degradation of organic compounds, both species act in a symbiotic way. In a consortium, the catabolic degradation of organics by bacteria is done by getting electrons from algae and the mineralization end products being used by algae for their photoautotrophic growth. Figure 11.1 provides an overview of the phycoremediation for various emerging contaminants.

4.1 Pharmaceutical Products

Water streams adjacent to the urban areas receive considerably large amount of wastewater from wastewater treatment plants which contains residues of various pharmaceuticals. In recent years, several studies have been published on the spatial and temporal monitoring of water streams for various kinds of pharmaceuticals such as antibiotics, analgesics, antimicrobials, antidiabetics, antineoplastics, anticonvulsant, antiepileptics (e.g., carbamazepine), antipsychotics, antihistamines, antianxiety, anti-inflammatory drugs, antidepressants, beta-blockers (e.g., metoprolol), beta-sympathomimetics, cytostatics and estrogens (e.g., 17b-estradiol) and hormonal compounds, lipid regulators (e.g., clofibrinic acid), stimulants, X-ray contrast media and antiepileptic drugs, etc. (Adler et al. 2001; Buser et al. 1999; Hirsch et al. 1999; Kuch and Ballschmiter 2000; Sedlak et al. 2000; Ternes 1998). These pharmaceuticals are excreted directly or partially in metabolized form by human beings and passed into the environment through wastewaters, as most of the wastewater treatment systems are not typically designed for the removal of traces of such contaminants. Therefore, the removal of such residues from wastewaters is challenging and of serious concern. Intense research and technological advent is the need of the day for the removal of pharmaceutical residues from water and wastewater.

Separation and detection of most of the pharmaceutical residues, with concentrations ranging from few ng/L to µg/L, are two of the major challenges. Nevertheless, these residues exert deleterious effects on aquatic organisms either individually or due to the combined effect of the mixtures. However, due to the unavailability of exact information on mode of action and fate of various pharmaceuticals in the aquatic ecosystem, systematic understanding of their potential ecotoxicological effects is sparse (Cleuvers 2003; Webb 2001). While evaluating ecotoxicological potential of ten prescription drugs, Cleuvers (2003) reported that the acute toxicity of most of the individual pharmaceuticals was moderate, while in combinations it was comparatively more toxic. The concept of independent action should be used for the ecotoxicological risk assessment for the algal tests; however, the acute toxicity of individual pharmaceuticals is very unlikely, therefore chronic combination effects of substances are of concern (Cleuvers 2003).

While assessing the ecotoxicity of three pharmaceuticals and personal care products (ciprofloxacin, triclosan, and Tergitol NP 10) to the natural algal communities of the receiving natural bodies, Wilson et al. (2003) reported marked shifts in the algal community and significant difference in the biomass yield. They also reported the potential influence on the structure and function of algal communities which may result in changes in the natural food web structure.

The phycoremediation of most of the drugs and personal care products is mediated through its cellular metabolism and transformation by mostly cytochromes P450 (P4503A and 2C8 families) in first phase and catabolic biotransformation of such xenobiotics by various metabolic isozymes in second phase (Stresser et al. 2000). Such isozymes facilitate by conjugation of secondary metabolites with reduced glutathione (Stresser et al. 2000; Thomas et al. 1976). Glutathione reductase, responsible for the maintenance of lipid peroxidation and glutathione levels, due to elevated oxidative stress during oxidative biotransformation, also needs to be monitored (Laville et al. 2004; Peakall 1992). Monitoring of metabolic markers such as glutathione S-transferases (GSTs) activity in microalgal and cyanobacterial cells, for the assessment of their phycoremediation potential, can be applied for the screening of suitable species for bioremediation purposes. Vernouillet et al. (2010) investigated phycoremediation potential of a green alga, *Pseudokirchneriella subcapitata*, for an antiepileptic drug (carbamazepine) by monitoring of glutathione-S-transferase (GST), GR activity, and LPO levels. The cellular concentration of carbamazepine in *Pseudokirchneriella subcapitata* was found more than twofold of initial exposure concentration. Such accumulation also results in more than 50 % reduction in cytochromes

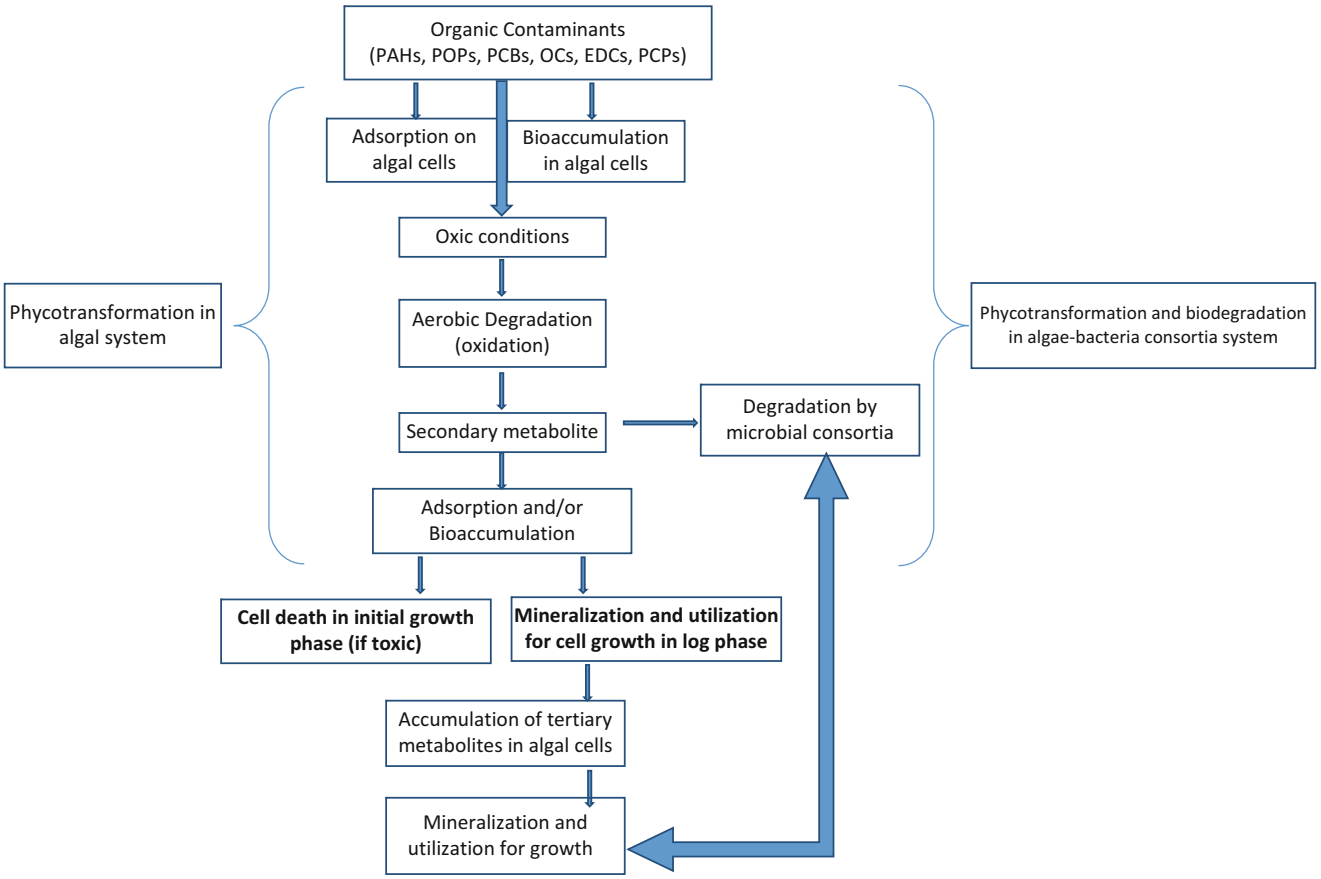

Fig. 11.1 Generalized overview of phycotransformation and degradation of organic contaminants

P450 3A4-like activity in *P. subcapitata*, but glutathione reductase activity was increased up to 40 % which demonstrated that direct exposure to such contaminants results in strong modulation of biochemical biomarkers in aquatic organisms (Vernouillet et al. 2010). In the process of phycoremediation of pharmaceuticals and personal care products, bioaccumulation by algae and cyanobacteria is the primary mechanism. However, it depends on several factors such as toxicity, ionization state, and lipophilicity of the chemical compound as well as sensitivity of tested species (Delépée et al. 2004; Vernouillet et al. 2010; Voutsas et al. 2002).

4.2 Personal Care Products

The ecotoxicological consequences of continuous release and environmental exposure of personal care products (PCPs) are poorly understood. The major contaminant used in PCPs is triclosan (5-chloro-2-(4-dichlorophenoxy)-phenol) (TCS), an antimicrobial compound which has been majorly used in a variety of PCPs such as various cosmetics, soaps, toothpaste, etc., over the last 40 years (Katz et al. 2013). It is also used as a preservative in various consumer products such as textiles, countertops, cutting boards, etc. (Cooney 2010; Savage 1971). Contamination of aquatic bodies and the surrounding environment is resulted through continuous release of inadequately treated domestic wastewaters containing traces of TCS. WWTPs are the major sources of TCS in surrounding water bodies (Fair et al. 2009; Fernandes et al. 2011; Kumar and Xagoraraki 2010). Previous studies have demonstrated that even at very low concentrations, TCS poses serious adverse effects to the phytoplankton, microalgae, cyanobacteria, invertebrates, and fish (Dann and Hontela 2011; DeLorenzo et al. 2008; Jacobs et al. 2005; Perron et al. 2012; Wilson et al. 2003). However, there are various physicochemical and environmental factors such as lower solubility and photolytic degradation and transformation, which regulate the distribution, toxicity, and ecological risk of PCPs (Wong-Wah-Chung et al. 2007) (US EPA 2011). Studies have also demonstrated that TCS easily gets adsorbed to the organic matter thus getting accumulated in sediments (Fernandes et al. 2011; Kumar et al. 2010; Orvos et al. 2002; Ying et al. 2007). In a study of spatial distribution of triclosan, Katz et al. (2013) evidenced that WWTP effluent is an important source of TCS as the annual accumulation rates of TCS in the sediments of Greenwich Bay of Rhode Island,

USA, exceeded the calculated annual discharge of TCS from the local WWTP. Therefore, the regulation and control of TCS exposure to the environments can be regulated primarily through the better management of WWTPs.

Phthalate esters (1,2-benzenedicarboxylic acid) are commonly used as plasticizers and also used in a wide range of products such as pharmaceuticals, detergents, and personal care products. Phthalate esters are known to possess endocrine-disrupting potential for human beings. Babu and Wu (2010) reported the degradation and mineralization of three phthalate esters, i.e., diethyl phthalate, di-*n*-butyl phthalate, and dimethyl phthalate by cyanobacteria *Anabaena flos-aquae* by transesterification on the side chains of phthalate esters instead of de-esterification. In this study, two pathways, i.e., $C_{16} \rightarrow C_{14} \rightarrow C_{12} \rightarrow C_{10} \rightarrow C_8$ and $C_{16} \rightarrow C_{15} \rightarrow C_{13} \rightarrow C_{11} \rightarrow C_9$, following first order kinetics were demonstrated and proposed for phthalate esters degradation by cyanobacteria. Table 11.1 lists some phycoremediation applications for these PPCPs.

4.3 Surfactants

As per estimates, globally more than 1.5–2 million tons of synthetic detergents are produced per year (de Wolf and Feijtel 1998). The extent of environmental discharge of detergents and its degradation by-products depends on the effectiveness of STPs and WWTPs, its adsorption to the sewage sludge and more importantly chemical structure, and the persistence of the detergent molecules (de Wolf and Feijtel 1998; Ojo-Omoniyi 2013). Linear alkyl benzene sulfonate (LAS), alkylphenol polyethoxylates, nonylphenol ethoxylates, alkyl ethoxysulphates, etc., are some of the anionic surfactants most widely used in industrial and domestic detergents. Residual surfactants find their way to the natural water bodies through STPs and WWTPs effluents and enter to the hydro-geological cycle. Biological degradation of LAS is comparatively simpler than the branched, nonlinear alkyl benzene sulfonate (ABS), e.g. dodecylbenzene sulfonates (Gledhill 1974; Nomura et al. 1998; Ojo and Oso 2009). Biodegradation of LAS occurs primarily through w-oxidation of methyl group followed by b-oxidation, i.e., oxidative cleavage of C2 units of alkyl chain (Cook 1998). Sulfo-phenyl carboxylic acids (SPACs) are formed in the primary degradation phases and get further mineralized by desulfonation (Field et al. 1992). Dialkyltetralin sulfonates (DATS) and iso-LAS, which account for 10–15 % as impurities in commercial LAS, are also found to be degraded and being used as a sulfur source by bacteria in various studies (Cook 1998; Field et al. 1992; Kölbener et al. 1995).

Alkylphenols and nonylphenol, which are the precursors of alkylphenol polyethoxylates and nonylphenol ethoxylates, are widely used in detergents and several other industrial products and have been identified to exert estrogenic effects. Nonylphenol (NP) has been identified for its potential endocrine disruptor- and xenoestrogen-like activity (Maguire 1999). While studying the biodegradation of xenoestrogen nonylphenol by *Cyclotella caspia*, Liu et al. (2013) reported 37.7 % removal in 192 h at initial concentration of 0.18 mg/L of nonylphenol. The degradation rate was found to be decreasing with the increasing concentration of the nonylphenol, and at 0.22 mg/L, the degradation reduced to 6.7 % due to its toxicity. In natural seawater, degradation of alkyl ethoxysulfates (anionic surfactants) takes place in two phases. In first phase, cleavage of ether bonds by means of hydrolytic reaction, followed by ω- and β-oxidations of the secondary metabolites in second phase (Sibila et al. 2008). More than 96.5 % degradation of anionic surfactant Empicol® ESB 70/SP was observed after 124 days by Sibila et al. (2008).

4.4 Persistent Organic Compounds

Persistent organic pollutants are ubiquitous in nature due to "grasshopper effect" (Koziol and Pudykiewicz 2001). Moreover, due to two or more enantiomers and chirality, more than 25 % of organic compounds pose serious threat in the biosphere (Williams 1996). The major challenge with the persistent organic pollutants (POPs) to the human being is higher carcinogenic potential of these contaminants. Most of the organohalogenated and organochlorinated compounds such as PAHs, PCBs, and chlorinated pesticides pose serious threat to the flora and fauna and are potentially carcinogenic to the human beings and wild life (IARC 1983). Therefore, once these contaminants find their way to the food chain, they get accumulated to the higher trophic levels and pose serious threats to the human beings. The potential collateral effect of POPs to the nontarget organisms is not well understood as such contaminants behave differently individually, whereas the toxicity increases/decreases several folds due to synergistic and antagonistic effects of co-occurring POPs.

Due to the complexity of the environmental matrices, degradation pattern, and ultra-trace levels of emerging contaminants, the identification and quantification of their occurrences is an intricate process. Though tremendous efforts have been made in the past few decades, the removal of such contaminants has not yet been completely understood by the scientific community. Since most of these parent contaminants and their metabolites have different chemical properties such as molecular weight, normality, polarity, oxidation-reduction state, they follow completely different environmental degradation/removal pathways. Therefore, today the major challenge is the development of suitable techniques for identification and quantification. Moreover, the development of eco-friendly and

Table 11.1 Bioremediation/biotransformation in algae and cyanobacteria of pharmaceuticals and personal care products

Compounds	Nature of compounds	Algae	Remarks	Reference
Carbamazepine	Antiepileptic drug	*Pseudokirchneriella subcapitata*	Bioaccumulation and biotransformation	Vernouillet et al. (2010)
Fluoxetine and its metabolites norfluoxetine, propranolol, lidocaine, and trimipramine	Pharmaceuticals with an aliphatic amine group	*Scenedesmus vacuolatus*	Toxicity of aliphatic amine-based pharmaceuticals toxicokinetic effect rather than toxicodynamic effect	Neuwoehner and Escher (2011)
Tetracycline	Veterinary antibiotics	*C. vulgaris*	Removal of antibiotics through lphotodegradation which depends on shallow geometry of HRAPs	de Godos et al. (2012)
Bisphenol A (BPA; 2,2-bis (4-hydroxyphenyl)propane)	Potential endocrine disruptor	*Nannochloropsis* sp.	13–34 % removal by *Nannochloropsis* sp. and was 25–53 % *C. gracilis* in 6 days under light conditions.	Ishihara and Nakajima (2003)
		C. gracilis		
Bisphenol A (BPA; 2,2-bis (4-hydroxyphenyl)propane)	Potential endocrine disruptor	*Chlorella fusca*	85 % degradation under light conditions and production of monohydroxybisphenol A (secondary metabolite)	Hirooka et al. (2005)
Ibuprofen, acetaminophen caffeine	Pharmaceuticals	*Stigeoclonium* sp. diatoms, *Chlorella* sp., *Monoraphidium*	Up to 90 % removal in the high-rate algal pond (HRT of 8 days)	Matamoros et al. (2015)

environmentally sustainable techniques for the removal of such residues from water and wastewater is the topmost priority in the scientific communities.

4.4.1 Polyaromatic Hydrocarbons

In aquatic systems, several microorganisms such as bacteria, fungi, protozoa, and some of the microalgae species possess the bioremediation potential for PAHs, PCBs, and other POPs such as chlorinated organic compounds and utilize these compounds as a source of carbon and energy (Brusseau 1998). However, in the degradation of complex aromatic hydrocarbons, asphaltenes is very slow due to its low hydrophobicity; moreover, the shorter- (<C10) and longer-chain alkanes (C20–C40) remain difficult to degrade (Brusseau 1998; Guiliano et al. 2000; Yuste et al. 2000). Studies have demonstrated that certain microalgae species possess some enzymes which facilitate and enhance the degradation potential of microbes. Study of Tang et al. (2010) is in accordance with the above statement. While studying the biodegradation of aliphatic and aromatic hydrocarbons, Tang et al. (2010) observed significant degradation of alkanes (46 %), alkylcycloalkanes (51 %), and monoaromatic alkylbenzenes (33 %) by *S. Obliquus* GH2. Enhanced degradation of PAHs (81 %) of crude oil with the consortia of four bacteria (*Sphingomonas* GY2B, *Burkholderia cepacia* GS3C, *Pseudomonas* GP3A, and *Pandoraea pnomenusa* GP3B) and axenic *Scenedesmus obliquus* GH2 was clearly demonstrated.

In recent years, various studies have demonstrated bioaccumulation, biotransformation, and biodegradation potential of several algal species for various organic contaminants. However, majority of literature is available of microalgal bioaccumulation of organic and inorganic pollutants, and limited studies have been done of phycoremediation of such contaminants other than nutrient removals. Selective phytoplankton, diatoms, and microalgal species have shown the potential of biodegradation of organic contaminants, especially biotransformation of the complex organic compounds in lower carbon compounds. Such secondary or tertiary metabolites are easily degraded by resident consortia of microbes such as bacteria and fungi (Walker et al. 1975). Jacobson and Alexander (1981) reported the degradation (meta-cleavage) of dehalogenate 4-chloro-3,5-dinitrobenzoic acid to 2-hydroxymuconic semialdehyde by non-axenic cultures of *Chlamydomonas* sp. Such transformation is not possible with the only bacterial consortia, which clearly indicates that phycotransformation plays crucial role in the biodegradation of complex organics. Walker et al. (1975) also reported significant degradation of saturated aliphatic hydrocarbons (38–60 %), aromatic compounds (12–41 %) of crude oil by *Prototheca zopfii*. In a recent review, Semple et al. (1999) summarized various studies on catabolic sequences of degradation pathway in phycoremediation of organic contaminants. They highlighted the phycoremediation potential of microalgae for polyaromatic hydrocarbons. At high initial concentrations, PAHs get accumulated in algal cells and pose toxicity at initial growth phases, but in log phases it becomes ineffective, whereas, on exposure of lower concentrations, algae and cyanobacteria are capable of phycotransformation of PAHs (Cerniglia et al. 1979, 1980; Soto et al. 1975). The 1-naphthol, 4-hydrox-4-tetralone, cis-naphthalene dihydrodiol, and trans-naphthalene dihydrodiol are the major metabolites of microalgal phycotransformation of naphthalene (Cerniglia et al. 1979, 1980). *Scenedesmus obliquus* was found to possess the ability to desulfonate the 1-naphthalene which releases sulfonate naphthalene sulfonic acids and being used as sulfur for growth (Luther 1990; Luther and Soeder 1987), whereas amino substituents of aminonaphthalenes and amino and nitrobenzoates are used as nitrogen sources by chlorophyte algae. Such studies clearly indicate

that algal bacterial consortia are well capable of phycotransformation and accelerated the degradation of polyaromatic hydrocarbons. Tikoo et al. (1997) reported pentachlorophenol mineralization and degradation by three *Chlorella* species. Various studies have reported mineralization of exogenous phenol in aqueous medium (Ellis 1977; Semple and Cain 1996; Semple et al. 1999). Phycoremediation by conversion of phenols to the corresponding catechols by eukaryotic alga *Ochromonas danica* was reported by Semple and Cain (1996). They observed further degradation of these intermediate compounds by the meta-cleavage of aromatic ring in axenic culture of eukaryotic alga *O. danica*. Pinto et al. (2002) reported up to 70 % removal of phenolic compounds by two green algae, *Ankistrodesmus braunii* and *Scenedesmus quadricauda.*

From above examples it is clear that in phycoremediation of PAHs, algae produce various kinds of exudates and enzymes such as dioxygenase and cytochrome P-450 monooxygenases which oxidize or hydrolyze and/or transform the aromatic compounds in various secondary or tertiary metabolites. Such metabolites either get accumulated in algal cells itself or undergo further degradation by other microorganisms of the native environment (Cerniglia et al. 1979, 1980; Schoeny et al. 1988; Warshawsky et al. 1995). It has been observed that the degradation of complex organic compounds by consortia of algae and other biodegrading microbes is more effective in the removal in comparison to the algal systems alone (Meulenberg et al. 1997).

