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Marimuthu Prashanthi
Rajakumar Sundaram *Editors*

Integrated Waste Management in India

Status and Future Prospects for Environmental Sustainability

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Editors

Integrated Waste Management in India

Status and Future Prospects for Environmental Sustainability

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Editors
Marimuthu Prashanthi
Department of Environmental Management
Bharathidasan University
Tiruchirappalli, Tamil Nadu
India

Rajakumar Sundaram
Department of Marine Biotechnology
Bharathidasan University
Tiruchirappalli, Tamil Nadu
India

ISSN 1863-5520 ISSN 1863-5539 (electronic)
Environmental Science and Engineering
ISSN 1431-6250
Environmental Science
ISBN 978-3-319-27226-9 ISBN 978-3-319-27228-3 (eBook)
DOI 10.1007/978-3-319-27228-3

Library of Congress Control Number: 2016931343

Printed on acid-free paper

This Springer imprint is published by SpringerNature
The registered company is Springer International Publishing AG Switzerland

Preface

"Solid wastes are the discarded leftovers of our advanced consumer society. This growing mountain of garbage and trash represents not only an attitude of indifference toward valuable natural resources, but also a serious economic and public health problem"

—Jimmy Carter

Waste Management is a mammoth task in India, which stands complicated with the increase in urbanization, changing lifestyles, and increase in consumer behavior. The current practice of uncontrolled dumping of waste in open areas of towns/cities have created serious environmental and public health problems. Financial constraints, institutional weaknesses, insufficient manpower and collection systems, improper choice of technology, and public apathy toward Municipal Solid Waste (MSW) have contributed to making the situation worse. Annual increase in waste generation is around 5 % each year. India produces 42.0 million tons of municipal solid waste annually at present. However, the collection efficiency is between 50 % and 90 % of solid waste generated.

The Government of India has brought in the movement of Swachch Bharat—Clean India Mission to clean the cities and villages and to improve hygiene, yet waste is strewn around in cities and lack of personnel to collect and discard waste is evident. Beyond the dumping of solid waste into streets and gutters is the problem of open defecation, adding to more disease spread and unsanitary conditions in India. Although more NGOs have come forward to clean up the discarded wastes through routine management, scheduled collection, proper segregation and disposal, the rate of waste generation is ever increasing, adding to more problems in the collection schedules. Sensitization programs on sanitation and hygiene show a positive response, however, if these schemes are coupled with methods for management of household waste, it would be a boon to India.

In recent years, there has been outstanding research work seeking the right approach to waste minimization and appropriate management using several techniques. This book is an outcome of research contribution from several authors, delivering results to manage the present-day waste accumulation. The approaches include bioremediation, microbial degradation, environmental friendly waste disposal, facing health challenges due to waste accumulation, economic reasoning, and energy recovery from waste. Much more work is to be done and explored and more

efforts have to be put forth into bringing these technologies for common use of the public and to create awareness among them. Future cities or smart cities should focus on zero waste discharge through recycling of waste and to derive energy from the waste.

April 2016

Marimuthu Prashanthi
Rajakumar Sundaram

Contents

Part I Applied Waste Management

Impact of Water Pollution on Coconut Cultivation in Vellore District, Tamil Nadu 3
K. Sivakumar and M.P. Parvez Ahmed

Solid Waste Management in Vellore District, Tamil Nadu. 13
A. Royal Edward Williams and S. Kumar

Site Suitability Analysis for Solid Waste Management Using Multi Criteria Analysis 19
Sanhita Ghosh and Sachikanta Nanda

Part II Alternate Clean Energy Fuel Technologies

Nitrate Reduction by Denitrifying *Bacillus Cohnii* Isolated from Sewage Treatment Plant 35
T.K. Poornima Priyadharsani, D. Suriyaprakasham, P. Prakash and K. Thamaraiselvi

A Mini Review on Cyanophycin: Production, Analysis and Its Applications. 49
J. Aravind, T. Saranya, G. Sudha and P. Kanmani

Utilization of Cheese Industry Whey for Biofuel–Ethanol Production 59
G. Rajarajan, A. Irshad, B.V. Raghunath, G. Mahesh kumar and A. Punnagaiarasi

Anaerobic Digestion of Wastewater and Municipal Sludge to Isolate Potent Methanogen Using BMP Assay. 65
Ayesha Sulthana, K.C. Latha and S. Balasubramanian

Part III Waste Management Strategies

A Statistical Based Experimental Approach for Biodegradation and Optimization of Heavy Metals 85
S. Umadevi, S. Aalfin Emmanuel, P.M. Ayyasamy and S. Rajakumar

Wastewater Treatment Studies on Free Water Surface Constructed Wetland System 97
G. Midhun, L. Divya, Jessen George, P. Jayakumar and S. Suriyanarayanan

Recycling of Distillery Spent Wash and Their Effect on Growth of Sesame (*Sesamum*) Crop 111
V. Karthika, G. Rajannan and P. Thangavel

ISO 14001: Environmental Management System 117
R. Mayamurugan

Part IV Bioremediation and Environmental Sustainability

Utilization of Medicinal Oil Effluent for Lipase Production by *Penicillium citrinum* MKF3 125
N. Vinod Kumar, Mary Esther Rani, R. Gunaseeli and N.D. Kannan

Waste Water Sludge Treatment Using Rice Bran 133
P. Geetha and M. Kokila

A Novel Lipopeptide Bacterial Biosurfactant for Bioremediation 139
C. Elizabeth Rani Juneius and Jayasundari

Bioremediation of Ethylbenzene by Soil Column Study and Bioreactor Study for Polluted Soil and Water Samples Using Optimized Bacterial Consortium 155
S. Ashok, V. Akila, P.M. Ayyasamy and S. Rajakumar

Bio-degradation of Reactive Dyes by Indigenous Bacteria Obtained from Textile Effluent Contaminated Site 169
P. Muthukumaran, M. Priya, P. Vignesh and J. Aravind

Hydrolytic Enzyme Profiling of *Bacillus Subtilis* COM6B and Its Application in the Bioremediation of Groundnut Oil Mill Effluent 179
P. Kanmani, J. Aravind and K. Kumaresan

Management of Grey Water with Bio-beds Using Phytotechnology 191
N.D. Shrinithivihahshini, R. Chithra Devi, R. Viji, D. Mahamuni and M. Sheela Mary

Optimization of *Bacillus* sp. (KTSMBNL-16) for Decolourization of Dye AV90 Using Batch Studies 207
P. Prakash, M. Dexilin, B. Gowri Manogri and K. Thamaraiselvi

Part V Implications of Waste on Human Health and Community Participation in Waste Management

Role of NGO's in Protecting Environment and Health 221
M. Loganathan and Jothi Narendiran

A Study on Self-sacrificing Role Played by Garbage Pickers in Cleaning up the Environment 229
J. Jacob Stanley Inbaraj and M. Elangovan

Impact of Dairy Effluent on Environment—A Review 239
B.V. Raghunath, A. Punnagaiarasi, G. Rajarajan, A. Irshad, A. Elango and G. Mahesh kumar

Treatment of Waste Water from Meat Industry 251
A. Irshad, S. Sureshkumar, B.V. Raghunath, G. Rajarajan and G. Mahesh Kumar

Waste Management in Food Packaging Industry 265
G. Mahesh Kumar, A. Irshad, B.V. Raghunath and G. Rajarajan

Part I
Applied Waste Management

Impact of Water Pollution on Coconut Cultivation in Vellore District, Tamil Nadu

K. Sivakumar and M.P. Parvez Ahmed

Abstract In this paper the study on impact of water pollution with respect to coconut cultivation was done. Water is regarded as polluted when it is changed in quality or composition, directly or indirectly as a result of human activities. Coconut is the benevolent provider of the basic needs of millions of people across the globe, for their livelihood security. Coconut is grown in more than 93 countries of the world and Indonesia, Philippines, India are the major producing countries of the world. Forty-seven (47) of the seventy-seven ACP (African, Caribbean and Pacific) member countries produced 4.59 million tons of coconuts in 2009 on 1.7 million hectares. This represented 7.4 % of world production. Coconut in India is mostly a small holders crop contributing to about Rs. 83,000 million annually which is about 2 % of the contribution of agriculture and allied sectors with more than 10 million farming families dependent on the crop for their livelihood. India is one of the largest producers of coconut. The purpose of this study is to analyze the impact of water pollution on coconut production in Vellore District, Tamil Nadu. The important objectives of this study are to analyze the area, production and productivity of coconut in the study area and the need for fresh water irrigation for coconut cultivation. In order to obtain the objectives of this study, the secondary data was used for the period 2000–2013. This study is descriptive type in nature where simple percentage tables and diagrams were used for analysis purpose.

Keywords Water pollution · Coconut cultivation · Irrigation · Water quality

1 Introduction

Water is one of the most important needs of living things. It is second only to the air we breathe. Air, water and food are the three natural resources which determine the existence of human beings. Water is used for agriculture, industrial and domestic

K. Sivakumar · M.P. Parvez Ahmed (✉)
Department of Economics, Sacred Heart College, Tirupattur, Tamil Nadu, India
e-mail: parvezahmed.mp@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_1

purpose. Water is also used for drinking, cooking, washing and bathing, called water for personal demand. The water used for drinking purpose must be free from all types of contaminations. Like air and food, water is also contaminated by various pollutants.

Water is regarded as "polluted" when it is changed in its quality or composition, directly or indirectly as a result of human activities. So that it becomes useless or less suitable for drinking, domestic, agricultural, fisheries or other purposes for which it would otherwise be quite suitable in its natural or unpolluted state. Pollutants bring about physical and chemical changes not only in the surface water but also in the ground water. The water is fit for consumption if it has biological organisms like guinea worms, tape worms, cholera and typhoid creating bacteria and excess chemicals such as fluoride, iron, arsenic and nitrate. All human activities, agriculture, industrial and domestic cause more pollution in water than natural sources (Das 2008; Gangali and Maulick 2013).

1.1 Coconut Cultivation

Coconut (*Cocosnucifera*) plays a significant role in the agrarian economy of India. Apart from the importance of copra and coconut oil which is widely used in the manufacture of soaps, hair oil, cosmetics and other industrial products, the husk is a source of fibre which supports a sizable coir industry. The tender nut supplies coconut water, a popular thirst quencher of health and hygienic value. Coconuts are typical single trunked palms which can reach up to 50–100 ft in height. They are believed to be largely crossed pollinated and produce fruits (nuts/seeds) which are ovoid in shape, up to 15″ long and 12″ wide. Coconuts are seed propagated. They are usually planted 25 ft apart in all directions and can be intercropped with staples like corn and even other tree crops. They mature within 2–7 years and the first fruit appears one year after flowering. One tree can yield an average 70–150 coconuts per year. The palms remain productive for 50–100 years and yields are highest between 10 and 20 years old (Lathika and Ajithkumar 2005).

The most important and economically valuable produce of coconut palm is its fruit popularly known as *nut*. It is made up of an outer excerpt, a thick fibrous fruit coat known as husk; underneath lays the hard protective endocarp or shell. Lining the shell is a white aluminous endosperm or *coconut meat* and the inner cavity is filled with a clear sweet refreshing liquid called *coconut water*. The kernel of a matured nut is the most precious product used for edible purpose. The dried kernel or *copra* is the richest source of edible oil and a by-product coconut oil cake, a source of vegetable protein used as an ingredient for livestock feed. The shell as such is used for fuel purpose, shell gasified as an alternate source of heat energy, making handicrafts, ice-cream cups and other commercial products like shell powder, shell charcoal and activated carbon. The husk yields fibres, which is converted into coir and coir products viz., coil carpets, coir geo-textile, coir composite, coir safety belts, coir boards, coir asbestos and coir pith. *Coir pith,* a secondary by product obtained during

defibring process is used as soil conditioner and mending all types of soils (Devadas 2006).

Coconut is grown in more than 93 countries of the world notably Indonesia, Philippines, India are the major producing countries of the world. In 2008, 4.75 million tons were produced on 1.6 million hectares (7.7 % of world production). The average production in this group 2.6 tons/ha was way below the world average of 5.2 tons/ha. Forty seven of the seventy seven ACP (African, Caribbean and Pacific) member countries produced 4.59 million tons of coconuts in 2009 on 1.7 million hectares. This represented 7.4 % of world production. The reduction was as a result of storm damage as well as the wave of lethal yellowing and Red Palm mite infestation (Gopalakrishna et al. 2010).

Coconut in India is mostly a small holders crop contributing to about Rs. 83,000 million annually which is about 2 % of the contribution of agriculture and allied sectors with more than 10 million farming families dependent on the crop for their livelihood. India is one of the largest producers of coconut. Traditional areas of coconut in India are the states of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. However, several states like Assam, Gujarat, Madhya Pradesh, Bihar, Tripura, Manipur, Nagaland and Arunachal Pradesh have emerged as non-traditional areas for the cultivation of coconut. In Vellore district of Tamil Nadu, coconuts are grown in more than 22,720 ha with an estimated 125.8 million nuts during 2012–13 with an average productivity of 5537 nuts per ha.

In India, coconut plays an important role in the social, economic and cultural activities of the people with a production of 12.83 billion nuts from an area of 1.93 million ha under coconut. Coconut provides livelihood to millions of farm families either directly or indirectly. Coconut occupies more than 40 % of the area under cultivation, but the productivity is low, because of the incidence of pests and diseases. The disease has attained a serious proportion in some of the localities, especially hilly tracts with high humidity. Since coconut is a perennial crop, the loss due to the disease is very high (Mohana and Basheer 2007).

Mathew (2007) states that the biggest obstacle to the competitive of India's coconut sector is low rate of returns from the coconut holding, and reduces input-output realization especially in the traditional coconut growing states. The severe problem of price induced market instability of coconut and coconut products on accounts of seasonal variations in the production, market arrivals, the demand and influence of the prices of other cheaper vegetable oils and fats are the other factors responsible for the pessimistic growth of domestic coconut industry. The resultant import surge of cheaper palm oil and palm kernel oil has reducing the coconut oil market.

According to Sulochana (2008) report, the coconut tree or *cocosnucifera* has multiple uses, besides being an important oil seed crop. Its raw nut and edible copra are important items of food. Coconut is an indispensable items offered in divine oblation. Tender coconut water is a refreshing unadulterated cool health drink. The coconut shell and husk are main raw materials for the manufacture of handicraft

articles. Coconut milk, the extract of the solid coconut endosperm, plays an important role in the cuisines of South Asia and in the food industries. The trunk of the tree is used as building material, fuel and for making utensils, furniture, etc. In short, every part of the coconut tree is of great utility and hence it is rightly called '*Kalpavirksha*' or the '*tree of heaven*'.

Jesitha (2008) outlined the major problems confronting coconut growers. Production problems are that the coconut is generally grown in small land holding and homestead gardens mixed with other crops. Majority of the coconut growers have small size of coconut holding. Average management conditions though coconut palms start bearing between the age of 7 and 12 years, and yield stabilization takes place only around the age of 20 and yield declines around the age of 50. Since most of the trees are below the age group, the yield is low which results in declined productivity. The prevalence of root (wilt) disease is the major problem affecting the production and productivity of very serious disease of coconut palm.

A study on the water pollution on coconut cultivation has expressed concern at the presence of high levels of Total Dissolved Solids (TDS) in groundwater. The crisis of water availability and pollution is worsening day by day. On the other hand, the groundwater level is going down rapidly, and the quality is getting worse. Vellore District faces serious problems of environmental pollution, water pollution from untreated community and industry flows into our rivers and streams, etc. In order to avoid the above problems, the following study has been chosen and objectives of the present study are to analysis the cultivation of coconut in the study area during study period; to find out the total impact of water pollution on coconut cultivation in the study area; and to study the trend and performance of coconut cultivation in the study area.

2 Methodology of the Study

This study is based on secondary data. Secondary source of data were collected from various sources i.e. journals, newspaper, books, electronic sources, publications from Govt. of India, Govt. of Tamil Nadu, Pollution Control Board of Tamil Nadu, etc. The time series data which is relevant to the study have been collected for the period from 2000–01 to 2012–2013. Simple percentage tables, regression analysis and diagrammatic representation have been used for analysis purpose.

2.1 Analysis of the Data

This chapter deals with secondary data analysis and interpretation focuses on the reality of the study area. To achieve the objectives of the study an analysis was made on the basis of collected data.

3 Results

3.1 Coconut Production in World Scenario

Coconut production countries in the world was taken as a variable for analysis, through which the growth rate of world scenario can be identified from the year 2000–01 to 2012–2013. It helps to get sufficient information to reduce bias in the study.

From Table 1 it is observed that the five major countries in the world are producing coconuts from the period of 2000–01 to 2012–13. The results shows that there is large flexibility in the production pattern of coconuts. In the year of 2001–02 to 2006–07, there is a positive increase in the level of coconuts production from 51,925,165 to 61,906,731 metric tons of world level coconuts production. After this period the level of coconuts production is flexible because of the productivity of coconuts has decreased as the quality of water is impaired due to domestic wastes and as the use of chemicals has increased.

3.2 Coconut Production in Indian Scenario

Coconut production states in India were taken as a variable for analysis, through which the growth rate of our nation from the year 2011 to 2012 is identified. It helps to get sufficient information as to where to reduce the bias in the study.

Table 1 The top 5 coconut producing countries year wise (2000–2013)

Year	Country wise coconuts production (in metric tons)					World level production
	Indonesia	Philippines	India	Brazil	Sri Lanka	
2000–01	15,815,000	13,146,040	8,670,000	2,130,821	2,104,440	51,925,165
2001–02	15,495,000	14,068,500	8,920,000	2,892,350	1,817,920	53,501,990
2002–03	16,145,000	14,294,200	8,630,000	2,978,490	1,947,120	54,195,192
2003–04	16,285,000	14,366,184	8,380,000	3,117,339	1,969,160	55,035,075
2004–05	18,250,000	14,824,585	8,829,000	3,118,937	1,683,400	57,447,031
2005–06	17,125,000	14,957,900	10,190,000	2,978,217	2,115,840	57,870,111
2006–07	19,625,000	14,852,900	10,894,000	2,831,004	2,180,440	61,906,731
2007–08	17,937,000	15,319,500	10,148,300	3,223,983	2,210,840	60,408,502
2008–09	19,000,000	15,667,565	10,824,300	2,960,049	2,168,280	61,381,382
2009–10	18,000,000	15,510,283	10,840,000	2,843,453	1,990,440	60,295,788
2010–11	17,500,000	15,244,609	10,280,000	2,943,651	2,057,320	58,882,610
2011–12	19,400,000	15,862,386	10,560,000	2,931,531	2,224,500	62,139,506
2012–13	18,300,000	15,353,200	11,930,000	2,820,468	2,200,000	61,965,166

Source Fact fish coconuts, production quantity world statistics and data

Table 2 State wise coconut production the Indian scenario

S. No.	2011–2012	Area ('000 ha)	Production ('000 MT)	Productivity (kg/ha)
1	Chhattisgarh	00.80	06.30	7875
2	Puducherry	02.10	20.00	9524
3	Maharashtra	21.00	120.00	5714
4	Goa	25.70	89.00	3463
5	West Bengal	29.10	252.90	8691
6	Orissa	53.90	258.00	4787
7	Andhra Pradesh	142.00	1270.00	8944
8	Tamil Nadu	430.70	4515.60	10,484
9	Karnataka	511.00	3784.60	7406
10	Kerala	766.00	3973.90	5188
	India	2070.70	14,940.00	7215

Source www.coconutboard.gov.in/presentation/statistics/statistics.aspx

Table 2 shows the coconuts production in different states from the period of 2011 to 2012 in India. The results revealed that the maximum level is 4515.60 metric tons at 430.70 k ha in Tamil Nadu, 3973.90 metric tons at 766.00 k ha in Kerala, 3784.60 metric tons at 511.00 k ha in Karnataka, 1270.00 metric tons at 142.00 k ha in Andhra Pradesh, followed by Orissa, West Bengal, Goa, Puducherry, and other states. The lowest coconut production level is 06.30 metric tons at 0.80 k ha in Chhattisgarh.

3.3 Coconut Production in Tamil Nadu

Coconut production districts in Tamil Nadu were taken as a variable for analysis; through which the growth rate of our State from the year 2012 to 2013 is identified (Fig. 1). It helps to get sufficient information where to reduce bias in the study.

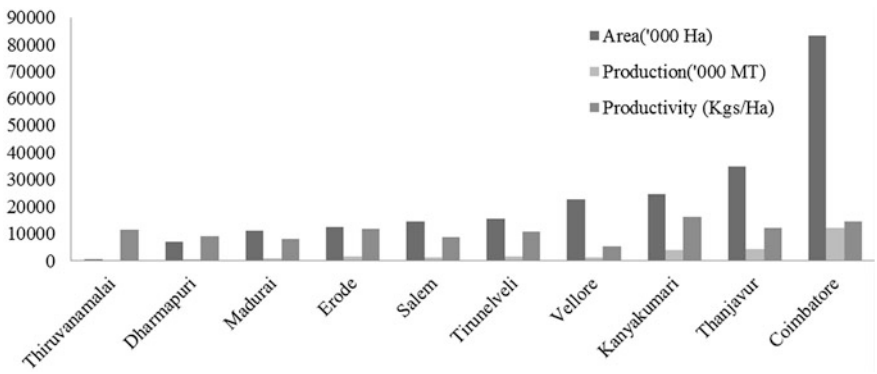

Fig. 1 District wise coconut production: Tamil Nadu scenario

Table 3 District wise coconut production in Tamil Nadu scenario

S. No.	2012–2013	Area ('000 ha)	Production ('000 MT)	Productivity (kg/ha)
1	Thiruvanamalai	688.00	79.00	11,483
2	Dharmapuri	7085.00	654.00	9231
3	Madurai	11,221.00	921.00	8208
4	Erode	12,623.00	1485.00	11,765
5	Salem	14,476.00	1286.00	8884
6	Tirunelveli	15,806.00	1703.00	10,775
7	Vellore	22,720.00	1258.00	5537
8	Kanyakumari	24,502.00	4007.00	16,354
9	Thanjavur	34,747.00	4230.00	12,174
10	Coimbatore	83,341.00	12,197.00	14,636
	Tamil Nadu	424,121.00	50,747.00	11,965

Source www.coconutboard.gov.in/presentation/statistics/statistics.aspx

From Table 3 it is observed that the coconuts production in different Districts from the period of 2012 to 2013 in Tamil Nadu. The results revealed that the maximum level is 12,197.00 metric tons at 83,341.00 k ha in Coimbatore, 4230.00 metric tons at 34,747.00 k ha in Thanjavur, 4007.00 metric tons at 24,502.00 k ha in Kanyakumari, 1703.00 metric tons at 15,806.00 k ha in Tirunelveli, followed by Vellore, Erode, Salem, Madurai, Dharmapuri, and other Districts. The lowest coconut production level is 79.00 metric tons at 688.00 k ha in Thiruvanamalai.

From Table 4, that the cultivation of coconuts from the period of 2000–2001 to 2012–2013 is observed. The results show that there is large flexibility in the pattern of coconut production in Vellore district. In the year 2003–04 to 2005–06 (Fig. 2), there is a positive increase in the level of coconuts production and after this period

Table 4 Year wise coconut production in Vellore district

Year	Area (ha)	Production (Lakh Nuts)	Productivity (Nuts/ha)
2005–2006	23,098	2765	11,971
2006–2007	22,569	2654	11,760
2007–2008	22,619	2172	9603
2008–2009	22,416	1964	8762
2009–2010	22,203	2274	10,242
2010–2011	22,292	2081	9336
2011–2012	22,680	2985	13,162
2012–2013	22,720	1258	5537
2013–14 (R)	24,005	2317	9662
2014–15 (R)	24,374	2357	9687
2015–16 (R)	24,742	2398	9711
2016–17 (R)	25,111	2438	9736
2017–18 (R)	25,480	2478	9761

Source www.coconutboard.gov.in/presentation/statistics/statistics.aspx

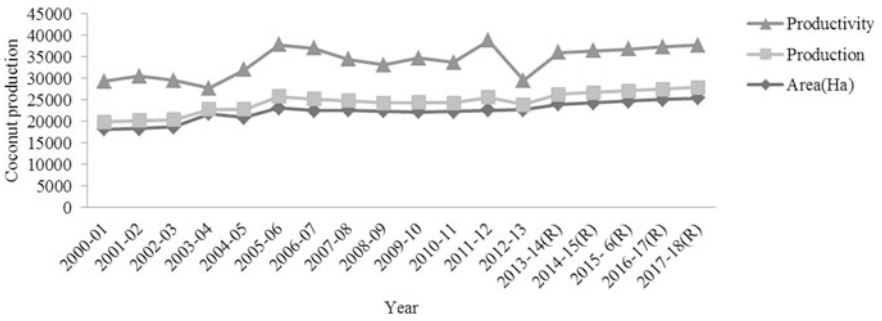

Fig. 2 Year wise coconut production in Vellore district

there is a decrease of coconuts production because the quality of water is low or water is polluted. In the year 2012–13, there is very low production of coconuts compared to past 10 years due to water being highly polluted.

3.4 Analysis of Water

Reports on variables on water quality parameters for the period of 2011–13 (Table 5) were taken for analysis, this could report whether the water is polluted or not.

Table 5 Report of water samples collected from Vellore district

S. No.	Parameters	Tolerance limit	2011 (June)	2012 (June)	2013 (June)
1	PH	5.5–9.0	7.9	7.6	7.9
2	Total suspended solids	100	180	144	196
3	Total dissolved solids	2100	8988	12,564	13,492
4	Chlorides	1000	4798	4549	6998
5	Sulphates	1000	930	2092	2926
6	Oil and grease	10	2.0	2.4	1.2
7	BOD 3 days at 27 °C	30	70	72	32
8	COD	250	504	634	399
9	Sulphides	2	14.0	1.6	4
10	Ammoniacal nitrogen	50	168	34	48
11	Phenolic compounds	1	1.2	0.22	<0.0005*
12	Hexavalent chromium	0.1	<0.01*	<0.01*	<0.01*
13	Total chromium	2	<0.003*	<0.003*	<0.003*
14	Percent sodium	–	37.6	42.2	82.1

*The above table-5 highlights that the samples collected in the study area, sampled well water is totally contaminated in all the parameter such as pH, Total suspended Solid, Total Dissolved solid, Chloriedes, Sulphates. BOD, COD, etc. are very high when compare to tolarence limit prescribed by Tamil Nadu Pollution Control Board. The ground water sources is not suitabel for irrigation purpose which ends with reduction in coconut cultivation in the study area

Source tnpcb.tn.gov.in

Note BOD—Biochemical oxygen demand; COD—Chemical oxygen demand

4 Discussion and Conclusion

The rate of coconut production is flexible because of the productivity of coconuts decreased due to impaired quality of water from domestic waste and increased usage of chemicals. The lowest coconut production level is 06.30 metric tons from 0.80k ha in Chhattisgarh. In TamilNadu, the lowest coconut production level is 79.00 metric tons at 688.00k ha in Thiruvanamalai. The tolerance limit of Total Suspended Solids (TSS) is only 100 but in year 2011, it is observed as 180, and in year 2012 as 144. The tolerance limit of Total Dissolved Solids (TDS) is 2100 but now it has increased to 13,492 in 2013.

This study helps the researcher to gain more knowledge on the water pollution risks, functions and its effects. The expected outcome of the research is to eradicate or control the water pollution through the control of discharges like drainage, sewage, effluents, etc. to increase the production and productivity of coconuts. Hence, the water used for irrigation purpose should be pollution free so as to increase the productivity of the coconut cultivation. However, our study reports that the contaminated water has affected only some productive areas of the study area.

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Solid Waste Management in Vellore District, Tamil Nadu

A. Royal Edward Williams and S. Kumar

Abstract Rapid growth of population and urbanization has resulted in increasing the volume of solid waste generation in the study area in particular and in India with 68.8 million tons/day. The improper disposal of solid waste becomes a major menace to the urban area and their surroundings. The management of Municipal Solid Waste (MSW) has become an acute problem to the society due to enhancement of economic activities and modernization. The composition of MSW is 51 % organic, 17.5 % recyclables (paper, plastic, metal and glass). The composition of MSW in the North, East, South and Western regions of the country varied between 50 and 57 % of organics, 16–19 % of recyclables, 28–31 % of inserts and 45–51 % of moisture. In Tamil Nadu, the estimated volume of solid waste is around 6404 tons/day and the per capita solid waste generation is 0.71 kg. The development along with population growth resulted in the accumulation of huge amount of solid waste including hazardous and toxic waste. The main purpose of this paper is to give a view of the solid waste management, practices and its implications on environment in Tamil Nadu.

Keywords Solid waste management · Issues · Practices · Environment

1 Introduction

Solid waste management is one of the most challenging issues in India then elsewhere at the global level which are facing a serious pollution problem due to the generation of Municipal Solid Waste (MSW), which has also increased tremendously with improved life style and social status of the populations in urban center.

A. Royal Edward Williams (✉) · S. Kumar
Department of Economics, Sacred Heart College, Tirupattur, Tamil Nadu, India
e-mail: royalriya_vas@yahoo.co.in

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_2

Each urban resident generates 350–1000 g solid wastes per day. Every day urban India generates 188.500 tons of MSW, 68.8 million tons per year solid waste generation and it increases by 50 % every decade. In 2015, the population will rise up to 50 lakhs and by 2025 it is expected to go up to 56 lakhs. The rapid growth of industrialization and population explosion in India has led to the migration of people from village to the cities which generates thousands of tons MSW generation day by day. Municipal solid waste generation (MSW) in urban India increased from 23.86 million tons per year to more than 39 million tons per year. The improper handling of wastes and open dumping affects human health and environment. Tirupattur town generated 12.50 MT of solid waste per day, of this nearly 10.00 MT of the solid waste is collected, transported and disposed daily which works to per capita generation of 250 g/day. The efficient present mechanism can collect 80 % of the total waste generated in the town. Of the total garbage collected, 48 % is the domestic waste, 42 % is the commercial waste and 10 % is the construction wastes among the domestic solid waste (Chandraleka 2014; Karthigarani and Elangovan 2014; Sathewaran 2014).

1.1 Major Issues of Municipal Solid Waste Management

The Ministry of Environment and Forests (MoEF) of the Government of India has issued Management and Handling rules in the year 2000 for scientific municipal solid waste management (MSWM), ensuring proper collection, segregation, transportation, processing and disposal of MSW and to upgrade of the existing facilities to arrest contamination of soil and ground water.

As per provision, CPCB has been assigned to monitor the implementation of these rules and the municipalities will be required to submit annual reports regarding the status of MSW in their areas to the CPCB. These rules are applicable to every municipal authority in India, which is responsible for MSWM.

In addition, there are municipal corporation acts in different states such as the Delhi municipal corporation act 1959, Uttar Pradesh municipal corporation act 1959 and Karnataka municipal corporation act 1976. These acts also deal with environment pollution caused by improper disposal of solid waste.

During the past two decades, India is facing a lot of problems in municipal solid waste management. The fact is that MSWM rules are not being effectively implemented in most of the local bodies accounting to about 4377 municipalities and municipal corporations spread throughout the country. But mega cities or few other cites also maintain the collection and storage of waste in a proper manner. The major focus of the study is to identify the average solid waste disposal at household level and the disposal method practiced in the study area (Gidde et al. 2008).

2 Analysis and Interpretation

The collected sample in the study area was analysed with the help of SPSS software. Only few variables were discussed below and the variables were significant role in the domestic solid waste at the household level. Among the variables, mode of solid waste disposal at the household level is primarily discussed.

3 Mode of Disposal

For this study, the mode of solid waste disposal with frequency and percentage was given in Table 1.

The Table 1 shows that 88.9 % of the people dumped the household waste in open places, commercial waste are dumped onto the road side. Only one respondent used to burn their domestic waste and around 10 % of the household disposed through other methods. The recycling method adopted for this study was given in Table 2.

Many of the responses in the study shows that domestic household waste cannot be reused. Only the commercial waste is reused by the government. But only one respondent said that they are recycling their domestic waste by converting into manure for their garden and the rest said that they simply dumped into the common area/bin. The material composition in domestic waste is given in Table 3.

This shows that various type of material is disposed as domestic waste. Among the samples, almost 67 % of the respondents stated that waste paper occupies the major portion of waste from their households. Next to this level carry bags stands second with 22.2 %, 1.4 % of their waste comes from food waste, fold cloths, old cloths, respectively and only 2.8 % their waste comes from medicine waste. The amount of waste disposal by individual is represented in Table 4.

Table 1 Mode of solid waste disposal

Mode of disposal	Frequency	Percentage
Dumped in open space	64	88.9
Burnt	1	1.4
Any other methods	7	9.7
Total	72	100.0

Table 2 Recycling method adopted

Do you recycle the waste?		
Response	Frequency	Percentage
Yes	1	1.4
No	71	98.6
Total	72	100.0

Table 3 Percentage of material composition in the domestic waste

Types of wastes material		
Items	Frequency	Percentage
Paper	48	66.7
Carry bags	16	22.2
Ball point pens	2	2.8
Buckets	1	1.4
Fold clothes	1	1.4
Old clothes	1	1.4
Waste food	1	1.4
Medicine	2	2.8
Total	72	100.0

Table 4 Average waste disposal (in kg)

Domestic waste (in kg)	Frequency	Percent
1	31	43.1
2	41	56.9
Total	72	100.0

The Table 4 shows the average disposal of household waste. Almost 57 % of the respondents are disposing nearly 2 kg on an average basis and 43 % of the respondents dispose only 1 kg. Table 5 shows that the various agencies involved in collecting the solid waste and garbage waste in the study area.

Among the samples, most of the respondents i.e., 85 % of them said that waste is collected by the municipal worker, next to this people pay a private collector to collect the waste and only four respondents said that NGOs are playing a vital role in collecting the solid waste and these NGOs convert the solid waste into wealth. The frequency of solid waste collection is given in Table 6.

Table 6 infers the regular collection of solid waste by the municipal workers on daily basis. Around 43 % of the respondents stated that the municipal workers are

Table 5 Agency involved in collecting the waste

Mode of collection	Frequency	Percent
Municipality	61	84.7
Private	7	9.7
Others	4	5.6
Total	72	100.0

Table 6 Regular of solid waste collection

Daily collection	Frequency	Percent
Yes	31	43.1
No	41	56.9
Total	72	100.0

Table 7 Willingness to pay for clean atmosphere

Willingness	Frequency	Percent
Yes	45	62.5
No	27	37.5
Total	72	100.0

collecting the waste daily and majority of the respondents i.e. 57 % of the sample respondents stated that workers are not collecting the waste regularly.

4 Respondent's Willingness to Pay

Some of the respondents are willing to pay for the waste disposal, the frequency is given in Table 7.

It is clear that almost 62.5 % of the respondents expressed their willingness to pay for cleaning the garbage waste in their surrounding area and to prevent the location from diseases. Only 37.5 % of the respondents have stated that they are not willing to pay as they are paying the house tax and hence they claim it is the responsibility of the Tirupattur municipality.

5 Impact of Improper Disposal of Solid Waste

Large quantities of solid waste are subjected to uncontrolled, unscientific and incomplete combustion which results in release of a number of toxic gases into the atmosphere which causes air pollution, acid rain etc. Large quantities of chemicals are quickly pushed into drains and rivers causing immense damage to human health. Dumping of agriculture solid waste and municipal solid waste will pollute soil, affect it's fertility and contaminate the ground water. Solid waste produces foul smell, breeds insects and mosquitoes besides deteriorates the aesthetic value of land. Solid waste changes the properties of air, soil and water.

6 Conclusion

Solid waste management is one of the serious problems in Tirupattur in particular and all over India and world in general. In Tirupattur town the accumulation of solid waste generation is about 12.50 MT per day, of these nearly 10.00 MT of the solid waste collected, transported and disposed daily which works to per capita generation of 250 g/day. Only one respondent in the sample is recycling their domestic waste for gardening and the rest of them were simply dumping in the open

space. As the income level of the respondent increases the usage of plastic bags and paper also increase and it ends in the form of waste. Nearly 62 % of the respondents are having the willingness to pay for clean environment.

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Site Suitability Analysis for Solid Waste Management Using Multi Criteria Analysis

Sanhita Ghosh and Sachikanta Nanda

Abstract Solid waste management is the most challenging problem of the current world. Due to rapid urbanization and growing population there is a huge increase in solid wastes which need to be disposed properly without causing any environmental problem or adverse effect on human. There are so many methods for waste disposal in practice, but sustainable solid waste management needs the selection of a suitable site along with suitable criteria of consideration. In the present study, with the help of geospatial techniques site suitability for solid waste disposal has been assessed cost effectively for Kattankulathur block of Kancheepuram district in Tamil Nadu. The satellite images and field data gives a complete idea about the surface criteria and Geographic Information system (GIS) helps us to analyse all the spatial and non-spatial data to get the desirable output. Toposheets, LANDSAT TM and ASTER DEM data have been used for generation of various layers like transportation, drainage, geomorphology, land use/land cover and geology in Arc GIS platform. By using Multi Criteria Analysis (MCA), different ranks and weightages have been assigned to all thematics according to their suitability. The different thematics have been overlayed and final site suitability map for solid waste disposal has been obtained. The entire block has been divided into five classes according to suitability criteria as very high, high, moderate, low and unsuitable for solid waste disposal.

Keywords Solid waste management · Site suitability · Geospatial techniques · Multi criteria analysis

S. Ghosh (✉) · S. Nanda
Department of Civil Engineering, SRM University, Kattankulathur, Tamil Nadu, India
e-mail: sanhitaghosh027@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_3

1 Introduction

Solid waste is the non-liquid waste produced by different human activities such as commercial, industrial, domestic, agricultural, biomedical, etc. There has been a significant increase in solid waste generation in India over the years from 100 g per person per day in small towns to 500 g per persons per day in large towns (Nishanth et al. 2010). The main environmental problem associated with urbanization is the amount of solid waste generated which exceeds the ability of natural environment to absorb or the authorities to manage.

There are several techniques for solid waste management including incineration, vermicomposting, sanitary landfill, dumping on land and ocean dumping, etc. But the accumulation of solid waste on ground in an unscientific way leads to emission of toxic gases (carbon-di-oxide, methane), contamination of groundwater, soil with hazardous substances. So, proper site selection along with proper method is very much essential for solid waste disposal. The site selection procedure is also very complicated as it comprises of different themes or parameters, e.g., geology, geomorphology, soil types, landuse pattern, slope, transportation, drainages along with types and sources of solid wastes. Geospatial techniques help to associate all the factors to find the appropriate site for waste disposal. It gives spatially complete and temporal information about the state of the Earth's surface which helps in the complete study about the ground features. Site selection for sanitary landfill therefore requires an extensive evaluation process in order to find the best available location (Sumathi et al. 2008). The location should be chosen according to Government laws, economic, environmentally safe and accepted by most of the stakeholders.

2 Materials and Methods

2.1 Study Area

The study area is Kattankulathur block of Kancheepuram district in Tamil Nadu, India. The area is situated between 12°36′58″N–12°53′48″N in latitude and 79°52′40″E–80°10′18″E in longitude. The total geographical area of the study area is 378.53 km^2. Palar river is flowing over the Southern part of the block. Sandy loam is the major soil group of study area. Temperature varies between 20 and 42 °C. Humidity is relatively high all over the year, 58–84 %. Average annual rainfall is between 1105 and 1214 mm. The location of study area is shown in Fig. 1.

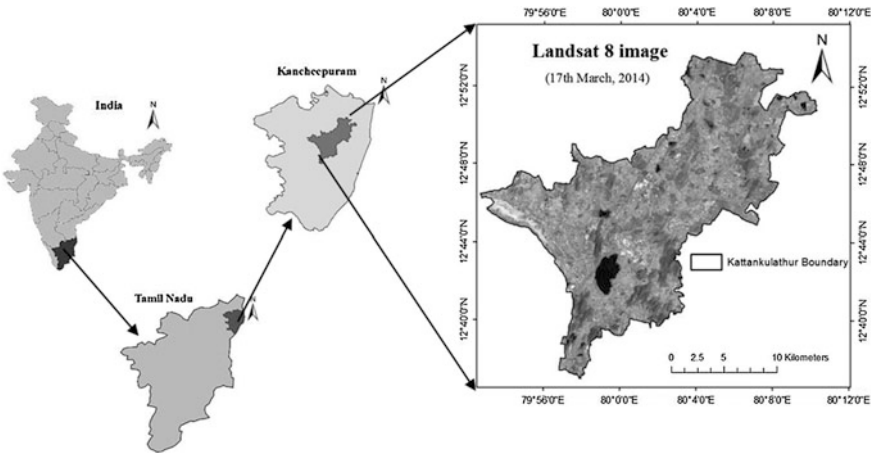

Fig. 1 Study area (Kattankulathur block)

2.2 Data Used

Spatial Data

i. Toposheets—57P/13, 57P/14, 66D/1, 66D/2 (SOI)
ii. Landsat 8 data (USGS, 30 m resolution)
iii. ASTER DEM (USGS, 30 m resolution)
iv. Geology Map (GSI—District resource map for Kancheepuram, TamilNadu)

Non-spatial Data

i. Soil data (NBSS)
ii. Population data (Census, 2011).

2.3 Generation of Thematics Maps

Different thematics have been created from the spatial and non-spatial data with the help of GIS software. Drainage map, transportation map have been prepared by digitization of toposheets (57P/13, 57P/14, 66D/1, 66D/2) of 1:10,000 scale collected from Survey of India (SOI) after geometric corrections in ArcGIS 10.2 software. Buffer maps of drainage and transportation have been created. The slope map has been created from contour map generated from the ASTER DEM data. Landuse and landcover, geomorphology maps have been delineated from Landsat 8

data (17th March, 2014) by visual interpretation. Geology, soil and population maps for the study area have been created by digitizing geometrically corrected district resource map for Kancheepuram district collected from Geological Survey of India (GSI), National Bureau of Soil Sciences (NBSS) and from population data of census, 2011, respectively.

2.4 Overlay of Thematics and Multi Criteria Analysis (MCA)

The study of solid waste management using geospatial techniques is based on Multi Criteria Analysis. Weightages to all the factors, such as landuse and land cover, geology, geomorphology, soil, etc. have been given as percentages as tabulated in Table 1. Ranks for each classes of each factor are according to their influence on solid waste disposal. All the vector layers have been converted to raster layer for overlay analysis. With the help of Arc GIS software Multi Criteria Analysis has been performed to generate the final suitability map.

3 Results and Discussions

3.1 Geomorphology

Geomorphology map of the study area prepared from Landsat 8 data has been shown in Fig. 2. There are residual hills, structural hills surrounded by pediments, pediment-inselberg complex. There is an occurrence of flood plain in the southern part of the study area. Deep, moderate and shallow buried pediments, linear/curvilinear ridge, sedimentary high land and tertiary upland are also present over the study area. Table 1 gives ranks of different geomorphological features according to their height, slope and rate of infiltration.

3.2 Geology

In case of waste management, geology is one of the important factors. The geology map (Fig. 3) of the study area demonstrates that Charnockite is the major rock type present in the study area. Sand, silt can be observed near the river basin. Green shale is present over some places. The ranks of geological features are mentioned in Table 1.

Table 1 Ranks and weightages assigned to the factors

Factors	Categories	Ranks	Weightage (%)
Geomorphology	Tertiary upland	5	20
	Residual hills, structural hills	4	
	Sedimentary high land	3	
	Deep buried pediment, moderate buried pediment, shallow buried pediment, pediment, inselberg, pediment-inselberg complex, linear/curvilinear ridge	2	
	Flood plain, river, water bodies	1	
Geology	Sand, silt	1	15
	Green shale	3	
	Charnockite	5	
Slope (in degree)	0–5	2	15
	5–20	5	
	20–40	4	
	40–60	3	
	60–90	1	
Soil	Clay	1	14
	Clay loam	2	
	Loamy sand	4	
	Sandy clay	3	
	Sandy clay loam	5	
	Sandy loam	1	
Distances from drainage (in m)	0–500	1	12
	500–1000	2	
	1000–2000	3	
	2000–3000	4	
	>3000	5	
Population density	<500	5	10
	500–1000	4	
	1000–2000	3	
	2000–3000	2	
	>3000	1	
Land use/land cover	Settlement	1	8
	Crop land	1	
	Natural vegetation	2	
	Deep water	1	
	Shallow water	1	
	Fallow land	3	
	Barren land	5	

(continued)

Table 1 (continued)

Factors	Categories	Ranks	Weightage (%)
Distances from roads (in m)	0–500	1	6
	500–1000	2	
	1000–2000	3	
	2000–3000	5	
	>3000	4	
Total			100

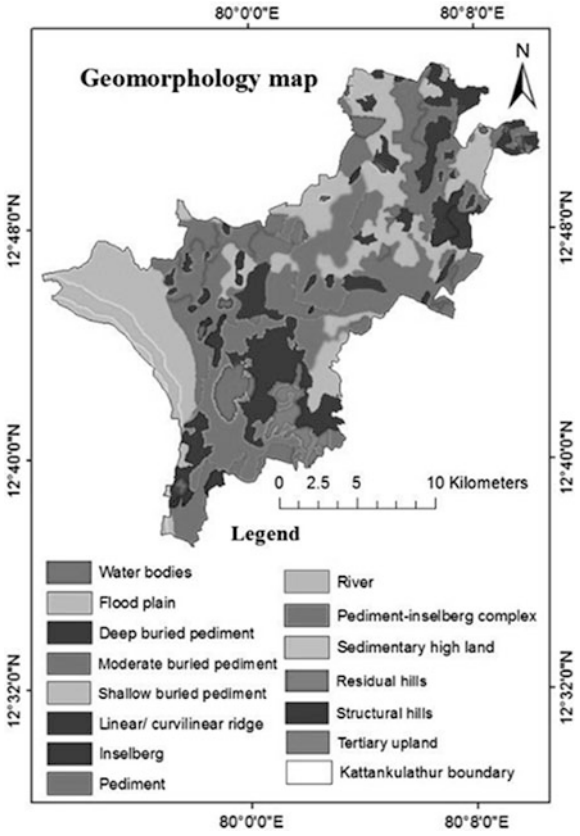

Fig. 2 Geomorphology map of study area

3.3 Slope

The slope map (Fig. 4) shows that most of the places of the study area have slope <20°. There are some residual hills with slope >60°. Rest of the places have slope between 20° and 60°. From the slope map it is clear that the study area is more or

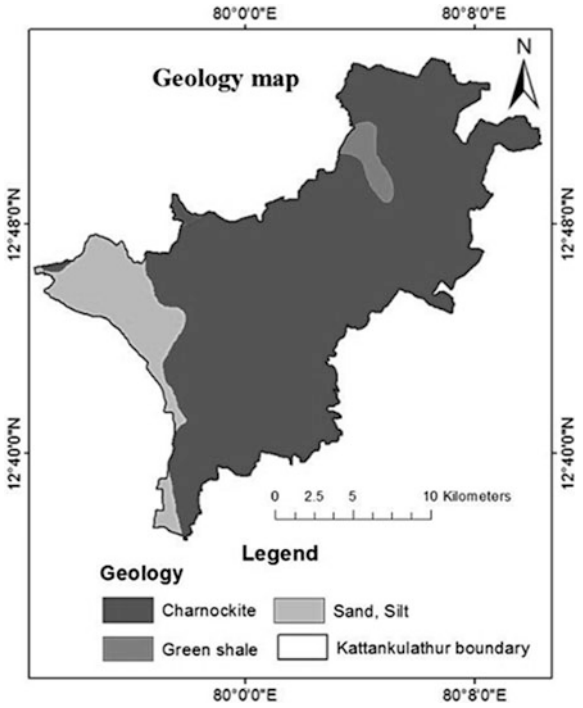

Fig. 3 Geology map of study area

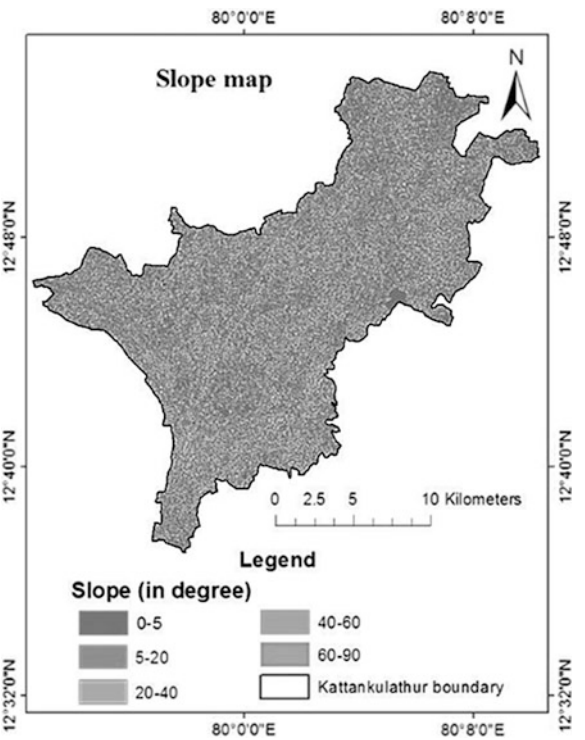

Fig. 4 Slope map of study area

less flat. Very steep or almost flat areas are not suitable for waste dumping, whereas moderate slope is more preferable (Oyinloye and Fasakin 2013).

3.4 Soil

Six different types of soil can be observed in the study area among which sandy loam occupies the maximum area which is almost 36.7 % of the total area (Fig. 5). Other groups present are clay, clay loam, sandy clay, sandy clay loam and sandy loam are also present there. Table 1 gives the ranks obtained by each category. Among the six types infiltration factor is high for sandy loam and less for clay. Very high and very less infiltration both are unsuitable for waste disposal, because it may cause water or air contamination. Sandy clay loam has moderate infiltration factor which has been considered as suitable.

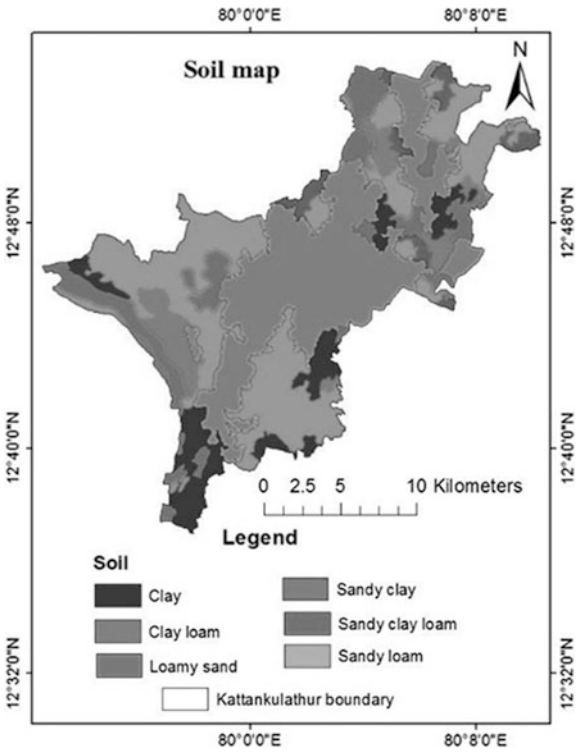

Fig. 5 Soil map of study area

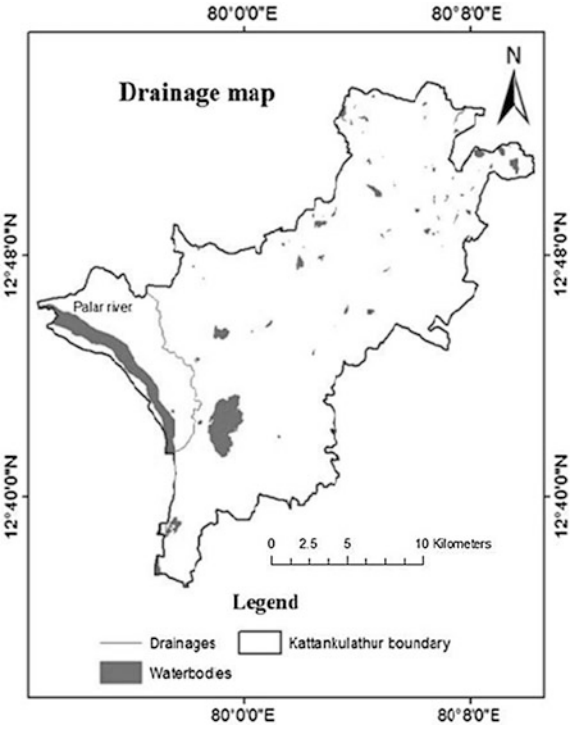

Fig. 6 Drainage map of study area

3.5 Drainage

In case of waste disposal, water contamination is one of the most critical issues. Waste disposal site should be chosen at a considerable distance from water bodies (Al-Hanbali et al. 2011). There are some lakes present over the area (Fig. 6). Some small drainage also can be observed. Main river which is flowing through the Southern part of study area is Palar river. Generally distances greater than 2000 m from the water bodies and drainages are considered as safe for waste dumping. Buffer of drainages has been shown in Fig. 7.