Degradation of crude oil is reported in various studies. Walker et al. (1975) reported extensive degradation of mixed hydrocarbon and n- and iso-alkanes of crude oil by *Prototheca zopfii*, isolated from the crude oil-contaminated matrix. Various algae species (green, red, and brown), diatoms, and cyanobacteria have shown promising biodegradation potential for organic contaminants. Cerniglia et al. (1979) and Cerniglia et al. (1980) did extensive studies on the degradation of naphthalene by various green, red, brown algal species and diatoms. The results revealed that most of the studied species have degradation potential; however, the extent of degradation mechanism is not fairly understood (Leahy and Colwell 1990). A detailed review compiled by Semple et al. (2009) on aromatic compounds biodegradation by microalgae is recommended for elaborated reading.

4.4.2 Polychlorinated Biphenyls (PCBs)

PCBs are relatively refractory to the biodegradation and are highly toxic, carcinogenic, and ubiquitous in the environment. Microbial and microalgal degradation of these contaminants are very difficult and depend on the extent of halogenation and location of halogen atom in such compounds (Campbell 1977; Saeger and Thompson 1980). It has been established that degradation of chlorinated benzenes and PCBs is done through reductive dehalogenation of such compounds under aerobic conditions (Bouwer et al. 1981; Colwell and Sayler 1978). Degradation of PCBs also mostly occurs via reductive dehalogenation/dechlorination and accelerated by photochemical transformation (Cerniglia et al. 1980; Matsumura and Benezet 1978). Dai et al. (2002) reported that orthochlorinated PCBs suppress dehydroxybiphenyl oxygenase, which is the key enzyme responsible for the microbial degradation. Lynn et al. (2007) reported significant alteration in the PCBs (2,2′,6,6′-tetrachlorobiphenyl) uptake by phytoplankton (diatom, *Stephanodiscus minutulus*) due to nutrient availability which directly affects its trophic transfer. However, such trophic transfer mainly depends on various factors such as algal species, type of organic compound, its environmental circulation, geochemistry, and bioavailability. Moreover, nutrient limitation significantly affects the trophic transfer of such contaminants in the aquatic environment. Removal of PCBs by uptake and accumulation in lipid stores by various algae and phytoplankton has been reported widely (Fitzgerald and Steuer 2006; Lara et al. 1989). Replantation of benthic microalgae in the natural systems accelerate the growth of aerobic microorganisms thus the aerobic activity in the biota (Yamamoto et al. 2008). Such condition leads conversion of anoxic sediment to oxic condition, and in turn the synergy of algae and aerobic bacterial system facilitates aerobic decomposition of organic contaminants. Uptake- and accumulation-based removal of several chlorinated hydrocarbons by marine phytoplankton has been reported widely (Harding and Phillips 1978). Commonly the major constrain in the in situ biodegradation of polychlorinated biphenyls is the lack of effective electron donors which can promote the degradation pathways (Chun et al. 2013). An application of an electric potential in to the PCB-contaminated soils or sediment matrix was found effective for enhancing electron donors/acceptors to the microorganisms (Chun et al. 2013). Therefore, an amalgamation of physical and biological approaches could be a cost-effective and environmentally sustainable option for the in situ remediation of PCB-contaminated sediments and soils. Table 11.2 lists some phycoremediation examples of these hydrocarbons.

4.4.3 Pesticides

Most of the organochlorine pesticides (OCPs) are persistent and highly toxic to flora and fauna, including human beings and wild life. "*According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 21 persistent organic chemicals are pesticides.*" The main mechanism of biodegradation of pesticides is the reductive dehalogenation/dechlorination, which is accelerated by photochemical transformation in autotrophic microorganisms and has been reported widely (Cerniglia et al. 1980; Matsumura and Benezet 1978; Miskus et al. 1965). In such reductive dehalogenation processes, microorganisms facilitate electron transfer from reduced organic compounds for the oxidation-reduction which results in the removal of halogen atoms from the complex halogenated compounds (Esaac and Matsumura 1980; Kobayashi and Rittmann 1982). In gen-

Table 11.2 Bioremediation/biotransformation in algae and cyanobacteria of hydrocarbon

Compounds	Nature of compounds	Algae	Remarks	Reference
14c Naphthalene	Aromatic hydrocarbon	*Agmenellum quadruplicatum*	Catalysis of cis hydroxylation of aromatic hydrocarbons	Cerniglia et al. (1979)
R-endosulfan and its oxidation product endosulfan sulfate	Cyclodiene insecticide	*Chlorococcum* sp.	95–99 % degradation in 30 days	Sethunathan et al. (2004)
		Scenedesmus sp.		
BaP		*Selenastrum capricornutum*	Metabolizes BaP to cis-dihydrodiols using a dioxygenase enzyme system to todiols, and quinones	Warshawsky et al.
Polyurethane		*Protothecazopfii*	Removal by immobilized and free cells	Ueno et al. (2008)
Fluoranthene		*Chlorella vulgaris, Scenedesmus platydiscus, Scenedesmus quadricauda, Selenastrum capricornutum*	Species-specific removal	Lei et al. (2007)
Pyrene				
Phenanthrene		*S. costatum* and *Nitzschia* sp.	Higher removal of mixture than the single compound.	Hong et al.
Fluoranthene			The presence of any PAH compound in the matrix enhances the degradation of the other PAH compounds	

eral, similar principle also works for algal degradation or transformation of chlorinated pesticides (Matsumura and Benezet 1978; Matsumura and Esaac 1979). However, in algal system, electrons required for the reductive dechlorination are produced and transferred from photosystem. Esaac and Matsumura (1980) reported that the reductive dechlorination of chlorinated compound mainly depends on its degree of chlorination and occurs only at 0.35 V and/or lower oxidation-reduction potential of a medium.

Other than carcinogenicity to the human beings, some of these contaminants, e.g., endosulfan, are also potential endocrine disruptors. In last few decades, various studies have been done on bioremediation of OCPs especially endosulfan by individual or mixed culture of bacteria and fungi (Guerin 1999; Kullman and Matsumura 1996; Sutherland et al. 2000). However, studies on phycoremediation of such compounds are scanty. Shivaramaiah (2000) reported the bioremediation potential of *Anabaena* sp., a blue-green alga for endosulfan. While studying biodegradation of a cyclodiene insecticide, α-endosulfan, Sethunathan et al. (2004) convincingly demonstrated that high-density cultures of *Chlorococcum* sp. or *Scenedesmus* sp. were capable of the biosorption and biotransformation of this α-endosulfan. It was noted that such phycoremediation process includes transformation of R-endosulfan to endosulfan sulfate and endosulfan ether. These metabolites were further removed by sorption in the algal cells. In this study, 60–70 % degradation was recorded in 20 days, whereas up to 99 % degradation of endosulfan was achieved in 30 days. Such type of phycotransformation coupled with phycosorption makes algae potential candidate for bioremediation of organochlorine pesticides. Kobayashi and Rittmann (1982) compiled interaction of eukaryotic algae with pesticides and reported that algae are capable of biotransforming of some of the organic contaminants.

Time-dependent environmental risk assessment is very important in the evaluation of algal biodegradation and biotransformation of pesticides, as some of the degradation byproducts or transformation products are more toxic to the biota than the parent chemical compounds. Cai et al. (2009) observed a significant biotransformation of an herbicide, diclofop-methyl, which gets hydrolyzed to diclofop after absorption in the cells of *Chlorella vulgaris* and further degraded intracellularly to 4-(2, 4-dichlorophenoxy) phenol. It was found that the 4-(2, 4-dichlorophenoxy) phenol was more toxic to *C. vulgaris* in comparison to the diclofop-methyl. Significant biotransformation of diphenyl ether herbicide by *Chlorocorrum* sp. of an algal bacterial consortium was reported by Wolfaardt et al. (1994). These studies have clearly demonstrated that in algal microbial consortia, other than bioaccumulation in the cells, most of the microalgal species perform biotransformation of the complex organic compounds to the simpler or low-carbon compounds and ultimately facilitate their degradation by other microbes present in the consortia/biota. Though there is no concrete evidence available, however, it can be hypothesized that in natural conditions the autotrophs such as algae and cyanobacteria provide oxic conditions during photosynthesis and nitrogen fixation which facilitate growth of a wide range of other microorganisms, and in turn these bacteria and fungi accelerate degradation in harmony with these autotrophs (Rao and Burns 1990; Sethunathan et al. 2004). Table 11.3 lists some examples of phycoremediation of pesticides. Figure 11.2 provides a generalized overview of phycotransformation and degradation of PCBs and OCPs contaminants.

Table 11.3 Bioremediation/biotransformation in algae and cyanobacteria of pesticides

Compounds	Nature of compounds	Algae	Remarks	Reference
Monocrotophos	Organophosphate insecticides	*Chlorella vulgaris, Scenedesmus bijugatus*	Degradaton	Megharaj et al. (1987)
Quinalphos		Cyanobacteria: *Synechococcus elongatus*, *Nostoc linckia*, *Formidiumtenue*		
Methyl Parathion				Megharaj et al. (1994)
DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane)	Organochlorine pesticide	*Anabaena* and *Nostoc*	Transformed to DDD [1,1-dichloro-2,2 bis (p-chlorophenyl)ethyl]benzene	Megharaj et al. (2000)
DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane)	Organochlorine pesticide	*Chlorococcum* spp.	Transformed to DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene)	Megharaj et al. (2000)
Fenamiphos (ethyl 4-methylthio-m-tolyl isopropyl phosphoramidate)	Organophosphorus pesticide	five different species of cyanobacteria and green algae	Transformation into oxidation product fenamiphos sulfoxide (FSO)	Cáceres et al. (2008)
Organophosphorus pesticide, fenamiphos (ethyl 4-methylthio-m-tolyl isopropyl phosphoramidate) and its metabolites fenamiphos sulfoxide, fenamiphos sulfone, fenamiphos phenol, fenamiphos sulfoxide phenol, and fenamiphos sulfone phenol	Organophosphorus pesticide and its metabolites	*Chlorococcum* sp. *Pseudokirchneriella subcapitata*	Biotransformation and bioaccumulation	Cáceres et al. (2008)
DDT, dieldrin, and photodieldrin	Organophosphorus pesticide	*Ankistrodesmus amalloides*	Metabolism of DDT to DDE and DDD	Neudorf and Khan (1975)

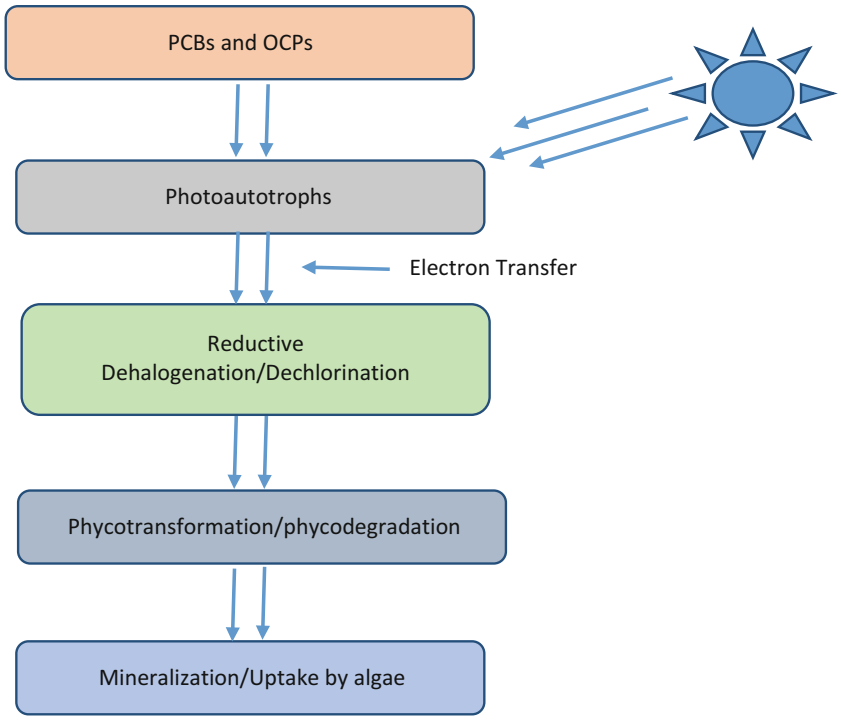

Fig. 11.2 Generalized overview of phycotransformation and degradation of PCBs and OCPs contaminants

5 Phycoremediation: Limiting Factors

The algal biotransformation and degradation, i.e., phycoremediation of emerging contaminants depend on various factors such as physiology of the selected species, their survival and growth, species density, tolerance potential, and prior exposure to the specific xenobiotic compound. The synergy and compatibility of selected species with other resident-competing microflora and fauna also play very important role (Corner 1981; Horvath 1972; Rosenzweig and Stotzky 1980). Various studies have demonstrated that the consortia of algae and bacteria were found to be more effective in bio-

transformation and mineralization of the organic contaminants in comparison of individual algal or bacterial degradation (Chekroun et al. 2014; Horvath 1972; Kobayashi and Rittmann 1982). Other than the physiology of algal sp., the concentration, physical and chemical properties i.e. hydrophobicity, solubility and volatility of the xenobiotics are also important and need to be considered while accessing the phycoremediation (Gibson 1978; Kobayashi and Rittmann 1982; Steen et al. 1980). The susceptibility of hydrocarbons degradation depends on their chemical form as well. Polar and high molecular weight compounds are less liable to degradation, whereas light aromatic and saturated compounds are comparatively more susceptible for degradation (Leahy and Colwell 1990). In case of petroleum hydrocarbons, physical state and the contaminated matrix both play crucial role in its biodegradation. In natural water system, petroleum hydrocarbons easily get dispersed, resulting the formation of a thin slick due to the action of wind and flow current, tidal oscillation. Such thin slicks provide high surface area thus higher degradation. In contrast thick slick or large mousse of hydrocarbons either in water or in soil inhibits the biodegradation due to low surface area (Colwell and Sayler 1978; Cooney 2010). In general, algal mineralization and degradation is proportional to the concentration, water solubility. Degradation of low molecular weight aromatic hydrocarbons such as toluene follows the Michaelis-Menten kinetics (Boethling and Alexander 1979; Pfaender and Bartholomew 1982), whereas the same is not applicable for insoluble hydrocarbons such as naphthalene and phenanthrene, having high molecular weight (Thomas et al. 1986; Wodzinski and Coyle 1974). The octanol-water partition coefficient for the organic compound is also crucial for degradation (Gibson 1978) (Fig. 11.3).

As far as halogenated complex and persistent organic contaminants are concerned, the degree of halogenation, number of halogen atoms, and their bioavailability and toxicity have direct potential effects on the phycoremediation process (Colwell and Sayler 1978; Gibson 1978; Kobayashi and Rittmann 1982; Leahy and Colwell 1990). There are several environmental factors such as temperature, light duration and intensity, pH and oxidation-reduction potential, salinity, and dissolved oxygen of the medium, which directly or indirectly affect the feasibility and success of phycoremediation process either by limiting growth and survival of the microalgae or by changing the geochemistry of the medium (Colwell and Sayler 1978; Gibson 1978; Kobayashi and Rittmann 1982). For example, temperature plays very important role in in situ biodegradation of PAH; therefore, the optimal degradation varies seasonally. Moreover, the solubility of the PAHs is temperature dependent (Bamforth and Singleton 2005). The solubility, thus the bioavailability, increases with the increase of the temperature (Kobayashi and Rittmann 1982; Leahy and Colwell 1990; Margesin and Schinner 2001). pH also plays critical role in the algal degradation of polyaromatic hydrocarbons. Degradation of PAHs is higher in acidic to neutral pH range in comparison to basic range (Wong et al. 2002). PHAs degradation is also affected by oxygen. Therefore, the rate of aerobic and anaerobic degradation of PAHs is greatly affected by the influence of oxygen (Bamforth and Singleton 2005).

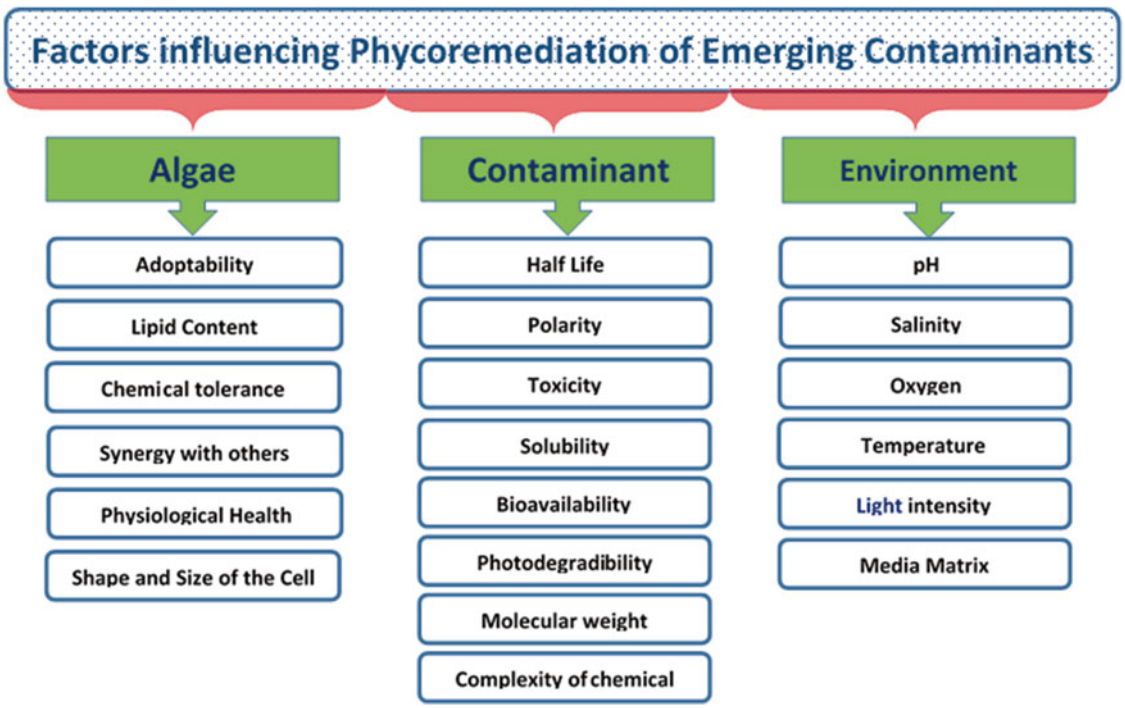

Fig. 11.3 Generalized overview of factors influencing phycoremediation of emerging contaminants

6 Conclusions

Though several incidences are available to date on degradation of various organic contaminants by individual algae and cyanobacteria or in conjugation of other native microbes such as bacteria and fungi, however, such mechanistic understanding of algal degradation pathways for individual type of contaminants needs to be elucidated. The biodegradation potential of mixotrophic cyanobacteria and microalgae species should be identified, and efforts should be made to improve the biodegradation potential of selected species by employing genetic engineering. Microbial consortium engineering by functional genomics, metabolic profiling, and other genetic engineering tools can help in improving the biodegradation potential of such microorganisms (Subashchandrabose et al. 2011, 2013).

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Carbon Dioxide Sequestration by Microalgae: Biorefinery Approach for Clean Energy and Environment

12

Abhishek Guldhe, Virthie Bhola, Ismail Rawat, and Faizal Bux

1 Introduction

Global warming is currently at a disturbing level owing to the rise in anthropogenic greenhouse gases of which CO_2 contributes up to 68 % of total emissions. Power plants are responsible for more than 22 % of CO_2 emissions worldwide as CO_2 is the primary emission of flue gases (Radmann et al. 2012). Flue gas emitted from fossil fuels usually contains N_2, CO_2, O_2, and water vapor as well as minor quantities of CO, NO_x, SO_x, and particulate matters (Kumar et al. 2011). Carbon dioxide mitigation strategies employed globally thus far can broadly be grouped into physical and biological methods (Kumar et al. 2010, 2011; Ho et al. 2011). However, due to various problems associated with physical techniques, there is a demand to develop other appropriate technologies (Kumar et al. 2010, 2011).

Biological carbon sequestration could offer advantages as an intermediate solution for reduced carbon emissions. Carbon dioxide, which is a necessary compound in the formation of complex sugars by green plants and microalgae through photosynthesis, has also shown much potential in significantly increasing the growth rates of most microalgal species (Kumar et al. 2010, 2011; Ho et al. 2011). This application could therefore prove useful for closed systems, using specific microalgal strains to maximize CO_2 conversion to biomass thus absorbing this greenhouse gas. Microalgal biomass could thus represent a natural sink for carbon. Furthermore, such systems could minimize capital and operating costs, complexity, and energy required to transport CO_2 to other places. However, further research is required on various fronts, such as: separation of flue gas or direct utilization, CO_2 capture, introduction of CO_2 to the photosynthetic system and the physiological response this would have on cells, supply and distribution of light, as well as temperature requirements (Kumar et al. 2010, 2011).