3.6 Population Density

A total of 50 *Panchayat* villages are there under Kattankulathur block. Highest population density has been found for Urapakkam and lowest for Thernmalpakkam. For solid waste disposal the low population density is to be considered as most suitable. Population density map and rank table have been given in Fig. 8 and Table 1 respectively.

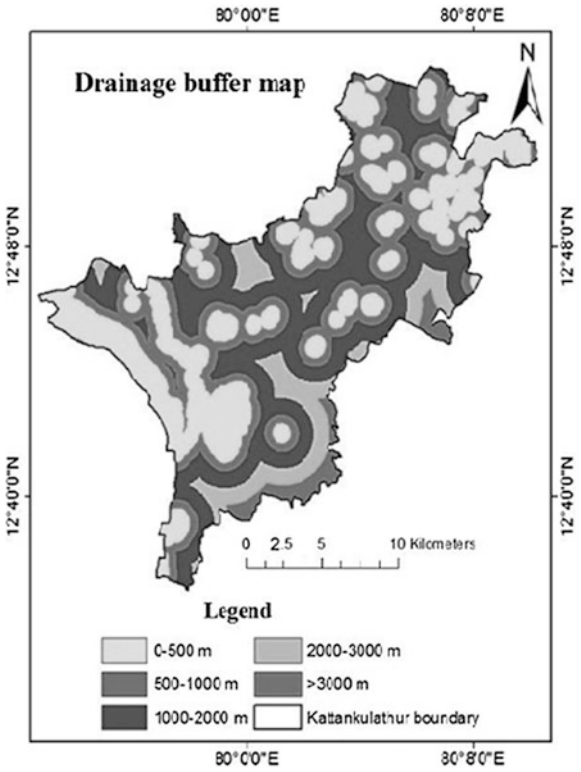

Fig. 7 Drainage buffer map of study area

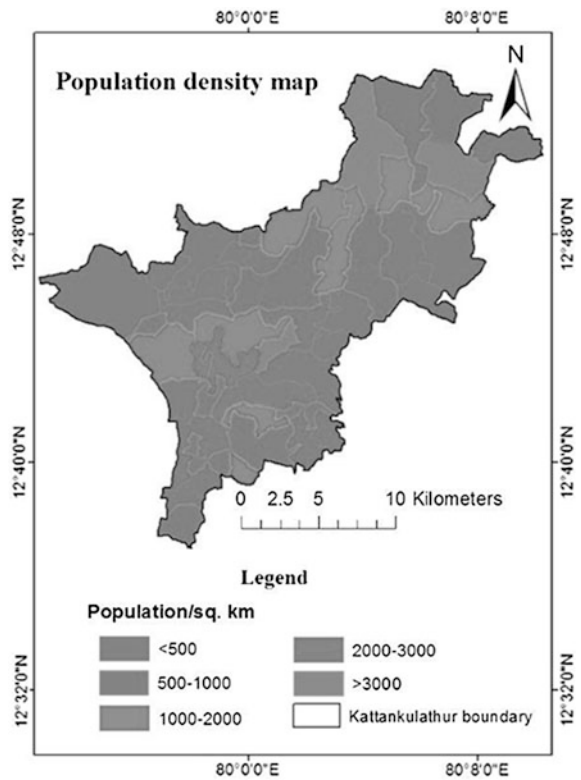

Fig. 8 Population density map of study area

3.7 Land Use/Land Cover

Land use and land cover map is given in Fig. 9. Seven types of land uses can be identified in the study area such as settlement, deep and shallow water body, crop land, natural vegetation, fallow land and barren land. For waste dumping, the site should be far away from settlement, agricultural area water bodies to avoid pollution and related health problems. The barren lands are more suitable for waste disposal. Ranks for each land use pattern are mentioned in Table 1.

3.8 Transportation

Kattankulathur is connected through many major and minor roads to the national highway (NH-45). The lengths of national highway, major roads and minor roads are 29, 150, 580 km respectively. Waste disposal sites should not be very near to the major roads. On the other hand transportation should be cost effective. 2000–3000 m distance from the major roads is considered to be safe and suitable for dumping (Ebistu and Minale 2013). Transportation and buffer maps are given in

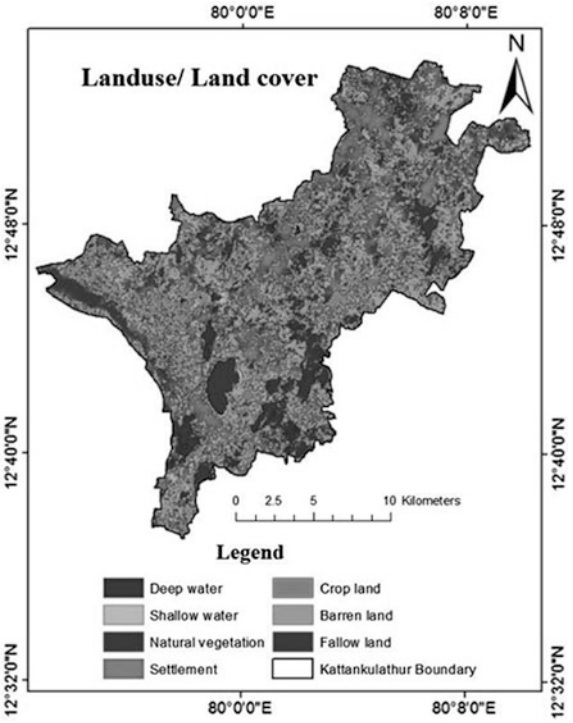

Fig. 9 Land use/land cover map of study area

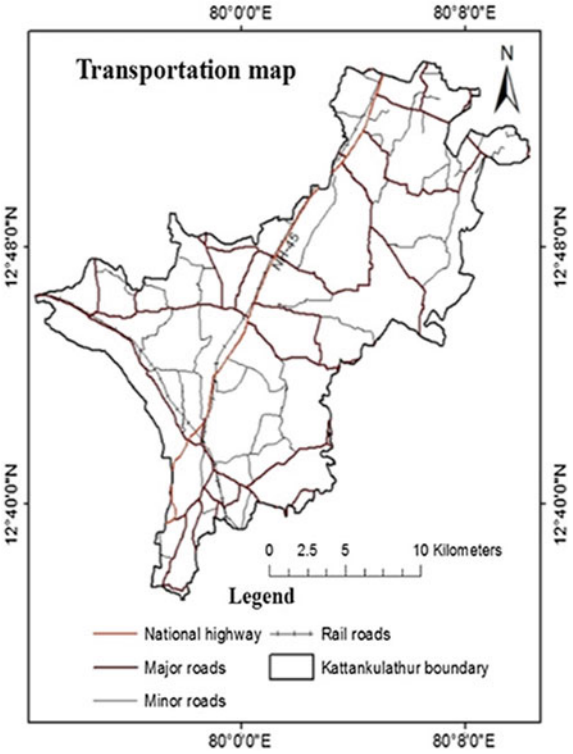

Fig. 10 Transportation map of study area

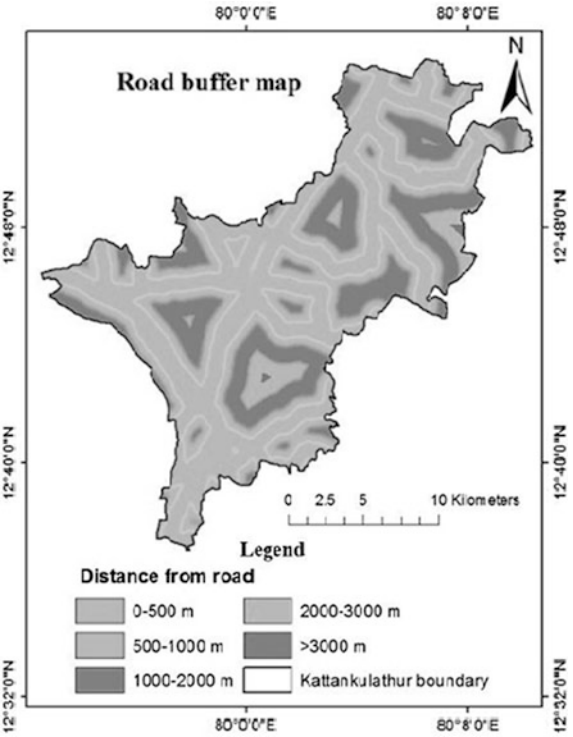

Fig. 11 Road buffer map of study area

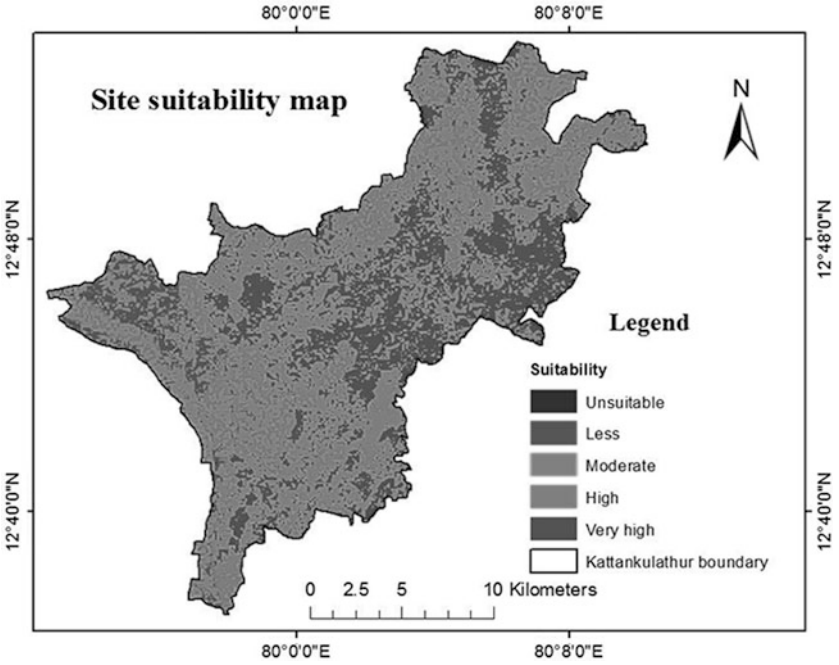

Fig. 12 Site suitability map for solid waste disposal

Figs. 10 and 11, respectively. Using weighted overlay analysis by Arc GIS software the final suitability map has been obtained which is shown in Fig. 12.

4 Conclusions

In this study, geomorphology, geology, soil, distance from drainages, population and transportation layers have been taken into consideration to make that procedure of solid waste disposal environmentally safe and economically feasible. The total area of Kattankulathur block has been divided into five classes according to their potentiality in solid waste management practice. Very less area (0.85 km^2) is unsuitable for waste disposal. Almost 179.16 and 111.05 km^2 area are categorised as highly and very highly suitable for solid waste management, respectively. Less and moderate suitable sites cover 17.81 and 69.66 km^2 area, respectively. Depending on the degradability of wastes the site selection may be variable but this study gives a complete idea about application of geospatial techniques in the field of solid waste management.

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Part II
Alternate Clean Energy Fuel Technologies

Nitrate Reduction by Denitrifying *Bacillus Cohnii* Isolated from Sewage Treatment Plant

T.K. Poornima Priyadharsani, D. Suriyaprakasham, P. Prakash and K. Thamaraiselvi

Abstract In this study, six bacterial colonies were isolated from the wastewater at Tiruchirappalli Corporation Sewage Treatment Plant, Panjappur, Tamil Nadu. Among the six bacterial isolates, only one has the capability to remove 90.6 % of nitrate within 120 h. The phenotypic and genotypic characteristics were identified using biochemical tests and 16S rRNA analysis. In the phylogenetic tree, the organism has 97 % similarity with *Bacillus cohnii* and the organism was confirmed as *B. cohnii.* Environmental factors such as pH, temperature, NaCl and incubation time showed the maximum nitrate reduction at pH 10, 45 °C, 5 % and 120 h, respectively. When compared with the control (without nitrate), the denitrifying bacteria expressed considerable amount protein on 57, 48, 43, 36, 33, 25, 18, 16, 14 and 12 kDa, this was probably attributed due to a higher degree of functional diversity among the bacteria.

Keywords Sewage treatment plant · *Bacillus cohnii* · Nitrate reduction · Denitrifying

1 Introduction

Water resource development exists worldwide and there is tremendous pressure in protecting it. One of its important goals is to protect surface water resources from anthropogenic activities. Discharge of wastewater into surface water bodies mainly in urban areas create lot of environmental health hazards to the public which is mainly due to improper management. Due to expansion of urbanization, the quality of many freshwater streams and ground water are deteriorating and it also leads to depletion of these water resources. It is observed that in the past three to four

T.K. Poornima Priyadharsani · D. Suriyaprakasham · P. Prakash · K. Thamaraiselvi (✉)
Molecular Bioremediation and Nano Biotechnology Laboratory, Department of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India
e-mail: kthamaraiselvi@hotmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_4

decades there is a gradual decrease in the quality of drinking water mainly due to high levels of nitrate concentrations. Many countries report that the level of nitrate has increased tremendously since 1950s. Nitrate pollution will cause serious impact on the development of national economy and people's daily life and it has to be considered seriously. Both nitrate and nitrite are naturally occurring ions which are part of the nitrogen cycle. The nitrate ion (NO_3^-) is the stable form of combined nitrogen for oxygenated systems. Although it is chemically inert, it can be reduced by microbial action. Nitrate is a natural compound present in all ecosystems. It is one of the chemicals essential to plant life but too much can be problem for plants and animals. Sources of nitrate into water bodies are mainly from surface run-off, surface flow or groundwater exchange, agricultural practice, domestic and industrial waste discharge, sewage and atmospheric nitrogen pollution (Vitousek et al. 1997; Carpenter et al. 1998; Howarth et al. 2000; National Research Council 2000; Smil 2001; Galloway and Cowling 2002).

The accumulation of anthropogenic nitrate has also caused hypoxia in western coast of India (Naqvi et al. 2000). In addition of causing ecological problems to the surface water bodies, nitrate in groundwater can persist for decades and accumulate to a high level, which affects water quality and threatens the safety of drinking water (Hallberg 1989; Nolan et al. 1997; Yang et al. 1998; Knobeloch et al. 2000).

Health impacts to humans and animal life occur from drinking water and/or consuming foods high in nitrate such as vegetables and meat. In humans, pregnant women are advised to avoid high nitrate water because reports shows possible connections to birth defects and miscarriages. Also, babies under six months may get a disease commonly called Blue Baby Syndrome, or medically called methemoglobinemia. Early human symptoms include a bluish tint to fingers, lips and other extremities. Other symptoms include headache, dizziness, lethargy, syncope, coma, arrhythmias, shock, and convulsions and even brain damage or death can occur (Rouse et al. 1999). Ruminant livestock tend to experience a similar illness to Blue Baby. If too much of hemoglobin changes to methemoglobin, the animal will begin to show signs of a lack of oxygen, lack of coordination, labored breathing, blue coloring of mucous membranes, rapid heart beat, abdominal pain and vomiting, blood discolored to chocolate-brown, and abortions (Rouse et al. 1999).

Correlations have also been made between high nitrate and toxicity in fish eggs, amphibian egg, and tadpoles. Fertilizers often increase the amount of nitrate and phosphate in surface water and groundwater resulting in accelerated algae and weed growth. When the algae and weeds decay, the decomposers remove oxygen from the water, which can kill fish and other aquatic life (Spencer 1975; Kinne 1984; Gleick 1993; Wetzel 2001; Rabalais 2002).

The WHO recommended maximum limit for nitrate concentration in drinking waters as 50 mg l^{-1} NO_3^-, equivalent to 11.3 mg l^{-1} as NO_3^-–N.

Denitrification is mainly sustained by denitrifying bacteria, although it has been seen in certain fungi. Nearly 130 species of bacteria including Archaeabacteria can

reduce NO_3^- to N_2 (Zumft 1992). Most of the denitrifying bacteria can survive in the presence of oxygen. However, denitrification was only induced in anaerobic conditions in which microorganisms use nitrogen oxides (NO_x) as electron acceptors. It is an alternative pathway to aerobic respiration (Enwall et al. 2005). Denitrifying bacteria also have the ability to degrade toxic organic matter in the environment. Song et al. (2000) isolated 33 strains of halobenzoate-degrading denitrifying bacteria from halobenzoate enriched soil and sediment samples. Rudolphi et al. (1991) studied enzymatic steps of *Pseudomonas* and *Paracoccus* strains that can metabolize methyl phenols and dimethylphenols in anaerobic condition along with reduction of nitrate. With the ability to degrade organic matter, denitrifying bacteria plays a crucial function in reducing organic carbon, thereby reducing nitrate in the wastewater and soils (Neef et al. 1996; Hallin and Pell 1998; Pai et al. 1999; Song et al. 2000). Reports show that *Achromobacter, Agrobacterium, Alcaligenes, Bacillus, Chromobacterium, Flavobacterium* and *Hyphomicrobium,* are responsible for denitrifaction (Otlanabo 1993).

The study has been designed to isolate and identify the denitrifying bacteria from sewage. And further more the protein expression in the presence and absence of nitrate in the isolates were also studied.

2 Materials and Methods

2.1 Sample Collection

Sewage water sample was collected from Tiruchirappalli Corporation Sewage Treatment Plant, Panjappur, Tamil Nadu, South India. The sample was collected in sterile plastic container and stored at 4 °C until use.

2.2 Isolation of Denitrifying Bacteria

To isolate the denitrifying bacteria, serial dilution was done in the range of 10^{-1}–10^{-9}. The samples from 10^{-4} to 10^{-7} dilution were inoculated in the medium containing peptone–0.5 g, yeast extract powder–0.3 g, NaCl–0.5 g in 100 ml of double distilled water. The medium was sterilized at 121 °C for 15 min. The serial dilutions were spread on plate of medium containing 2 % (w/v) agar and incubated at 40 °C for 24 h. The size and color of the colonies were recorded. Then the strain was purified by repeatedly streaking of single colonies in agar plates containing the above medium with 2 % agar. The plates were incubated for 24 h at 40 °C. Pure bacterial strain obtained was kept in LB slant at 4 °C.

2.3 *Identification of Denitrifying Bacteria*

2.3.1 Phenotypic Analysis

Biochemical and morphological tests namely gram staining, indole production, motility, methyl red, Voges Proskauer's, citrate utilization, triple sugar iron, catalase, urease, oxidase, ortho-nitrophenyl-β-galactoside (ONPG), nitrate, and starch hydrolysis were carried out to identify the denitrifying bacteria.

2.3.2 Genotypic Analysis

Bacterial culture was grown in nutrient yeast extract medium until stationary phase and centrifuged to form a pellet. The pellet was suspended in TE buffer and 10 % SDS was added and mixed thoroughly and incubated for 1 h at 37 °C. To this, 5 M NaCl and CTAB/NaCl was added, mixed well and incubated for 10 min at 65 °C. To the above, 1 volume of chloroform: Isoamylalcohol (24:1) mixture was added and centrifuged for 4-5 min. To the supernatant, isoproponal was added to precipitate the DNA. The contents were then gently shaken until precipitates of DNA appeared. The precipitated DNA was collected in fresh micro tubes and 70 % ethanol was added and centrifuged for 30 s at 10,000 rpm. Ethanol was decanted and the pellets were dried at 37 °C. The DNA was dissolved in TE buffer.

Agarose gel (0.8 %) was prepared in 1X TAE electrophoresis buffer and ethidium bromide dye (ethidium bromide stock 10 mg ml^{-1}) was added. Loading dye (1X) was added to DNA samples. The isolated DNA sample and DNA marker were loaded. The samples were run for 1 h at 100 V (Medox) and DNA was visualized by exposure to UV transilluminator (Gel Doc™XR+, Bio-Rad, USA).

The DNA was amplified using universal 16S rRNA primers - A 8F and A 1492R (Ocimum Biosolutions Ltd) (Table 1) (Stephen et al. 1996). The PCR reaction was performed in a 50 µl containing 0.1 ng of template DNA, PCR Master Mixer, 10 pmol concentration of each primer and 0.025 U of Taq DNA polymerase enzymes. The final volume was adjusted with sterilized Mili-Q water. A PCR thermocycler (Bio-rad) was used to amplify the reactions through an initial denaturation step consisting of 94 °C for 2 min followed by 25 cycles at 94 °C for 1 min, 52.3 °C for 1 min and with an extension of 72 °C for 1 min followed by an final extension temperature at 72 °C for 2 min. Amplified PCR products were stored at −20 °C.

The PCR amplified product was loaded on 1.4 % agarose in 1X TAE buffer at 50 V for 45 min and the PCR products were visualized in a UV transilluminator. Lambda DNA double digested with EcoR I and Hind III was used as a marker. Purification of amplified PCR product was done using a purification kit (HiYield™ Gel/PCR DNA extraction Kit-Real Genomics, Taiwan). The amplified DNA sample was mixed with TE buffer and its absorbance was read at 260 nm using UV–Visible spectrophotometer (Model UV-1700 Shimadzu). TE buffer served as a

Table 1 Morphological and biochemical characteristics of the isolated strain

S. No.	Biochemical characteristics	Results
1	Gram's staining	+
2	Shape	Rod
3	Motility	Motile
4	Indole	+
5	Methyl red	+
6	VP	−
7	TSI	A/A
8	Citrate	−
9	Urease	−
10	Oxidase	+
11	Catalase	+
12	Nitrate	+
13	ONPG	−
14	Starch hydrolysis	+
15	Isolated strain	Bacillus Sp.

+ Positive, − Negative, *A/A* Acid slant/acid butt

blank. An optical density (OD) of one corresponds to approximately 50 μg mg^{-1} of double standard DNA (Sambrook et al. 1989). Based on the OD value, DNA sample was quantified.

The 16S rRNA sequencing of for the amplified product was carried out at Xcelris Labs Ltd, Ahmadabad, India. The sequencing reactions were run on ABI-PRISM automated sequencer-ABI-3730 DNA analyzer (Applied Biosystems, USA). The nucleotide sequence was initially analyzed at Blast-n site in NCBI server (http://www.ncbi.nlm.nih.gov./BLAST) and corresponding sequences were downloaded. The alignment of the sequences was done using Clustal X software program. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches. The clock calibration of 0.01 (time $node^{-1}$ height) was used to convert distance to time. The phylogenetic tree was lineralised assuming equal evolutionary rates in all lineages. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distance tree was constructed using the aligned sequences by the neighbor joining method using MEGA 4 software (Tamura et al. 2007).

2.4 Protein Studies

The bacterial strain was grown in nutrient broth in absence and presence of nitrate (0.5 g l^{-1}). The pure culture was used to extract the protein. Both, the cells were harvested by centrifugation and further protein extraction was done using PBS (Phosphate buffer saline) buffer.

Protein concentration was estimated by Bradford method (1976) using bovine serum albumin as a standard at 595 nm.

Sodium Dedocylsulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) was performed on one dimensional 10 % polyacrylamide slab gel containing 0.1 % SDS. Gel was run on a miniature horizontal slab gel unit. A sandwich was made with two glass plates separated by spacer strips (1.5 mm thickness). The resolving gel mix was poured into the space between the glass plates. A stream of distilled water was layered onto the gel to exclude oxygen from inhibiting polymerization and to ensure a uniform flat gel surface. After decanting, 5 % stacking gel was prepared and poured over the separating gel. Teflon comb (1.5 mm thickness) was inserted to form wells. Care was taken not to trap any air bubbles while casting the gel. Teflon comb was removed after polymerization. The basal strip was removed and the glass plates with polymerized gel were fixed to the electrophoresis apparatus. Protein samples were mixed with equal volumes of sample loading buffer, kept in boiling water bath for 10 min and then loaded onto each well. After electrophoresis, gel were stained with coomassie brilliant blue at 0.025 % (w/v) in methanol: acetic acid: distilled water (4:1:5 v/v/v) at room temperature and destaining was done in methanol:acetic acid:distilled water (4:1:5 v/v/v). The apparent subunit molecular weight was determined.

2.5 *Batch Studies for Nitrate Reduction Using the Isolated Denitrifying Bacteria*

The bacterial isolate was inoculated into a series of conical flasks containing growth medium amended with 1 g l^{-1} of nitrate. The pH was varied from 2 to 14 using dilute HCl or NaOH. The cultures were kept under shaking condition at 40 °C for 24 h. After 24 h of incubation, the growth of the biomass was determined spectrophotometrically at 595 nm. The biomass was separated by centrifugation. The initial and final concentration of nitrate was calculated by estimating the concentration of nitrate at 410 nm spectrophotometrically (APHA-AWWA-WEF 1998).

For temperature effect, the temperature was varied from 25 to 60 °C and the experiment was conducted as before. Similarly the NaCl concentration was varied from 1 to 10 % and the study was carried as before.

Time dependent studies for nitrate reduction were carried out at optimized conditions (pH, temperature and NaCl concentration). The bacterial isolate was inoculated into a series of 50 ml conical flasks containing growth media amended with 1 g l^{-1} of nitrate. Culture was kept under shaking condition for 120 rpm. During the incubation period, nitrate concentration and biomass were monitored until nitrate reduction attains a saturation level.

3 Results and Discussion

3.1 Isolation and Identification of Denitrifying Bacteria

Six bacterial colonies were isolated from the sewage treatment plant, by using nutrient yeast extract medium. Under the batch tests, nitrate reduction rate were checked with different isolated bacteria. Of these, only one bacteria effectively reduced nitrate (86.7 %) within 120 h (Fig. 1a). Figure 1b shows hydrolysis of starch in the medium by the isolated bacteria. Further studies were continued by denitrifying bacteria using this strain.

The isolate which reduced nitrate was identified as *Bacillus* sp by morphological and biochemical characteristic (Table 1).

The molecular weight of genomic DNA was determined as ~1500 bp. A distinct PCR product of the ~1400 bp size was produced when target DNA from isolated strain was used (Fig. 2). From this analysis, it was determined that 25 cycles and 0.1 ng of genomic DNA is required for successful amplification.

The 16S rRNA sequences of this strain showed 97 % of sequence similarity with *B. cohnii* strain (FJ161349) and was assigned as *B. cohnii* KTSMBNL07 in NCBI.

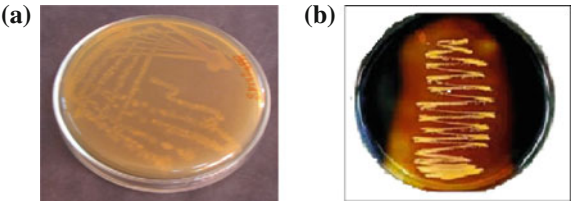

Fig. 1 **a** Growth of denitrifying bacteria. **b** Starch hydrolysis by isolated bacteria

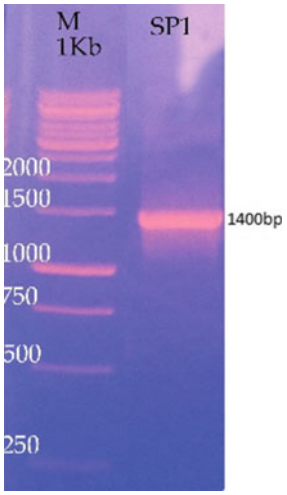

Fig. 2 Agarose gel electrophoresis for the PCR amplified product of 16S rRNA

3.2 *Nitrate Reduction by Denitrifying Bacteria*

Nitrate steadily increased with increase in pH and a maximum of 86.80 % was observed at pH 10 (Fig. 3). Decreased in reduction was seen at lower (2–4) and higher pH (12–14). Mostly for the denitrifying bacteria, optimum pH is in the alkaliphilic range of pH 8–10. The pH plays a vital role in all biological reactions. In denitrification, alkalinity was produced during the conversion of nitrate to nitrogen gas resulting in an increase in pH and as nitrate acts as an electron acceptor in the metabolism for generation of energy.

Wang et al. (1995) concluded that many enzymes were involved in the denitrification process and that enzyme kinetics were pH dependent. It has been reported that the pH is responsible for denitrification using a pure culture of *Pseudomonas denitrificans*. It was also reported that the optimum pH for the biological reduction of NO_3 (7.35–7.45) was slightly higher than the biological reduction of nitrite (7.12–7.20) and also the optimum pH does not change with NO_2^- or NO_3^- concentration. Furthermore, pH also affects the accumulation of denitrification intermediate products (e.g., NO, N_2O).

Temperature is also an important factor in determining the rate of reduction reaction (Das and Chandran 2011). Maximum nitrate reduction (82.28 %) was observed at 45 °C (Fig. 4).

The general denitrification rates have been reported to be directly proportional to the effective temperature (Carpenter et al. 1998). Rates of denitrification reactions increased with temperature in a manner similar to that of rates in nitrification reactions. Some bacteria can continue to carry out the processes at temperature upto 60 °C. These bacteria were relatively rare and as a result, reactions were not usually carried out at such high temperatures despite the expected increased in reaction rate. The denitrification rate is in the range of 10–35 °C. As the temperature increased, nitrate reduction was found higher in the biological denitrification process (Amatya et al. 2009). Most of the denitrifying bacteria are mesophiles that grow in the range of 20–45 °C.

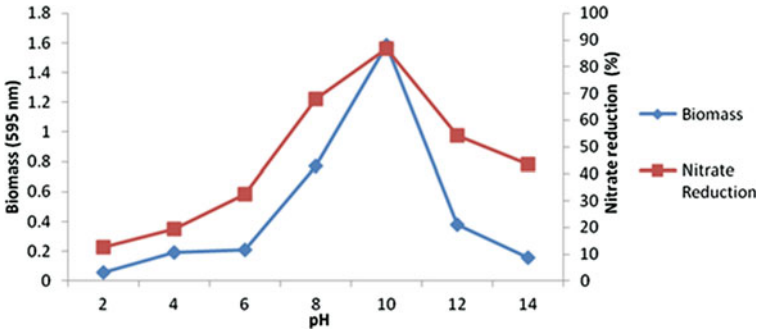

Fig. 3 Effect of pH on nitrate reduction by denitrifying bacteria

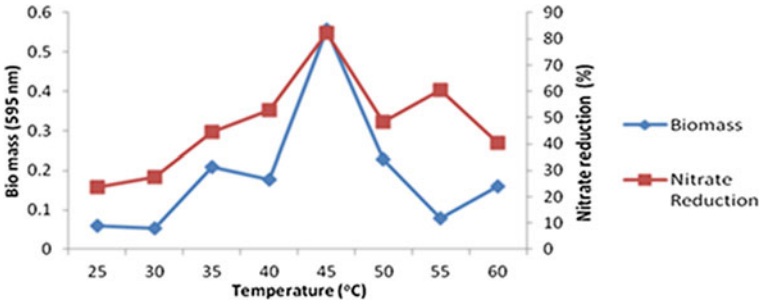

Fig. 4 Effect of temperature on nitrate reduction by denitrifying bacteria

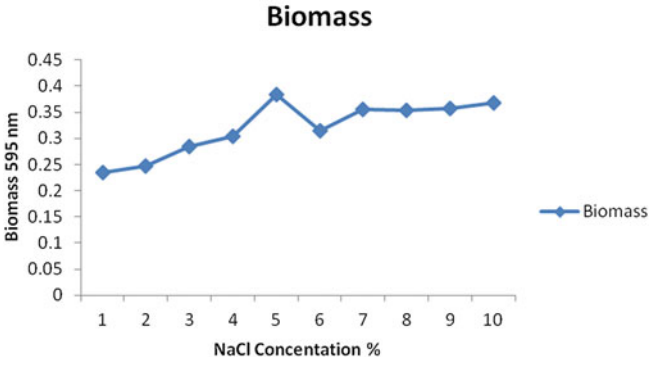

Fig. 5 Effect of NaCl concentration on denitrifying bacteria

Denitrifying strain *B. cohnii* was grown at different NaCl concentrations and the growth was higher in 5 % of NaCl when compared to others (Fig. 5).

The time dependent curve for nitrate reduction process of denitrification is shown in Fig. 6. After 24 h of incubation, the reduction efficiency was 35.7 % and further at 48, 72, 96, 120 h it was 39, 50, 70.8 and 90.6 % of NO_3^-, respectively.

3.3 Protein Studies

The amount of the protein present in whole cell fractions was 20 µg/10 µl. Protein from the *B. cohnii* was separated by 10 % SDS-PAGE. The nitrate treated bacterial strains exhibited various kDa proteins such as 57, 48, 43, 36, 33, 25, 18, 16, 14 and 12 kDa but in untreated bacterial strains the proteins were suppressed (Fig. 7).

Many bacteria have more than one of the three types of nitrate reductases which comprise the soluble assimilatory nitrate reductase and two dissimilatory reductases. It was further subdivided into the respiratory and the periplasmic nitrate

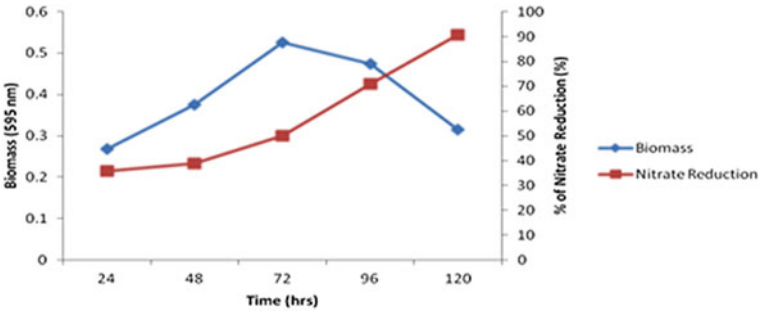

Fig. 6 Effect of time on nitrate reduction by denitrifying bacteria

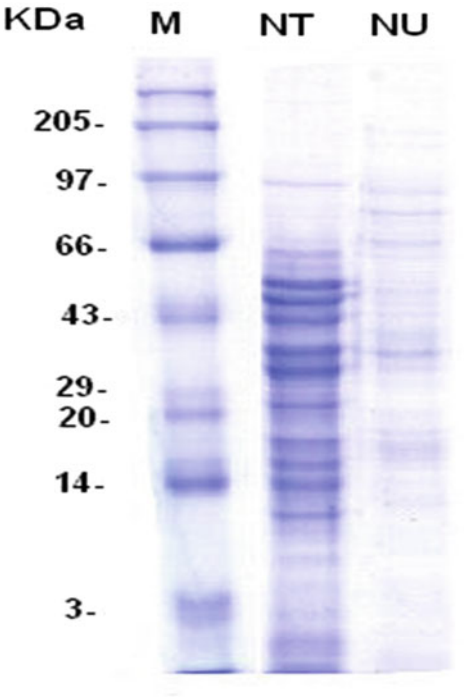

Fig. 7 Protein profile of denitrifying bacteria in the presence and absence of nitrate. M - Marker, NT - Nitrate treated, NU - Nitrate untreated

reductases. Nitrate reductase has been characterized from the denitrifiers *Halomonas halodenitrificans*, *Thiobacillus denitrificans*, *Rhodobacter sphaeroides* IL106, *Bacillus licheniformis* and *Bacillus stearothermophilus* (Hochstein and Tomlinson 1988). In dissimilatory reduction, the nitrate was mostly reduced by aerobic bacteria, where one or both of the ionic nitrogen oxides was converted into gaseous nitrogen oxides, which could be further reduced to dinitrogen gas. Reduction of nitrate into nitrite is by periplasmic proteins such as 57 kDa (narH),

48 kDa (nosD), 25 kDa (narJ), 18 kDa (napB) and 12 kDa (napD) (Berks et al. 1995; Hoeren et al. 1993; Holloway et al. 1996; Zumft et al. 1990, 1992; Blasco et al. 1992; Dubourdieu and DeMoss 1992; Grove et al. 1996; Siddiqui et al. 1993). In the nitrite reduction, further dissimilation process was done by some periplasmic proteins of denitrifying bacteria such as 43 kDa (nirF), 36 kDa (nirK) and 33 kDa (nosF) (de Boer et al. 1994; Kawasaki et al. 1995; Palmedo et al. 1995; Chen et al. 1996; Glockner et al. 1993; Nishiyama et al. 1993; Toffanin et al. 1996; Holloway et al. 1996; Zumft et al. 1990). The reduction of nitrous oxide to ammonium gas or nitrogen gas had been lead by dissimilatory process. Some periplasmic nitrate reductase enzyme such as 16 kDa (NorC) and 14 kDa were involved in nitrite to nitrogen gas reduction (Barthikas et al. 1997; de Boer et al. 1994; Zumft et al. 1994).

4 Summary and Conclusion

Wastewater treatment process is dependent on microbial activities for the reduction of pollutants. Denitrifying bacteria are important for the reduction of nitrogen compounds from waste water. This study is an initiative for developing new methods by microbes for the treatment of nitrate from domestic and industrial effluents. The isolated *B. cohnii* can be a preferred additive when compared to chemical agents used in nitrate removal. The application of microbes is advantageous, since it is simple, convenient, easy and economical. The present investigation concludes that denitrifying *B. cohnii* isolated from sewage could be employed as an effective reduction method for nitrate from aqueous solution.

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A Mini Review on Cyanophycin: Production, Analysis and Its Applications

J. Aravind, T. Saranya, G. Sudha and P. Kanmani

Abstract Cyanophycin or Cyanophycin Granule Polypeptide (CGP), a non-ribosomally synthesized amino acid polymer, consists of equimolar amounts of arginine and aspartic acid. It can be soluble under acidic (pH < 2) or alkaline (pH > 9) conditions. Cyanophycin has many advantages: high viscosity, high solubility and complete biodegradability. Because of its soluble and polymeric nature, CGP can be used in the fields of medical biotechnology for scaffold production. It has been proposed as an eco-friendly alternative for poly (acrylic acid) as water softening agents. CGP is usually produced by different species of cyanobacteria and also by some heterotrophic bacteria like *Acinetobacter* sp., *Bordetella bronchiseptica, Clostridium botulinum, Desulfitobacterium hafniense*, etc. Due to the low yield of CGP by cyanobacteria, bacterial strains are commercially preferred for industrial scale. *Acinetobacter* have been found to have high accumulation (46 % of cell dry weight) of CGP. Bacterial strains are grown in different production medium and the productivity of cyanophycin was analyzed. It has been found that addition of arginine to the medium has enhanced CGP accumulation. Characterization of cyanophycin can be performed by SDS-PAGE, HPLC and Mass spectroscopy analysis. Native PAGE analysis reveals that CGP occurs in the range of 21–29 kDa. Cyanophycin has potential applications in the fields of biomedicine, agro-chemistry, pharmacy and personal care.

Keywords Cyanophycin · *Acinetobacter* sp. · Cyanophycin production · Cyanophycin applications

J. Aravind (✉) · T. Saranya · G. Sudha · P. Kanmani
Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India
e-mail: dr.j.aravind@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_5

1 Introduction

Cyanophycin or multi-L-arginyl-poly (L-aspartic acid), a non-protein, non-ribosomally produced amino acid copolymer composed of equimolar amounts of aspartic acid and arginine. It consists of a poly-aspartate backbone and arginine residues which are linked to the β-carboxyl group of each aspartic acid by their α-amino groups (Simon and Weathers 1976).

The polypeptide comes from different species of cyanobacteria and has also been found in some heterotrophic bacteria. CGP is synthesized under conditions of imbalanced growth and contains five nitrogen atoms in every building block, and thereby constitutes an ideal intracellular nitrogen reserve (Mackerras et al. 1990). It occurs as insoluble inclusions in the cytoplasm and serves as a storage compound for carbon, nitrogen and energy (Simon and Weathers 1976; Sherman et al. 2001). The biopolymer is sequestered in intracellular granules of the microorganism, and is thus commonly also referred to as the Cyanophycin Granule Polypeptide (CGP) (Simon and Weathers 1976).

Cyanophycin usually accumulates during the stationary phase induced by a lack of a nutrient other than nitrogen and disappears when balanced growth resumes (Allen 1984; Simon 1987b). The cyanophycin content is low in exponentially growing cells, but increases during stationary phase to amounts of 8–18 % (w/w) of the cell dry mass (Feng et al. 2007). It also accumulates when nitrogen-starved or nitrogen-repleted cells are given a usable nitrogen source (Allen et al. 1980; Lawry and Simon 1982). In nitrogen-starved cells which were given a nitrogen source, cyanophycin accumulated transiently, and growth did not recommence until cyanophycin had been degraded again (Mackerras et al. 1990; Allen et al. 1980). Because of the isopeptide bonds, cyanophycin is resistant to proteolytic enzymes and requires special hydrolytic enzymes for degradation.

Purified CGP can be chemically converted to a derivative with reduced arginine content or even to poly (aspartic acid) (Joentgen et al. 1998). The latter is produced by the chemical industry as a biodegradable substitute for non-biodegradable polyacrylic acid (Alford et al. 1994), which is used for many technical and medical applications (Roweton et al. 1997; Joentgen et al. 1998; Schwamborn 1998). The biocompatibility and complete biodegradability makes cyanophycin, an ideal candidate for many applications in human life in the fields of biomedicine, agro-chemistry, agriculture, personal care and pharmacy (Obst and Steinbuchel 2004).

2 Structure of Cyanophycin

The molecular mass of CGP ranges from 25 to 100 kDa, as estimated by SDS-PAGE. Upon MALDI-TOF analysis, CGP gave an average value of approximately 29.5 kDa for the $[M + H]^+$ ion, with a maximal intensity at m/z = 29,361 and

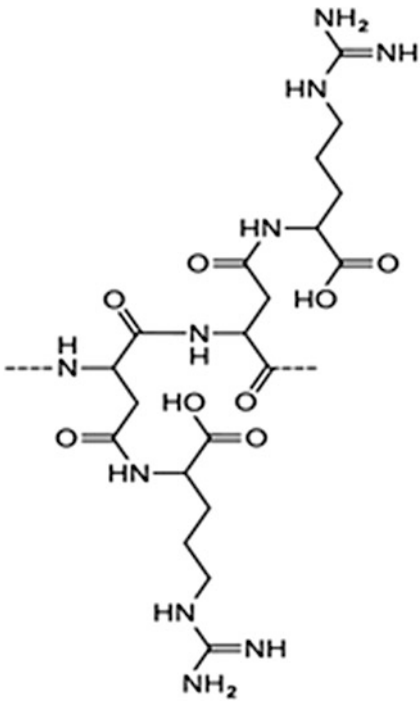

Fig. 1 Structure of cyanophycin

a broadness of about 1100 m/z (Ziegler et al. 2002). At isoelectric point (IP) 4.75, CGP may lose some of the arginyl residues and at IP = 6.1, molar ratio of aspartic acid:arginine in CGP is 1:1.

Circular Dichroism (CD) studies reveals that cyanophycin exhibited CD spectrum in far UV region and posses 50 % β-sheet, 45 % random coil and 0–5 % α-helix as secondary structure, but these spectral structures abolished at alkaline pH and at high concentrations of urea, further CD is not applicable for samples which scatter light. Raman spectral analysis elucidated that insoluble form of cyanophycin posses some β-sheet and no α-helix (Simon 1987a). Figure 1 is the two dimensional structure of cyanophycin.

3 Solubility of Cyanophycin

Cyanophycin has unusual soluble properties, it is insoluble in water at physiological pH and is soluble under acidic (pH < 2) or alkaline (pH > 9) conditions (Lang et al. 1972). It is also insoluble in H_2O containing metal ion, EDTA, sodium deoxycholate and organic solvents like methanol, ethylene glycol, dimethyl sulfoxide, formamide and dimethyl formamide. It is soluble in alkali metal (or) alkaline earth metal salts when added at high concentrations in an inorganic salt solution. It is not

affected by proteolytic enzymes such as proteases, pepsin, pronase, chymotrypsin, α- or β-carboxypeptidase, clostripeptidase B and leucin aminopeptidase (Simon and Weathers 1976).

4 Cyanophycin Producing Organisms

Cyanophycin was first discovered by Borzi in cyanobacteria, and it was described as a polymer that exclusively occurs in cyanobacteria. However, recently, the occurrence of genes encoding proteins homologous to cyanobacterial cyanophycin synthetases has been described for other eubacteria not belonging to the cyanobacterial family (Schwamborn 1998). Cyanophycin functions as an intracellular energy reservoir within cyanobacteria under nutrient limitations. The synthesis of cyanophycin can be directed by cyanophycin synthetase without the participation of ribosomes. The gene coding cyanophycin synthetase, cphA was identified in 44 prokaryotes by genetic analysis over the genomic sequences of 570 strains. Among them, different cphA genes have been cloned from *Anabaena variabilis, Synechoncystis* species and *Acinetobacter baylyi*, and expressed in various organisms, such as *Escherichia coli, Saccharomyces cerevisiae*, *Pichia pastoris, Corynebac-terium glutamicum, Ralstonia, Eutropha, Pseudomonas putida* and plants.

Because of the low polymer content and their slow growth rate, cyanobacteria are inappropriate organisms for an industrial scale production of cyanophycin (Mooibroek et al. 2007; Sallam et al. 2009). By gene transfer cyanophycin synthesis was introduced into various heterotrophic microorganisms like *E. coli, Ralstonia eutropha, P. putida*, yeast, etc. that accumulate cyanophycin in their cells up to 50 % of their dry weight (Aboulmagd et al. 2001). However, fermentation processes using cyanobacteria are not efficient and can result in very low yields. In addition, the requirement for addition of arginine in the medium makes the cost of growing large quantities of product prohibitive. As such there is a strong interest in finding alternative sources of the potentially valuable cyanophycin.

5 Screening of Cyanophycin Producing Organisms

Leeds Acinetobacter medium (LAM) is a differential medium developed to selectively support the growth of *Acinetobacter* sp. (Jawad et al. 1994). LAM contains cefsulodin and cephradine to inhibit the growth of Gram-negative bacteria and vancomycin to prevent Gram-positive growth. LAM also contains sucrose and fructose, which have not fermented by *Acinetobacter* sp., hence resulting in pink coloration of the medium upon growth of *Acinetobacter* sp. (McConnell et al. 2011).

The phenol red in the medium serves as a pH indicator. The base of the medium is turned mauve by the growth of *Acinetobacter* sp., which is due to the high alkalinity produced in the medium upon growth of the organisms, which changes the color of the phenol red indicator to mauve. This is due to the liberation of ammonium ions from complex nitrogenous materials present in the medium. The carbohydrates present in the medium are also extensively decomposed, but the alkalinity produced by aerobic organisms is greater than the acidity (Hugh and Leifson 1953; Stanier et al. 1966).

Acinetobacter sp. usually produces circular, convex, smooth, opaque colonies with entire margins of 1–2 mm in diameter after 24 h of incubation at 30 °C in air (McConnell et al. 2011). They possess high growth rate at 30 °C and at low pH. With respect to nutritional properties, the *Acinetobacter* sp. resembles many species of the aerobic pseudomonads (Stanier et al. 1966). Under conditions of homogeneous aeration, *Acinetobacter* overgrows the pseudomonads either because of greater growth rate or because of its occurrence in greater numbers in water and soil.

6 Production of Cyanophycin

Acinetobacter calcoaceticus strain ADP1 was cultivated at 30 °C in complex Luria-Bertani (LB) medium or in a Tris-HCl-buffered mineral salts medium (MSM) with sodium glutamate and ammonium sulphate as carbon and nitrogen source respectively. However, only small amounts of CGP were detected in the cells (the maximum amount was 1.4 % [wt/wt] of the total protein), and these also occurred only if there was phosphate limitation (Krehenbrink et al. 2002). Ammonium limitation gave the lowest CGP contents, as expected, since nitrogen is the major constituent of the CGP molecule.

Variation of the initial pH of the basic medium did not enhance the accumulation of CGP significantly. Addition of chloramphenicol, an inhibitor of ribosomal protein biosynthesis known to stimulate CGP synthesis (Simon 1973a), had no significant effect on the CGP content of the cells after 20 h of cultivation. Only in cells cultivated for 40 h in Erlenmeyer flasks without baffles was a markedly higher CGP content measured in the presence of chloramphenicol (Elbahloul et al. 2005a). Varying the nitrogen-to-carbon ratio in MSM by using sodium glutamate and ammonium sulfate as the carbon and nitrogen sources, respectively, and varying the concentrations of these two nutrients in the medium had no significant effect on the amounts of CGP accumulated by cells of *A. calcoaceticus*. Addition of arginine had a substantial positive effect on accumulation of CGP. In the absence of aspartate in the medium, and with the addition of 15 and 30 mM arginine, the cells accumulated CGP at levels that were about 23 or 28 % of the CDM, respectively. A high ratio of arginine to ammonium sulfate resulted in a high CGP content and with 75 mM arginine and 10 mM ammonium sulfate in the medium, CGP content as high as 41 % (wt/wt) of the CDM was obtained. *A. calcoaceticus* has a high capacity for

CGP synthesis and accumulation. The only conditions under which significantly larger amounts of CGP were accumulated were addition of arginine as a carbon source at relatively high concentrations (75 mM) (Elbahloul et al. 2005a).

Research has indicated that besides the usual carbon and nitrogen sources, the provision of a source of amino acids is vital to achieve high product yields (Elbahloul et al. 2005a; Frey et al. 2002). Products known as peptones are typically added to the growth medium for this purpose. Commercially available peptones are expensive. An agro-industrial co-product with high amino acid content would thus be attractive for use in biotechnological production of CGP. A co-product stream from a potato-processing plant (trade name: Protamylasse), which contains large amounts of arginine, aspartate and asparagine, was shown to support CGP synthesis (Simon 1973a). The application of protamylasse as a sole and complete medium could make the biotechnological production of CGP economically feasible and reliable, because the costs of protamylasse medium are much lower than those of other complex media or mineral salt media, and also eco-friendly, because it provides a beneficial application of this residual of the starch industry. A 1.8 mM sterile solution of $CaCl_2 \cdot 2H_2O$ was added to reduce the phosphate concentration in the medium. *A. calcoaceticus* ADP1 cultures were inoculated from a preculture previously grown at 30 °C at pH 7.5 for 46 h (Elbahloul et al. 2005b).

Acinetobacter sp. accumulated only a little CGP when grown on protamylasse under the applied conditions which may be due to the presence of other amino acids beside arginine that could negatively interfere with CGP biosynthesis (Simon 1973a). Moreover, the synthesis of CGP in bacterial strain depends on arginine feeding as the sole carbon source or on supplementation of aspartic acid and arginine, respectively. Protamylasse contains more aspartic acid and asparagine than arginine, which is not optimal for CGP synthesis in *Acinetobacter* sp. Cultivation experiments with different concentrations of protamylasse revealed that high CGP synthesis and a high cell density were achieved using 6 % (vol/vol) protamylasse. If higher conentration of protamylasse used, it reduced the cell density and CGP content by 33 and 95 %, respectively (Elbahloul et al. 2005b). The use of hydrolysates of meat and bone meal (MBM) as the source of amino acids from unmarketable animal tissues such as slaughterhouse by-products and dead stock for CGP production by a recombinant *E. coli* containing a cloned cyanophycin synthetase gene (Garcia et al. 2006). The crude CGP yields extracted from cells grown on casamino acids were 70–82 mg/L of culture, amounting to about 4 % of the cell mass (Solaiman et al. 2011).

7 Analysis of CGP

Characterization of cyanophycin is a big challenge, because cyanophycin standard is not commercially available and cyanophycin itself is very polydispersed. Quantification of cyanophycin can be done in several ways. The number and size of cyanophycin granules can be determined using light microscopy and electron

microscopy. The amount of polymer can also be determined by amino acid analysis after complete hydrolysis with HCl. This method provided qualitative as well as quantitative information about cyanophycin, but it requires specific equipment and is time-consuming. A sensitive method for quantification of cyanophycin is NMR spectroscopy. HCl extracts of pellets consisting of broken cyanobacterial cells were found to contain cyanophycin sufficiently pure for NMR analysis. The polymer can be quantified by integration of the NMR peak representing the protons attached to the δ-carbon atoms of its arginyl moieties; calibration of the peak area with purified cyanophycin is required. Cyanophycin has been determined from rather crude samples by high pressure liquid Cyanophycin was extracted from cells by simple acid extraction method. SDS-PAGE showed that the apparent molecular masses of CGP molecules were in the range from about 21 to 29 kDa. Proteins and cyanophycin were stained with Serva Blue R. The concentrations of soluble protein were determined as described by (Bradford 1976). Matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectroscopy of the polydispers material gave an average value of approximately 29.5 kDa for the $[M + H]^+$ ion, with a maximal intensity at m/z = 29,361 and a broadness (full width at half-maximal intensity) of about 1100 m/z (Ziegler et al. 2002).

Cyanobacterial cells were fixed with 2.5 % (vol/vol) glutaraldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.3) for 45 min. After three washes with PBS for 20 min, cyanobacterial cells were postfixed in 1 % (wt/vol) osmium tetroxide in 0.1 M PBS (pH 7.3) and washed once with PBS for 20 min. Then the remaining water was removed by using a graded water-ethanol series (30, 50, 70, 90 and 96 % and absolute ethanol); each step last for about 15 min. To obtain thin sections of cells, the samples were embedded in Spurr resin without propylene oxide (Spurr 1969). Moreover, isolated CGP granules were shown to have similar staining properties and showed a similar staining reaction with the dyes acetocarmine and neutral red as granules of CGP-accumulating cyanobacterial cells that were stained in situ with the same reagents. The dye Amido Black 10B stained the isolated CGP granules, and methylene blue showed a weak staining reaction despite its inability to stain the granules in the living cells (Lang et al. 1972).