A. Guldhe (✉) • V. Bhola • I. Rawat
Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa
e-mail: abhiguldhe@gmail.com

F. Bux
Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa

2 Environmental Implications of Carbon Dioxide Gas

CO_2 is the major contributor of the greenhouse gases (GHG). Greenhouse gas emission is primarily responsible for the global warming. The rise in the temperature is associated with many environmental implications and disturbance in the climate. Global warming is directly related to glacial melting and rise in the ocean level. Climate changes are also associated with the reduced agricultural productivities. Irregular rainfall due to climate change causes the water shortage. Environmental and climate irregularities are also associated with the species extinctions. The United Nations has established the Kyoto Protocol setting the objective of decreasing the GHG emissions by 5.2 % based on 1990 emissions (Pires et al. 2012). Thus, it becomes imperative to reduce the CO_2 levels in the atmosphere for sustainable future of the planet.

3 Existing Technologies for CO_2 Sequestration and Their Limitations

There are numerous techniques that are currently been employed globally to limit the amount of CO_2 escaping into the atmosphere. However, over the years there has been much debate on the selection of the most appropriate technology. Carbon dioxide alleviation strategies that are currently been applied worldwide can broadly be grouped into physical or biological techniques. Physical-based methods essentially comprise three important steps: capture, transpor-

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tation, and storage. Physical methods begin with the collection of CO_2 from a fixed source. Examples of fixed sources that release large amounts of CO_2 daily into the atmosphere include power plants and cement manufacturing facilities. After capture of a sufficient amount of CO_2, the gas is then converted into a supercritical fluid. Conversion to a supercritical fluid enables simple transportation by ship or pipeline to a safe place of storage. Common storage methods utilized include injection into deep geological or oceanic trenches and mineralization (Khoo et al. 2011; Pires et al. 2012). These disposal techniques are energy intensive and expensive, require large amounts of land, and eventually lead to CO_2 leakage over time. Hence, these methods are often considered unsustainable (Stewart and Hessami 2005). This technology, however, still remains a popular choice, even with the abovementioned drawbacks. This could be attributed to the fact that this technique allows communities to sustain their current carbon-based infrastructure while aiming to lessen the outcomes of CO_2 on global warming (Pires et al. 2012). Communities at large are also often nervous and skeptical to venture out and try new techniques.

Another popular alternative would be to make use of the accumulated CO_2. For instance, precipitated calcium carbonate (PCC) can be formed by managing the reaction of CO_2 with lime. This can then be utilized as a replacement for titanium dioxide or kaolin in the production of paper products. Carbon dioxide can also be used in the production of paint, plastic, solvent, and packaging. However, this would use relatively minute amounts of CO_2, when compared to the large quantities released into the atmosphere annually.

Monoethanolamine (MEA) scrubbing is a popular method whereby CO_2 is removed from flue gases during the combustion process. This method involves a chemical absorption process together with the use of a MEA solvent. During this procedure, the MEA solution makes contact with the flue gases and mixes in the absorber. The MEA solution that is now rich in CO_2 is then transported to a stripper. It is then reheated to discharge almost pure CO_2. This captured pure CO_2 can then be utilized for various industrial processes. The MEA solution can be recycled to the absorber. This process requires large equipment sizes and high regeneration energy requirements; therefore, it is considered to be uneconomic. Other technologies that have been investigated (such as membrane separation, cryogenic fractionation, and adsorption using molecular sieves) are even less energy efficient for them to be considered economically viable (Stewart and Hessami 2005).

A carbon fiber composite molecular sieve was developed by the Oak Ridge National Laboratory. This carbon monolith was capable of separating CO_2 from CH_4, CO_2 from air, and CO_2, CO, H_2S, and H_2O from a mixture of gases. This separation technology displayed some potential as it proved to be cost effective, produced minimal waste, and could be adapted to numerous carbon sequestration strategies (Stewart and Hessami 2005).

Desiccant adsorption is another process that can be employed. This is often termed a pressure and temperature swing adsorption (PTSA) that could possibly be applied to electric power plant flue gas. Carbon dioxide can be adsorbed at near normal pressure using zeolite or alumina as the desiccant. Target removal efficiencies of 90–99 % purity of CO_2 removed were achieved using this technology. A major problem with this method, however, is the reaction of the desiccant with SO_X present in the flue gas (Stewart and Hessami 2005).

Biological CO_2 fixation, which can be accomplished during the photosynthesis of terrestrial plants and photosynthetic microorganisms, seems to be the only economical and environmentally viable technique of the future without the aforementioned shortcomings (Kumar et al. 2010, 2011; Ho et al. 2011). Even though terrestrial plants fix around 500 billion tons of CO_2 per annum, they are anticipated to play a minor role (3–6 %) in the overall reduction of atmospheric CO_2 (Skjanes et al. 2007). Microalgae and cyanobacteria have rapid growth rates as opposed to terrestrial plants; their CO_2-fixation efficiency is about 10–50 times better, and they are also known to have higher tolerance to extreme environments (Costa et al. 2000; Ho et al. 2011). These microorganisms have therefore come to the forefront of studies as they offer greater possibility in the long run. The biological mitigation of CO_2 using autotrophic microalgae offers numerous advantages: no additional CO_2 is created, while nutrient utilization can be accomplished in a continuous manner leading to the production of biofuels and other secondary metabolites (Kumar et al. 2010, 2011). In 2010, Sydney confirmed that carbon uptake by microalgae is essentially dependent on the metabolic activity of the particular microalgal strain. However, research has shown that microalgae supplied with higher levels of CO_2 (>5 %) respond much better (on a biomass basis), as opposed to microalgae exposed to ambient air only (Kumar et al. 2010, 2011; Ho et al. 2011). Microalgae have the ability to generate approximately 280 tons of dry biomass per ha per year by utilizing only 9 % of the freely available solar energy. During this process, roughly 513 tons of CO_2 can be sequestered (Sydney 2010). A study by Borkenstein and Knoblechner in 2011 investigated the growth of *C. emersonii* for 30 days with both pure CO_2 and flue gas. Results revealed that when the *Chlorella* strain was supplied with CO_2 containing flue gas, it produced 2.06 g L^{-1} biomass.

4 Bio-mitigation of CO_2 by Microalgae

4.1 CO_2 Capture

Microalgae are capable of accumulating large amounts of inorganic carbon in their cytoplasm. Most often, these carbon concentrations are much higher when compared to concentrations on the outside. This process is referred to as a CO_2-concentrating mechanism (CCM). An important factor in

photosynthesis is the CO_2 concentration. If there is a very high concentration of CO_2, this would increase the mass transfer mechanism from the gas mixture to the medium. This would eventually lead to a drastic drop in pH. The pH reductions often adversely affect the growth and productivity of most microalgal species. Oxygen, produced during photosynthesis, is another important factor that can hinder microalgal growth. Therefore, it is imperative that gas is regularly removed from a microalgal system and not allowed to accumulate (Pires et al. 2012).

4.2 Fate of CO_2 in Microalgal Physiology

Photosynthesis occurs in two stages within microalgae cells. The first stage only occurs when cells are exposed to light and involve light-dependent reactions. This step utilizes light energy to produce the energy-storage molecules adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). Dark reactions (carbon-fixation reactions) transpire during the second stage of photosynthesis. These reactions can occur both in the presence and in the absence of light. It is at this stage that energy-storage products produced during light reactions are employed to capture and reduce CO_2 (Calvin 1989). The two major photoactive complexes (photosystem I (PSI) and photosystem II (PSII)) transfer sunlight into the electron transport chain using the excited chlorophyll dimer (Calvin 1989; Iverson 2006; Ho et al. 2011). Photosynthesis starts in the PSII complex. An electron is then transferred to the primary electron acceptor molecule once a chlorophyll molecule at the center of the PSII complex attains ample excitation energy. This process is referred to as photoinduced charge separation. An electron transport chain is then responsible for transporting electrons across the membrane. PSI then takes up the electrons from PSII and moves them via the P700 dimer of chlorophyll, which is oxidized from light-excited antenna chlorophyll to strongly reducing ferredoxin and NADPH (Cerveny et al. 2009; Ho et al. 2011). During photophosphorylation, energy harvested during the light reactions can be stored by the formation of ATP (Yang et al. 2000). Research has found that 1.3 ATP molecules are created per pair of electrons moving through the photosynthetic electron transport chain (Yang et al. 2000).

Dark reactions or carbon-fixation reactions involve the Calvin cycle (Calvin 1989; Iverson 2006; Yang et al. 2000). It is during this cycle that CO_2 is converted into the enzyme RuBisCO (Ribulose 1,5-bisphosphate carboxylase/oxygenase). Ribulose 1,5-bisphosphate carboxylase/oxygenase is also involved in oxygenase activity and forms glycolate 2-phosphate as an end product. This product and its synthesis consume considerable amounts of cellular energy, yet it is of no use to the cell. The oxygenase activity of RuBisCO is known to hinder around 50 % of biomass formation (Giordano et al. 2005; Kumar et al. 2011).

Photosynthesis in microalgae is often measured as rates of carbon accumulation or O_2 evolution. Either measurement can be converted into the other using the photosynthetic quotient (Pires et al. 2012). In 2010, Jacob-Lopes et al. studied the influence of photoperiods on the rates of CO_2 sequestration using a cyanobacterial strain in both BGN medium and refinery wastewater. They observed a linear decrease in biomass productivity during a longer dark period when cultivated in BGN medium. When the strain was grown in refinery wastewater, a photosynthetic quotient of 0.74 was recorded. This reading basically means that 1 g of CO_2 utilized corresponds to the release of 0.74 g of O_2. This study showed that the gas-exchange pattern within a system is greatly influenced by the intermittent light cycle. During the dark phase, the microalgal cells were able to consume organic carbon through heterotrophic metabolism and release CO_2 in the process.

Chlorococcum littorale is an extremophile that has the ability to grow well at CO_2 levels up to 60 % (Miyachi et al. 2003). Research conducted on this microalga demonstrated that when exposed to high levels of CO_2, there is a rapid state transition of the photosynthetic apparatus (Demidov et al. 2000; Miyachi et al. 2003; Solovchenko and Khozin-Goldberg 2013). A state transition is often caused due to a reduction in the plastoquinone pool owing to accumulation of NADPH. This shift in the photosynthetic apparatus from state I to state II causes an increase in the cyclic electron transport over PSI. The additional ATP generated during this change is used to sustain pH homeostasis in the microalgal cell (Miyachi et al. 2003). In 2004, Muradyan et al. stated that CO_2-intolerant species often exhibit signs of PSI damage when exposed to high CO_2 conditions as they lack the state transition response (Solovchenko and Khozin-Goldberg 2013).

4.3 Microalgal Strains for CO_2 Sequestration

Microalgae and cyanobacterial species that are often employed for CO_2 sequestration include *Anabaena* sp., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Chlorella* sp., *Chlorococcum littorale*, *Scenedesmus* sp., and *Spirulina* sp. (de Morais and Costa 2007; Ota et al. 2009; Packer 2009; Chen et al. 2010; Chiang et al. 2011; Ho et al. 2011). Green microalgae that are efficient carbon sequesters usually belong to the genera *Chlorococcum*, *Chlorella*, *Scenedesmus*, and *Euglena*. In 1970, Seckbach and Libby isolated microalgal species from the abovementioned genera. These strains were even able to tolerate pure (100 %) CO_2. Research on the *Scenedesmus* culture showed that this strain was capable of flourishing under a 100 % CO_2 level cell concentration increased for up to 30 days, achieving 3.65 g L^{-1}. This proved to be a considerable increase in cell

concentration, when compared to the 1.19 g L^{-1} recorded during exposure to atmospheric CO_2 (0.036 %).

In another study, A *Chlorella* TX 71105 strain was supplemented with pure CO_2 at a rate of 3.3 mL min^{-1} over a 28-day period. It was observed that during the first 6 days, the effluent gas collected contained more than 96 % O_2. On the 12th day of cultivation, an increase in temperature from 37 to 39 °C was recorded. A 32 % CO_2 concentration was noted in the effluent gas on the 13th day. The gas flow was then turned off, and the culture was not supplied with CO_2 for 3.5 h. There were no adjustments to the experiment during the last 6 days. For the duration of this period, it was observed that the effluent gas contained 18 % CO_2. When this strain was grown under 41, 71, and 100 % CO_2, the mean biomass concentration noted was 3.15, 2.71, and 2.49 g L day^{-1}, respectively. These cell concentrations achieved are reasonably similar to those obtained with other *Chlorella* species (Geckler et al. 1962).

In 2011, Zhao et al. did a comparative study of the growth and CO_2 biofixation of a *Chlorella* sp. under two different modes of cultivations. Results obtained suggest that closed cultivation significantly enhanced microalgal performance with regard to growth and carbon biofixation. During conditions of closed cultivation, specific growth rate and CO2-fixation rate were observed to be 1.78 and 5.39 times higher, respectively, when compared to that of open cultivation. Closed systems permit effective gas bubble motion, which plays an essential role in displacing dissolved O_2 buildup. Under appropriate cultivation modes, *Chlorella* sp. display much potential as effective carbon sequesters. Kurano et al. (1995) demonstrated that at a 20 % CO_2 concentration, *C. littorale* was able to attain a high cell concentration of 4.9 g L^{-1}. A short lag phase prior to active photosynthesis was observed when this strain was exposed to CO_2 concentrations of greater than 20 %. It is important to note that the performance of microalgal strains is not solely influenced on CO_2 concentrations, but also on experimental and culture conditions (culture medium, temperature, light intensity, as well as reactor design). Variation in any of these conditions could have an adverse effect on the CO_2-fixation efficiency of the strains (Ho et al. 2011).

5 Making CO_2 Available for Microalgae

5.1 Cultivation Systems

Once an appropriate strain has been selected, the next step would be suitable cultivation of the microalgae. It is essential that artificial propagation of microalgae both mimics and enhances the optimum natural growth conditions (Brennan and Owende 2010; Vasumathi et al. 2012). Open ponds and closed photobioreactors (PBR) have been extensively exploited for the growth of microalgae (Molina et al. 2001; Suh and Lee 2003; Chisti 2008; Brennan and Owende 2010). However, with regard to utilizing microalgae for CO_2 mitigation, there has been ongoing debate as to which would be a better cultivation system. Raceway ponds are the most popular growth systems as they are cost effective and relatively simple to maintain. These systems, however, utilize CO_2 much less efficiently when compared to PBRs as there is a significant loss of CO_2 to the atmosphere (Borowitzka 1999; Chisti 2007; Brennan and Owende 2010).

Over the years, much research into PBR technology has been conducted to overcome some of the important problems linked with open pond production systems. Photobioreactors are more advantageous than open systems as they allow for culture of single species of microalgae for long durations with lower risk of contamination (Brennan and Owende 2010). Due to higher cell mass productivities when using a PBR system, harvesting costs are also often reduced. Carbon dioxide is also utilized more effectively using these systems (Chisti 2007; Brennan and Owende 2010; Vasumathi et al. 2012). Even though a great deal of work has been done to design and produce PBRs for microalgal propagation, and effective CO_2 consumption, more research is still needed to improve PBR technologies and know-how of microalgal cultures. For the efficient mass cultivation of microalgae for carbon sequestration, PBR design and development is possibly one of the first major steps that should be undertaken (Ugwu et al. 2008).

Photobioreactors equipped with unique designed light systems have been examined for effective CO_2 sequestration and greater biomass productivities (Lee 2001). In 2003, Suh and Lee designed and operated an internally illuminated airlift PBR. This reactor was constructed to study the light distribution and to ultimately maximize the photosynthetic efficiency to promote greater carbon uptake by a *Synechococcus* sp. Another important feature when developing a closed reactor is the volumetric gas transfer coefficient. By increasing the gas transfer coefficient, cell growth rate can be enhanced (Kumar et al. 2011). A series of trials was conducted by Zhang et al. in 2002 to comparatively investigate gas transfer in different PBRs at varying CO_2 percentages. From the results obtained, they were able to conclude that a decrease in the CO_2 concentration from the inlet gas stream leads to an increase in the gas transfer coefficient. Furthermore, it is imperative that the CO_2 solubility within the cultivation media is established as this will give the researcher an idea on the amount of CO_2 available for growth.

Borkenstein and Knoblechner (2011) investigated the growth of *C. emersonii* in 5.5 L airlift PBRs using both flue gas and pure CO_2. The experiment lasted 30 days. At the end of experimentation, it was noted that there was no significant

difference in biomass yields obtained. When the *Chlorella* sp. was supplied with CO_2 containing flue gas, it produced 2.00 g L^{-1} biomass, and when cultivated with pure CO_2, a biomass yield of 2.06 g L^{-1} was recorded. However, an important conclusion to this study was that when supplied with flue gas, *C. emersonii* was able to grow as successfully as when it was purged with pure CO_2.

5.2 Parameters Affecting CO_2 Uptake

Carbon dioxide can be an effective supplement to promote microalgal growth. However, high levels of CO_2 (5 %) can often hinder growth of certain microalgal strains. High percentages of CO_2 can cause acidification of the cellular content, which eventually hampers growth and productivity (Lee and Lee 2003; Solovchenko and Khozin-Goldberg 2013). In 2000, Watanabe et al. demonstrated that microalgal cultures that grew well at CO_2 levels between 5 and 10 % had significant reductions in their growth rates at CO_2 percentages above 20 %. Elevated levels of CO_2 often lead to drastic drops in pH, due to the formation of large amounts of bicarbonate. pH can drop to 5 or even lower in some cases. Extreme decreases in pH cause an environmental stress that leads to a biological reduction in the ability of microalgal cells to sequester CO_2. Microalgal growth is only slightly affected when there is a small drop in pH, but extreme pH changes could possibly inhibit all growth (Kumar et al. 2011; Solovchenko and Khozin-Goldberg 2013). Screening studies have identified microalgae that are capable of tolerating and flourishing under CO_2 concentrations between 30 and 70 % (Hanagata et al. 1992; Iwasaki et al. 1996; Sung et al. 1999). In 2003, Olaizola demonstrated that microalgal growth could even be sustained at a 100 % CO_2 level. This can only be achieved if changes in pH were monitored strictly and CO_2 was only supplied to the strain on demand.

Light is a necessary requirement for photosynthesis. The photosynthesis-irradiance response (P-I) curve accurately depicts the relationship between light and photosynthesis. This curve has three distinct regions: light-limited photosynthesis, light-saturated photosynthesis, and photoinhibition (Ralph and Gademann 2003). Optimum light intensity is essential for effective CO_2 fixation and biomass production. Light intensity below the optimum becomes the limiting factor for microalgal growth, whereas too high light intensity causes photoinhibition to microalgal cells. Photoinhibition occurs when there is damage to the PSII repair mechanism – this also causes inactivation of other systems (electron carriers, oxygen-evolving systems, and the related D1/D2 proteins). Light intensity is dependent on numerous factors (wavelength, cell concentration, and the penetrating distance of light as well as then geometry of the system) (Kumar et al. 2011).

5.3 Novel Techniques for Facilitating Supply

The CO_2-fixation rate can be improved if research was conducted on the Calvin cycle, PEP carboxylase, and/or through synthetic pathways (Rosgaard et al. 2012; Gimpel et al. 2013). Previous research efforts have met with varying degrees of success. Studies have focused on the following aspects: engineering RuBisCO to promote higher catalysis rates of carboxylation and decreases in the oxygenation reactions, improving the activation state of RuBisCO, accelerating the regeneration phase of the Calvin cycle, and enriching CO_2 around RuBisCO in an attempt to inhibit the oxygenase reaction. From these studies it was concluded that the challenge lies in the activity of RuBisCO for carbon flux through the carbon flux when the media is not enriched with CO_2 or during extreme temperature/light conditions. *Chlamydomonas reinhardtii* are ideal candidates for RuBisCO engineering as they are RuBisCO deficient and therefore able to complete their life cycle heterotrophically. *Chlamydomonas* sp. has been engineered for the efficient exploitation of energy, carbon and nitrogen. This has been accomplished using the nuclear genome of an MRL1-deficient strain and expressing the rbcl mRNA maturation factor MRL1 at varying levels. As opposed to the wild type, results for the deficient strain illustrated that RuBisCO could sustain phototrophic growth even when it was lowered up to 15 %. Based on these findings, it can be concluded that depending on the culture conditions (light intensity or CO_2 concentration), an inducible promoter for MRL1 could successfully be applied to modify RuBisCO accumulation (Rosgaard et al. 2012; Gimpel et al. 2013).

As discussed above (section on photosynthesis), an increase in the PSI/II ratio suggests that microalgal species require an increase in their PSI light-harvesting antenna in order to grow at high CO_2 concentrations. However, it must be noted that these changes can often be reversible. A reduction in the ATP demand caused by the overall acclimation of the microalgal cell to high levels of CO_2/or a drop in the CCM activity often leads to the ratio returning to its original level (Miyachi et al. 2003; Solovchenko and Khozin-Goldberg 2013).

6 Integrated Biorefinery: From Waste to Value Addition

An integrated biorefinery has been considered as the sustainable and economical approach for the cultivation of microalgae for generation of biomass. In this approach maximum outputs and goals can be achieved in the single integrated cultivation system (Singh et al. 2015). Wastewater can be utilized as the growth medium for microalgae; flue gases can be

used to provide the CO_2. The microalgal biomass thus generated can be utilized for a number of purposes such as biofuels (biodiesel, bioethanol, biomethane, etc.), pigments (carotenes), therapeutic biomolecules, animal or fish feed, etc.

6.1 Waste Utilization: Flue Gas and Wastewater

The ability to fix atmospheric CO_2 via photosynthesis by microalgae is 10 times more efficient than the terrestrial plants (Pires et al. 2012). Carbon is key component which constitutes 36–56 % of dry matter of microalgal cell. For per kg of dry biomass generation, 1.3–2.4 kg CO_2 is fixed by microalgae (Van Den Hende et al. 2012). Microalgae can be cultivated by supplying CO_2 from flue gases. Direct flue gases or CO_2 separated from flue gases can be applied for cultivation of microalgae. Direct use of flue gases is energy- and cost-saving approach. Microalgal strain should be resistant toward the high percentage of CO_2 (15 %) and presence of SO_X and NO_X for application of flue gases in the cultivation (Maeda et al. 1995). Maeda et al. (1995) investigated several microalgal species for flue gas application and observed a *Chlorella* sp. strain with high growth rate at temperature of 35 °C and 15 % CO_2 concentration. Table 12.1 depicts the microalgal species cultivated using flue gases. Flue gases composed of several compounds like CO2, SO_X, NO_X, CO, C_xH_y, halogen acids, and particulate matter. Some of these contents could be toxic for the microalgal growth. Microalgae can be grown in open or closed system. Delivery of the flue gases to the cultivation system and its proper mixing have several challenges which need to be addressed for successful implementation of this technology. This biorefinery approach of utilization of flue gases for CO_2 can minimize environmental concerns as well as earn carbon credits.

Industrial and domestic wastewater discharges to the environment are adding organic and inorganic nutrients, pathogens, heavy metals, suspended solids, and oxygen-demanding material to the water bodies. Freshwater resource is limited to cater industrialization and socioeconomic developments. Cultivation of microalgae in open ponds needs around 11–13 million L ha^{-1} $year^{-1}$ freshwater (Chinnasamy et al. 2010). In biorefinery concept, utilization of wastewater for cultivation of microalgae serves dual purpose of biomass generation as well as nutrient removal from the final effluent (Rawat et al. 2011; Singh et al. 2014). The microalgal biomass can be utilized for several purposes, viz., energy, value-added products, and feed production. Utilization of wastewater for microalgal cultivation reduces the freshwater footprint and also provides treated water for other uses. Microalgal growth needs inorganic nutrients like nitrogen and phosphorous. Nutrient supplementation is the contributor to the cultivation cost. This cost is reduced if the wastewater is utilized as the source. Various microalgal strains have been investigated using wastewater nutrient medium (Ramanna et al. 2014).

6.2 Utilization of Microalgal Biomass: Energy, Bioproducts, and Feed

Microalgal biomass can be utilized for several applications like renewable energy generation, bioproducts such as pigments and therapeutic biomolecules, and also as fish or animal feed. In biorefinery approach, focus is on deriving as many products as possible from the microalgal biomass. Multiproduct approach is favorable for economics, as microalgal biomass produces lipids, carbohydrates, and proteins which can be used for various purposes. Biodiesel is most studied biofuel from the microalgae. Microalgae have capabilities of fast growth rates and high lipid accumulation (Guldhe et al. 2015). Microalgal lipids are used as feedstock for biodiesel production. Anaerobic digestion of microalgae for biomethane generation is also another approach of biofuels generation. Either whole microalgal biomass or the lipid-extracted residue can be used for biomethane generation from microalgae. Lipid-extracted algae are nitrogen rich as protein content is high; thus, it can be co-digested with other carbon-rich substrates such as primary sewage sludge to maintain the desired C/N ratio (Sahu et al. 2013). Carbohydrate constituent of microalgae can be used for bioethanol production. Several microalgae can store energy in the form of starch, which can be used as a substrate for bioethanol generation. Biohydrogen is considered as the cleaner renewable fuel. Microalgae can be utilized for biohydrogen generation. Microalgae can photosynthetically produce the biohydrogen or microalgal biomass can be used as substrate for dark fermentation using bacteria for biohydrogen production (Batista et al. 2014).

Microalgae are diverse group of organisms which produces numerous secondary metabolites. Primary metabolites like carbohydrates, lipids, and proteins from microalgae can be used to produce numeral commercial products. The secondary metabolites from microalgae like fatty acids, sterols, carotenoids, phycocolloids, lectins, mycosporine-like amino acids, halogenated compounds, polyketides, and toxins have also numerous commercial applications (Singh et al. 2015). In an integrated biorefinery approach, microalgal biomass can be utilized for generation of various products of therapeutic and cosmetic significance, health food, coloring agents, and aquaculture and animal feed. The biomolecules from microalgae having therapeutic and cosmetic significance are high-value products. A biorefinery based on CO_2 sequestration, wastewater utilization for growth of microalgal biomass for energy, bioproducts, and feed generation is a sustainable and economical approach.

Table 12.1 Microalgae grown using flue gases and their biomass yields

Microalgae	CO_2 concentration (%)	Biomass (gL^{-1} d^{-1})	References
Chlorella sp. MTF7	25	0.48	Chiu et al. (2011)
Scenedesmus obliquus SJTU-3	10	0.155	Tang et al. (2011)
Chlorella pyrenoidosa SJTU-2	10	0.144	Tang et al. (2011)
Chlorella sp.	6–8	19.4–22.8[a]	Doucha et al. (2005)

[a]Biomass in gm^{-2} d^{-1}

7 Challenges and Future Prospects for CO_2 Sequestration by Microalgae

The CO_2 sequestration by microalgae is an environmentally friendly method, as the CO_2 captured is converted to valuable biomass while the oceanic or geological storage of CO_2 only delays its release in the environment. However, cultivation of microalgae and CO_2 supply to cultivation system have several challenges which need to be addressed. The mass transfer coefficient of CO_2 is low, and thus mass transfer from gaseous phase to liquid phase is a major bottleneck in application of this technology. Microalgae can be cultivated in closed or open system. In closed system maintaining the high flow rate could alleviate this mass transfer problem. Efficient mixing and aeration in open pond system facilitate the CO_2 mass transfer (Pires et al. 2012). Delivery of the CO_2 to the microalgal cultivation system is also cost incurring and thus needs economical and efficient technologies to overcome this barrier. When the flue gases are used for CO_2 supply, it composed of several other components which could be toxic to the microalgal growth. Thus, further investigation is important assessing the effect of each component from the flue gases on the microalgal growth physiology. The CO_2 sequestration technology by microalgae is still in its early stages. Engineering of the photosynthetic mechanism of microalgae could also improve the CO_2 capture efficiency. Designing efficient closed or open cultivation system at the site of CO_2 generation such as power plants and cement factories could reduce the transportation cost. Biorefinery concept where waste refusals from industry are used to cultivate microalgae and subsequent biomass utilized for maximum applications could make CO_2 sequestration by microalgae economically feasible.

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Remote Sensing Strategy for the Study of Algal Monitoring

13

Laxmi Kant Sharma, Binita Kumari, and Saurabh Kumar Gupta

Abbreviations

ASTER	Advanced Spaceborne Thermal Emission and Reflection Radiometer
ALI	Advanced Land Imager
AVHRR	Advanced Very High Resolution Radiometer
Hyperion	First spaceborne hyperspectral sensor onboard Earth Observing-1(EO-1)
IKONOS	High-resolution satellite operated by GeoEye
IRS-1C/D-	LISS Indian Remote Sensing Satellite/ Linear Imaging Self Scanner
IRS-P6-	AWiFS Indian Remote Sensing Satellite/Advanced Wide Field Sensor
Landsat-4 5 TM	Thematic Mapper
Landsat-7 ETM+	Enhanced Thematic Mapper Plus
MODIS	Moderate Resolution Imaging Spectroradiometer
HABs	Harmful algal blooms
MERIS	Medium Resolution Imaging Spectrometer
AVIRIS	Airborne Visible/Infrared Imaging Spectrometer
SeaWiFS	Sea-viewing Wide Field-of-view Sensor
NOAA	National Oceanic and Atmospheric Administration
Chl a	Chlorophyll a

L.K. Sharma (✉) • B. Kumari • S.K. Gupta
Centre for Land Resource Management, Central University of Jharkhand Brambe, Ranchi, Jharkhand 835205, India
e-mail: laxmikant1000@yahoo.com

1 Introduction

Applications of satellite remote sensing have given very powerful insight to the study of aquatic and freshwater ecosystems because it provides sophisticated information for the management of water conditions and resources. The concept of harmful algal blooms (HABs) expansion in inland water bodies has been increasing worldwide; therefore, algal monitoring and study have their importance and challenge today. Lots of efforts and steps are taken for proper monitoring and management of algal blooms and reducing their expansion; it's a global concern. The impact of the blooms has an effect on human health, ecosystems, and marine mammals, with evidence of economic losses in the fishing, aquaculture, and recreation industries (Camen et al. 2001). In August 2000, nine people in Washington State became ill from PSP (paralytic shellfish poisoning) after consuming recreationally harvested shellfish from closed waters of Carr Inlet in South Puget Sound.

In India evidence of occurrence of algal communities that have 101 cases has been identified; therefore, for the purpose of monitoring, a lot of programs are organized with the participation of many institutions and government agencies like the Bathythermograph (XBT) program; Ballast Water Management Program, India (BAMPI); Ministry of Earth Sciences (MOES); and CSIR–National Institute of Oceanography (NIO) (D'Silva et al. 2012). Under this program, Port Baseline Biological Surveys (PBBS)-like programs are trendy in the port region of some parts of India. It is found that flourishing growth of numerous species of green, red, and brown algae occurs along the southeast coast of Tamil Nadu from Rameswaram to Kanyakumari; also, algal community expansion has been a concern in the Cheriyapaniyam and Kiltan in Lakshadweep. The mission adopted the technique which is commonly used for identification of the Eutrophication Status of the Maritime Area. The NOAA (National Oceanic and Atmospheric Administration) testified the first valua-

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tion on the load and effect of nutrient concentration in US estuaries – National Estuarine Eutrophication Assessment (NEEA). The Helsinki Commission that is also called HELCOM developed many tools and methods for calculation of eutrophication in the Baltic Sea. From the marine study, the red tide phenomena and HABs of the middle Gulf of Oman and outer Arabian Sea have been recorded since 1987 (Thangaraja et al. 2007); there is the detection of the blue-green algae, *Trichodesmium* spp., in coastal Arabian Sea water using Landsat satellite data (Chaturvedi et al. 1986).

1.1 Algal Blooms in Water Bodies

An observable and sticky symptom of eutrophication within water bodies is speedy expansion and growth of phytoplankton leading to fading and pollution of contaminated water. These events are referred as bloom. For estimation of productivity of algal community, we have to combine ancillary data taken from temperature, salinity, and tissue nutrients and get biomass from it. The main impact of eutrophication is the rapid expansion in the amount of algae community in water bodies and wetlands, and a coastal marine ecosystem leads to increase in biomass accumulation. The incident shows highly in cyanobacterial population which have been testified as toxic and harmful worldwide for natural water bodies, and similar trends are reported here both for phytoplankton for causing turbidity in reservoirs and for top algae community in water bodies and rivers. These blooms are responsible for water quality contamination and harm aquatic life, which can lead to bad taste, discoloration, bad odor, decreased amount of oxygen, toxicity, fish kills, and food web alterations (Paerl et al. 2001). The anthropogenic sources like increase in urbanization, agricultural activities, and industrialization cause increment in discharge of phosphorus (P) and nitrogen (N) which are the main nutrient loads in waters, and the increase amounts, proportions, and chemical composition of these nutrients lead to algal bloom (Pearl 2008). The blooms that are not removed from the food web and by other natural means increase sediment load at the bottom of water bodies and are responsible for biological oxygen demand (BOD). Therefore, N and P load of water must be removed by two methods: single nutrient removal like nitrogen and both N and P removal. There are also other removal techniques to overcome these nutrient loads; these are hydrologic manipulations, reducing the water dwelling time via flushing and artificial mixing (Pearl 2008).

1.2 Harmful Freshwater Algal Blooms (HABs)

There are many harmful phytoplanktons present in water and are capable of forming blooms, an example of which is blue-green algae (or cyanobacteria) which are the most notorious bloom formers. Other examples are scum-forming genera like *Anabaena*, *Nodularia*, *Microcystis*, and *Aphanizomenon*, as well as *Oscillatoria* and *Cylindrospermopsis*. They are subsurface bloom formers and have ability to tolerate nutrient-enriched conditions. These Blooms flourish in extremely productive waters by being able in the direction of speed travels, between top bright surface waters and nutrient-rich area at benthic region. In addition, a lot of detrimental species are tolerant of extremist environmental situation, including incredibly periodic nutrient deprivation, high temperatures, and waterlessness. A lot of the most harmful cyanobacterial bloom genera (e.g., *Nodularia*, *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*) are capable of setting up atmospheric nitrogen, enabling them to periodically dominate under nitrogen-limited environment. The significance of cyanobacteria is that they produce organic compounds, including those that are poisonous from top trophic-level consumers, like zooplanktons, to uppermost consumer in the food web. The adjustments in the abundances of other organisms are also related to variations in this phytoplankton in the water bodies.

1.3 Overview of Remote Sensing

Remote sensing tool has many attributes for exploring, mapping, and monitoring the environment. Spatial variations complicate the study of recurring and long-term trends of biological conquest. Remote sensing, however, by way of its broad view has the potential on the way to bring the relevant information. Satellite imagery is availably designed for nearly every one of the humankind. The scenery of satellite imagery permits keeping track of dynamic features of landscaping and consequently can provide a means to detect key land cover alterations and measure the rates of change. Remote sensing tool is applied in a variety of fields of science and technical investigations as well as used for monitoring purposes. It covers the area of meteorological studies to measure atmospheric pressure, water vapor, and wind velocity. In oceanographic studies, its utility is toward measuring sea surface temperature, wave energy, mapping ocean currents, and its spectra. It is also applied in the fields of glaciology, disaster management, geodesy, geology, cartography, landscape, planning activities, oil and mineral studies, environmental monitoring and exploring, hydrology, and forestry and in defense. There

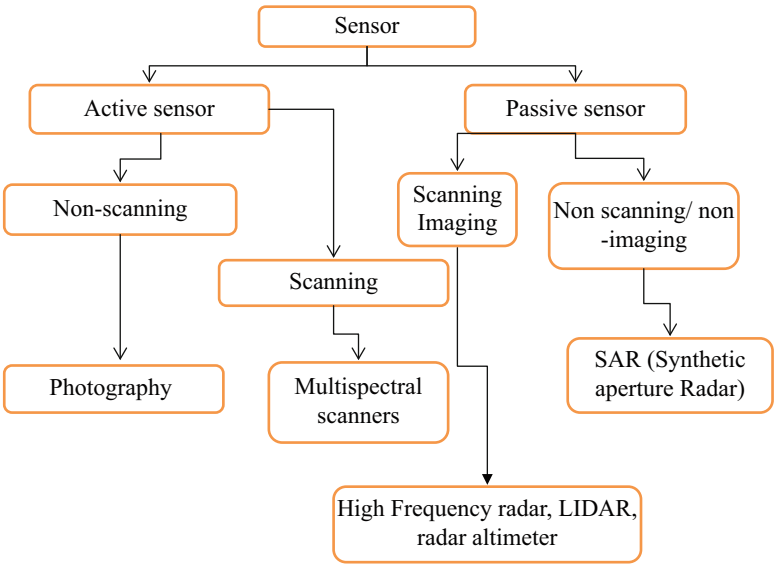

Fig. 13.1 Types of sensors (Source: Adapted from Harvey 2008)

are many sensors available in satellites and airborne instruments which are capable of getting information from a targeted area. There are two types of sensors (Fig. 13.1: Types of sensors): active and passive. Active sensors have their own source of light for illumination like radar and passive sensors do not have their own source of light; they detect energy from naturally occurring energy, i.e., sun, like Landsat sensors. All satellite and airborne sensors utilize both active and passive methods of sensing, for example, camera. Another example of active remote sensing is radar instrument to monitor speed of vehicles which is mainly used by police.

2 Remote Sensing in Algal Monitoring and Detection

Satellite remote sensing has a benefit over traditional monitoring strategies for monitoring algal blooms because it offers synoptic coverage and real-time consistency of the data captured plus help when there is unavailability of field data. The reflectance properties of various algal types and estimation of chlorophyll present in the each type of algal bloom can predict the algal bloom characteristics from absorbance spectra (400–600 nm) of each pigment and help in monitoring purposes and identification; remote sensor used for it are TM imagery, AVIRIS, and hyperspectral remote sensing (Richardson 1996). The algal community blooms increased in the eastern boundary along the coasts of Washington-Oregon in California, Northern Peru, Namibia, and southwestern part of India; the research demarcated the expansion of harmful algal blooms (HABs) from different satellite sensors and its utility in applying the identification of chlorophyll concentration and reflectance properties for the algal bloom expansion locally and globally (Klemas 2012).

Satellite and aboveground measurements of spectral reflectance (ocean color) assists in monitoring chlorophyll concentration and absorption of phytoplankton; the surface radiances refer to the changes into reflectance, calculated from reflectance values from the remote sensing sensors for classifying chlorophyll and other properties of water. There are many sources of ocean color data from where information can be collected (Table 13.1). For remote sensing of single species of algae (e.g., *Oedogonium*), a study that was conducted on color infrared film observed from aerial photography helped in detecting regions of *Oedogonium* progression which are clearly segregated as a light orange color within the red-colored *Myriophyllum* beds (Michael et al. 1974). To minimize errors in interpretation, atmospheric correction must be accomplished for detection of algal communities from any sensors. The remote sensing survey was conducted to establish visual interpretation of the lake and to calculate the execution of observational and semi-expository calculations in hypertrophic water. For incessant change detection applications in inland waters, MERIS is the ideal sensor. Procedures for estimating chlorophyll a, total suspended solids (TSS), Secchi disc depth (zSD), and other measurements are derived simultaneously which are collected from in situ measurements from MERIS. The review illustrated the current remote sensing techniques, diverse algorithms for detecting HABs, and its spatial scale. With the use of visual interpretation, parameter retrieval, and spectra investigation along with spatial-temporal pattern analysis, the detection of HABs can be frameworked. The research describes the improved way of cyanobacterial sprout transport applying preoperational hydrodynamic model and high temporal resolution satellite imagery. The experiment defines the bloom position and extent of the region; for this purpose a geographic center was calculated and assigned to the bloom

Table 13.1 Details of sources of ocean color* data, information, and products

Satellite	Details	Web source
NASA OceanColor Web	Daily, weekly, monthly, and seasonal climatology	www.oceancolor.gsfc.nasa.gov
	Gathering, processing, calibration, validation, archive, and distribution of ocean-related products from a huge number of operational, satellite-based remote sensing missions providing ocean color, sea surface temperature, and sea surface salinity data	
GlobColour/ HERMES	Merging of MERIS, SeaWiFS, and MODIS	www.hermes.acri.fr
	Daily, weekly, and monthly	
	Extraction of ocean color data for user-defined areas is possible and a free GlobColour subscription service allows users to systematically obtain near real-time products at 1 km spatial resolution for a specific area	
NOAA CoastWatch	Provides access to multiple satellite ocean remote sensing data and products	www.coastwatch.noaa.gov
ESA	It is mainly designed to discover out more about the globe, its instantaneous space environment, solar system, and the cosmos and in addition to develop satellite-based technologies and services	www.envisat.esa.int
MyOcean	Provides access to a range of regional and global ocean color data, including GlobColour	www.myocean.eu.org or
		www.hermes.acri.fr

*Ocean color refers to information in relation to the optical properties of the ocean; it includes parameters like concentration of chlorophyll *a* and suspended sediments
Source: Dean (2007)

from satellite data. The position of cyanobacteria is determined by the image pixel which contains the species information and may link between different images for its better interpretation.

3 Sensors and the Modeling Techniques Used for Algal Remote Sensing

For monitoring purposes along with the study of algal population and their growth, remote sensing sensors are available and used as source of gathering information regarding modeling such that all ease the task. A number of modeling techniques demonstrate that multispectral sensors such as Landsat or MODIS, AVHRR, AVIRIS, and SeaWiFS are ineffectual in differentiating waters subjugated as a result of cyanobacteria within water bodies (details of sensors given in Table 13.3). The sensors meant for the study of algal communities have their own characteristics along with some limitations and advancements. MERIS satellite sensors permit the detection of phycocyanin absorption close to 630 nm, along with a little rise in reflectance spectra that settles to 650 nm which is characteristic of waters dominated by cyanobacteria. Consequently, MERIS can be used in identifying cyanobacteria and other species of algae in the event that they are available in moderately substantial amounts (Table 13.2). This is due to the fact that their spectral band pattern will not allow the identification of absorption features produced via phycocyanin pigment (present within cyanobacteria), other than several additional spectral features that are characteristic of cyanobacterial population. Recently, scientific studies show that monitoring of surface water is capable of taking advantage of remote sensing practice which provides a synoptic view over big areas and repeated acquisitions. For this, in situ data are needed for the calibration and justification of satellite-based products. Both MODIS and MERIS imageries are well suited on behalf of regional assessments of chlorophyll and other optically associated water quality features of large inland lakes, other than their coarse spatial resolution which to a great extent possesses restriction for lakes that can be considered. The spatial resolution derived from Landsat satellites facilitates lakes <4 ha appearing in area to be assessed; however, its low spectral resolution confines it toward assessments of water transparency (Olmanson et al. 2011). Yet, there is proof that remote sensing imagery and analysis possibly will be exploited and meant for the implementation of the EU-WFD (Bresciani et al. 2011). On the other hand, the detection of emerging blooms may perhaps not be possible because the phycocyanin absorption feature merely results in being detectable via MERIS as soon as chlorophyll a concentrations access certain standards of approximation (depending on species). MODIS and MERIS imageries have been used regularly and are meant for global-scale assessments of oceanic and sea chlorophyll (Reinart and Kutser 2006; Kutser et al. 2006; Hansson and Hakansson 2007; Kratzer et al. 2008; Park et al. 2010) other than barely a few fields of study that have examined their use in support of studies of coastal waters and lakes (Alikas and Reinart 2008; Tarrant et al. 2010; Guanter et al. 2010; Matthews et al. 2010; Binding et al. 2011; Olmanson et al. 2011) (Table 13.3).