The Sakaguchi reagent, which reacts with free arginine, but in addition also reacts with protein-bound arginine residues, forming napthol–arginine complexes (Messineo 1966), was later successfully employed to stain CGP, thus indicating its high arginine content. On the basis of the Sakaguchi reaction, (Simon 1973b) developed an assay for the quantitation of CGP granules in cyanobacteria. Cyanophycin granule polypeptide (CGP) was analyzed quantitatively by the method of (Simon 1973a), using purified CGP as the standard in the arginine assay. The amino acid composition of cell extracts was qualitatively determined by two-dimensional paper chromatography after hydrolysis with 6 N HCl at 105 °C for 24 h. Amino acids were separated on Whattman no. 1 paper by using butanol-acetic acid-water (3:1:1, vol/vol/vol) in one dimension and 80 % phenol-water-34 % ammonium hydroxide (176:24:1, vol/vol/vol) in the other dimension. Aspartic acid and arginine were quantitatively determined in hydrolysates by separating them by paper chromatography with the latter solvent system. Spots corresponding to

aspartic acid and arginine were cut out, eluted with 0.1 N HCl, and assayed for free alpha amino groups (Simon 1973a).

For gel chromatography, the cyanophycin granules were dissolved in 6.0 M urea-0.1 M H_3PO_4 (pH 2.7) and run on a Sephadex G-200 column that had been equilibrated with the same buffer. Granule protein in the column elute was assayed by determination of the amount of arginine present. Blue Dextran-2000 was used to determine the void volume, and molecular weight was estimated (Simon 1971). Free amino acids and dipeptides were detected by High pressure liquid chromatography (HPLC) system. The column was a 300 Å reverse phase (C18) support and an A-316 pre column filter with A-130 filters. Despite its broad molecular weight range, CGP eluted as a single sharp peak proportional to CGP content, at about 58 min. CGP was well resolved from other proteins in crude cell extracts. With the chromatographic method, small amounts of CGP were readily detected in as little as 5-ml aliquots of cultures containing 1.0 mg cell protein (Newton and Tyler 1987).

8 Applications

Cyanophycin has recently attracted the attention of the scientific community as a biodegradable replacement for petrochemical based industrial products. Cyanophycin is a source for the constituent N-functionalized precursor chemicals. The direct application of cyanophycin itself is unknown right now; however, as a starter chemical it can be converted to many other important chemicals. Cyanophycin can be hydrolyzed to its constituent amino acids: arginine and aspartic acid. These amino acids can be utilized directly in food and pharmaceutical applications. Cyanophycin also can be hydrolyzed to a derivative with reduced arginine content or even to poly-aspartic acid.

The major market targeted for CGP's use is the vast water treatment industry, in which CGP's poly (aspartic acid) backbone polymer can serve as an environment-friendly substitute for poly (acrylic acid) commonly used as a water softener (Joentgen et al. 1998). Other potential applications of CGP and its associated poly (-Asp) backbone are as metal ion-exchange systems, as materials for hydrogels (Solaiman et al. 2011), as a biotechnological source of versatile and important platform chemicals, nutriceuticals (Sallam and Steinbuchel 2010), as biopolymers and in some medical applications and as an additive in the paper, paint and oil industries (Joentgen et al. 1998).

8.1 Medical Applications

Besides being a potential natural source for poly-aspartic acid, dipeptides derived from CGP has been used in biomedical applications (Sallam and Steinbuchel 2010).

Dipeptides play an important role in medicine and pharmacy, e.g., as an additives for malnourished patients, as treatment against liver diseases, or as aid for muscle proliferation (Sallam et al. 2009). Arginine, the second constituent amino acid derived from cyanophycin has positive effects on humans in physiological and immunological processes, as growth inductor or as a tumor cell inhibitor (Neubauer et al. 2012).

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Utilization of Cheese Industry Whey for Biofuel–Ethanol Production

G. Rajarajan, A. Irshad, B.V. Raghunath, G. Mahesh kumar and A. Punnagaiarasi

Abstract Large quantities of whey are produced as a by-product during the manufacture of cheese and casein, and this must be disposed of or processed in an environmentally acceptable way. The key to the utilisation of this resource has been changing the perception of whey from a 'waste material' to an 'opportunity' for further processing. In our country, there has been a substantial increase in the production of cheese resulting in an increased accessibility of whey. India's annual production is estimated at 650,000 tons of paneer and yielding 3.3 million tons of whey are produced per annum. By effectively utilizing the whey for the production of ethanol, the cost spent on the treatment of dairy waste to reduce the biochemical oxygen demand can be minimized and the ethanol produced can be utilized as bio-fuel for the automobiles and power generation.

Keywords Waste utilization · Cheese · Paneer · Whey · Ethanol · Bio-fuel

1 Introduction

The disposal of whey is a worldwide problem. Large quantities of whey are produced as a by-product during the manufacture of cheese and casein, and this must be disposed of or processed in an environmentally acceptable way. About 9 L of whey is generated for every kilogram of cheese manufactured (Jelen 2003; Onwulata and Huth 2008). Approximately 47 % of the 115 million tons of whey produced world-wide every year are disposed of in the environment (Greiter et al. 2002). Since most of the components are of small molecular weight and soluble, they can quickly deplete oxygen levels in natural water systems. The COD (Chemical Oxygen Demand) of raw whey is about 60 kg m^{-3} (Leite et al. 2000).

G. Rajarajan (✉) · A. Irshad · B.V. Raghunath · G. Mahesh kumar · A. Punnagaiarasi
Department of Livestock Products Technology (Dairy Science),
Veterinary College and Research Institute, Namakkal, Tamil Nadu, India
e-mail: rajarajanvet@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_6

The key to the utilisation of this resource has been changing the perception of whey from a 'waste material' to an 'opportunity' for further processing.

Cheese whey contains about 7 % solids comprising of about 10–12 % proteins, the rest being lactose (74 %), minerals (8 %) and fat (3 %) (Morr 1989). The major whey proteins are a-lactalbumin, ß-lactoglobulin, bovine serum albumin, and the heavy and light chain immunoglobulins. Other minor but important proteins include lactoferrin and lactoperoxidase (Onwulata and Huth 2008). Whey protein is used in many food applications because of its functionality and nutritive value. Deproteinated whey or serum has been found, as an opportunity for ethanol production. Sweet wheys, such as those derived from the production of cheese, were already being used to make lactose powders. Acid wheys from the production of lactic and sulphuric casein are not suitable for this (as they contain high levels of sulphate ions and lactose acid), but it was seen that they could be used to ferment the lactose to ethanol. The technology to process deproteinated whey into ethyl alcohol was developed in Europe about 20 years ago. The recovery of ethanol from a previously waste stream is a good example of technology being applied to rectify a problem. The products and their used are illustrated in Fig. 1.

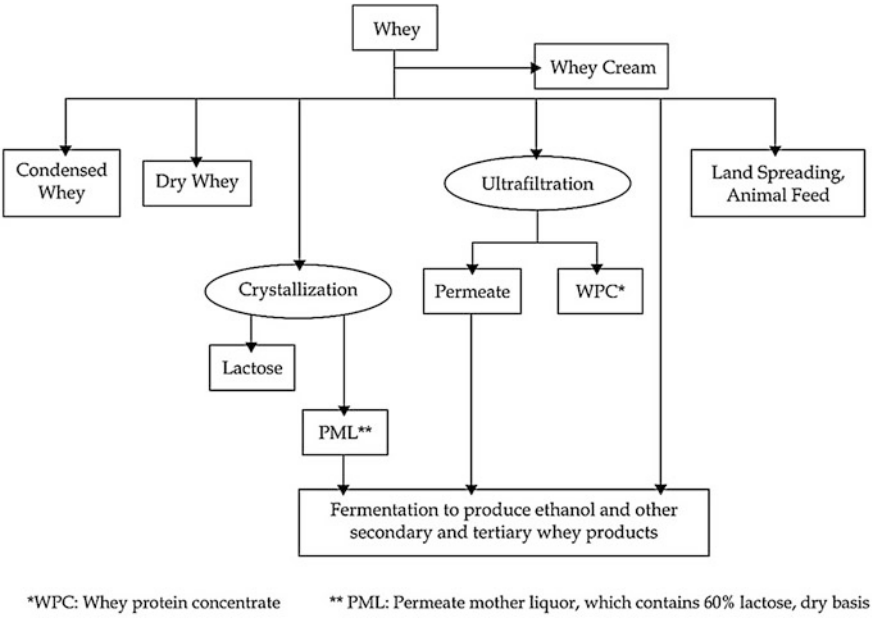

Fig. 1 Major products and uses derived from cheese whey

2 The Manufacturing Process

Ethanol production processes may vary between plants, but they all share some basic principles and steps (Audic et al. 2003) (Fig. 2). After whey protein has been harvested from whey by ultrafiltration, the remaining permeate is concentrated by reverse osmosis to attain higher lactose content for efficient fermentation. Lactose in whey permeate is fermented with some special strains of the yeast *Kluyveromyces marxianus* that are efficient in fermenting lactose. The yeast is added to the fermenting substrate and pumped to the fermentation vessels (Dale 2007). Once the fermentation has been completed, yeast is separated from the fermented substrate and the remaining liquid (beer) is moved to the distillation process to extract ethanol. This ethanol is then sent through the rectifier for dehydration. If the resulting anhydrous ethanol is intended for fuel, it is denatured by adding gasoline to prevent misuse. The effluent remaining in the liquid after ethanol has been removed from the beer (stillage) and the bio-mass (spent yeast) may be discharged to a treatment system, digested for methane gas, sold as feed, or further processed into food, feed, or other products. The process is represented in Fig. 2.

The distilleries receive serum or deproteinated whey from lactic casein, mineral acid or total milk protein (TMP) production processes. If the whey is derived from lactic casein manufacture the lactose content is only about 4 % because about 20 % of the lactose in the milk has been converted to lactic acid as a result of the natural fermentation process. Whey derived from sulphuric acid casein manufacture or TMP production is slightly higher in lactose. The lactose is yeast fermented and the resultant ethanol is distilled off and then purified to one of eight grades depending on its intended end use.

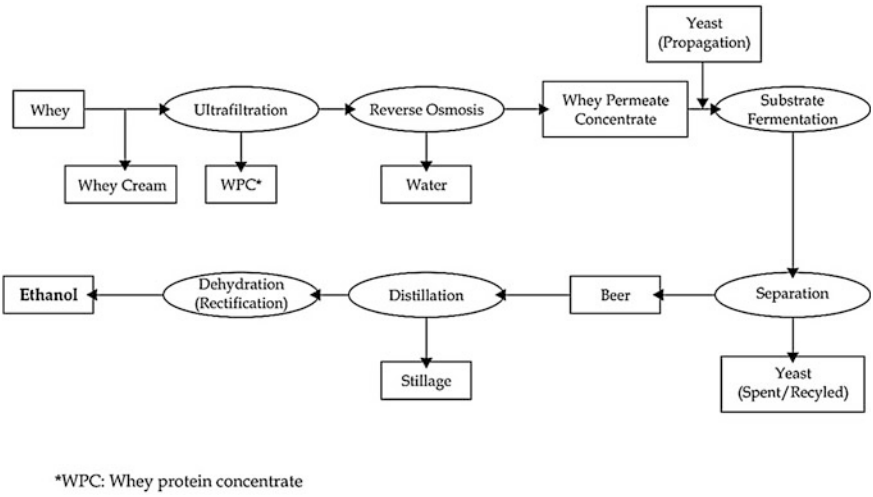

Fig. 2 Basic steps of whey-ethanol production

2.1 Cooling the Serum

Temperature of whey on receipt at the distillery is normally greater than 60 °C. At this temperature the serum is essentially free of bacterial contamination, thus the first stage of the process is to cool the serum to the fermentation temperature using a plate heat exchanger. Yeast is added to this cooled serum and pumped to the first of three fermentation vessels.

2.2 Fermentation

The fermentation takes about 24 h, with the fermenting serum passing from one vessel to the next. In-process control involves monitoring the processing flow rate ($m^3\ h^{-1}$) and also serum specific gravity, which declines from about 1.022 to 1.008 kg L^{-1} as the fermentation progresses. The decline in specific gravity reflects the change of lactose to ethanol with the evolution of carbon dioxide.

2.2.1 The Chemistry of the Process

Yeast is grown up (or propagated) in separate vessels. Air is used to promote the growth of yeast biomass growing on the serum. After the serum is cooled, yeast from the vessel is used to inoculate the cool serum. The yeast used is *Kluveromyces fragilis* (Dale and Moelhman 1997). This yeast produces β-galactosidase, the enzyme required to split lactose (a disaccharide) into its component sugars which are glucose and galactose (Eq. 1; Fig. 3).

$$Lactose + Water \rightarrow Galactose + Glucose \rightarrow 4Ethanol + 4CO_2 \qquad (1)$$

The fermentation temperature is determined by the processing speed required, but it is kept as low as possible to minimize bacterial contamination of the process.

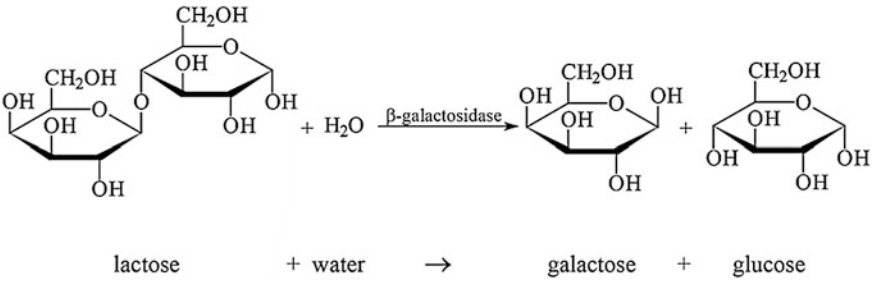

Fig. 3 Basic chemistry of whey-ethanol production

The maximum conversion of reactants to products is 51 % and this percentage is used as the basis of the fermentation efficiency calculation (Mawson 1994). Milk Serum has been found to have everything required by the yeast to grow so no further additions to the fermentation are required.

Once the fermentation has been completed yeast is removed from the fermented serum using separators or by decantation. When the yeast has been removed from the fermented serum the liquid is called beer. The beer is stored prior to the extraction of ethanol by distillation.

2.3 Distillation

Once the fermentation has been completed, yeast is separated from the fermented substrate and the remaining liquid (beer) is moved to the distillation process to extract ethanol. This ethanol is then sent through the rectifier for dehydration. If the resulting anhydrous ethanol is intended for fuel, it is denatured by adding gasoline to prevent misuse. The effluent, remaining liquid after ethanol has been removed from the beer (stillage) and the bio-mass (spent yeast) may be discharged to a treatment system, digested for methane gas, sold as feed or further processed into food, feed, or other products (Hamilton 2006).

3 Uses of Ethanol Derived from Whey

The ethanol and its derivatives are the primary product of fermentation reaction, their names and their use in industries are given in Table 1.

Table 1 Ethanol derivatives and their industrial uses

Name	Origin of name	Industrial uses
EA95	Ethyl alcohol 95 %	Industrial solvent and coloured methylated spirits
95WS	95 % white spirit	Industrial solvent; white vinegar; medicines; surgical spirit; food colourings; food flavourings
NS	Neutral spirit	Higher quality deodorants, perfumes and cosmetics; food colourings; food flavourings; alcoholic beverages
XNS	Extra neutral spirit	Alcoholic beverages; top quality deodorants and perfumes
EA99	Ethyl alcohol 99 %	Paint, printing ink and packaging industries; industrial solvents
AA	Anhydrous alcohol	Some aerosol products; hospital applications; pharmaceuticals
HGAA	High grade aerosol alcohol	Aerosols—especially hair care products; pharmaceutical cosmetics

4 Conclusion

In India, there has been a substantial increase in the production of *paneer*, resulting in an increased accessibility of whey. India's annual production is estimated at 650,000 tons of *paneer* and yielding 3.3 million tons of whey are produced per annum. By effectively utilizing the whey for the production of ethanol, the cost spent on the treatment of dairy waste to reduce the BOD can be minimized and the ethanol produced can be utilized as bio-fuel for the automobiles and power generation.

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Anaerobic Digestion of Wastewater and Municipal Sludge to Isolate Potent Methanogen Using BMP Assay

Ayesha Sulthana, K.C. Latha and S. Balasubramanian

Abstract Municipal sludge production is the inevitable residue generated in enormous amounts by the wastewater treatment plants. Sludge treatments at lower handling costs are the requisites to combat the growing population, rising production of municipal sludge and increasing energy demands. Anaerobic digestion of municipal sludge ranks to be an excellent treatment in view of sludge utilization and biogas production. Numerous researches have been conducted till date to enhance the anaerobic digestion processes of sludge and to upgrade the biogas production. Applications of microbial additives are the most promising technology among the various biogas production enhancement technologies. In the present study, isolation of potent methanogens was carried out from the lab scale reactor containing anaerobically digested municipal sludge. The raw wastewater and municipal sludge (9:1) mixture was anaerobically digested after the inoculation of pure bacterial isolates to screen the potent methanogen through simple and reliable Biochemical Methane Potential (BMP) assays for a period of 45 days. Amongst the nine isolates (DWH1, DWH2, DWH3, DWH4, DWH5, DWH6, DWH7, DWH8 and DWH9), one isolate (DWH9) showed interesting results by producing maximum cumulative biogas (3.64 l kg^{-1} VS). Molecular characterization of the potent methanogen was carried out and the phylogeny was confirmed by the 16S rDNA sequence, the isolate was confirmed to be *Methanobacterium* sp. AS1.

Keywords Municipal sludge · BMP assay · *Methanobacterium* sp. AS1 · 16S rDNA

1 Introduction

Management of the inevitable production of municipal wastewater sludge in substantial amount is a serious hindrance and a long-term challenge faced worldwide. In India, the population is more than 1.2 billion, which is virtually 17.5 % of the

A. Sulthana · K.C. Latha · S. Balasubramanian (✉)
Department of Water and Health, JSS University, Mysore, Karnataka, India
e-mail: sbalasubra2001@rediffmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_7

world's population (Provisional Population Totals India: Paper 1: Census 2011). Increase in population is boosting the unmanageable production of municipal waste, cities and towns having a population of more than 50,000 generate 38,254 million liters of wastewater per day. According to the Central Pollution Control Board (CPCB), there are 269 sewage treatment plants (STPs) in India, out of which only 231 STPs are operational (Kamyotra and Bhardwaj 2011). By the year 2020, when the country thrives to rank as the industrialized nation, there will be a telling effect on the increase in generation of waste (CPCB 2004; Sharma and Shah 2005).

Sludge generation in huge volumes is obvious, when municipal wastewater treatment plants are practicing stricter effluent quality standards to reduce the nutrient emissions. Municipal sludge processes demands 60 % of the total costs of wastewater treatment plants (Ahmed and Henzea 2008). The possibility of minimizing the sludge generation could have been successful, if municipal wastewater treatment plants could consistently treat the low strength wastewater (BOD <500–1000 mg l^{-1}) through anaerobic digestion (AD) processes. Along with the significant reduction in sludge generation, this anaerobic wastewater treatment would have been a tremendous development in biogas production at lower energy exigencies (Switzenbaum and Jewell 1980). However, anaerobic wastewater treatments are not considered applicable due to the diluted solids of low strength wastewaters.

Sludge treatment and disposal has been studied for the past years to safeguard the environment and human health (Hultman and Levlin 1997; Levlin 1999). Eventually, it can be expected that sludge management focuses on the recovery and reuse of valuable products from sludge and a complete solution to the toxicity of sludge at acceptable costs. In this regard, sustainable energy recovery from sewage sludge is gaining importance (Rulkens and Bien 2004). Amongst the various sludge treatments (Sludge pasteurization, Dewatering, Composting, Incineration and Landfilling) (Metcalf and Eddy 1991), anaerobic digestion (AD) is deemed to be an outstanding economic process in view of biogas production and sludge utilization (Johnson et al. 2003; Appels et al. 2008; Mukharje 2007).

The energy generated from sewage sludge through anaerobic digestion is solely based on its composition, the primary sludge originating from the bottom of primary clarifier is composed of simple, digestible carbohydrates and fats (Gary et al. 2007), it has higher biodegradability and biogas production rate than the activated sludge derived from the secondary treatment, as it is composed of complex carbohydrates, proteins and long chain hydrocarbons (Rittmann and McCarty 2000; Sato et al. 2001; Speece 2001). The highest portion of the biogas production depends upon the biodegradability of the sludge (El-Mashad and He 2003). Therefore, in the present study, partially digested municipal sludge from the facultative lagoon of Vidyaranyapuram STP was used to increase the strength of raw wastewater for anaerobic digestion.

The anaerobic digestion process is comprised of three important steps:

(1) Hydrolysis: Extracellular enzymes hydrolyze the organic compounds (polysaccharides, proteins, and fats),
(2) Acidification: Products of the hydrolysis are converted into hydrogen, formate, acetate and higher molecular-weight volatile fatty acids (VFA), and
(3) Methanogenesis: Conversion of hydrogen and carbon dioxide or acetate to methane (CH_4) (Schink 1997; Angelidaki et al. 2003).

The microbiology involved in the above three steps of anaerobic digestion has been extensively investigated from the past decades. Three physiological groups (hydrolyzing bacteria, acetogenic bacteria and methanogenic bacteria) of bacteria involved in the anaerobic conversion of organic matter to methane have been validated.

Biochemical methane potential (BMP) assay is an extensively practiced technique to assess the production of biogas by the given weight of certain substrate in anaerobic conditions, particularly to characterize the methane potential efficiency of wastewater (Speece 1996; Angelidaki et al. 2009). In fact, anaerobic digestion is a technology older than 100 years, it progressively emerged from an airtight vessel and a septic tank, to a temperature-controlled digester, and finally to a high rate reactor (McCarty 1982). However, minimal laboratory infrastructure, low cost, less space occupying set-up, minimal maintenance and monitoring are the requisites of BMP assays, they are evident for more accurate and repeatable results. Thus, they are efficiently used as alternatives to assist with site specific design criteria (Moody et al. 2009). A considerable group of studies was carried out under specific conditions to determine the methane yielding potential of numerous agricultural and municipal wastes (Nallathambi 1997; Nohra et al. 2003; Gunaseelan 2004; Kirk and Bickert 2004; Kirkeby et al. 2006; Labatut and Scott 2008; Lovanh et al. 2008; Kusch et al. 2008; Lansing et al. 2010; Alaru et al. 2011; Thaniya and Sohgrathok 2012). Typically, most of the biogas plants show unexpected turn down in the production of biogas when influenced by the operational parameters; therefore several, studies are conducted to improve the overall efficiency of anaerobic digestion process in the biogas plants. To promote the biogas production under various operating conditions, numerous techniques such as pre-treatment to anaerobic digestion, diverse modifications of the operational parameters of anaerobic digestion, providing favourable nutritional supplements of microorganisms and application of additives have been intensively studied and practiced (Sanders and Bloodgood 1965; Lettinga et al. 1980; Nyns 1986; Wilkie and Colleran 1986; Yadvika et al. 2004; Santosh et al. 2004; Azbar et al. 2008; Li et al. 2010; Wei et al. 2010). Biological additives such as crop residues, weeds, various plants and microbial cultures have been used abundantly to promote the performance of biogas plants. However, the suitable additive is anticipated to be actively dependent on the variety of substrates.

Biogas production from cattle dung was upgraded up to 8.4–44 % by vitalizing the activity of certain enzymes produced by a number of microbial additives such as bacteria and fungi strains (Attar et al. 1998; Tirumale and Nand 1994;

Potivichayanon et al. 2011). There was an increase in the production of biogas by 8.4–44 %, when the anaerobic digestion of cattle dung was influenced by a variety of cellulose degrading enzymes produced by actinomycetes and mixed consortia (Tirumale and Nand 1994; Attar et al. 1998; Yadvika et al. 2004). Anaerobic digestion of cattle excreta and sugar cane bagasse resulted in higher production of biogas, when the pre-treatment of sugarcane bagasse was carried out for 3 weeks under ambient temperature along with the *Phanerochaetechrysosporium* microbial additive (Geeta et al. 1994). The methane yield was enhanced up to 8.1–86.4 % in case of thickened activated sludge, whereas 0–24 % in case of thickened activated sludge and primary sludge mixture, when the sludge was treated with cell lysate during the period of anaerobic digestion (Doheanyos et al. 1997). From the aforementioned studies, it is evident that the application of microbial additives will enhance the biogas production. Therefore, this present study chiefly focuses to isolate and evaluate the potent methanogen through BMP assay.

2 Materials and Methods

2.1 Study Area and Sample

Collection Vidyaranyapuram STP (latitude 12.273681°–12.270031°N and longitude 76.650737°–76.655947°E) is assigned to the southwest drainage district 'B' of Mysore district (area 128.42 km^2, latitude 11°45′–12°40′N and longitude 75°57′–77°15′E) situated at south Karnataka, India. About 435 TPD (Tonnes per Day) of solid waste and 140 MLD (Million Liters per Day) of sewage is generated in the Mysore district, out of which only 200 TPD of waste is processed at Vidyaranyapuram Sewage Treatment Plant (STP), Mysore (Bennur 2011). Therefore, the waste collected by Vidyaranyapuram STP is more than 50 % of the waste generated by the Mysore district; thus it was selected as the study area. The Mysore City Corporation (MCC) determined to build six biogas plants at different parts of the city to shun the dissimilarities of the generation and processing of waste as well as to reduce the amount of burden on the centralized Vidyaranyapuram STP, (Arvind 2012). However, about 2, 50,000 cum of waste is disposed at the dumpsite of Vidyaranyapuram from past 5 to 6 years as this plan could not be accomplished (Javeriya et al. 2013).

Neighbouring to the solid waste dumpsite at the foot hills of Chamundi Hills, the biological treatment plant with a sewer length of 7000 m and capacity of 67.65 MLD is situated. The Vidyaranyapuram STP consists of two facultative aerated lagoons each having a surface area of 50,544 m^2 (312 m length × 162 m width) and volume of 76,904 m^3 (312 m length × 162 m width × 3.5 m depth) with a mean detention time of 11.8 days along with sedimentation basins. To ascertain the less accumulation of sludge and foul odour, these lagoons are successfully empowered by 36 blowers of 20hp for surface aeration. In addition to that, the STP

Table 1 Characterization of sample mixture (RW:MS)

S. No.	Parameters	Raw wastewater: Municipal sludge (9:1)
1	pH	6.45
2	EC	7.0
3	COD	10,880 mg l^{-1}
4	TS	69,300 mg l^{-1}
5	TDS	2700 mg l^{-1}
6	VS	96.52 %
7	N	920 mg l^{-1}
8	P	0.18

dwells with two maturation ponds each having a surface area of 24,940 m^2 (172 m length × 145 m width) and the volume of 37,410 m^3 (172 m length × 145 m width × 1.5 m depth) with the mean detention time of 2.5 days.