Results from these concluding studies indicate that MODIS along with MERIS compromises good potential for monitoring colored dissolved organic material (CDOM) and

Table 13.2 Sensor used for the species identification

Sensor used	Worked on
TM, AOCI, SeaWiFS, AVIRIS	Chlorophytes (green algae)
	Ankistrodesmus falcatus, Schroederia setigera, Staurastrum natatura
	Chrysophytes (diatom sp.)
	Melosira granulata
	Cryptophytes
	Cryptomonas pusilla, Cryptomonas ovata
	Cyanophytes (blue-green algae)
	Anabaena flos-aquae, Aphanizomenon flos-aquae
	Chlorophytes (green algae)
	Schroederia setigera
	Chrysophytes (diatoms)
	Fragilaria brevistriata, Fragilaria crotonensis
	Cryptophytes
	Cryptomonas pusilla
	Cyanophytes (blue-green algae)
	Anabaena flos-aquae, Aphanizomenon flos-aquae, Microcystis aeruginosa
Landsat series satellite, ALI, IKONOS, MODIS, MERIS, AVHRR, CZCS, SeaWiFS	*Cyclotella cryptica, Aphanizomenon flos-aquae, Aphanizomenon flos-aquae, Anabaena circinalis, Nodularia spumigena, Scenedesmus obliquus*
AAHIS, AVIRIS, Proto, CRESPO, IKONOS, Landsat-ETM+, SPOT-HRV	Mapping the algae, sand, and coral extent
MODIS, AVHRR, SeaWiFS, MERIS	*Microcystis*, *M. aeruginosa*

Table 13.3 Characteristics of sensors used in algal remote sensing

Satellite sensor	Bands	Resolution		
Landsat		Spatial	Temporal	Radiometric
Thematic Mapper (TM)	Six multispectral bands:	30 m for multispectral 120 m for thermal	16 days	8-bit
	(1) 450–520 nm (2) 520–600 nm			
	(3) 630–690 nm (4) 760–900 nm			
	(5) 1550–1750 nm (6) 2082–350 nm			
	One thermal band: 10,400–12,500 nm			
Enhanced Thematic	Six multispectral bands:	30 m and 15 m for panchromatic	16 days	8-bit
Mapper Plus (ETM+)	(1) 450–515 nm (2) 525–605 nm			
	(3) 630–690 nm (4) 750–900 nm			
	(5) 1550–1750 nm (6) 2090–2350 nm			
	One thermal band: 10,400–12,500 nm			
	One panchromatic band: 520–900 nm			
Coastal Zone Color Scanner (CZCS)				
	Five multispectral bands:	825 m for all bands	26 days	8-bit
	(1) 433–453 nm (2) 510–530 nm			
	(3) 540–560 nm (4) 660–680 nm			
	(5) 700–800 nm			
	One thermal band: 10,500–12,500 nm			
SeaWiFS				
	Eight multispectral bands:	1.1 km for all bands	1–2 days	10-bit
	(1) 402–422 nm (2) 433–453 nm			
	(3) 480–500 nm (4) 500–520 nm			
	(5) 545–565 nm (6) 660–680 nm			
	(7) 745–785 nm (8) 845–885 nm			

(continued)

Table 13.3 (continued)

Satellite sensor	Bands	Resolution		
Landsat		Spatial	Temporal	Radiometric
IKONOS				
	Four multispectral bands:	4 m for multispectral	<3 days	11-bit
	(1) 445–516 nm (2) 506–595 nm	1 m for panchromatic		
	(3) 632–698 nm (4) 757–853 nm			
	One panchromatic band: 450–900 nm			
QuickBird				
	Four multispectral bands:	2.44 m for multispectral	1–5 days	11-bit
	(1) 450–900 nm (2) 520–595 nm	0.61 m for PAN		
	(3) 630–690 nm (4) 760–900 nm			
MERIS				
	15 Multispectral bands:	From 300 to 1200 m	3 days	12-bit
	(1) 407.5–4175 nm (2) 437.5–447.5 nm			
	(3) 485–495 nm (4) 505–515 nm			
	(5) 555–565 nm (6) 615–625 nm			
	(7) 660–670 nm (8) 677.5–685 nm			
	(9–15) 700–905			
MODIS				
	36 Multispectral bands:	250 m for bands 1 and 2	1–2 days	12-bit
	(1) 620–670 nm (2) 841–876 nm	500 m for bands 3–7		
	(3) 459–479 nm (4) 545–565 nm			
	(5) 1230–1250 nm (6) 1628–1652 nm			
	(7) 2105–2155 nm (8) 405–420 nm			
	(9) 438–448 nm (10)483 493 nm			
	(11) 526–536 nm (12) 546–556 nm			
	(13) 662–672 nm (14) 673–683 nm			
	(15) 743–753 nm (16) 862–877 nm			
	(17–36) 890–14,385 nm			

Sources: Jensen (2007), McClain et al. (1992, 1998), Hooker et al. (1992), and Rocchio (2010)

chlorophyll. MERIS is maybe best suitable among these sensors used for keeping track of coastal inland water quality, through a full resolution of approximately 260×300 m by the side of nadir and 15 spectral bands invisible and NIR wavelengths. MERIS and MODIS satellite imageries help further research to determine their functionality for addressing issues for keeping track of cyanobacteria blooms. This kind of research would include the diligence of algorithms developed and validated in concerned lake from airborne imagery and in situ data.

4 Remote Sensing Approaches Adopted Worldwide

It is very important to know and get all the information about the previous and ongoing research worldwide for monitoring of algal bloom with the help of remote sensing. It is also valuable to consider methodology adopted, type of sensor needed, study outcomes, study area, and contributions of the many scientists in the field of remote sensing for the study of algal blooms (Table 13.4). Many studies and researches have already been done and many approaches are still in a way to come with great advancements and upgrades.

The improved quality of the data products of MERIS is relevant for the algal bloom detection and monitoring system (Folkestad 2005). By the help of optical methods like aerial photography or satellite imagery in Indian River Lagoon (Florida, USA) (Riegl et al. 2005). Spectral reflectance data are useful for remote sensing of shallow habitats and used to monitor their health in Western Atlantic subtropical/tropical region (Thorhaug et al. 2007).

Table 13.4 Methodologies adopted for monitoring algal community

Author	Methodology	Study	Sensor/data	Study area
Dustan et al. (2001)	Change detection analysis	The spatial variations of the reef community decreased in the early 1980s at consistent scales with well-known ecological changes to the coral community	Landsat TM	Largo, Florida
Nayak and Bahuguna (2001)	Spatial analysis	Mapping and extent of stressed area	Indian Remote Sensing Satellite (IRS)	Many coastal regions of India
Kutser et al. (2003)	Reflectance analysis	It provides the ability of optical remote sensing to distinguish between the various building blocks of a coral reef ecosystem	Hyperion HyMap ALI	Great Barrier Reef (GBR), near Townsville
Hochberg and Atkinson (2003)	Reflectance, discrimination, spectral mixture analysis	Available satellite sensor that is insufficient for assessment of global coral reef condition but that it is both essential and possible to design a sensor system suited for various works	AAHIS AVIRIS Proto CRESPO IKONOS Landsat-ETM+ SPOT-HRV	Reefs around the world
Tang et al. (2004)	Oceanographic studies, chlorophyll analysis, and wind detection	Better understanding of the biological oceanography of HABs	SST imagery QuikSCAT data and satellite data of chl a	Southeastern Vietnam
Folkestad (2005)	Specific inherent optical properties (SIOP)	MERIS improved quality of the data products relevant for the algal bloom detection and monitoring system	SeaWiFS MODIS and MERIS	Norwegian Coastal
Riegl et al. (2005)	Optical methods like aerial photography or satellite imagery	A high level of settlement (60 %) with the actual distribution of algae. The study conducted including the actual distribution of algal blooms	QTC survey that acquires acoustic data and AUW-5600 video imagery	Indian River Lagoon (Florida, USA)
Li et al. (2006)	Band ratio detection, chlorophyll analysis	A method for real-time mapping of algal blooms in turbid beachfront waters utilizing the remote detecting reflectance of red band and near-infrared band	AVHRR sensor on the NOAA series satellite	China
Reinart and Kutser (2006)	Chlorophyll concentration analysis, bio-optical modeling, and comparative analysis of different	The great ability of the MERIS and MODIS fine determination groups to identify cyanobacterial blossom quantitatively is shown	Hyperspectral sensor SeaWiFS, MODIS/ Aqua and MERIS	Western part of the Gulf of Finland
Thorhaug et al. (2007)	Spectral reflectance analysis	Spectral reflectance data useful for remote sensing of shallow habitats and used to monitor their health.	UniSpec and spectral reflectance data	Western Atlantic subtropical/tropical
Lekki et al. (2009)	Reflectance analysis	The largest area of variance is in the HSI showing a higher reflectance of blue light than the in situ measurement	Hyperspectral imager (HSI)	Great Lakes
Tyler et al. (2010)	Spectral data and using lookup table approaches. Acoustic	This is an advanced objective for remote sensing and will usually be constrained to the shallowest 10–15 m of the reef and used for habitat mapping and for predicting bloom types	High-resolution satellite (e.g., QuickBird), Airborne, Acoustic, CASI, or HyMap	Reef
Ontract (2011)	Acoustic survey, data processing, interpreting the classification of the training dataset recall, SAV coverage maps, classifying hydroacoustic records discriminant	Understood the goal of founding an accurate, well-organized, and temporally reliable manner for acoustically mapping drift macroalgae biomass	Sonar equipment	Indian River Lagoon

(continued)

Table 13.4 (continued)

Author	Methodology	Study	Sensor/data	Study area
Shanmugam (2011)	Advancement of new algorithms for reflectance analysis and chlorophyll concentration	To be talked in order for the major monitoring sequencers to help safeguard marine ecosystems and to safeguard and preserve sustainable development, the economy of the country, and the environment of the region	High-resolution MODIS/Aqua level 1A	Oceans worldwide
Hamylton et al. (2012)	Classic ordinary least squares and spatial autoregression techniques	Spatially lagged model, assume a vital part in deciding benthic front of the Aldabra tidal pond	Using GIS techniques (water level variation) and satellite remote sensing data (water depth)	Aldabra lagoon
Simon (2012)	Algorithm used to make the use of reflectance properties of algal community, spectral analysis	The new algorithm has the promise to classify and monitor the examined algal blooms	MODIS-Aqua ocean color data	Coastal waters around India
Riha (2013)	A novel model-based inversion algorithm using neural network technique	Algae expansion having chlorophyll fluorescence detected by the sensors of satellite help in measuring chlorophyll concentration	MERIS	Baltic Sea

5 Challenges and Limitations of Remote Sensing for Algal Monitoring

5.1 Challenges

- Identification and acquirement of satellite imagery for specific HABs
- Extracting of thresholds and expressive terms for the study of blooms
- Extraction of cyanobacteria and chlorophyll indices for cell counts
- The HAB models accounting for variation in chlorophyll abundance within a species
- Information gathering for tributary-specific and regional-specific HAB
- Forecasting models for the bays and tributaries for the study

5.2 Limitations

- Data acquisition is inadequate to specific missions to survey specific sites.
- Moving algal communities in water bodies sometimes do not coincide to the data acquisition process, creating difficulties in monitoring and proper observations.
- Sometimes, remote sensing approach is unable to correlate with in situ measurement due to disappointment in environmental adjustment; this failure is due to divergence between the optical properties.
- More advancement may be needed for all prospective and skills also compensate errors.
- The major concern is the data consistency in data acquisition.
- It is difficult to identify a cause of the variation at the largest spatial scale (we had only three replicate sites) and to correlate the sensitivity of a site with environmental variables like coastal, geomorphology, height, and orientation.
- The biomass and productivity data does not resolve the influence of water movement on productivity.
- Available satellite sensor is insufficient for appraisal of worldwide coral reef status, yet it is both essential and possible to design a sensor system suited to the task.
- Other major difficulties lie in the assortment of remote sensing imagery of suitable spectral, spatial, and temporal resolution for HAB study. Spectral resolution is an additional main worry for remote sensing of algal community.

6 Conclusion

The applicability of many remote sensing sensors in the field of monitoring algal communities is discussed in the articles, and it also comprises previous literature about remote sensing study for algal bloom. Although the use of remote sensing techniques for the study related to algal species is increasing rapidly, some techniques fail in accurate monitoring issues and are difficult and costly. Remote sensing plays vital role in

detection and monitoring of algal community, which seems to be very harmful and expanding rapidly in water bodies. Since algal bloom causes much harm like eutrophication of lakes, it leads to bad quality of water and also harms aquatic organism associated with it. Therefore, its monitoring and detection is very important. Harmful algal bloom is one of the global concerns, and a lot of efforts are taken to minimize the load of nutrients and reduce the expansion of algal blooms in water bodies. For this purpose, the monitoring programs and researches are carried out for detecting and mapping the extension of algal blooms. Some satellite sensor and data are easily available for the study of algal blooms, but with the advancement in remote sensing, a lot of powerful sensors and technologies are developed but extra charge is required for investigating the algal communities. The chlorophyll present in the algal cell pigments, the color, and the reflectance and absorption properties of algae are the principle source of information required for algal community explorations. The sensor of MERIS is considered useful as its chlorophyll observation bands are very accurate to keep track of chlorophyll concentration found in algae.

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Life Cycle Assessment of Algal Biofuels

14

Dipesh Kumar, John Korstad, and Bhaskar Singh

1 Introduction

First-generation biofuels are produced directly from food crops (e.g., biodiesel from soybean or rapeseed and ethanol from corn or sugarcane) and have a number of associated problems. However, the most problematic issue with first-generation biofuels is the "fuel vs. food" controversy. Production of biofuels from crop plants has resulted in an increase in diversion of crops from the global food market towards biofuel generation and resultant escalation of food prices.

Second-generation biofuels have been developed to overcome the limitations of first-generation biofuels. They are produced from non-food crops, therefore eliminating the main problem with first-generation biofuels, but they can potentially cause large-scale land use and land cover changes as vast land area would be required for meeting the growing demand of biofuels. Land use changes have the potential to offset the greenhouse gas balance of second-generation biofuels. High water, fertilizer, and pesticide use are major concerns.

Biofuels from algae are among the third generation of biofuels and have several advantages over first- and second-generation biofuels including higher productivity over terrestrial counterparts. Research has shown that microalgae are comparatively more suited as feedstock for large-scale biofuel production than their terrestrial counterparts. Suitability of algae as biofuel feedstock is attributed to its (1) higher CO_2 assimilation rate and photosynthetic efficiency, (2) high lipid accumulation, (3) minimal competition with food crops, (4) minimal land use changes, (5) ease to cultivate and metabolically manipulate, and (6) ability to utilize wastewater and saline water for growth. Although algae offer immense potential for exploitation as a biofuel feedstock, cultivation of algae, concentration and harvesting of biomass, extraction of lipids, and processing of biomass to biofuel remain highly energy-intensive processes. It is necessary to estimate material and energy inputs and associated environmental impacts of algal biofuels based on the concept of LCA in order to determine its suitability over fossil fuels and biofuels from non-algal biomass.

2 Life Cycle Assessment (LCA)

As with most of the industrial products (including biofuels), the impact associated with only the end use of product does not give a true picture of its environmental performance as impacts associated with its production, transportation of extracted raw materials and end product to the point of use or distribution, and its final disposal into the environment may have negative impacts. Therefore, it is vital to also consider processes upstream of product use for their environmental performance in order to get a holistic view of the impacts. LCA has emerged as a tool of choice for assessing the environmental performance of a production chain, process, or policy throughout its life cycle—from extraction of raw materials, production of energy used to create the product, production of goods and services, transportation to the point of use or distribution system, product use, to its final disposal into the environment. LCA is a systematic set of procedures for compilation and examination of the inputs and releases of energy and materials and the associated cumulative environmental impacts directly attributable to the functioning of a product or process throughout its life cycle (i.e., consecutive and interlinked stages of a product or process system from the extraction of resources to its final use disposal). Thus, LCA provides a holistic and comprehensive evaluation of environmental burdens associated with a production system or service and can help avoid a narrow outlook on environmental concerns (Fig. 14.1).

D. Kumar • B. Singh
Centre for Environmental Sciences, Central University of Jharkhand, Ranchi 835205, Jharkhand, India

J. Korstad (✉)
Department of Biology and Chemistry, Oral Roberts University, 7777 South Lewis, Avenue, Tulsa, OK 74171, USA
e-mail: jkorstad@oru.edu

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LCA is based on comparison of alternative products, products from alternative sources, or different production techniques in terms of their environmental benefits. LCA is mostly used to compare different processes, products, or services that deliver similar functions. However, LCA can also be used as an independent tool that can help identify hotspots in a production system (Gasafi et al. 2003). When comparing and selecting between different alternative options, LCA allows the decision-makers to select alternative options that are most environment friendly. LCA studies can scrutinize all of the steps based on inputs and outputs and thus allow us to identify major environmental burdens associated with individual steps and also highlight improvement opportunities to increase the environmental sustainability of the process system. Some stages of a product's life cycle may be more troublesome for the environment than the other and thus suggest stepwise improvement opportunities. A full LCA involves a "cradle-to-grave" approach which includes all of the stages of a product, process, or activity encompassing extraction of raw materials from the environment and its processing; manufacturing, transportation, and distribution; use, reuse, and maintenance; recycling; and final disposal into the environment. The basic principle behind LCA is based on the perspective that all stages of a product life cycle are interdependent and one operation or activity leads to the next.

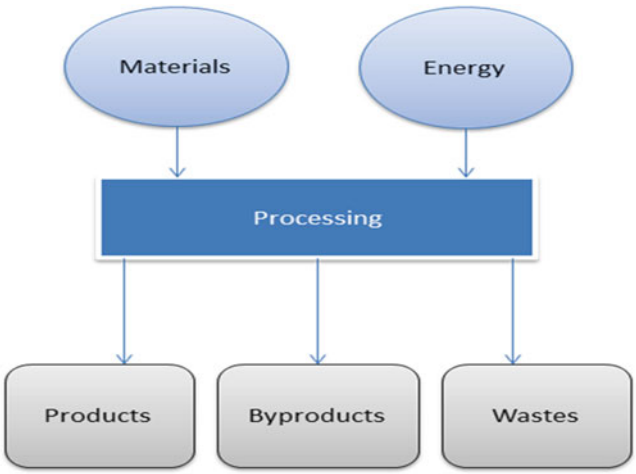

Fig. 14.1 General life cycle inputs and outputs

LCA enables managers to estimate the cumulative impacts resulting from all individual stages in a product life cycle, as it includes impacts usually not considered in traditional approaches of impact assessment such as raw material extraction, material transportation, product manufacturing, ultimate product disposal, etc. LCA therefore provides a comprehensive and analytical view of environmental aspects of the system and an accurate picture of the true environmental trade-offs in product and process systems.

LCA involves the following four types of activities in sequential order: (1) goal and scope definition of the LCA, (2) collection of life cycle inventory data on material and energy flow and environmental releases, (3) life cycle impact assessment based on inventory data, and (4) analysis of the major findings to support decision-making.

LCA can provide a comprehensive analysis of environmental impacts associated with a process or a production chain throughout its life cycle, but *data* and *knowledge* limitations imply that LCA entails selection of a "system boundary" that delineates processes included in the analysis versus those excluded which is usually based on a cut-off value. Cut-off criteria are often included in LCA studies for boundary delineation. For example, Sills et al. (2012) included life cycle inventory of all relevant energy and material inputs, with a 5 % cut- off for each unit process. Since LCA is a relative approach, it involves a reference system against which all of the products or processes delivering similar functions are compared to ascertain their environmental friendliness over alternatives. Biofuels are compared against their fossil fuel-based counterparts (e.g., petrol vs. ethanol, diesel vs. biodiesel, etc.).