To carry out the following study the sample was collected from the sampling point 1 (untreated raw wastewater influent flowing from the screening chamber to facultative aerated lagoon 1) and sampling point 2 (accumulated municipal sludge in the facultative aerated lagoon 2). Both the samples [sample: 1 (raw wastewater = RW) and sample: 2 (municipal sludge = MS)] were mixed at the ratio of 9:1, followed by the characterization of the sample mixture (chemical oxygen demand (COD), nitrogen (N), phosphorus (P), total solids (TS), total dissolved solids (TDS), volatile solids (VS), electric conductivity (EC) and pH) (APHA 1998) (Table 1).

2.2 *Enrichment and Isolation of Methanogen*

Enriched Thioglycollate broth (HiMedia) (Anderson et al. 2005; Murray et al. 2007) was used for the isolation and screening of potent methanogenic bacteria. Around 1 g of anaerobically digested sample (RW 9:1 MS) was taken from the lab scale reactor, which was previously set to conduct the studies on operational parameters (pH: 7 and Room temperature) of anaerobic digestion for 45 days. Then the sample was seeded into a test tube containing 9 ml of autoclaved enriched thioglycollate broth and incubated at 37 °C in McIntosh and Filde's anaerobic jar for 72 h to enhance the growth of the anaerobic methanogenic bacteria. After the period of incubation, the dilution 10^{-1} (w/v), that is the broth containing bacterial growth was agitated manually for the uniform distribution of cells and was further serially diluted to 10^{-9} (w/v). The 0.1 ml of the 10^{-9} dilution was inoculated on to nutrient agar plates by pour plate method and was incubated at 37 °C in McIntosh and Filde's anaerobic jar for a period of 48 h. After successive incubation, pure isolated colonies were observed; randomly nine bacterial colonies were selected amongst the obtained isolated colonies. The nine bacterial isolates were labelled as

DWH1, DWH2, DWH3, DWH4, DWH5, DHW6, DWH7, DWH8 and DWH9; the same were maintained on agar slants as stock cultures. The bacterial isolates were further used to assess their potential for the biogas production using BMP assay.

2.3 Experimental Setup for Potent Methanogen Screening

The biochemical methane potential assays were performed in triplicates for each bacterial isolate, by considering 250 ml serum bottles as lab scale reactors (Fig. 1). A working volume of 180 ml sample mixture (RW 9:1 MS) was dispensed into each 250 ml serum bottle and was autoclaved at 121 °C for 30 min. The lab scale reactors containing sterile sample mixtures (RW 9:1 MS) were inoculated with 40 ml nutrient broth containing 24 h old bacterial isolate and the headspace was flushed with Nitrogen gas to ensure the anaerobic ambience.

The lab scale reactors were labelled with respective bacterial isolates and were stoppered with rubber corks with one opening connected to biogas scrubber bottles of 600 ml volume through the infusion tube. Biogas scrubber bottles containing 500 ml NaOH (1M) with phenolphthalein as pH indicator are meant to absorb the carbon-di-oxide (CO_2) so that only methane (CH_4) is pumped into the 600 ml capacity displacement bottle containing methyl orange coloured double distilled water to ease the recording of biogas volume in the graduated cylinder. Once the scrubber solution (NaOH 1M) is saturated by CO_2 absorption, the pink colour of phenolphthalein indicator fades, and this is an indication to replace the solution with the freshly prepared scrubber solution.

Fig. 1 Biochemical methane potential assay setup for methanogen screening

The complete set up was air tightened and the anaerobic condition was maintained by sealing with M-seal adhesive to avoid air contamination. All the lab scale reactors were incubated at room temperature by agitating the mixture manually two times a day, the data recording and monitoring of biogas production was conducted for 45 days.

2.4 Characterization of Potent Methanogen

The phenotypic characterization of bacterial isolate producing maximum biogas was carried out by studying the colony characteristics, by performing Gram's staining and by determining the cell morphology through Scanning Electron Microscopy (SEM). The molecular characterization of the isolate was performed by DNA extraction and purification through phenol-chloroform method, followed by Polymerase Chain Reaction (PCR) amplification using the primers designed by comparing 16S rRNA sequences of *Methanobacterium* sp. available at NCBI database: 397F (5′-CAGGCCTAACACATGCAAGTC-3′) and 398R (5′-GGCGGATGTGTACAAGGC-3′). The amplification reactions were performed in a 50 µl volume by mixing template DNA (50 ng) with the 5 µl polymerase reaction buffer (10x); 1.5 µl dNTP mix (10 mM), 2.0 µl forward and reverse primers (100 ng μl^{-1}) each, and 1.5 U of Taq polymerase (0.25 µl), 2.0 µl $MgCl_2$ (1.5 mM) and 35.75 µl milli-Q water. Amplification of DNA was carried out with the following conditions: An initial denaturation at 95 °C for 3 m, 30 cycles of denaturation for 30 s at 94 °C, annealing 56 °C for 30 s and extension 72 °C for 60 s; and a final extension at 72 °C for 6 m. The amplified product was run on a 0.8 % agarose gel containing ethidium bromide (1 µl ml^{-1}), along with 1 kilo base pair DNA ladder, at a constant voltage and visualized under UV light. The amplified PCR product (800 bp) was outsourced to Chromous Biotech Pvt. Ltd., Bangalore for 16S rDNA sequencing. The phylogenetic analysis was carried out by using MEGA5 (Tamura et al. 2011).

3 Results and Discussion

3.1 Microbial Consortia Versus DWH9 Bacterial Isolate

The main aim of the study is to evaluate the methane potential of the bacterial isolate in the naturally existing conditions, that is the existing initial pH of the sample mixture was found to be 6 and the set up was maintained at room temperature (27 °C). The microbial consortia present in the biochemical methane potential assay set up of initial pH 6 of control 1 and the control 2, includes archaea, bacteria and other microorganisms, which are capable of carrying out anaerobic

Table 2 Comparison of cumulative biogas yield

S. No.	Inoculum	COD (mg l^{-1})	VS (%)	pH	T (°C)	Days	Cumulative biogas yield (l kg^{-1} VS)	Percentage
1	Microbial consortia (control 1)	6880	61.04	6	27	45	2.46	–
2	Microbial consortia (control 2)	10,880	96.52	6	27	45	2.54	3.25
3	DWH9 (bacterial isolate)	10,880	96.52	6	27	45	3.64	47.96

T Temperature

degradation of municipal sludge and wastewater to yield biogas at room temperature (27 °C). Whereas, the another biochemical methane potential assay set up of initial pH 6, maintained at room temperature (27 °C) inoculated with 24 h old pure bacterial isolate (DWH9) yielded high cumulative biogas in comparison with the microbial consortia (Table 2).

The Chemical Oxygen Demand (COD) and volatile solids is used to quantify the amount of organic matter in waste and predict the potential for biogas production. During anaerobic digestion the biodegradable COD present in organic material is preserved in the end products, namely methane and the newly formed bacterial mass (Mes et al. 2003). However the high COD and VS of sample mixture of control 2 could show only 3.25 % higher cumulative biogas yield in comparison with the COD of the sample mixture of control 1. Whereas, the high COD and VS of the sample mixture of DWH9 bacterial isolate showed 47.96 % higher cumulative biogas yield in comparison with the same sample mixture of control 2 setup. This clearly states that the DWH9 bacterial isolate has enhanced the biogas yield in comparison with the microbial consortia of control 1 and control 2 setup.

Linear regression analysis was performed to determine the influence of DWH9 bacterial isolate, microbial consortia of control 1 and control 2 setup on the cumulative biogas yield. The following regression models were determined (Table 3), where X is the number of days the BMP assay was performed and Y is the cumulative biogas yield by the microbial consortia of control 1, control 2 and DWH9 bacterial isolate.

Table 3 Linear regression model summary for comparison of biogas production between microbial consortia and DWH9 isolate

S. No.	Model	a (intercept)	b (slope)	R	R square
1	Microbial consortia (control 1)	10.378	0.957	0.940	0.884
2	Microbial consortia (control 2)	4.177	2.191	0.968	0.936
3	DWH9 isolate	2.889	3.273	0.965	0.932

Model 1: The Y-intercept a = 10.378 is the value of dependent variable when X = 0 (cumulative biogas yield of Microbial consortia of control 1), the slope value b = 0.957, this model shows that the cumulative biogas yield increases by 0.957 ml with each additional day. The correlation coefficient R^2 determined the relationship between cumulative biogas yield and number of days is 0.884, which is 88.4 % of the variance in cumulative biogas yield is related to the number of days.

Model 2: The Y-intercept a = 4.177 is the value of dependent variable when X = 0 (cumulative biogas yield of Microbial consortia of control 2), the slope value b = 2.191, this model shows that the cumulative biogas yield increases by 2.191 ml with each additional day. The correlation coefficient R^2 determined the relationship between cumulative biogas yield and number of days is 0.936, which is 93.6 % of the variance in cumulative biogas yield is related to the number of days.

Model 3: The Y-intercept a = 2.889 is the value of dependent variable when X = 0 (cumulative biogas yield of DWH9 isolate), the slope value b = 3.273, this model shows that the cumulative biogas yield increases by 3.273 ml with each additional day. The correlation coefficient R^2 determined the relationship between cumulative biogas yield and number of days is 0.932, which is 93.2 % of the variance in cumulative biogas yield is related to the number of days.

Analysis of variance (ANOVA) showed that all the three linear regression models are statistically significant and the residual are normally distributed (Tables 4, 5, and 6).

From the above interpretation of models, it is observed that the Y-intercept value of the cumulative biogas yield of microbial consortia of control 1 (a = 10.378) and control 2 (a = 4.177) was higher than the Y-intercept value of DWH9 isolate (a = 2.889), this is the result of optimum Inoculum to substrate ratio (ISR) of microbial consortia. ISR affects the occurrence and duration of lag phase (extracellular hydrolysis) and methanogenesis (Raposo et al. 2011; Xu et al. 2013), the ISR is a major parameter affecting the process of anaerobic digestion (Angelidaki

Table 4 ANOVA (Microbial consortia of control 1)

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	6944.348	1	6944.348	328.057	**
	Residual	910.230	43	21.168		
	Total	7854.578	44			

**Statistical significance at $P < 0.01$

Table 5 ANOVA (Microbial consortia of control 2)

Model		Sum of squares	df	Mean square	F	Sig.
2	Regression	36,430.436	1	36,430.436	629.913	**
	Residual	2486.864	43	57.834		
	Total	38,917.300	44			

**Statistical significance at $P < 0.01$

Table 6 ANOVA (DWH9 isolate)

Model		Sum of squares	df	Mean square	F	Sig.
3	Regression	81,327.767	1	81,327.767	587.880	**
	Residual	5948.653	43	138.341		
	Total	87,276.420	44			

**Statistical significance at $P < 0.01$

et al. 2009; Jensen et al. 2009). The optimum inoculum to substrate ratio of DWH9 isolate was dependent on the period of incubation, therefore the cumulative biogas yield was later picked up by the isolate, which can be observed by the higher slope value b = 3.273 in comparison with the slope value of microbial consortia of control 2 b = 2.191 and microbial consortia of control 1 b = 0.957. However, the cumulative biogas yield is also influenced by the depletion of organic matter in the sample mixture, resulting in the interspecific competition of nutrients amongst the microbial consortia.

From the above interpretations, it was concluded that the DWH9 bacterial isolate could successfully enhance the cumulative biogas yield (Fig. 2). Therefore, the DWH9 bacterial isolate was further characterized by 16S rDNA sequencing for species identification.

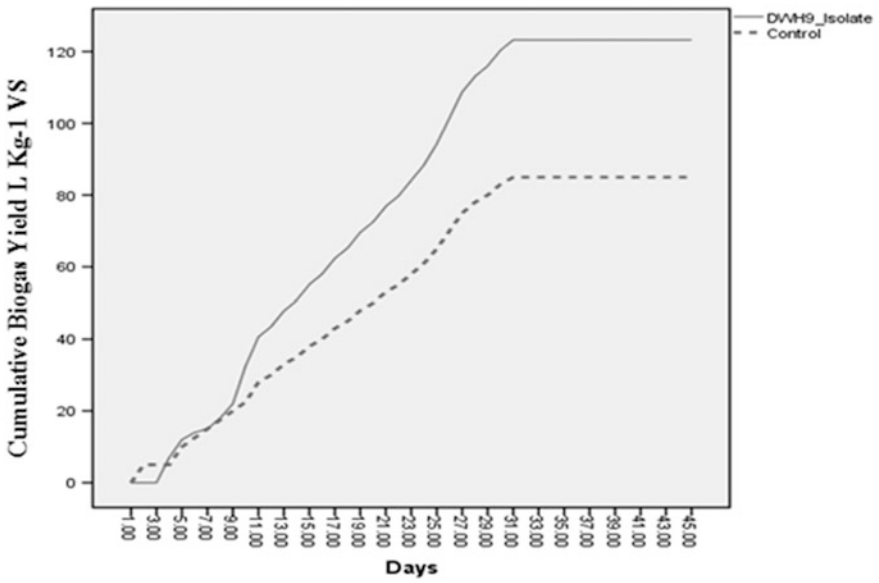

Fig. 2 Effect of methanogen isolate on biogas yield

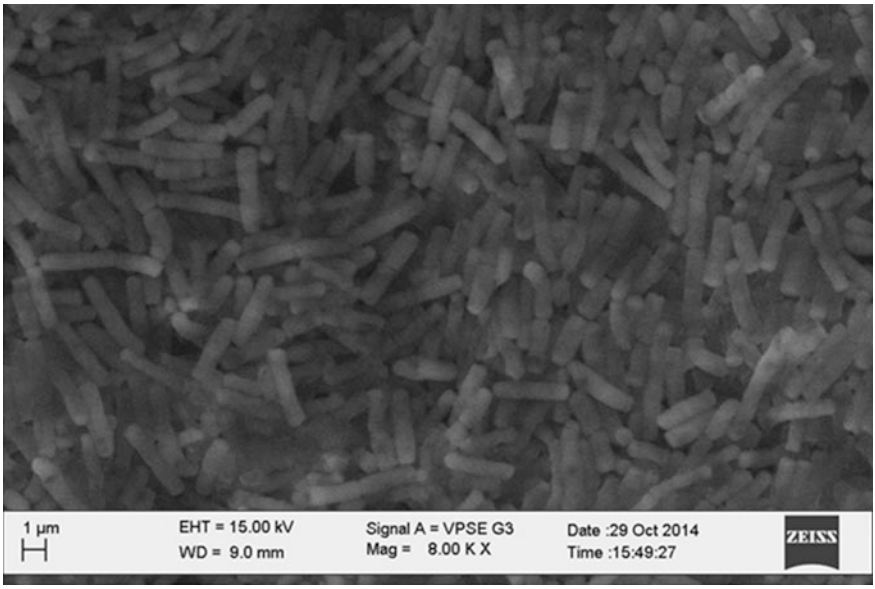

Fig. 3 Scanning electron micrograph of DWH9 isolate (*Methanbacterium* sp.)

3.2 Cell Morphology

The bacterial colonies of the isolate DWH9 were creamish white, smooth, opaque and circular in shape with a diameter of 0.7–1 mm. The isolate was observed to be gram positive *Bacilli* and the scanning electron micrograph of DWH9 isolate confirms the cell morphology (Fig. 3).

3.3 16S rDNA Sequencing

Basically, 16S rRNA gene is the most common housekeeping genetic marker to resolve the phylogenetic and evolutionary relationships among bacteria and archaea (Corless et al. 2000; Hofman-Bang et al. 2003; Deng et al. 2008). The 16S rRNA gene exists in almost all bacteria, therefore, its transformation and activity is worthy of comparison (Hofman-Bang et al. 2003). Specific primers can be designed to confirm the phylogeny of the microorganisms at their genus and species level by targeting the conserved and variable regions of the large 16S rRNA gene of 1500 bp (Patel 2001; Zhang and Fang 2006). More than 90 % of the genus identification and 65–83 % of the species identification was based on the 16S rRNA gene sequencing studies, whereas, 1–14 % of the isolates remained unidentified even after targeting the same gene (Clayton et al. 1995; Mignard and Flandrois 2006; Woo et al. 2003).

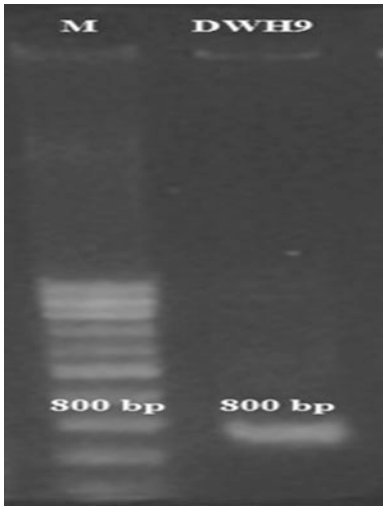

Fig. 4 Agarose gel electrophoresis of 16S rDNA PCR amplicons

However, the recognition for targeting 16S rRNA gene can be clarified by the existing 16S rRNA genetic databases. Therefore, in the present study, the 16S rRNA gene was the target gene to determine the phylogenetic relationships and the polymerase chain reaction based on 16S rRNA gene gave an amplicon size of 800 bp (Fig. 4), thus confirming that the isolate belongs to methanogenic Archaea. The identification was further confirmed by 16S rDNA sequencing and phylogenetic analysis.

3.4 Phylogenetic Analysis

All the living entities on earth are classified under three domains namely archaea, bacteria and eukaryota, based on the 16S and 18S rRNA gene sequences and this is the foundation for the construction of phylogenetic tree of life (Woese et al. 1990; Madigan et al. 2006). The diversified group of methanogens belong to the phyla Euryarchaeota of the Archaeal domain. Till date, the characterized methanogens are further categorized into five orders: Methanobacteriales, Methanomicrobiales, Methanosarcinales, Methanococcales and Methanopyrales (Garrity and Holt 2001). Methanogens belonging to the phylogenetic orders of Methanobacteriales, Methanomicrobiales and Methanosarcinales have been reported in higher amounts in anaerobic digesters in comparison with the methanogens belonging to the order Methanococcales seem to play a relatively minor role in anaerobic digesters (McHugh et al. 2003; Li et al. 2008; Cardinali-Rezende et al. 2009). Whereas, the methanogens of the order Methanopyrales have not detected so far in biogas plants. The methanogens of the order Methanobacteriales are further classified into two families Methanobacteriaceae containing three mesophilic genera Methanobacterium,

Methanobrevibacter and Methanosphaera, and one thermophilic genus *Methanothermobacter* and *Methanothermaceae* containing one hyperthermophilic genus *Methanothermus*, based on the 16S rRNA gene sequence similarities below 89 % (Liu 2010).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 2.50000000 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (1000 replicates is shown next to the branches (Dopazo 1994; Rzhetsky and Nei 1992). The evolutionary distances were computed using the number of differences method (Nei and Kumar 2000) and are in the units of the number of base differences per sequence. The analysis involved 18 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 81 positions in the final data set. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). The isolate DWH9 had highest similarity of 99 % with the strain *Methanothermobacter defluvii* (NR 028248). Based on the molecular characterization and phylogenetic analysis, the isolate DWH9 was identified as *Methanobacterium* sp. AS1 (Fig. 5). The occurrence of Methanobacterium genera is extensively distributed in anaerobic habitats such as aquatic sediments and animal intestines (Garrity and Holt 2001). The hydrogenotrophic methanogens of the order Methanobacteriales produce methane by the utilization of CO_2 or methyl compounds as the main substrates and H_2, formate and secondary alcohols act as the electron donors. They appear to perform a major activity in anaerobic digesters under thermophilic conditions (Leven et al. 2007; Krakat et al. 2010).

The16S rDNA sequence of the isolate DWH9 has been deposited in the Gene Bank and the accession number KM379099 was obtained. Seventeen additional 16S rRNA sequences of the methanogens *Methanothermobacter defluvii* (NR 028248), *Methanobacterium flexile* strain GH (NR 116276), *Methanobacterium movens* strain TS-2 (NR 116289), *Methanobacterium thermoaggregans* (AF095264), *Methanobacterium thermoaggregans* strain: DSM 3266 (AB679267), uncultured *Methanobacterium* sp. clone BQI15-1 (EU154438), *Methanobacterium beijingense* strain 8-2 (NR 028202), *Methanobacterium formicicum* strain Mb2 (JN 205053), *Methanobacterium uliginosum* strain P2St (NR 104694), Methanobacterium *ferruginis* strain Mic6c05 (NR 113045), Methanobacterium petrolearium strain Mic5c12 (NR 113044), *Methanobacterium arcticum* strain M2 (NR 115811), *Methanobacterium oryzae* (AF028690), *Methanobacterium oryzae* strain FPi (NR 028171), *Methanobacterium lacus* strain 17A1 (NR 117917), *Methanobacterium aarhusense* (AY386124) and *Methanobacterium* sp. MC-20 (JF812256) were included in phylogenetic analyses and the phylogeny was confirmed (Fig. 5).

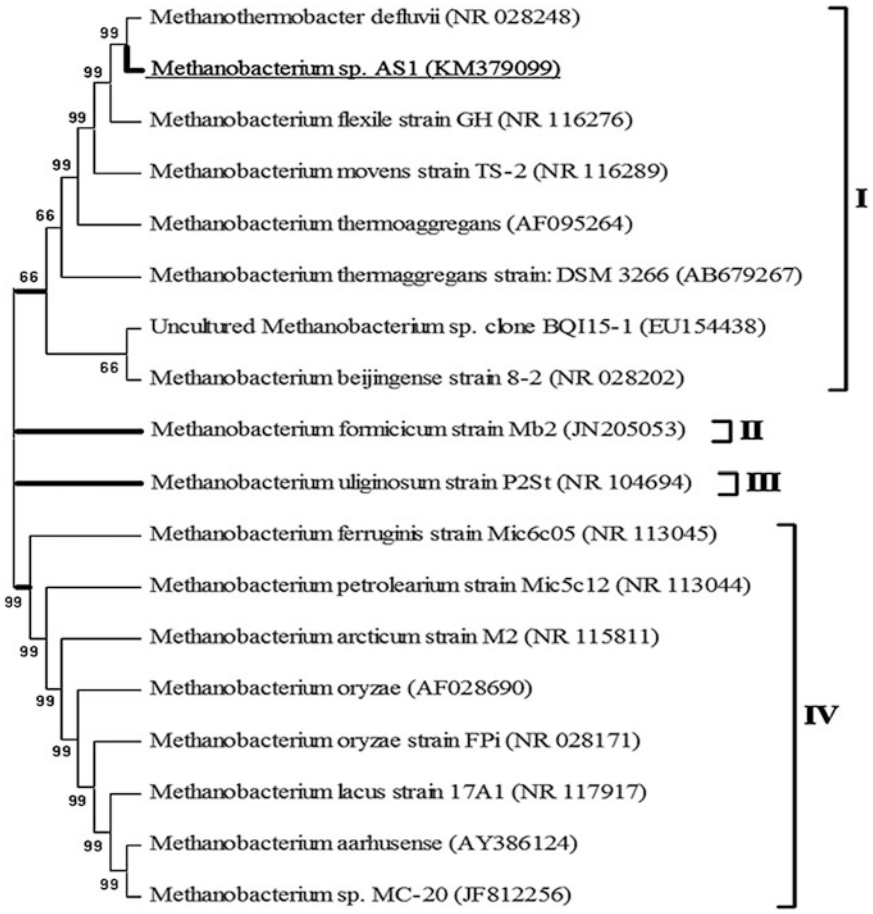

Fig. 5 Phylogenetic tree of the isolate DWH9 in relation to *Methanobacterium* sp.

4 Conclusion

This study is an initiative to fortify the low strength wastewater through the safe and profitable utilization of municipal sludge. Microbial culture was the preferred additive to accomplish the proposed study, therefore isolation and screening of potent methanogen was carried out from a lab scale reactor. BMP assay employed to conduct the experiments found to be simple, convenient, easy to operate, favourable and economical, when compared to the sophisticated anaerobic digesters of huge capital investments. Anaerobic digestion of RW:MS (9:1) sample mixture and biogas production was enhanced (up to 47.96 %) by the DWH9 isolate, which was later confirmed to be *Methanobacterium* sp. AS1 (KM379099) through 16S rRNA gene sequencing. The practical implication of this finding at the study area will possibly defeat the municipal sludge handling issues and energy crisis.

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Part III
Waste Management Strategies

A Statistical Based Experimental Approach for Biodegradation and Optimization of Heavy Metals

S. Umadevi, S. Aalfin Emmanuel, P.M. Ayyasamy and S. Rajakumar

Abstract The accumulation of many heavy metals especially lead, mercury, cadmium, arsenic and nickel from leather, textile, dye industries etc. into estuaries becomes a greater cause of heavy metal magnification. These heavy metals enter the food chain when it is absorbed by phytoplanktons and all the organisms which consume it would have health effects. In this work, the water samples were collected from estuaries of Poombuhar, Tamil Nadu. Thin layer chromatography was done for the confirmation of heavy metals present in the sample. The metal tolerant bacteria from the samples were isolated and tested for degradation capacity of the heavy metals. The bacterial consortium was prepared based on the antagonistic tests for the better results. The degradation level with respective combinations which provides higher degradation of heavy metals was observed. The selected combination of bacteria had optimized with starch, cellulose, glucose, pH and inoculum size by design of experiment. This bunch of experiment provides the peak level of degradation using bacteria with specific nutrients. In this study, seven microbial combinations reduced heavy metals from the effluent samples with the help of statistical predictions. It may helpful to develop affordable ecofriendly technology for the environment.

Keywords Heavy metals · Biodegradation · Bacteria · Thin layer chromatography · Design of experiment

S. Umadevi (✉) · S. Aalfin Emmanuel · S. Rajakumar
Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
e-mail: umadevi.bioinfo@gmail.com

P.M. Ayyasamy
Department of Microbiology, Periyar University, Salem, Tamil Nadu, India

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_8

1 Introduction

Heavy metals occur naturally in marine environments as trace elements. Trace metals have shown to be significantly hazardous pollutants in aquatic environments, even at very low concentrations. The accumulated heavy metals in sediments may enter the food chain and lead to environmental pollution, decreases biodiversity, affects ecosystem and human health (Wade et al. 2007). While some of the heavy metals are purely toxic with no known cellular role (Shi et al. 2002), other metals are essential for life at low concentration but become toxic at high concentrations (Koropatnick and Leibbrandt 1995). Heavy metal toxicity can result in damaged or reduced central nervous function, lower energy levels and damage to blood composition, lung, kidney, liver and other vital organs. Long term exposure may result in slowly progressing physical and neurological degenerative process that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are not uncommon and repeated long term contact with some metals or their compounds may even cause cancer (Goering et al. 1999). So it is vital to understand the scenario of the ecosystem for the environmental health regards.