There are four major algal biofuel LCA approaches, including (1) well-to-wheel approach in which cultivation of algae, harvesting of algal biomass, extraction of lipids, processing into biofuels to its end use, and disposal all are included in LCA inventory; (2) well-to-gate approach in which life cycle stages are limited up to biomass production only; (3) pump-to-wheel approach in which only the end use of the biofuel is included; and (4) well-to-pump approach in which LCA is limited to biofuel production and does not consider its end use and disposal (Fig. 14.2). LCA is structured around a functional unit which provides a reference to which all of the inputs and outputs are related. However, a

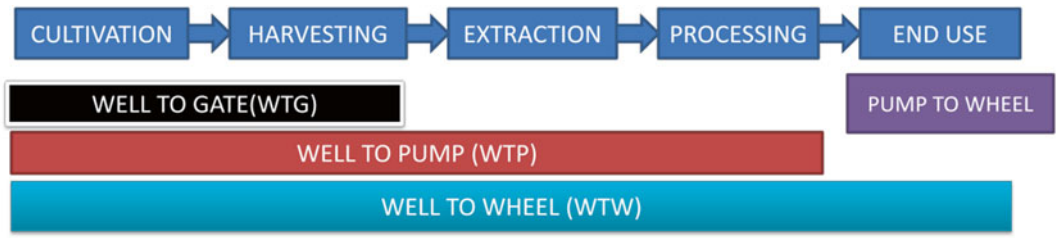

Fig. 14.2 Different LCA approaches for algal biofuels

variety of functional units exist which complicate the comparability among different studies. A diversity of functional units exist for a biofuel production system and may include volume of algal oil or biofuel produced (e.g., 1 L of biofuel), mass (e.g., 1 kg of biofuel or biomass produced), energy content of product (e.g., 1 MJ of biodiesel), energy released upon combustion (e.g., combustion of 1 MJ of methane), distance traveled (e.g., 10 km of traveling), etc. All of the inputs and outputs are compared against the functional unit such as energy required for producing 1 L of biodiesel or amount of greenhouse gasses (GHGs) released per liter of biodiesel produced.

Due to the absence of any large-scale industrial system dedicated to biofuel production, real-life data are unavailable and most of the studies are based on certain assumptions and on extrapolation of lab scale experimental data and software-based modeling.

Accounting for various by-products is an important issue which can sometimes provide misleading results depending on the by-product allocation method employed as it can affect the values of sustainability indicators on which assessment is based. Different allocation approaches include allocation by energy; allocation by economic value; use of residual algal biomass as animal feed, organic manure, and raw material for fermentation or anaerobic digestion; use of glycerol (by-product of transesterification) as an industrial or commercial chemical; recycling of waste heat; etc. Several studies have shown that coproduct allocation by several means is required for net gains in energy.

Although ISO standards (ISO 14044 and ISO 14040) exist which specify the structure for implementing LCA studies and a general approach and methodology to be followed, many important elements are up to the particular researcher, such as coproduct allocation, boundary delineation, impact categories to be included, and selection of a functional unit. This has created a problem in terms of comparability of different LCA studies. Different researchers have used different impact indicators, functional units, boundary delineation criteria, coproduct allocation strategies, different assumptions, and different modeling system and procedures which have resulted in a range of values for impact indicators, even for a particular production chain. Sills et al. (2012) in their work on LCA of algal biofuel production emphasized the need to conduct quantitative uncertainty analysis to better understand variability in LCA results.

Often with algae biofuel LCA the only aspect considered is the climate change impact, usually based on the global warming potential of environmental releases. This impact is clearly important; however, it means that other impacts are often overlooked and is not compliant with ISO 14040/44 (UNEP 2009). To assess the overall environmental sustainability of transportation fuels, LCA practitioners must address other impact categories like energy return on investment (EROI), climate forcing (global warming potential), other pollutant emissions and impacts, impact on water resources, land use changes, nutrient needs, ozone depletion potential (ODP), acidification, eutrophication potential, human and ecological health impacts, and other external costs. Social impacts and economic factors are usually not considered in LCA as accounting for these is difficult; however, several software-based modeling procedures are available which can account for these factors as well (UNEP 2009).

2.1 Algae Cultivation

2.1.1 Appropriate Species

The success of biofuel production from algae is dependent on many factors. Selection of an appropriate algal strain is especially important. An appropriate species and strain should have a clear-cut advantage over others and should (1) have high lipid productivity, (2) be robust and able to survive the stresses common in open ponds and photobioreactors, (3) be able to outcompete wild strains in open pond production systems, (4) have high CO_2 absorption capacity, (5) have limited nutrient requirements, (6) be tolerant to a wide range in temperatures resulting from the diurnal cycle and seasonal variations, (7) provide valuable coproducts, (8) have a fast productivity cycle, (9) have a high photosynthetic efficiency, and (10) have self-flocculation characteristics. These are very demanding conditions.

No known algal strain is capable of meeting all of these requirements. But certain species have a clear advantage over others, which cannot be ignored. Certain cyanobacteria have an inherent capability for atmospheric nitrogen fixation. Therefore, their ability to thrive even under environments lacking readily available nitrogen sources such as ammonia, nitrate, or urea is a potential growth advantage, besides offering economic savings in terms of nitrogenous fertilizer use. However, since nitrogen fixation is an energy-demanding process, biomass and oil production might be reduced.

There are algal species which are known to accumulate high levels of lipids under nitrogen-starved media conditions. Lipid accumulation in microalgae occurs when a nutrient (which is typically nitrogen) is exhausted from the medium or becomes the growth-limiting factor. Under limited nitrogen availability proliferation of algae is hampered but carbon assimilation by the cell is not affected, and it is converted to triacylglycerol (TAG) lipids that are stored within cells, thereby increasing their concentration. Lipids can be processed into biodiesel and "green" diesel via

transesterification and hydrotreating, respectively. TAGs are the best suited lipids for transesterification into biodiesel (Gong and Jiang 2011).

Different species may have different growth rates under identical conditions, and hence selection of an appropriate species is vital. Species having higher productivity or lipid accumulation are more feasible for biofuel production as costs related to infrastructure, nutrients, and water requirements remain virtually the same but decrease inputs in terms of energy requirement per unit of biofuel produced over species having low productivity. Selection of an appropriate species should be based on the composition of biomass under a given growth mode (auto-/hetero-/mixotrophic), culture system (open/closed ponds), nutrient availability (with/without N stress), and the intended product.

2.1.2 Autotrophic Growth Mode

Algae are chlorophyll-bearing cells that are capable of photosynthesizing carbohydrates using CO_2 and water in the presence of photosynthetically active radiation from sunlight or an artificial source. Algae have a comparatively higher photosynthetic efficiency (fraction of light energy that is fixed as chemical energy during photoautotrophic growth) than terrestrial plants owing to their simpler structure. This allows microalgae to achieve a higher productivity rate. During exponential growth phase microalgae can double their biomass in periods as short as 3.5 h (Chisti et al. 2007; Spolaore et al. 2006). Use of solar radiation is economically superior to artificial illumination, but spatial and temporal variability in amount of sunlight is problematic. Besides economic constraints associated with artificial illumination, its environmental performance depends on the local energy mix. Bioelectricity generated from direct combustion of unutilized algal biomass can be used to power fluorescent lamps for providing artificial photosynthetically active radiation in a biorefinery-based approach. Biological H_2 production can be achieved via algal biophotolysis using solar radiation. This can be achieved by inducing sulfur stress which inhibits O_2 mobility, which otherwise disrupts the conversion of H^+ to H_2 (Melis and Happe 2001). This leads to biological H_2 production that is one of the cleanest fuels. Combining hydrogen production through algal biophotolysis with other algal biomass-based production systems can significantly help improve energy return on investment (EROI) and other sustainability parameters of biomass-based production chains.

2.1.3 Heterotrophic Growth Mode

A number of microalgae are capable of growing heterotrophically on organic substrates and thus do not depend on sunlight for energy. Carbon in some form is necessary to provide the energy and carbon skeletons for cell growth. Heterotrophic algae derive their energy from organic substrates (often provided in the form of acetate or glucose) (Vazhappilly and Chen 1998). Other carbon sources include carbohydrates such as fructose, sucrose, lactose, and starch. C/N ratio is an influencing factor which affects cellular lipid content as it controls the switch between lipid and protein syntheses (Gordillo et al. 1998). Nitrogen deficit (high C/N ratio) in the culture media triggers lipid accumulation (Pal et al. 2011). Several researchers have suggested higher technical viability of heterotrophic production mode compared to photoautotrophic methods (Graverholt and Eriksen 2007; Xiong et al. 2008; Chojnacka and Noworyta 2004). Miao and Wu (2006) reported lipid content of 55 % when *C. protothecoides* was grown heterotrophically and only 15 % when grown photoautotrophically under similar conditions. Hence, heterotrophic cultivation of some algae could result in higher biomass production and high lipid accumulation in cells. Generally, an organism used for heterotrophic production should possess the following characteristics: (1) the ability to divide and metabolize in the dark, (2) the ability to grow on inexpensive media, (3) short or no lag phase when inoculated to fresh media, and (4) the ability to tolerate hydrodynamic stresses in fermenters and related peripheral equipment.

In microalgal culture, heterotrophic growth can be a cost-effective alternative to photoautotrophic growth. This mode of culture eliminates the requirement for light and, hence, offers the possibility of greatly increasing cell density and productivity (Chen et al. 1996). Heterotrophic algal cultivation can be carried out at large scale in stirred tank bioreactors or fermenters. Although technically viable, the energy required for producing an organic carbon source for algal growth and the related environmental impact can potentially offset the benefits obtained. Hence, exploration and development of an organic carbon source from waste materials is important.

2.1.4 Mixotrophic Growth Mode

Some algae are mixotrophic (i.e., they have the ability to photosynthesize and acquire exogenous organic nutrients heterotrophically) (Lee 2001). Certain algal species like the cyanobacteria *Spirulina platensis* and the green alga *Chlamydomonas reinhardtii* are well-known examples possessing this ability (Chen et al. 1996). This means that light is not an absolute limiting factor for algal growth. This allows for the integration of both photosynthetic and heterotrophic components during the diurnal cycle and during limited light availability conditions. This reduces the impact of biomass loss during dark respiration and decreases the amount of organic substances utilized during growth (Brennan and Owenda 2010). Chojnacka and Noworyta (2004) studied *Spirulina* sp. and reported improved growth rates over both autotrophic and heterotrophic cultures when compared to mixotrophic culture.

2.1.5 Open Pond Production Systems

Open ponds are shallow (usually 25–35 cm deep) circuits, raceways, or tanks wherein the contents of the pond are cycled continuously around the circuit by the action of a paddlewheel. Even mixing of inputs is achieved by a paddlewheel. Inocula produced in smaller ponds or photobioreactors are fed into open ponds to cultivate algae. Open ponds are the cheapest production system employed for large-scale algal cultivation. They do not necessarily compete with arable land since they can be installed in areas with little crop production potential (Chisti et al. 2008). They also have lower energy input requirement (Rodolfi et al. 2008), and regular maintenance and cleaning are easier and therefore may have the potential to return large net energy production (Ugwu et al. 2008). In all open pond systems the amount of sunlight, temperature, nutrient level, and water chemistry will change with the seasons and fluctuations in weather, thus impacting growth. Frequent cleaning and maintenance to deal with challenges from climate, competitors, grazers, and pathogens are inherent challenges associated with open pond production system. Some areas of the world will provide more uniform environments that reduce the complexity of pond management. However, even in the most favorable climates, continuous operation of raceway ponds for 365 days of the year without significant intervention (i.e., draining, cleaning, refilling, and inoculating) is unlikely to be achievable. Continuous operation of a paddlewheel to keep the contents in suspension is an energy-intensive process. Capturing H_2 produced by algae-mediated biophotolysis can be a difficult and energy-intensive affair. CO_2 utilization rates for open systems are comparatively lower than other systems because of its diffusion into the atmosphere and poor mass CO_2 transfer rates. This can result in lower biomass production (Ugwu et al. 2008).

2.1.6 Photobioreactors

Photobioreactors consist of an array of glass or plastic tubes (with a diameter of 0.1 m or less) in which the tubular array captures sunlight and can be aligned horizontally, vertically, inclined, or as a helix. Photobioreactors are designed to overcome some of the major problems associated with the open pond production systems as photobioreactors permit culture of single species of microalgae for prolonged durations with lower risk of contamination. These systems are more appropriate for sensitive strains as the closed configuration better assures control of potential contamination. Photobioreactors are more efficient than open pond system in terms of biomass productivity. Owing to the higher cell mass productivities attained, harvesting costs can also be significantly reduced. Algal cultures are recirculated either with a mechanical pump or airlift system. The airlift system allows CO_2 and O_2 to be exchanged between the liquid medium and aeration gas as well as provides a mechanism for mixing. Mixing and agitation are important to encourage gaseous exchange in the tubes. However, the costs of closed systems are substantially higher than open pond systems (Carvalho et al. 2006). This system, although more efficient than open systems, has considerably higher energy demand.

In a comparative life cycle energy analysis of the *Nannochloropsis* biomass production in photobioreactors and open ponds, Orlando et al. (2010) reported EROI >1 of biomass and lipid produced for photobioreactors and open ponds but that the open pond performed better than photobioreactors with EROI values of biomass and lipid produced 2.56 and 7.01, respectively. The total energy input for producing biomass in open ponds and photobioreactors was 450 and 729 GJ/year, respectively.

2.1.7 Hybrid Production Systems

Hybrid production systems combine distinct growth stages in photobioreactors and in open ponds. The first stage of growth is usually in a photobioreactor where carefully controlled condition allows for optimal growth, and this is followed by cultivation in open systems in which the algae can be subjected to nitrogen stress for enhanced lipid accumulation or sulfur stress to produce H_2 gas. Rodolfi et al. (2008) described a hybrid production system in which cultivation was carried out in photobioreactors followed by open ponds. 22 % of the plant was dedicated to a photobioreactor under N-sufficient conditions and 78 % dedicated to open pond under N-deficient conditions. He estimated lipid production equivalent to be 90 kg ha^{-1} day^{-1} (10 and 80 kg ha^{-1} day^{-1} in the first and second stage, respectively).

2.1.8 Stirred Tank Bioreactors or Fermenters

Stirred tank bioreactors or fermenters are suitable for heterotrophic algae cultivation. The scale-up possibilities are much simpler for these systems than photobioreactors, as growth is independent of light, which enables smaller reactor surface-to-volume ratio. High cellular densities are achievable as these systems allow for a high degree of growth control and consequently lower harvesting cost. The setup cost is comparatively lower, but the initial production of organic carbon sources is energy intensive (Christi et al. 2007).

2.1.9 Water

Like all life, water is a major and important constituent of algal cells. Fresh water, saline water, and even wastewater (usually after secondary treatment) can be used to meet cultivation water demand. This is in contrast to cultivation of terrestrial plants, which usually have high fresh water demand, and only a limited number of plants can be grown using saline water. Although large quantities of water are usually required for algal cultivation in open ponds, this water is essentially recyclable and can be used repeatedly. However, evaporation loss in open ponds has a cooling effect

and adds to an increase in the water footprint. Further, inputs from precipitation and runoff must be regulated to prevent drastic changes in culture media composition. Several algal species flourish in salt water that is readily available in coastal localities. Wastewater has been successfully employed to cultivate algae for biofuel production. Wastewater after secondary treatment is usually fit for culturing algae, and this can be used as a bio-treatment method for wastewater treatment. Biofuel production in conjunction with wastewater treatment can minimize the impacts associated with chemical remediation and provide economic returns (Christenson and Sims 2011).

Yang et al. (2010) reported that using fresh water requires 3726 kg of water to produce 1 kg of algal biodiesel if harvested water is not recycled. He reported a decrease in water demand by 84 % if recycling of water is practiced, while wastewater and saline water usages can decrease the water demand by 90 %. The O_2 released by microalgae assists aerobic bacteria in biodegrading pollutants, thus lowering the BOD and COD of wastewater. After harvesting and dewatering of algal biomass, the remaining water can be used several times. But this would necessitate the use of pumps which can significantly affect the energy balance of the system.

2.1.10 Nutrients

Nitrogen, sulfur, and phosphorus are among the major nutrients ("macronutrients") required for algal growth in relatively large amount. Nitrogen-based synthetic fertilizers are derived almost exclusively from ammonia. Ammonia is synthesized via the Haber process where nitrogen and hydrogen react in the gas phase to form ammonia. Steam reforming of methane produces H_2, which reacts with nitrogen present in air to form ammonia. Natural gas serves both as feedstock and process heat source and can be sourced from a variety of sources. Gasification (partial oxidation) remains the second preferred option after steam reforming to obtain hydrogen. Energy associated with ammonia production dominates the life cycle energy usage for the nitrogen fertilizers most relevant to algae. Jensen and Nielsen (2003) reported industry energy input averages as 36 MJ kg-ammonia^{-1} for European plants and 38 MJ kg-ammonia for the US operational plant energy use. Production plant age is a defining factor in estimating energy efficiency (Johnson et al. 2013). Urea, ammonium monophosphate, ammonium diphosphate, ammonium polyphosphate, ammonium nitrate, and ammonium sulfate are all derived from ammonia. Urea is manufactured by reacting ammonia with carbon dioxide. Kongshaug (1998) reported average Western Europe urea process energy at 4.13 MJ kg-urea^{-1}, and Davis and Haglund (1999) reported the total energy for older, less efficient plants to be 4.58 MJ/kg-urea. Process energy breakup is 3.70 MJ kg-urea^{-1} for process heat from steam and 0.53 MJ kg-urea^{-1} for electricity. The nitrogenous solution consisting of urea and ammonium nitrate (UAN) is produced by blending urea(s) and ammonium nitrate(s) or by adding urea into hot ammonium nitrate. Typically, UAN is prepared from 39 to 45 % ammonium nitrate and 31 to 36 % urea and contains approximately 28 to 32 % N by weight. Nitrate is usually manufactured from nitric acid (ammonium nitrate, calcium nitrate, nitro phosphate, and potassium nitrate).

The oxidation steps in nitric acid production release heat that can be used to produce steam to be used for other purposes including electricity generation (Johnson et al. 2013). Davis and Haglund (1999) reported direct energy inputs for nitric acid production to be 0.032 and 1.47 MJ kg-HNO_3^{-1} for electricity and steam heat export, respectively. Sulfuric acid manufacturing is a highly exothermic process. Phosphoric acid production requires heat for evaporation, so heat integration between the two processes is desirable (Table 14.1).

Higher nonrenewable energy demand leads to higher emission of greenhouse gases (GHGs). Handler et al. (2012) reported greenhouse gas (GHG) emissions associated with nitrogen fertilizers range from 2.6 kg CO_2e kg-N^{-1} to 16 kg CO_2e kg-N^{-1} depending on fertilizer and its nitrogen content, where mass of CO_2 equivalent (CO_2e) is the global warming potential of all emissions (Table 14.2).

Water remaining after harvest of algal biomass can be reused several times, and after lipid extraction and anaerobic digestion of the algal biomass the residual biomass can be effectively recycled back to cultivation medium to meet some of the nutrient demand, and this can reduce the energy demand and related emissions associated with synthetic fertilizers. Wastewater resources are often loaded with excess

Table 14.1 Values of direct material and energy inputs in fertilizer production (Johnson et al. 2013)

Product	Material input	Total direct energy input
Ammonia	–	37.0 MJ/kg-ammonia
Urea	Ammonia 0.567 kg/kg-urea	5.16 MJ/kg-urea
Nitric acid	Ammonia 0.288 kg/kg-HNO_3	0.032 MJ/kg-HNO_3
Ammonium nitrate(s)	Ammonia 0.213 kg/kg-AN, Nitric acid 0.787 kg/kg-AN	0.99 MJ/kg-AN
UAN	Ammonia 0.567 kg/kg-product, Ammonium nitrate 0.788 kg/kg-product	0.018 MJ/kg-AN
Monoammonium phosphate	Phosphoric acid 0.53 kg/kg-MAP, Ammonia 0.133 kg/kg-MAP	0.43 MJ/kg-MAP
Diammonium phosphate	Phosphoric acid 0.477 kg/kg-DAP, Ammonia 0.220 kg/kg-DAP	0.37 MJ/kg-DAP
Sulfuric acid	–	0.109 MJ/kg H_2SO_4

Table 14.2 Noncombustion process emissions associated with fertilizer production (Johnson et al. 2013)

Fertilizer	Air pollutant	g-emission/kg-product
Ammonia	CO	4
	VOC	4.7
Nitric acid	N_2O	7.8
Phosphoric acid	CO	3.9e−2
	VOC	3.0e−2

nutrients, which can potentially reduce the dependence on synthetic fertilizers if used for culturing algae. This brings about wastewater treatment and reduces impact on freshwater resources simultaneously (a "win-win" or mutually beneficial process). In their LCA uncertainty analysis, Sills et al. (2012) reported nonrenewable energy demands for cultivation of algae ranging from 1.7 to 4.9 (low productivity 2.4–16 g m^{-2} day^{-1}), 0.94 to 1.8 (base productivity of 17–33 g m^{-2} day^{-1}), and 0.7 to 1.3 MJ (high productivity of 34–50 g m^{-2} day^{-1}) per MJ biofuel produced.

Yang et al. (2010), in their LCA study, reported a nutrient demand of 0.33 kg nitrogen, 0.71 kg phosphate, 0.58 kg potassium, 0.27 kg of magnesium, and 0.15 kg sulfur for producing one liter of algal biodiesel using freshwater without recycling. They reported a decrease in nutrient demand by 55 % in harvested water that is recycled, and if wastewater or saline water is used, nutrient requirement is minimal except for phosphates.

2.1.11 Carbon Dioxide (CO_2)

During normal photoautotrophic growth, CO_2 dissolved in the water is captured along with sunlight by microalgae to produce carbohydrate via photosynthesis. CO_2 from three sources can be provided during cultivation—atmospheric CO_2, CO_2 from industrial emission or coal-fired thermal power plants, and CO_2 from carbonates (Na_2CO_3 and $NaHCO_3$). Limited biomass productivity can be achieved via utilization of atmospheric CO_2 because of lower concentration (390 ppm). Higher CO_2 usually translates into higher productivity under optimal growth conditions. CO_2 concentration up to 150,000 ppm can be easily utilized by most microalgal species. Therefore, waste CO_2 from combustion processes can be effectively sequestered by microalgae; however, only a small number of algal species are tolerant to the high levels of SOx and NOx that are usually present in flue gases. Cooling of flue gas stream is also often a prerequisite.

Algal species are also known to assimilate CO_2 from soluble carbonates such as bicarbonate and carbonate of sodium ($NaHCO_3$ and Na_2CO_3). These compounds raise the alkalinity of water and can bring about cost-effective concentrating with chemical flocculants. Higher pH can also reduce contamination possibilities due to other unwanted species (Wang et al. 2008). Several authors have reported improved environmental performance of coal-fired thermal power plants if algal cultivation is utilized for biomitigation of CO_2 and biomass cultivation.

2.1.12 Light

Light is not an absolute limiting factor for algal cultivation as heterotrophic and mixotrophic growth modes are well established. Sunlight has spatiotemporal variability in its availability. Hence, artificial illumination of the culture medium can be performed depending on absorption characteristics of algal pigments. Light penetration in open ponds is limited, which can affect biomass production. Thus, photobioreactors are more suited for photoautotrophic growth. However, photobioreactors are expensive and more energy intensive than open ponds. Heterotrophic growth in fermenters is independent of light, and high lipid accumulation can be achieved, but production of carbon source is energy intensive (Fig. 14.3).

Large-scale biofuel production would require sustainable industrial algal cultivation pathways which at the same time should be economically viable. Researchers have come up with different cultivation practices to enable industries to choose the pathway most suited to them involving a combination of approaches having different ratings for environmental sustainability and economic feasibility. There are a variety of cultivation pathways, involving different light requirements (solar, artificial, or dark), different growth modes (auto-, hetero-, or mixotrophic), different culture system (open pond, photobioreactor, fermenter, or a combination of these at different stages of growth), nitrogen/sulfur stress at times, etc. Thus, different combinations of approaches yield different values for sustainability indicators (e.g., EROI, GHG balance, or water footprint). Therefore, a holistic evaluation approach is vital. Selection of a particular approach would depend largely on the type of industry and the intended end product. The concept of biorefinery would be of great help to achieve economical production of a number of products and can potentially help offset environmental trade-offs associated with a particular production chain.

2.2 Harvesting Algal Biomass

There are a variety of harvesting methods for microalgae, and the choice is dependent on characteristics of the microalgae (e.g., density, size, and the value of the target products). Harvesting of algal biomass involves solid–liquid separation steps and is a challenging part of the production chain that can have a high impact on the resultant LCA. Harvesting may account for as much as 20–30 % of the total cost of production (Gudin and Therpenier 1986) and a proportionate energy input requirement. The selection of

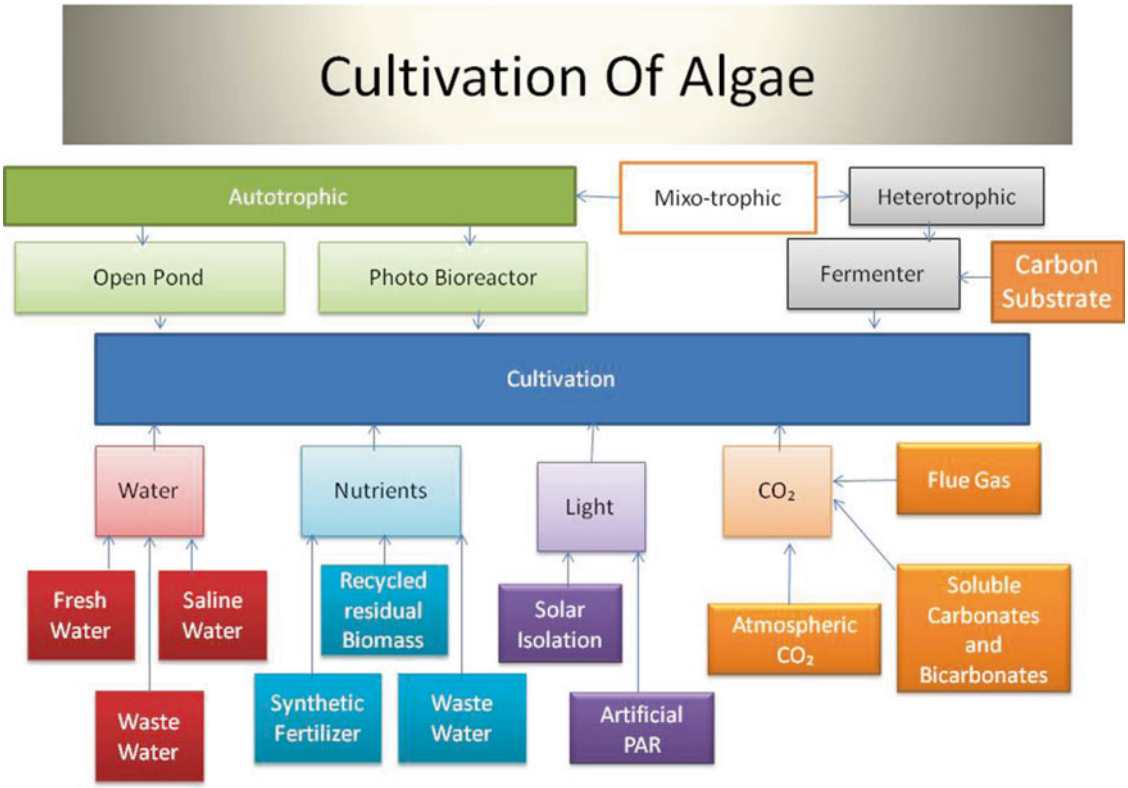

Fig. 14.3 Algal cultivation options

an appropriate harvesting technology is crucial to economic production of algal biomass. Algal species having spontaneous settling or bio-flocculation characteristics are inherently suited for easy, effective, and environmentally friendly harvesting. Certain species such as the cyanobacterium *Spirulina*, which have a long spiral shape (20–100 mm long), can be concentrated with the relatively cost-efficient and energy-efficient microscreen harvesting method (Benemann and Oswald 1996). Harvesting methods usually employed include flocculation, filtration, flotation, and centrifugal sedimentation, some of which are highly energy intensive. Microalgae cells carry a negative charge that prevents natural aggregation of cells in suspension. Addition of multivalent metal salts like ferric chloride ($FeCl_3$), aluminum sulfate ($Al_2(SO_4)_3$), and ferric sulfate ($Fe_2(SO_4)_3$) neutralizes or reduces the negative charge and thus promotes flocculation and subsequent sedimentation under gravity. Most of the chemical flocculants are most efficient under alkaline conditions, and thus careful monitoring and control of media pH is vital. Acoustically induced aggregation involves use of ultrasound to optimize the aggregation efficiency and subsequent sedimentation. Harvesting by flotation is based on the trapping of algal cells using dispersed micro-air bubbles and, therefore, unlike flocculation, does not require any addition of chemicals. Some strains naturally float at the surface of the water as the algal lipid content increases (Bruton et al. 2009). Centrifugation is one of the most efficient harvesting methods in which harvesting efficiencies of >95 % and increased slurry concentration by up to 150 times is achievable. Centrifugation recovery is preferred for harvesting of high-value metabolites and extended shelf-life concentrates for hatcheries and nurseries in aquaculture. The process is rapid but highly energy intensive (Grima et al. 2003). In addition to these high energy costs, other disadvantages include potentially higher maintenance requirements due to freely moving parts.

Biomass filtration under pressure or suction is most appropriate for harvesting of relatively large (>70 mm) microalgae such as *Coelastrum* and *Spirulina*. Mohn (1988) reported a concentration factor of 245 times the original concentration for *Coelastrum proboscideum* to produce sludge with 27 % solids using biomass filtration as the harvesting technique.

2.3 Dehydration of Harvested Biomass

The harvested biomass slurry (typically 5–15 % dry solid content) is perishable and must be processed rapidly after harvest. Dehydration or drying is commonly used to extend the viability depending on the final product required. After the separation of algal cells from the liquid phase, the algal biomass has to be dried using methods like thermo-drying or lyophilization ("freeze drying"); both are rather energy-demanding procedures. Dehydration or drying is usually required to extend the

viability of harvested biomass depending on the final product required. Several methods are available for drying the harvested biomass including sun, low-pressure shelf, spray, drum, fluidized bed, and freeze drying. Sun drying does not require fossil fuel energy but is both weather and volume dependent. It is the cheapest dehydration method, but the main disadvantages include long drying times, need for large drying surfaces, and risk of material loss. Spray drying is commonly used for extraction of high-value products, but it is relatively expensive and can cause significant deterioration of some algal pigment. Freeze drying is equally expensive, especially for large-scale operations, but it eases extraction of oils. Intracellular elements such as oils are difficult to extract from wet biomass with solvents without cell disruption but are extracted more easily from freeze-dried biomass. Drying of harvested biomass can be very energy demanding depending on several factors. Energy extraction methods for wet algae include hydrothermal liquefaction, fermentation, and anaerobic digestion. These energy extraction methods do not require biomass drying and thus have higher upstream EROI values for processing into biofuels. Biomass processing pathways that require dry algal biomass include direct combustion, pyrolysis, gasification, and transesterification to biodiesel. Sills et al. (2012), in their "well-to-wheel" LCA study, reported that the thermal drying of harvested biomass for base productivity (25 g m^{-2} day^{-1}) required 1.8 MJ of nonrenewable energy per MJ of biofuel (biodiesel in this case) produced. Thus, competitive wet lipid extraction techniques must be explored to minimize impacts associated with algal dewatering and drying processes.

2.4 Extraction of Oil from Algal Biomass

Extraction methods are aimed at removing lipids from algal biomass, later to be processed into lipid-based biodiesel, renewable diesel, jet fuel, and similar products. The remaining biomass that is rich in other bio-molecules can further be processed into other biofuel via different conversion processes. Alternatively, biofuels can also be produced via treatment of intact algal cell. Various methods are available for extraction of oil from algae, each with its advantages and disadvantages.

2.4.1 Heated Oil Extraction

Benemann and Oswald (1996) proposed mixing algae wet paste from a gravitational thickener with heated oil and then combining centrifugal dewatering with oil extraction in a three-phase centrifuge which could separate oil, water, and solids (i.e., residual biomass). In this extraction, a fraction of the oil is returned to the heater and then to extraction, and the remainder is used for biofuel production.

2.4.2 Mechanical Extraction

Mechanical treatments, such as ultra-sonication (disruption with high-frequency sound waves) and homogenization (carried out by rapid pressure drops), may be used to disrupt cell walls and lead to enhanced oil recovery. In mechanical press, algal biomass is subjected to high pressure, resulting in ruptured cell wells and release of oil. This method is easy to use, no solvent is required, and a large percentage (70–75 %) of the oils are extracted from the algal biomass.

2.4.3 Ultrasonic-Assisted Extraction

Ultrasonic-assisted extraction can be used which is based on cavitation, which occurs when vapor bubbles of a liquid form in an area where pressure of the liquid is lower than its vapor pressure. These bubbles grow when pressure is negative and compress under positive pressure, which causes a violent collapse of the bubbles. If bubbles collapse near cell walls, damage can occur and the cell contents are released. Advantages of this method over other extraction methods include lower extraction time, reduced solvent consumption, greater penetration of solvent into cellular materials, and improved release of cell contents into bulk medium. This can extract almost 76–77 % of the oils.

2.4.4 Solvent Extraction

Algal oil can be extracted using chemicals. Organic solvents such as benzene, cyclohexane, hexane, acetone, and chloroform, when mixed with microalgae biomass, degrade algal cell walls and extract the oil which has a high solubility in organic solvents. Solvents used in this method are relatively inexpensive, results are reproducible, and the solvent is recycled. 60–70 % of the oil is extracted by this method.

2.4.5 Supercritical Fluid Extraction

This method is more efficient than traditional solvent separation methods. Supercritical fluids have increased solvating power when they are raised above their critical temperature and pressure points. It produces highly purified extracts that are free of potentially harmful solvent residues, and extraction and separation are quick as well as safe for thermally sensitive products. This can extract almost 100 % of the oils. In the supercritical fluid CO_2 extraction, CO_2 is liquefied under pressure and heated to the point that it has properties of both a liquid and a gas. This liquefied fluid then acts as the solvent in extracting the oil.

2.4.6 Enzymatic Extraction

In this process water is used as solvent with the cell wall degrading enzymes to facilitate an easy and mild fractionation of oil, proteins, and hulls. The oil is found inside plant cells, linked with proteins and a wide range of carbohydrates like starch, cellulose, hemicellulose, and pectin. The cell content is surrounded by a rather thick wall that has to be opened so the protein and oil can be released. Thus, when opened by enzymatic degradation, downstream processing makes fractionation of the components possible to a degree which cannot be reached when using a conventional technique like mechanical pressing. This is the biggest advantage

of enzymatic extraction process over other extraction methods. But the cost of this extraction process is estimated to be much higher than most popularly used solvent-based extraction methods. The high cost of extraction serves as a limitation factor for large-scale utilization of this process.

2.5 Algal Biomass to Biofuel Conversion Technologies

2.5.1 Gasification

In gasification, partial oxidation of algal biomass into a combustible gas mixture is carried out at high temperatures (800–1000 °C) (Clark and Deswarte 2008). This process involves partial oxidation of biomass with oxygen and water (steam) to generate syngas (a mixture of CO, H_2, CO_2, N, and CH_4) (Demirbas 2001). Syngas is a low calorific value gas (typically 4–6 MJ m^{-3}) that can be burned directly or used as a fuel for gas engines or gas turbines. Hirano et al. (1998) estimated a marginal positive energy balance of 1.1; this low value is attributed to the energy-intensive centrifuge process during biomass harvesting. EROI value for gasification technology is dependent on factors such as biomass harvesting and drying.

2.5.2 Thermochemical Liquefaction

Thermochemical liquefaction is a low-temperature (300–350 °C), high-pressure (5–20 MPa) process aided by a catalyst in the presence of hydrogen to obtain bio-oil from algal biomass.

Reactors for thermochemical liquefaction and fuel-feed systems are complex and therefore expensive but have advantages in their ability to convert wet biomass into energy. The process utilizes the high water activity in subcritical conditions to decompose biomass materials down to shorter and smaller molecular materials with a higher energy density. Thermochemical liquefaction is a process that can be employed to convert wet algal biomass material into liquid fuel, and thus energy investment for drying is not required. In a similar study, Minowa et al. (1995) obtained an oil yield of 42 % dry wt. from *Dunaliella tertiolecta*, giving a HHV of 34.9 MJ kg^{-1} and positive energy balance of 2.94:1. These results indicate that thermochemical liquefaction is a viable option for the conversion of algal biomass to liquid fuel.

2.5.3 Pyrolysis

For biomass to liquid fuel conversion, pyrolysis is deemed to have the potential for large-scale production of biofuels that could replace petroleum-based liquid fuel (Demirbas 2006).

Pyrolysis is the conversion of biomass to bio-oil, syngas, and charcoal at medium to high temperatures (350–700 °C) in the absence of air. Flash pyrolysis conditions utilizing moderate temperature (500 °C) and short hot vapor residence time (about 1–2 s) have a biomass-to-liquid conversion ratio of 95.5 % (Demirbas 2006). However, there are technical challenges as pyrolysis oils are acidic, unstable, viscous, and contain solids and chemically dissolved water (Chiaramonti et al. 2007). Therefore, the process oil requires upgrading hydrogenation and catalytic cracking to lower oxygen content and remove alkalis. Compared to other conversion technologies, research on pyrolysis of algal biomass is quite extensive and has achieved reliable and promising outcomes that could lead to commercial exploitation. Miao and Wu (2004) used flash pyrolysis to enhance oil yield from *Chlorella protothecoides* after manipulating its metabolic pathway towards heterotrophic growth. The recorded oil yield of 57.9 % dry wt. basis from heterotrophic cultivation (HHV of 41 MJ kg^{-1}) was 3.4 times higher than achieved by phototrophic cultivation, and the results suggest that pyrolysis has potential in algal biomass to liquid conversion. Miao and Wu (2004) achieved bio-oil yields of 18 % (HHV of 30 MJ kg^{-1}) and 24 % (HHV of 29 MJ kg^{-1}) with fast pyrolysis of *C. protothecoides* and *Microcystis aeruginosa* grown phototrophically, respectively. Demirbas (2001), experimenting with *C. protothecoides*, showed that bio-oil yield increased with temperature increases up to a point and then decreased at higher temperatures. For example, the yield rose from 5.7 to 55.3 % with an increase from 254 to 502 °C and subsequently decreased to 51.8 % at 602 °C. They recorded a HHV from microalgae of 39.7 MJ kg^{-1} with temperatures ranging from 502 to 552 °C. Results indicate that bio-oils from microalgae are of a higher quality than those extracted from lignocellulosic feedstock.

2.5.4 Direct Combustion

In direct combustion process, biomass is burned in the presence of air just like any other fuel to convert the stored chemical energy in the biomass to hot gases, usually in a boiler, furnace, or steam turbine at temperatures >800 °C. Direct combustion is only feasible for biomass with moisture content <50 % dry weight. The heat produced must be used immediately as storage is not a viable option (Clark and Deswarte 2008). Direct combustion of biomass can be carried out for heat, power, and steam generation. Energy conversion by direct biomass combustion has the disadvantage of biomass generally requiring pretreatment processes such as drying, chopping, and grinding which incur additional energy demand and therefore cost (Goyal et al. 2008). Conversion efficiency in large biomass to energy plants compares favorably to that of coal-fired power plants but may incur higher cost due to high moisture content of biomass. Generation of combined heat and power (CHP) is desirable to improve overall plant efficiency. Net energy conversion efficiencies for biomass combustion power plants range from 20 to 40 %, with higher efficiencies obtained in larger systems (>100 MW) or when biomass is co-combusted in coal-

fired power plants (Demirbas 2001). There is little evidence of technically viable utilization of algal biomass in direct combustion, but a LCA suggested that coal-algae co-firing could lead to lower GHG emissions and air pollution (Kadam 2002). Further, flue gas CO_2 from coal-fired thermal power can be used to cultivate algae, which improves environmental performance of the combined system. Due to limited data, this area will require further research to determine viability.

2.5.5 Biophotolysis

As part of natural photosynthesis, photolysis of water produces hydrogen ions (H^+), oxygen, and electrons. While electrons are used in the electron transport chain, the remaining two are by-products of photolysis. H^+ can subsequently be converted to hydrogen (H_2) by a reversible reaction catalyzed by hydrogenase enzymes, but hydrogenase remains ineffective under aerobic conditions. Photosynthetic oxygen production causes rapid inhibition to the hydrogenase enzyme, and the photosynthetic H_2 production process is impeded. Consequently, microalgae cultures for H_2 production must be subjected to anaerobic conditions. Microalgae are capable of metabolic biohydrogen production via by direct or indirect photolysis. In direct photolysis of water, sunlight breaks water into H_+, e^-, and O_2. This is followed by hydrogenase-catalyzed reaction which recombines H^+ and e^- to produce H_2. In indirect photolysis, microalgae first produce hydrates that later produce hydrogen by dark anaerobic processes. Hydrogen is a clean fuel and has the highest energy content (142 KJ g^{-1}) per unit weight compared to other fuels (Das and Veziroglu 2008). This production process becomes limited with time, as H_2 yield will begin to level off after 60 h of production. The use of this production system does not generate toxic or environmentally harmful products but could yield value-added products as a result of biomass cultivation (Melis and Happe 2001) (Fig. 14.4).

Studies have shown that when algal cultures are deprived of sulfur, it induces anaerobic conditions and stimulates consistent H_2 production (Melis 2002). Several algal species have been used for experimental biohydrogen production including *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorococcum littorale*, and *Monas subcordiformis* (Ghasemi et al. 2012). Melis and Happe (2001) found that by using the two-stage photosynthesis process (where photosynthetic O_2 production and H_2 gas generation are spatially separated), a theoretical maximum yield of H_2 by green algae could be about 198 kg H_2 ha^{-1} day^{-1}.