Microbial activities in natural environment are the main process to remove or detoxify heavy metals and radionuclides (Fritsche and Hofrichter 2008). They have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms, such as adsorption, uptake, methylation, oxidation and reduction. Bacteria may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance (Fernandez et al. 2012). Based on this background this present study was focused to isolate the metal degrading bacteria and optimize the process by means of statistical analysis of effects of various nutrient and environmental variables.

2 Materials and Methods

2.1 Sample Collection

Collection of water samples has been done in four stations in estuaries region of Poombuhar, Nagapattinam district, Tamil Nadu, India. Its geographical coordinates are 13° 6′ 52″N and 80° 12′ 31″E. The samples 5 in number were taken in the sterilized air tight bottles and labeled.

2.2 Trace of Heavy Metals from Sample

2.2.1 Preparation of Cyanidin

Cyanidin was used as a dye to trace the heavy metals. It was extracted from calyces of Roselle plant (*Hibiscus sabdariffa* L.) based on the method of Ukwueze et al. (2009). Ten gram of dry calyces was ground to powder and macerated in 5 ml methanol:HCl mixture (85:15 % v/v) for 72 h and filtered. In filtrate 20 ml of conc. HCl was added and content was refluxed for 2 h at pH 5. The solution was then kept in a beaker and cooled until crystals settled out. The crystals were filtered out under suction and re-crystallized from hot methanol, air dried and weighed (Okoye et al. 2013).

2.2.2 Thin Layer Chromatography

Heavy metals identification was done through thin layer chromatography. Ethanol and cyanidin were used as a mobile phase and dye respectively. Sample was obtained till it reached its stationary level. Further retention factor (R_f) was calculated by the Eq. 1 (Okoye et al. 2013).

$$R_f = \frac{\text{Distance from Baseline travelled by solute}}{\text{Distance from Baseline travelled by solvent}} \quad (1)$$

2.3 Isolation and Identification of Bacteria

2.3.1 Isolation of Bacteria from Sample

An accurately weighed 1 ml of sample was dispersed in 100 ml of sterile distilled water and serially diluted. Further, 10^{-3}, 10^{-4} and 10^{-5} serially diluted samples were plated on Vaatanen nine-salt solution (VNSS) agar. After incubation at 37 °C for 18 h, the visible colonies were again sub-cultured on nutrient agar plate. This procedure was continued till pure bacterial colonies were isolated (Suhelen et al. 2001).

2.3.2 Identification of Bacteria

Isolated bacterial colonies were identified by Gram's staining, colony morphology, motility test and biochemical characterization including catalase, oxidase and oxidative fermentative according to Bergey's manual (Buchanan and Gibbons 1974).

2.4 Heavy Metal Degradation

2.4.1 Selection of Metal Degrading Bacteria

Bacteria added to the sample was separated with cyanidin complex and kept for 24 h of incubation. Optical density (OD) value in ppm for each sample was obtained using UV-Spectrophotometer. In the frequency of 389.6, 396.8, 357.8, 360 and 401, the metals Pb, Hg, Cd, As and Ni were determined respectively (Okoye et al. 2013).

2.4.2 Antagonistic Tests for Bacterial Consortium

In order to obtain maximized degradation rate by bacteria, antagonistic test was done to construct the bacterial consortium in Muller-Hinton agar medium. Overnight inoculated culture was used in well-cut method and analyzed by the formation of zone of inhibition after the incubation at 37 °C for 16–18 h (Lima-Filho et al. 2002).

2.5 Optimization of Metal Degradation

2.5.1 Design of Experiment

Design of experiment (DOE) is a statistical application to find out the interactive effects of multiple factors that could effect on grant result. In this study, the experiment was designed using statistical software Minitab 17. The test levels of the 6 different variables were chosen and given in Table 1. Based on the design module, experiments were carried out by Plackett-Burman design (PBD) to identify significant parameters of heavy metals degradation (Tripathi and Srivastava 2012). For this study, best 12 combinations were constructed through DOE.

2.5.2 Quantification of Various Compositions

Triplet experiment was carried out by inoculating 1 ml (1OD) stationary phase culture into 100 ml Bushnell Hass Medium with selected 12 combinations as per

Table 1 Actual values of test variables

Test variables	Starch % (w/v)	Cellulose % (w/v)	Glucose % (w/v)	pH	Inoculum size % (v/v)
Min. level	0.1	0.1	0.1	3	5
Max. level	1.0	1.0	1.0	11	10

the PBD and maintained at room temperature. After the incubation of 24 h, samples were analyzed in UV-Vis photospectrometer at respective OD values for each metal (Okoye et al. 2013).

2.5.3 Analysis of Optimization Data

The statistical significance of the variable and model was also determined by analyzing factorial for the selected 12 combinations using Minitab 17. Experimental values were selected as response and the predictions were made for the effects of starch, cellulose, glucose, pH and inoculum size. The estimated effects and global solution for the optimal degradation were the output of this programme. The factorial plots were made to identify the effects of each parameter graphically (Tripathi and Srivastava 2012).

2.5.4 Validation of Optimized Values

In the validation study, the maximum degradation level as estimated by Minitab 17 was verified by testing the predicted culture condition/optimal point of bacteria under static incubation condition. The degradation level was observed after incubation by UV-Vis spectrophotometry.

3 Results and Discussion

3.1 Heavy Metal Concentration in Sample

The four water samples were collected randomly from estuaries of Poombuhar, Tamil Nadu. In order to trace the presence of heavy metals in sample thin layer chromatography was performed. Ethanol was used as a solvent and the significant result was obtained. The R_f (X 100) value of thin layer chromatography (TLC) was compared with literature values. The R_f value of the extract (570) showed no significant variation from the authentic values (545). The average OD value of Pb, Hg, Cd, As and Ni was 0.5286, 0.4760, 0.1233, 0.1876 and 0.1959, respectively. These values show that estuaries at Poombuhar have heavy metal contamination.

3.2 Isolation and Identification of Bacteria

After the overnight incubation, seven morphologically different bacterial colonies were isolated and identified in the order of *Flavobacterium* sp., *Bacillus* sp., 3 *Micrococcus* spp., *Cytophaga* sp. and *Bacillus* sp. were labeled as A, B, C, D, E, F and G respectively.

3.3 Heavy Metal Degradation

3.3.1 Identification of Metal Degrading Bacteria

The OD value taken after incubation of 7 bacterial cultures with water sample shows that, all the bacteria were metal degrading. Maximum degradation of Pb (10.14 %), Hg (20.17 %), Cd (39.17 %), As (30.70 %) and Ni (38.74 %) was obtained by the bacterium F, A, F, G and B, respectively.

3.3.2 Bacterial Consortium on Heavy Metal Degradation

The antagonistic test results were observed by means of inhibition zone after incubation period and are represented in Table 2.

To maximize the degradation rate, all the 63 possible combinations based on antagonistic study results (A + B, A + C, …, A + G, A + B + C, …, A + B + C + D, …, A + B + C + D + E + F + G, which excludes the antagonistic effect) were inoculated with sample. Each heavy metal was highly degraded by different bacterial consortium. The results were graphically represented in Fig. 1.

Among the bacterial colonies *Flavobacterium* and *Bacillus* were effective in lead, cadmium, arsenic and nickel degradation. Whereas, *Micrococcus* and *Cytophaga* effectively degraded mercury.

3.4 Optimization of Metal Degradation

3.4.1 Design of Experiment

In order to optimize the degradation rate, the nutrients viz. starch, cellulose and glucose; pH and inoculum size were designed as in Table 1 in chapter "A Statistical Based Experimental Approach for Biodegradation and Optimization of Heavy Metals". The lower and higher values of these combinations were used for optimization, but statistically they were filtered out to twelve best combinations (Table 3) using DOE.

Table 2 Antagonism among the bacterial isolates

Bacteria	A	B	C	D	E	F	G
A	–			*		*	*
B	*	–	*			*	*
C	*		–			*	
D	*		*	–		*	*
E	*				–		*
F	*	*		*		–	*
G	*	*			*	*	–

* indicates formation of zone

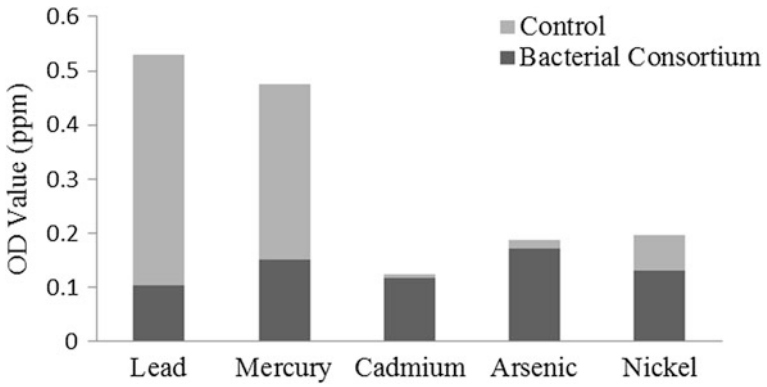

Fig. 1 Effect of bacterial consortium on metal degradation

Table 3 Experiments based on DOE for optimization of degradation

Combination	Starch % (w/v)	Cellulose % (w/v)	Glucose % (w/v)	pH	Inoculum size % (v/v)
1	0.1	0.1	0.1	11	10
2	1.0	1.0	1.0	11	10
3	1.0	1.0	1.0	11	5
4	0.1	0.1	0.1	3	5
5	0.1	1.0	1.0	3	10
6	0.1	1.0	0.1	3	5
7	1.0	0.1	1.0	11	5
8	1.0	1.0	0.1	11	5
9	1.0	0.1	0.1	3	10
10	0.1	0.1	1.0	11	10
11	1.0	1.0	1.0	3	10
12	1.0	0.1	1.0	3	5

3.4.2 Quantification of Various Compositions

Based on the 12 given experimental combinations by DOE, the study was carried out. The results were quantified using UV-Vis spectrophotometer with the complex of cyanidin and graphically illustrated in Fig. 2. For each heavy metal, highly degrading bacterial consortium was identified.

The results show that, the combination 2 gives the maximum degradation of Cd, As and Ni, whereas combinations 3 and 11 gives better results for Hg and Pb degradation correspondingly.

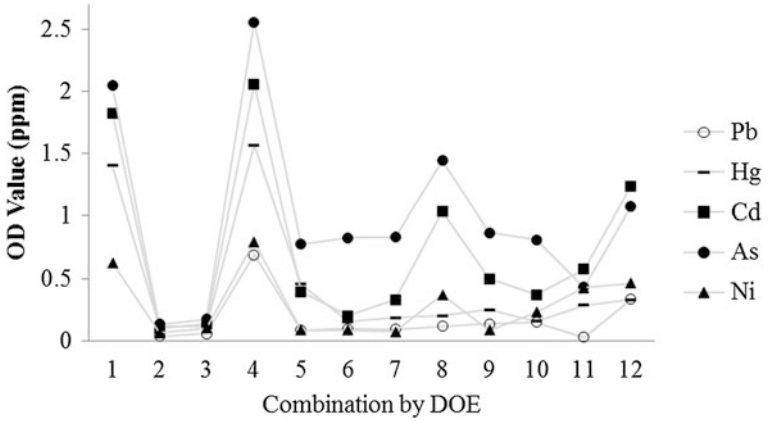

Fig. 2 Metal degradation by bacterial consortium

3.4.3 Effect of Optimization Experiment

The experimental results were further evaluated statistically and the best possible solution for the optimized degradation was predicted. The correlation coefficient in terms of R^2 for degradation of Pb, Hg, Cd, As and Ni were 0.671, 0.548, 0.459, 0.65 and 0.28, respectively. The percentage effect of variables on Pb, Hg, Cd, As and Ni were graphically illustrated in Figs. 3, 4, 5, 6 and 7 respectively.

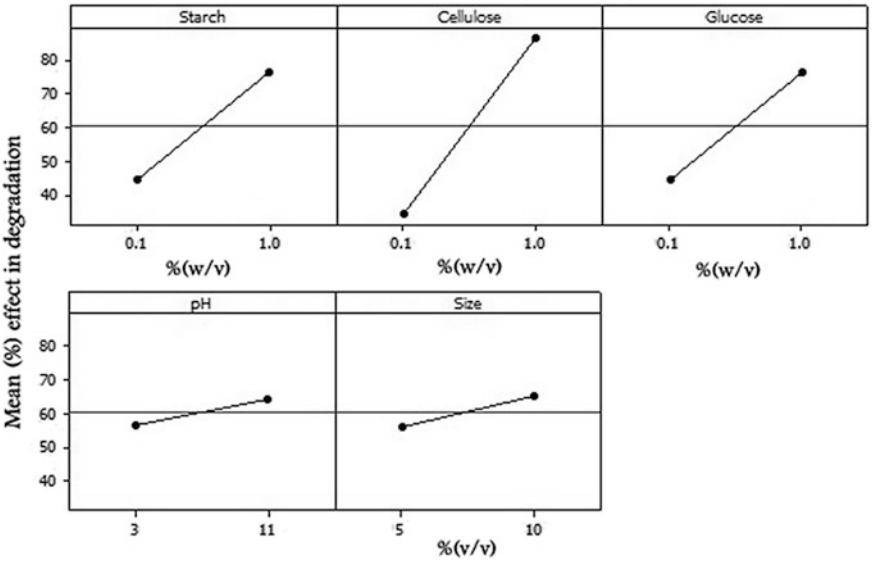

Fig. 3 Effect of various parameters on lead degradation

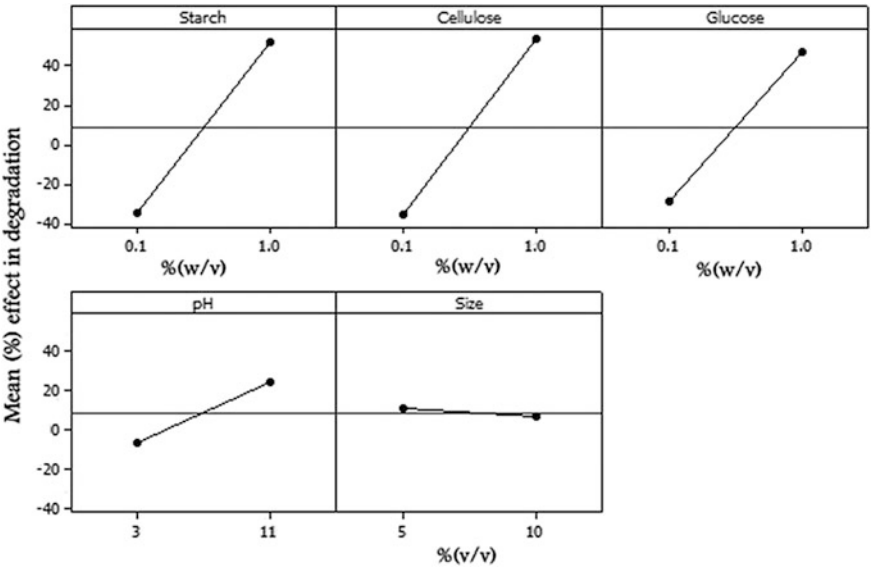

Fig. 4 Effect of various parameters on mercury degradation

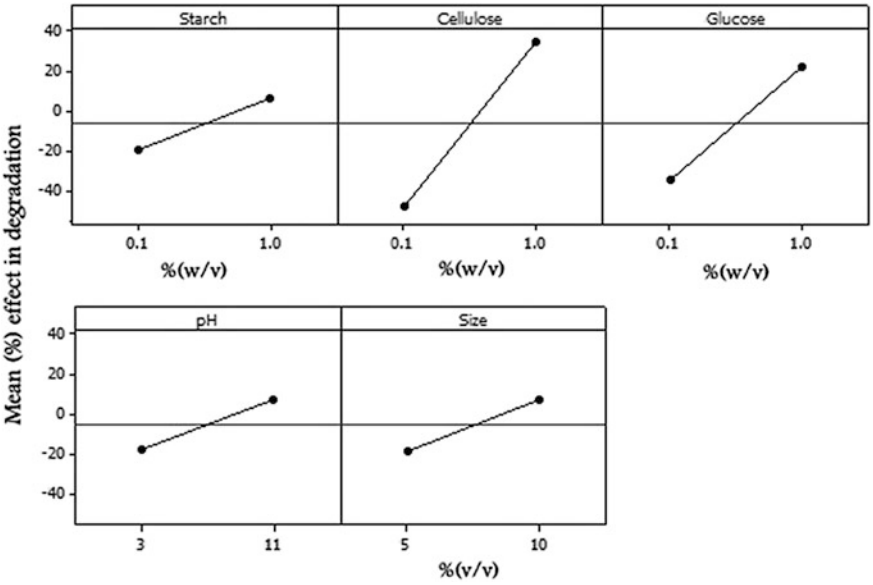

Fig. 5 Effect of various parameters on cadmium degradation

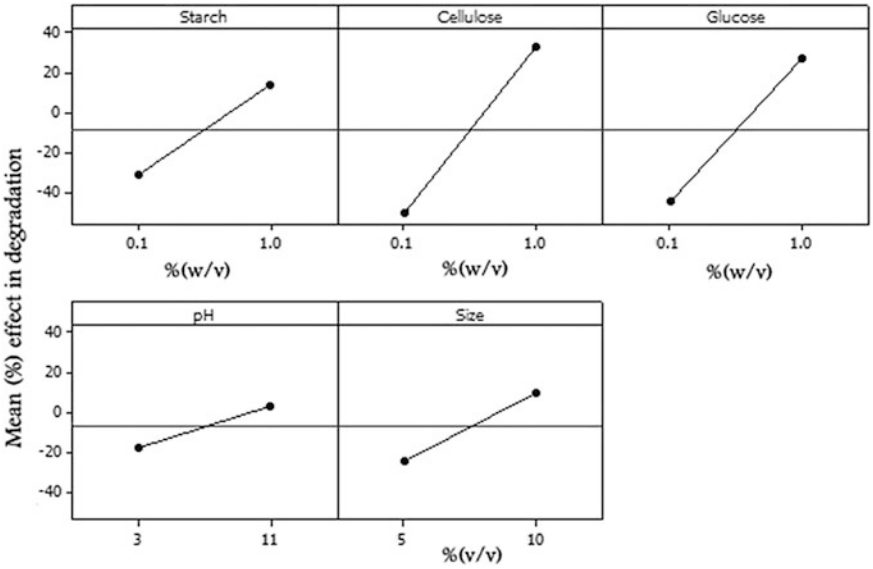

Fig. 6 Effect of various parameters on arsenic degradation

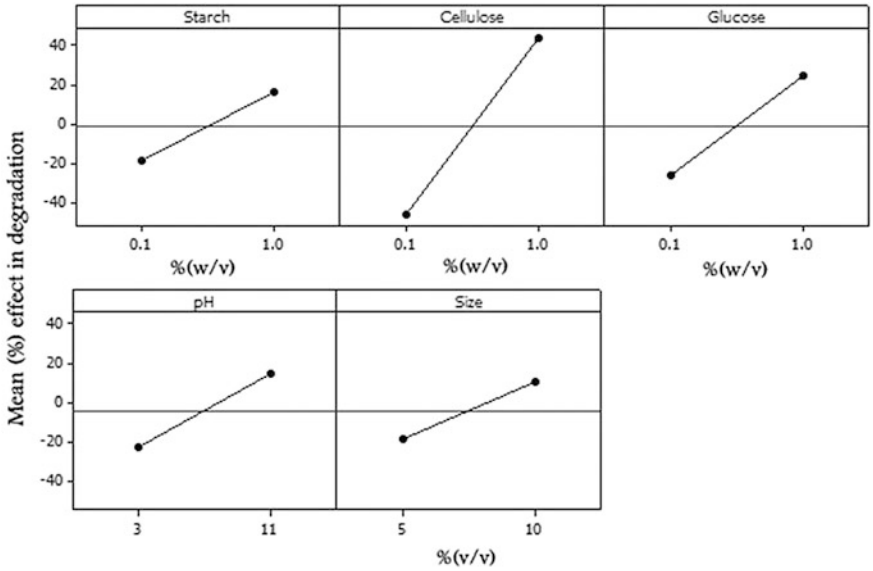

Fig. 7 Effect of various parameters on nickel degradation

The statistical modeled estimation reveals that degradation of lead, cadmium, arsenic and nickel would be optimal when starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 10 (v/v%). Mercury degradation would be optimal when starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 5 (v/v%).

3.4.4 Validation of Optimized Values

The final validation of the optimized value shows that there is a significant decrease in the heavy metal concentration using flawless combinations of bacteria and nutrients (Fig. 8).

The optimized experimental condition shows that 95.59 % degradation in lead by the bacterial consortium of *Flavobacterium* sp. and *Bacillus* spp. (B and G) with the nutrient combination of starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 10 (w/v%). This rate is higher than the previous research, which was reported that 94.32 % degradation of lead by Ameer and Rajaganesh (2014). The *Micrococcus* sp. (C) and *Cytophaga* sp. degraded mercury by 83.95 % with the nutrient combination of starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 5 (w/v%). This result shows that it is much efficient (18.4 % higher) than the results reported by Rajendran et al. (2003). But, in case of cadmium their experiment shows 27.82 % degradation. Whereas in the present study, cadmium concentration in the water sample was degraded into 19.63 % by the bacterial consortium of *Flavobacterium* sp., *Bacillus* sp.(B), *Micrococcus* spp. (D and E) with the condition starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 10 (w/v%). Nevertheless, the result was effective when introduce the optimal growth condition in comparison to the control used.

The identified species *Flavobacterium*, *Bacillus* (B), *Micrococcus* (C and E) and *Cytophaga* degraded arsenic upto 35.55 % under the condition when starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 10 (w/v%).

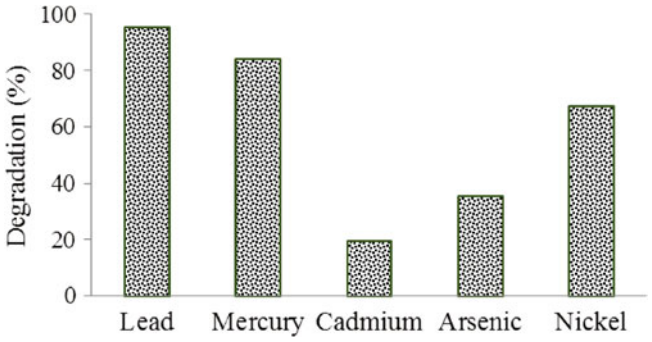

Fig. 8 Degradation effects of predicted parameters on heavy metals

Nickel was 67.39 % degraded by *Flavobacterium*, *Bacillus* (B), *Micrococcus* (E) and *Cytophaga* degraded arsenic upto 35.55 % at the optimal experimental condition, starch = 1 (v/v%), cellulose = 1 (v/v%), glucose = 1 (v/v%), pH = 11 and inoculum size = 10 (w/v%). The results show the significantly higher (23.43 %) than reported by Rajendran et al. (2003). In the present study, the possibility of reduction is much in lead and in mercury than the other heavy metals; however the degradation effect was significant subject to every heavy metal. This result shows the toxic metals namely lead, mercury, cadmium, arsenic and nickel can be removed partially as possible and reduce the water pollution in estuaries.

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Wastewater Treatment Studies on Free Water Surface Constructed Wetland System

G. Midhun, L. Divya, Jessen George, P. Jayakumar and S. Suriyanarayanan

Abstract Scarcity of safe drinking water is a major problem faced by mankind in recent years. Because of increase in population and over exploitation of natural water resources, fresh water resources are declining day by day. Considering the above scenario, protection of the available limited fresh water resources from deterioration in quality deserves utmost importance. This study aims to evaluate the wastewater treatment efficiency of a constructed wetland system using a laboratory scale model of the wetland system with the common wetland plant 'Reed' which is commonly found in the wetlands of Kerala. The treatment efficiency of the model wetland system was studied with reference to the parameters pH, Conductivity, TSS, TDS, DO, BOD, COD, TKN, Nitrate, and Sulphate. The result of this study indicates that the constructed wetland system showed a removal efficiency of 87.36, 57.93, 83.7, 86.6, 36.66, 98.28 and 61.83 % respectively for TDS, DO, BOD, COD, TKN, nitrate and sulphate. From the present study it was concluded that there is a scope for development of constructed wetland system using the wetland plant 'Reed' as a low cost system for wastewater renovation.

Keywords Waste water treatment · Constructed wetland · Reed · Treatment efficiency

1 Introduction

Water is one of the most important resources needed for the development of a healthy life. The exponential growth of population and industrialization will lead to a huge lack of this valuable resource unless we start to use it in a sustainable way.

G. Midhun · L. Divya · J. George · S. Suriyanarayanan (✉)
Department of Water and Health, JSS University, Mysore, Karnataka, India
e-mail: sunsjk@jssuni.edu.in

G. Midhun · P. Jayakumar
Environmental Studies Division, Centre for Water Resources Development and Management, Kozhikode, Kerala, India

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_9

To achieve this target, a high level of responsibility towards water usage is required, and it must be recycled considering its pollution content in order to maintain water quality and protect our environment. Constructed wetlands are essentially inspired by natural processes taking place in naturally occurring wetlands (Sheik 2005). Today constructed wetland is recognized as a reliable wastewater treatment technology and they represent a suitable solution for the treatment of many types of wastewater (Vymazal 2011). At first natural wetland are used in treating wastewater. But there are some operational difficulties in using the natural wetland as a wastewater treatment medium (Brix 1995). Wetland are the most simple and inexpensive technique for wastewater treatment (Awaleh and Soubaneh 2014). The operational limitations associated with natural wetland systems are the difficulties in hydraulic control and potential interference of the wastewater constituents on wild life habitat and ecosystems (USEPA 2000).

Constructed wetland is an opposite of natural wetland where it is defined as engineer-made equivalent of natural wetlands, and designed to reproduce and intensify the wastewater treatment processes that occur in natural wetlands (Hammer 1994). They were first introduced to treat wastewater by Siedel in 1952 in Germany (Vymazal 2008). Basically constructed wetland treatment systems consist of four major components which are soil or gravel, water or shallow pond, aquatic plant or macrophytes and also microorganism. In general, constructed wetland has been used to be a good solution to treat the polluted water and restored the ecosystem health (USEPA 2000).

Wetlands are some of the most biologically diverse and productive natural ecosystems in the world. While not all constructed wetlands replicate natural ones, it makes sense to construct wetlands that improve water quality and support wildlife habitat. Constructed wetlands can also be a cost-effective and technically feasible approach to treating wastewater (Robert and Robert 2004). Wetlands are often less expensive to build than traditional wastewater treatment options, have low operating and maintenance expenses and can handle fluctuating water levels (Bama et al. 2013). Additionally, they are aesthetically pleasing and can reduce or eliminate odors associated with wastewater.