2.5.6 Transesterification

Biodiesel is produced via a reaction called transesterification that involves transformation of glycerol-based ester derived from biomass-based lipids into monohydric alcohol-based ester usually known as fatty acid methyl ester (FAME). After extraction of lipids from algal biomass, it is transesterified using an alcohol (usually methanol) and a catalyst. Oleaginous microalgae are an attractive non-edible biodiesel feedstock having oil productivity significantly higher (5000–100,000 L ha^{-1} a^{-1}) than terrestrial feedstock. Some microalgae respond to nitrogen stress and certain other chemical and physical stimuli through the accumulation of intracellular triglycerides, thus accumulating higher amounts of lipid than in the absence of such stimuli. Heterotrophic algal culture has been reported to accumulate higher amounts of lipids than photoautotrophic culture. Unlike terrestrial oilseeds, microalgae are cultivated in dilute aqueous suspensions that make lipid recovery complicated. Biomass harvesting, dewatering, and drying and lipid extraction are challenging prospects. Microalgae, when grown outdoors in open ponds, have typical cell density and productivity ranging from 0.5 to 2 g dry biomass L^{-1} and 10 to 40 g m^{-2} d^{-1}, respectively (Doucha et al. 2005). Although higher biomass densities (5–200 g L^{-1}) can be achieved in photobioreactors (Doucha et al. 2005) and fermenters (Xiong et al. 2008), dewatering and drying remain energy- and cost-intensive processes (Molina et al. 2003). Biomass drying and organic solvent use for oil extraction could lead to significant energy and cost debt. Biodiesel has remained the biofuel of choice for conducting LCA studies, but comparison between studies is limited due to different boundary delineation criteria, different function unit, different assumptions, and other variables. Yang et al. (2010) reported that about 400 kg kg^{-1} biodiesel of freshwater must be used for culture even if sea-/wastewater serves as the culture medium, irrespective of the amount of harvested water recycling. Frank et al. (2012) reported that in baseline studies with assumed 25 g m^{-2} d^{-1} productivity and 25 dry wt.% lipids, per million BTU of biodiesel produced 55,400 g CO_2 equivalent compared to 101,000 g for fossil diesel having low sulfur content. Woertz et al. (2014) estimated total well-to-wheel GHG emissions for algal biodiesel to be 28.5 g CO_2e/MJ of biodiesel. Total energy requirement for well-to-tank was reported to be 1.2 MJ per MJ biodiesel produced. Cumulative well-to-wheel energy requirement (including energetic costs of production for methanol and hexane for biodiesel production and oil extraction) was estimated to be 2.2 million J/MJ biodiesel (Fig. 14.5).

2.5.7 Fermentation

Alcoholic fermentation of algal biomass yields ethanol, which is compatible with gasoline engine vehicles. Raw material required for alcohol fermentation is carbohydrate. Some microalgae (*Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, and *Spirulina*) are known to contain >50 % of the dry weight as starch, cellulose, and glycogen, which are raw materials for ethanol production (Singh et al. 2011). Microalgae such as *C. vulgaris* are a good source of ethanol due to their high starch content (ca. 37 % dry wt.), and up to 65 % ethanol conversion efficiency has been recorded

Fig. 14.4 Biophotolysis types

Direct Photolysis:

$2H_2O \rightarrow 2H_2+O_{2\rightarrow}$

Indirect Photolysis:

$12H_2O+6CO_2 \rightarrow C_6H_{12}O_6+6O_2$

$C_6H_{12}O_6+12H_2O \rightarrow 12H_2+6CO_2$

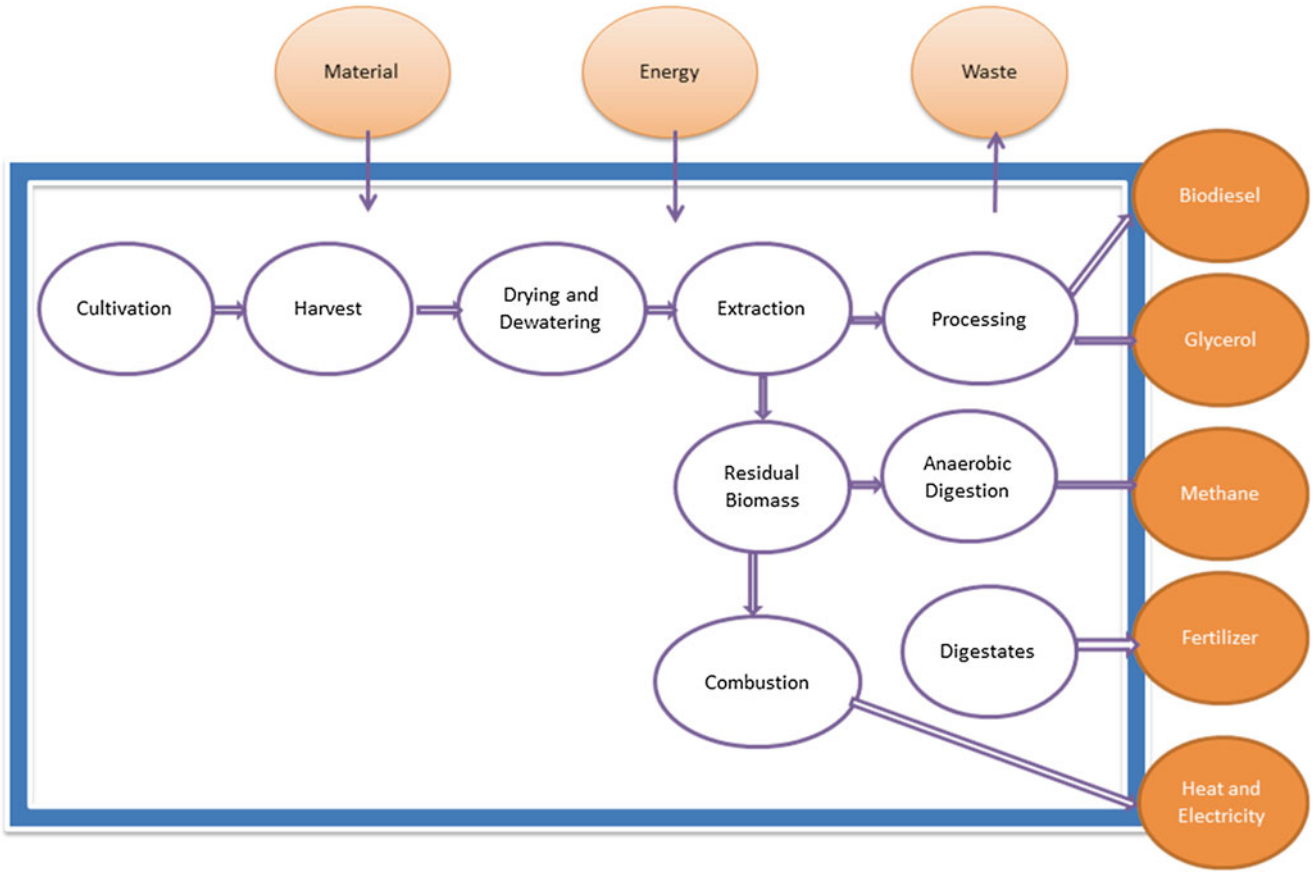

Fig. 14.5 Biodiesel production pathway and residual processing options

(Hirano et al. 1998). Chemical reaction is composed of enzymatic hydrolysis of complex carbohydrates followed by fermentation of simple sugars. The biomass is ground down and the starch is converted to sugars, which is then mixed with water and yeast and kept warm in large tanks called fermenters (Demirbas 2001). Yeast breaks down the carbohydrate and converts it to ethanol. Distillation is required to remove the water and other impurities in the diluted alcohol product (10–15 % ethanol). The concentrated ethanol (95 % volume for single distillation) is drawn off and condensed into liquid form, which can be used as a supplement or substitute for petrol in cars (Demirbas 2001). The solid residue from the process can be used for cattle feed or for gasification.

2.5.8 Anaerobic Digestion

Anaerobic digestion is the conversion of biomass into a biogas—a combustible mixture consisting primarily of methane and carbon dioxide, with traces of other gases such as hydrogen sulfide (H_2S). Anaerobic digestion of biomass proceeds via breakdown of organic matter to produce biogas, having an energy content of about 20–40 %, which is the lower heat-

ing value of the feedstock. One major advantage with anaerobic digestion is its ability to process high moisture content (80–90 %) biomass. This excludes the energy-intensive process of dewatering and drying of biomass. Anaerobic digestion of biomass proceeds in three sequential stages—hydrolysis, fermentation, and methanogenesis. In hydrolysis, the complex compounds are broken down into soluble sugars. Then, fermentative bacteria convert these into alcohols, acetic acid, volatile fatty acids, and a gas containing H_2 and CO_2, which is metabolized into primarily CH_4 (60–70 %) and CO_2 (30–40 %) by methanogens in methanogenesis. Microalgae having high proportion of proteins can result in low C/N ratios that can affect the performance of the anaerobic digester. This problem may be resolved by co-digestion with a high C/N ratio product (e.g., waste paper). Yen and Brune (2007) achieved a significant increase in methane production with the addition of waste paper to algal biomass. High protein content in the algae can also result in increased ammonium production, which can inhibit anaerobic microorganisms. Besides carbon, nitrogen, and phosphorus, which are major components in microalgae composition, other nutrients such as iron, cobalt, and zinc are also found (Grobbelaar 2004) and are known to stimulate methanogenesis.

2.5.9 Jet Fuel

Aviation fuels account for approximately 8 % of global petroleum usage and account for approximately 2 % of total anthropogenic CO_2 emission (end use). Jet fuels can be derived from biomass-based sources. Algal lipid serves as feedstock for jet fuel in addition to biodiesel and green diesel. Jet fuels can be produced from algal oil by removing the oxygen molecules (to raise the heat of combustion) and converting olefins to paraffins by reacting it with hydrogen and removing metals and heteroatoms like oxygen, nitrogen, and sulfur (increases the thermal stability of the fuel). This is followed by selective cracking/isomerization which produces jet-range paraffins (improves the freeze point) (Rahmes et al. 2009). O'Neil et al. (2015) used olefin metathesis of alkenones (a type of lipid composed of long chains with 37–39 carbon atoms) derived from *Isochrysis* which cleaved carbon-carbon double bonds present in alkenones to produce compounds containing 8–13 carbons, which can be used as jet fuel.

2.5.10 Green Diesel

Green diesel can be produced from triglycerides present in algal biomass via its hydroprocessing, which involves (1) hydrocracking and (2) hydrogenation to produce hydrocarbons having C_{15}–C_{18} chain (a liquid mixture within the boiling point range of fossil diesel). This is different from biodiesel, which is composition-wise an ester, while green diesel consists of hydrocarbons, mainly heptadecane and octadecane. Hydroprocessing of triglycerides to green diesel requires temperatures around 300 °C, pressure of 5 MPa of hydrogen, and a bifunctional solid catalyst in a continuous-flow process. Hydroprocessing is superior to transesterification in terms of energy requirement for drying the harvested algal biomass as it can process wet biomass (Fig. 14.6).

2.5.11 Greenhouse Gas Balance of Algal Biomass-Based Energy and Energy Carriers

Depletion of fossil fuels and its impact on climate are the driving forces for exploration and development of biofuels. Algae have higher photosynthetic efficiency than their terrestrial counterparts and are capable of biomitigation of CO_2. Since captured CO_2 is converted into biomass and biomass-based energy and energy carriers, algal biofuels do not cause any net emission of CO_2. Algal biofuels can even sequester more CO_2 than its emission depending on the production technique employed. The GHG balance of algal biofuels is usually reported in terms of CO_2 equivalent emissions (Table 14.3).

2.6 Algal Biorefinery

The concept of an algal biorefinery is analogous to the petroleum refinery. It is a facility that integrates different biomass conversion systems to produce biofuels, heat, power, and other valuable chemicals of ecological, economic, and health benefit. It is a system that involves sustainable processing of algal biomass into a spectrum of biologically derived products including chemicals, food, feed, chemicals, and bioenergy including biofuel power and heat. Thus, the biorefinery process has immense potential for sustainable bio-based production systems in which principles of industrial ecology are applied. Material and energy that come out of one production system is used as input for other systems and thus involves interdependence between individual systems. Depending on the scale of operation and individual processes, biorefinery can be an independent system in which minimal external support/input is required. The interdependence can avoid waste output to the environment and can potentially minimize several impacts associated with individual production chains (Fig. 14.7).

The impacts associated with any particular production system are strongly correlated to the electricity input required for producing a particular biofuel. These impacts are further dependent on the nature of the electricity source and nonrenewable energy sources and may lead to significantly greater environmental impacts. These impacts can be minimized to some extent by producing some of the electricity at the biofuel production facility through employing some conversion technique and by using less energy-intensive processes. Numerous alternatives exist which need to be integrated

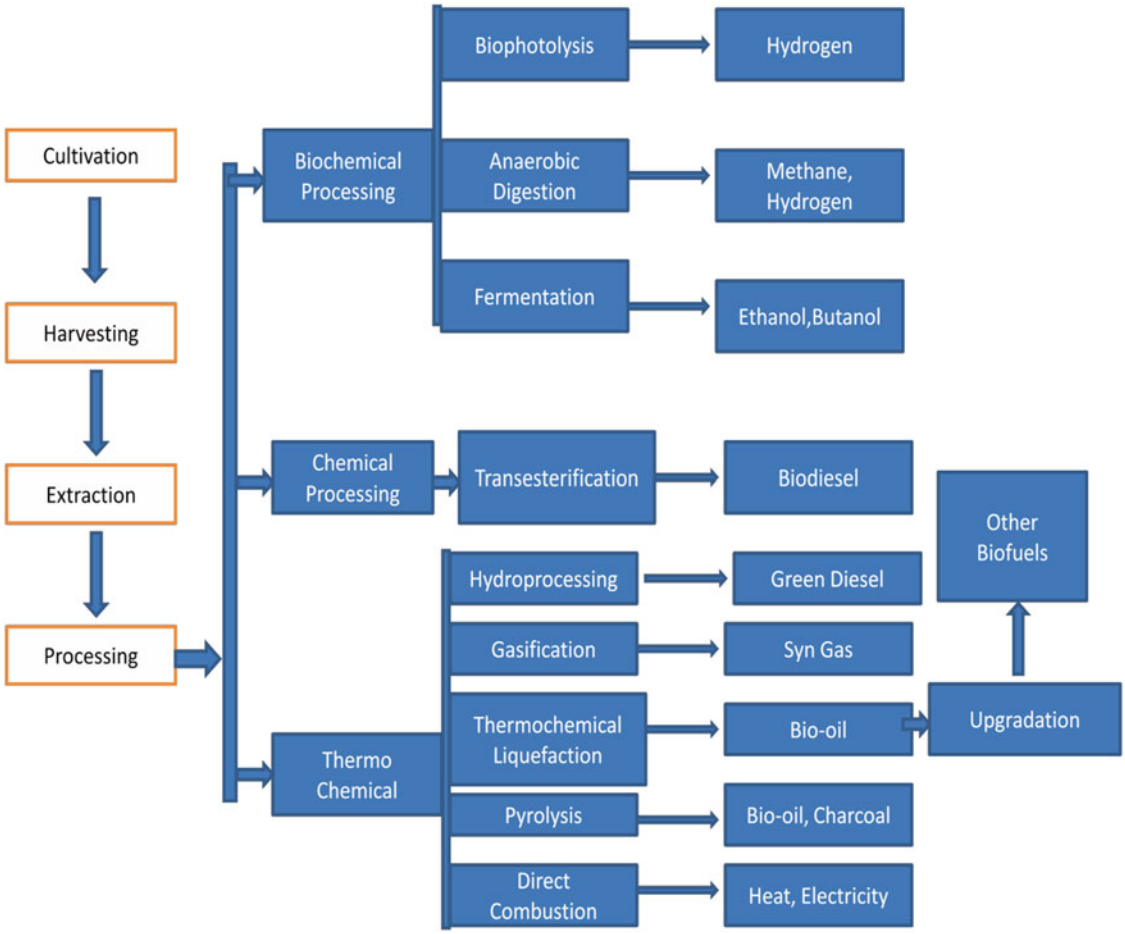

Fig. 14.6 Biomass conversion techniques

Table 14.3 Greenhouse gas balance of production and use of bioenergy from algae

Energy carrier	(g CO_2 eq/MJ)	Approach	Reference
Electricity	0.061	Flue gas CO_2 from coal-fired thermal power plant captured by algae (50 %) and used to produce electricity (WTW)	Kadam (2002)
Biodiesel	59.9	Open pond cultivation with N stress and dry extraction (WTW)	Lardon et al. (2009)
Biodiesel	18.5	PBR under greenhouse, waste heat from power plant as heat source (WTP)	Baliga and Powers (2010)
Biodiesel	−75.3 (without combustion)	PBR based on the GREET modeling (WTP)	Batan et al. (2010)
	−1.31 (with combustion)		
Biomass	56.8	Open raceway and chemical fertilizer (WTG)	Clarens et al. (2010)
Biodiesel	−18.0	Culture in two stages, GREET modeling (WTP)	Sander and Murthy (2010)
Biodiesel	13.6	Anaerobic digestion of extraction residue, digestates as fertilizer (WTW)	Stephenson et al. (2010)
Biodiesel	−0.729	Open ponds, CO_2 produced during the synthesis of nitrogen fertilizer used as carbon source (WTW)	Campbell et al. (2011)
Electricity	48.7	Direct combustion of algal biomass for bioelectricity production (WTW)	Clarens et al. (2010)
Biomethane	61.02	Open raceways, anaerobic digestion of biomass, digestates as fertilizer (WTW)	Collet et al. (2011)
Biodiesel	15.0	Open raceways, sea water (WTW)	Hou et al. (2011)
Biodiesel	310	Two-phase cultivation, first in photobioreactors then in open raceway (WTP)	Khoo et al. (2011)
Biodiesel	534 (base configuration)	Base configuration—open raceways, hexane extraction of dry algae, methanol transesterification, oilcakes as waste	Brentner et al. (2011)
	80.5 (best configuration)	Best configuration—PBR, extraction with in situ esterification by supercritical methanol, anaerobic digestion of oilcakes, digestates as fertilizers (WTP)	

Modified from Collet et al. (2013)

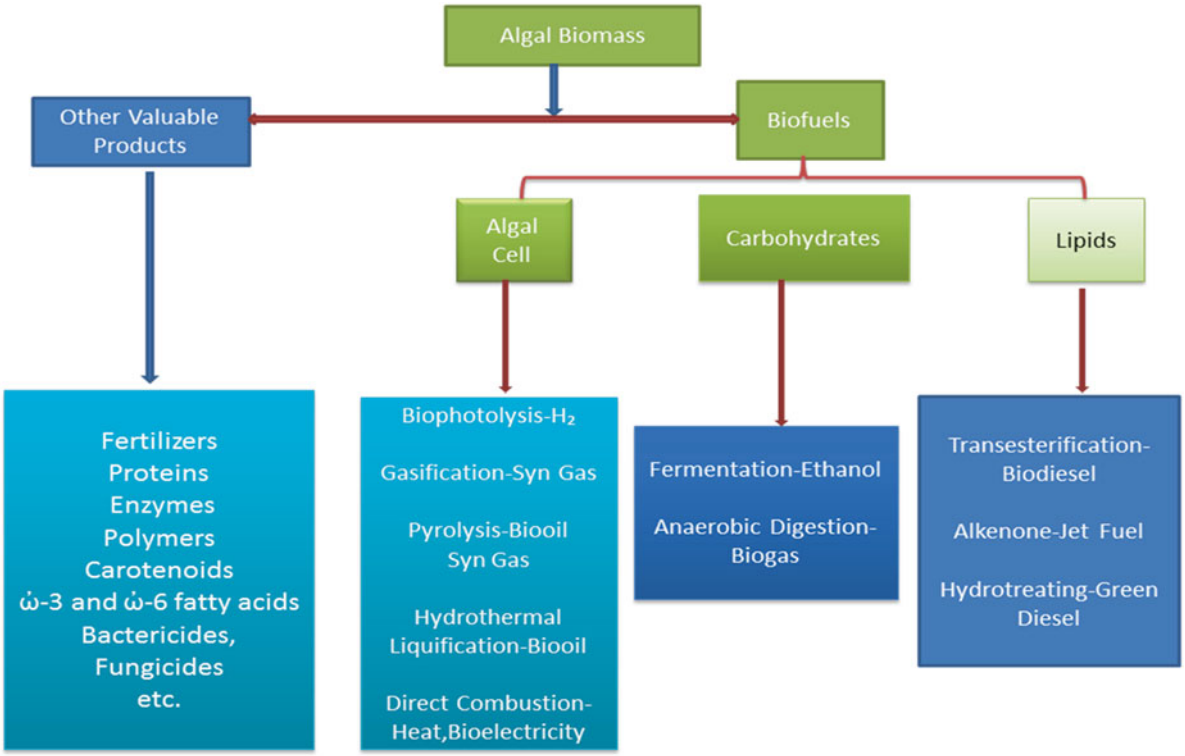

Fig. 14.7 Concept of algal biorefinery

judiciously for achieving a self-dependent biofuel production system which is sustainable in the long term (Subhadra 2010).

3 Conclusions

LCA is an analytical tool which can be employed to assess suitability of algal biofuels over their fossil fuel-based counterparts. Several types of biofuels can be produced from algae: bioethanol, biogas, biohydrogen, biodiesel, green diesel, jet fuel, etc. The performance of any biofuel is greatly dependent on the route taken for its synthesis. The various stages of any algal biofuel production system include cultivation of algae, harvesting of algal biomass, drying and dewatering of algal biomass extraction of lipids, and processing into biofuels. Each of these stages has multiple routes to choose from, and a careful selection of a particular combination of routes is critical which determines its performance on environmental front. GHG balance and EROI are widely used indicators in LCA studies to assess the overall suitability of a particular biofuel over its counterparts. In order to achieve environmental and economic goals of sustainable development, any production chain should use minimum amount of energy and should generate minimum amount of waste possible per unit of output. Algal biorefinery is an emerging system which is analogous to petroleum refinery and involves material, waste, and energy synergy at several stages in order to achieve environmental sustainability and economic feasibility.

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