2 Materials and Methods

2.1 Characterization of the Test Plant Species

In the present study we used the wetland plant ‘Reed’ (*Phragmites communis*) which is commonly found in wetlands of Kerala (Fig. 1).

Fig. 1 Reed plant

2.2 Preparation of Constructed Wetland

A laboratory scale wetland for the study was developed using three plastic trays each having a dimensions of 65 cm length, 45 cm breadth and 30 cm height (Fig. 2). These trays were filled with a mixture of wetland soil and sand in the ratio 2:1. Two of the trays were planted with the wetland plant 'Reed' in such a way that

Fig. 2 Experimental constructed wetland system

the plant density is 20 plants/m^2. The third tray was maintained without any plant to serve as a control. Sewage from Cannoly canal was used as input to the systems. Free water surface system (FWS) of flow in which the wastewater is allowed to stand above the soil media was adopted in the present study. An outlet with a ball valve is provided at the bottom of the trays to facilitate collection of the treated effluent.

2.3 Collection of Waste Water

Waste water for the study was collected from the Cannoly canal running across the heart of Kozhikode city. The city sewage finds way into this canal at several points along its path. The canal is now in a seriously polluted condition since free flow of water through the canal is interrupted during non-rainy months at several places due to deposition of sludge in the river bed. The wastewater collected from the canal is diluted twice before it is applied to the wetland for treatment studies.

3 Results and Discussion

The wastewater collected from the Cannoly canal was diluted twice before applying to the constructed wetlands. The waste waters were applied to the constructed wetland and the samples were taken for the analysis at a period of 4 days interval. The parameters analyzed during the period of study were pH, conductivity, total dissolved solids (TDS), suspended solids, dissolved oxygen, Biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate, sulphate, and total Kjeldahl nitrogen (TKN) (APHA 1998). Table 1 shows the characteristics of raw wastewater that was applied to the constructed wetland systems.

Table 1 Characteristics of raw wastewater

S.No.	Characteristics of wastewater	
	Parameters	Unit
1	pH	8.59
2	Conductivity	19.7 mS
3	TDS	12,700 mg/L
4	TSS	400 mg/L
5	DO	4.6 mg/L
6	BOD	511.1 mg/L
7	COD	537.6 mg/L
8	TKN	128 mg/L
9	NO_3–N	91.4 mg/L
10	Sulphate	760 mg/L

Table 2 Variation in pH in the experimental CWS

Retention time (days)	Wetland system 1		Wetland system 2		Control	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
4	8.59	3.45	8.59	3.40	8.59	3.58
8	8.59	3.44	8.59	3.25	8.59	3.56
12	8.59	3.43	8.59	3.23	8.59	3.52
16	8.59	3.39	8.59	3.23	8.59	3.52

3.1 pH

pH of the treated effluent from the wetland system was measured at 4th, 8th, 12th and 16th days from the date of application of wastewater. The variation in the pH value of the control and experimental sets are given in Table 2. The results indicate that vegetated system shows decrease in pH compared to unvegetated or control system. It may be due to nitrification process taking place in the system.

3.2 Conductivity

The treatment efficiency of the wetland system with regard to conductivity was studied and results are tabulated in Table 3 and Fig. 3. The conductivity of the wastewater sample is found decreasing in the wetland system. The unvegetated system does not show appreciable change in conductivity. The efficiency of reduction in conductivity increases from 74.5 to 84.6 % as the retention time increases. From the results it can be assumed that the conductivity of the water sample decreases with increase in retention time. The reduction in conductivity must be due to the absorption of ions by the plants.

3.3 Total Suspended Solids (TSS)

The waste water treatment efficiency of the wetland system with regard to TSS concentration was studied and the results were given in Table 4 and Fig. 4. All the

Table 3 Variation in conductivity in the experimental CWS

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mS)	Effluent (mS)	Treatment efficiency (%)	Influent (mS)	Effluent (mS)	Treatment efficiency (%)	Influent (mS)	Effluent (mS)	Treatment efficiency (%)
4	19.7	5.42	72.48	19.7	4.64	76.44	19.7	6.76	65.68
8	19.7	4.77	75.78	19.7	4.41	77.61	19.7	6.58	66.59
12	19.7	4.53	77	19.7	4.36	77.86	19.7	6.52	66.90
16	19.7	2.28	88.42	19.7	3.76	80.91	19.7	6.36	67.71

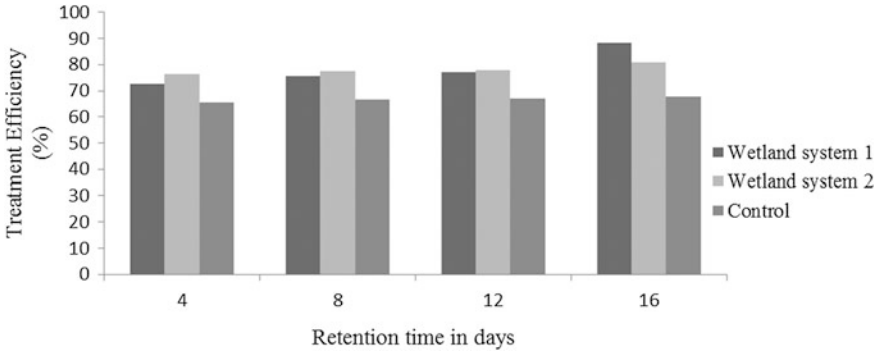

Fig. 3 Effect of CWS on conductivity

Table 4 Removal efficiency of TSS

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	400	220	45	400	210	47.5	400	380	5
8	400	170	57.5	400	160	60	400	190	52.5
12	400	90	77.5	400	80	80	400	130	67.5
16	400	20	95	400	30	92.5	400	90	77.5

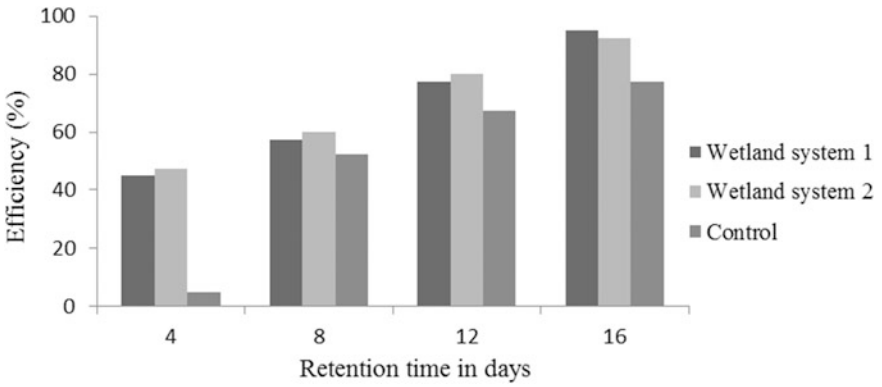

Fig. 4 Removal efficiency of TSS

three trays i.e. experimental and control sets show similar removal efficiency of total suspended solids. Hence it can be presumed that wetland plants have no significant role in treating suspended solids. The removal of suspended solids will be due to the effects of sedimentation, adsorption, and filtration taking place in the soil media.

Table 5 Removal efficiency of TDS

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	12,700	2830	77.71	12,700	2410	81.02	12,700	3560	71.96
8	12,700	2520	80.15	12,700	2300	81.88	12,700	3440	72.91
12	12,700	2360	81.41	12,700	2250	82.28	12,700	3410	73.14
16	12,700	1220	90.39	12,700	1990	84.33	12,700	3240	74.48

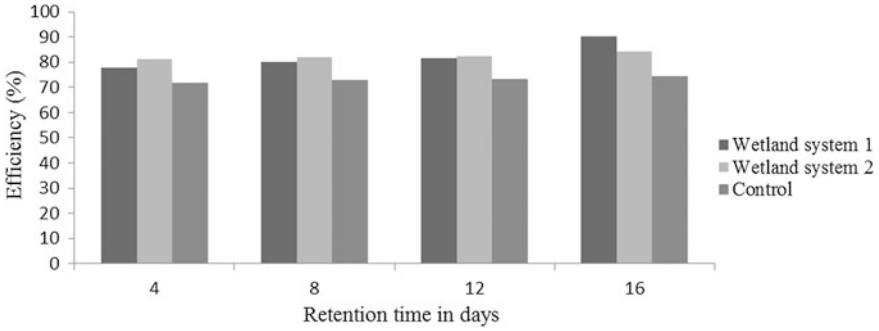

Fig. 5 Removal efficiency of TDS

3.4 Total Dissolved Solids (TDS)

The treatment efficiency of the wetland system in reducing dissolved solids concentration was tested and tabulated in Table 5 and Fig. 5. The experimental system shows a removal efficiency of 79.36 % initially and with increase in retention time the efficiency increased to 87.36 %. The control has no effect on the removal of total dissolved solids. From this it can be concluded that the wetland plants have an important role in the reduction of TDS. The reduction in the TDS can be attributed to the uptake of solids by the wetland plants.

3.5 Dissolved Oxygen (DO)

Variation in the effluent with regard to dissolved oxygen was given in Table 6 and Fig. 6. The increase in dissolved oxygen is due to the plant present in the experimental system. The Reed possesses deep roots system which transfer oxygen from the atmosphere to the roots and rhizomes and setup an aerobic environment in the rhizosphere. So the amount of dissolved oxygen concentration in the constructed wetland systems increases. Slight increase in DO was noticed in the control system. This can be due to the aerial oxidation.

Table 6 Variation in the dissolved oxygen content in CWS

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	4.66	5.39	15.66	4.66	5.46	17.16	4.66	4.9	5.1
8	4.66	5.93	27.25	4.66	5.99	28.54	4.66	5.19	11.37
12	4.66	6.79	45.70	4.66	6.86	47.21	4.66	5.26	12.87
16	4.66	7.33	57.29	4.66	7.39	58.58	4.66	5.33	14.37

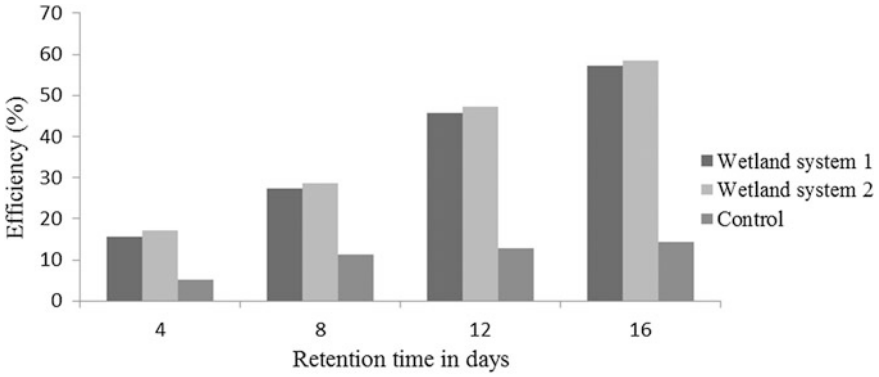

Fig. 6 Variation in the dissolved oxygen content in CWS

3.6 Biochemical Oxygen Demand (BOD)

Removal efficiency of BOD by the constructed wetland systems is shown in Table 7 and Fig. 7. The removal of biodegradable organic matter depends upon vegetation and substratum. BOD removal efficiencies of constructed wetland systems with plants were compared with unplanted ones. The experimental system with plants has shown a removal efficiency of 83.7 %. Results indicated that the BOD removal in the control system exposed to natural environmental conditions was very low.

Table 7 Removal efficiency of BOD

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	511.1	299.9	41.32	511.1	322.2	36.95	511.1	477.7	6.53
8	511.1	222.2	56.52	511.1	233.3	54.35	511.1	433.3	15.22
12	511.1	144.4	71.74	511.1	155.5	69.57	511.1	399.9	21.75
16	511.1	88.8	82.62	511.1	77.7	84.79	511.1	322.2	36.95

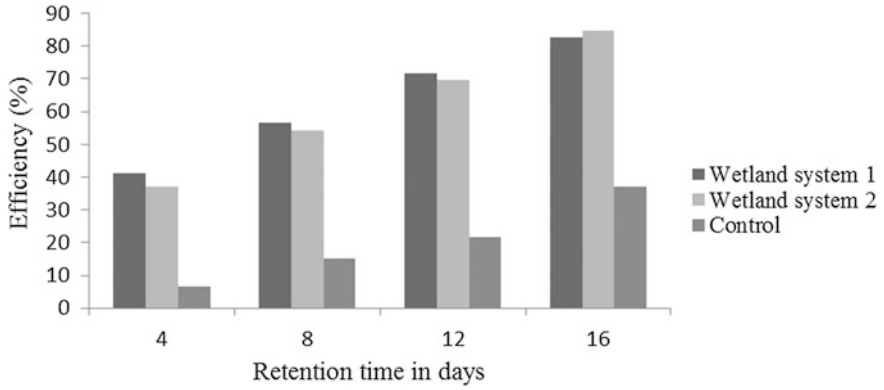

Fig. 7 Removal efficiency of BOD

3.7 Chemical Oxygen Demand (COD)

Removal efficiency of COD by constructed wetland systems was plotted in Table 8 and Fig. 8. The system with plants has a greater capacity in reducing COD compared to that with unvegetated system. The vegetated system has an efficiency of

Table 8 Removal efficiency of COD

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	537.6	131.2	75.59	537.6	137.6	74.40	537.6	352	34.52
8	537.6	128	76.19	537.6	134.4	75	537.6	323.2	39.88
12	537.6	86.4	83.92	537.6	96	82.14	537.6	284.8	47.02
16	537.6	70.4	86.90	537.6	73.6	86.30	537.6	243.2	54.76

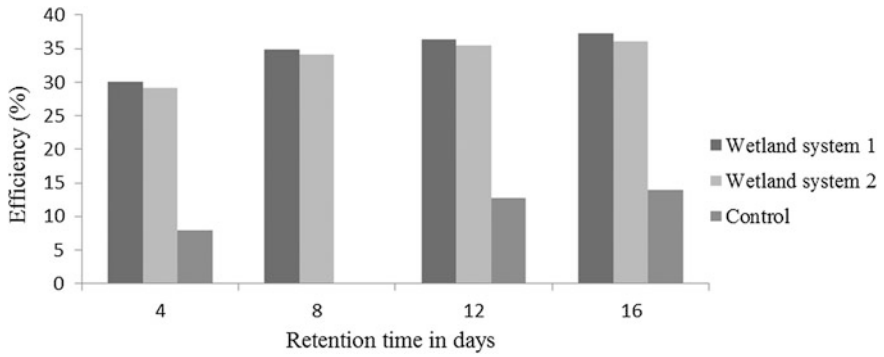

Fig. 8 Removal efficiency of COD

86.6 % in removing COD. The control shows no significant reduction in COD. This may be due to the plant's capability to break up carbonaceous matter. COD removal also depends upon hydraulic retention time. With increase in retention time COD removal efficiency also increases.

3.8 Total Kjeldahl Nitrogen (TKN)

Removal efficiency of Total Kjeldahl Nitrogen by the constructed wetland systems was analyzed and results are shown in Table 9 and Fig. 9. The results show that the constructed wetland systems have a potential to treat nitrogen. The control system shows a slight variation in the nitrogen level. TKN removals in the constructed wetland systems are most likely to be due to the accumulation of organic nitrogen in the sludge layers.

3.9 Nitrate (NO_3^-)

Results of Nitrate removal efficiency of the constructed wetland systems are shown in Table 10 and Fig. 10. The results indicate that nitrate is reduced significantly by

Table 9 Removal efficiency of TKN

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	128	89.6	30	128	90.72	29.12	128	117.6	8
8	128	83.44	34.81	128	84.28	34.15	128	113.68	11.18
12	128	81.48	36.34	128	82.6	35.46	128	111.72	12.71
16	128	80.36	37.21	128	81.76	36.12	128	110.04	14.03

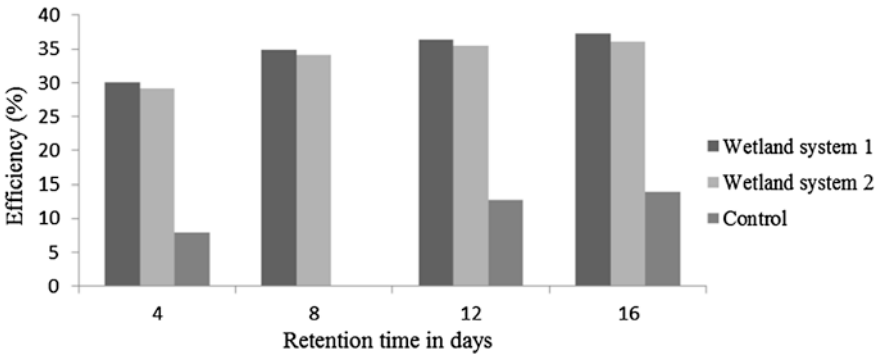

Fig. 9 Removal efficiency of TKN

Table 10 Removal efficiency of Nitrate-Nitrogen

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	91.4	43.26	52.66	91.4	43.83	52.04	91.4	81.4	10.94
8	91.4	16.35	82.11	91.4	16.72	81.70	91.4	47.7	47.81
12	91.4	2.60	97.15	91.4	2.98	96.73	91.4	4.66	94.90
16	91.4	1.36	98.51	91.4	1.78	98.05	91.4	3.53	96.13

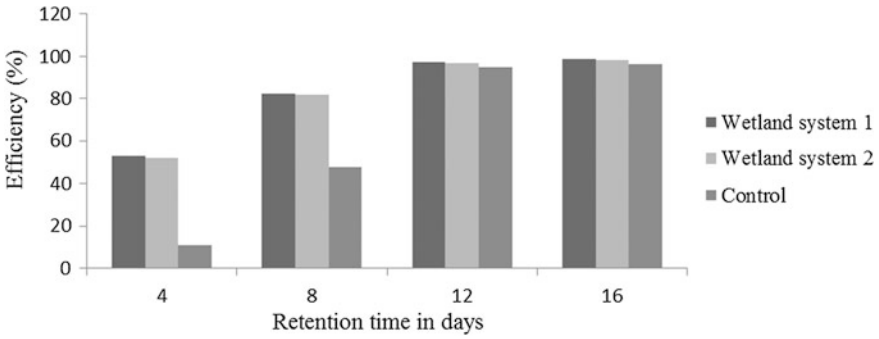

Fig. 10 Removal efficiency of nitrate–nitrogen

the constructed wetland systems compared to unvegetated system. The reduction in nitrate is due to the nitrification and denitrification process that take place in the rhizome. The constructed wetland system has an efficiency of 98.28 % in removing nitrate from the wastewater. This treatment rate was much greater than the conventional wastewater treatment process. The wetland has the potential to treat nitrate. Thus we can assume that wetland plants are good in removing nitrate from wastewater.

3.10 Sulphate (SO_4^-)

Removal efficiency of sulphate from wastewater in the constructed wetland is shown in Table 11 and Fig. 11. The results indicate that the constructed wetland systems with wetland plants gives significant removal of sulphate compared to unvegetated system. The wetland plants have an efficiency of 61.83 % in removing sulphate from the wastewater. From this we can presume that the wetland plants have a potential of removing sulphate from the wastewater. The unvegetated system shows no variation in the sulphate levels.

Table 11 Removal efficiency of sulphate

Retention time (days)	Wetland system 1			Wetland system 2			Control		
	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Treatment efficiency (%)
4	760	369	51.44	760	371	51.18	760	686	9.7
8	760	343	54.86	760	355	53.28	760	676	11.05
12	760	306	59.73	760	311	59.07	760	672	11.57
16	760	286	62.36	760	294	61.31	760	666	12.36

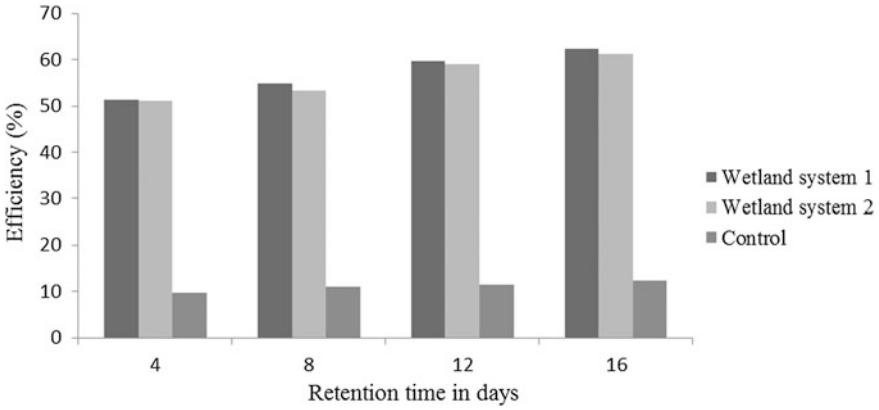

Fig. 11 Removal efficiency of sulphate (SO_4^-)

4 Conclusion

In the present study an effort has been made to evaluate the wastewater treatment efficiency of a constructed wetland system using a laboratory scale model of the wetland system with the common wetland plant 'Reed' which is commonly found in the wetlands of Kerala. For the study, wastewater collected from Cannoly canal to which several drains of Kozhikode city discharges is used. The wastewater is diluted and applied to the wetland system and effluent quality analyzed at 4 days intervals.

During the study carried out within the short period of about two months, the treatment efficiency of the model wetland system was studied with reference to the parameters pH, conductivity, total suspended solids, total dissolved solids, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, nitrate-nitrogen, sulphate and total Kjeldahl nitrogen. It is found that pH of the wastewater was altered from alkaline range to acidic range. This reduction in the pH may be due to the leachates from the soil media. The conductivity of the treated effluent was found to be reduced from 19.7 to 2.28 mS. It may be due to the

absorption of ions by the wetland plants. The removal of suspended solids in the wetland system showed that the wetland plants have no considerable role in the removal of suspended solids.

The constructed wetland system showed a removal efficiency of 87.36, 57.93, 83.7, 86.6, 36.66, 98.28 and 61.83 % respectively for TDS, DO, BOD, COD, TKN, nitrate and sulphate. The results obtained indicate the scope for development of constructed wetland system using the wetland plant 'Reed' as a low cost system for wastewater renovation. However further studies to establish the replicability of the results and to optimize the system will be needed to make the system suitable for adoption in the field.

Acknowledgment My sincere thanks to Executive Director, Center for Water Resources Development and Management, Calicut for granting me permission to execute my work in this reputed institute.

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Recycling of Distillery Spent Wash and Their Effect on Growth of Sesame (*Sesamum*) Crop

V. Karthika, G. Rajannan and P. Thangavel

Abstract Sugar industry is the second largest agro-based industry in India, which contribute substantially to the economic development of the country. Distillery effluent release 80 million kg of nitrogen and 520 million kg of potassium annually. A field experiment was conducted at Research and Development Farm, M/s Sakthi Sugars Ltd., Sakthi Nagar, Erode district, with different doses of distillery spentwash (DSW) along with inorganic N and P fertilizer without K using Sesame variety VRI (SV) 2 as test crop. Crop residue (6561 kg ha^{-1}) were recorded higher in treatment that received 100 % N through DSW and Seed yield (720 kg ha^{-1}), oil content (51.10) were recorded higher in treatment that received 75 % N through DSW and 25 % N through inorganic source. Based on the field experiments on sesame, it is concluded that the 75 % N through DSW and 25 % N through inorganic source based on crop requirement proved to be beneficial in increasing the crop yield and Quality parameter of the sesame crop.

Keywords Distillery spentwash · Crop residue · Sesame yield · Quality parameter

1 Introduction

Distilleries are one of the 17 most polluting industries listed by the Central Pollution Control Board of India (CPCB 2003). Indian distilleries employ various forms of primary, secondary and tertiary treatments of waste water. The typical treatment sequence is screening and equalization, followed by biomethanation. Ferti-irrigation and biocomposting with sugarcane pressmud are the most widely used options for effluent disposal (Ramana et al. 2002).

V. Karthika (✉) · G. Rajannan · P. Thangavel
Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
e-mail: sanjanakarthi@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_10

The spentwash is acidic (pH 3.94–4.30), dark brown liquid with high BOD (45,000–1,00,000 mg l^{-1}) and COD (90,000–2,10,000 mg l^{-1}), and produce obnoxious odour. Although it does not contain toxic substances, its discharge without any treatment brings about immediate discolouration and depletion of dissolved oxygen in the receiving water streams, in turn posing serious threat to the aquatic flora and fauna (Mane et al. 2006).

Spentwash is a rich source of organic matter and nutrients like nitrogen, phosphorus, potassium, calcium and sulphur. Hence while aiming for better crop production; their utilization has to be optimized for sustaining the environment. On an average, distillery effluents release 80 million kg of nitrogen and 520 million kg of potassium annually. Thus the availability of nutrients in distillery effluent and the possibility of substituting these for inorganic fertilizer in agriculture have a great promise (Joshi and Singh 2010). Sesame (*Sesamum indicum*) is an important ancient oil yielding crop. Rajannan et al. (1998) reported that fertigation of distillery spentwash at 40–50 times dilution increased the yield of sugarcane, banana, gingelly and rice.

2 Materials and Methods

A field experiment was conducted at Research and Development Farm, M/s Sakthi Sugars Ltd., Sakthi Nagar, Erode district, with different doses of distillery spentwash along with inorganic N and P fertilizer without K using Sesame variety VRI (SV) 2 as test crop. The experiment was formulated with six treatments with four replications, laid out in Randomised Block design.

2.1 Treatment Details

T_1: Absolute control
T_2: Control—100 % recommended dose of NPK
T_3: 25 % N through DSW and 75 % N through inorganic source based on crop requirement
T_4: 50 % N through DSW and 50 % N through inorganic source based on crop requirement
T_5: 75 % N through DSW and 25 % N through inorganic source based on crop requirement
T_6: 100 % N through DSW

While applying P, the available P in DSW and inorganic P was taken together to meet the P requirement of crop. Potassium was skipped in DSW applied treatments.

2.2 Field Experiment—Sesame

2.2.1 Application of Spentwash and Fertilizers

As per the treatment schedule the calculated quantity of biomethanated distillery spentwash for pre-sown application was uniformly applied to the plots before sowing. After 15 days, the treated plots were inverted manually with spade to facilitate aeration and oxidation and then plots were formed. In all the plots, entire phosphorus and half dose of nitrogen as per the treatments were applied as basal while remaining nitrogen as per the treatments was applied in two equal splits on 30th and 45th day after sowing. Potassium fertilizer was not applied to the plots treated with distillery spentwash because spentwash is rich in potassium. For T_1 (control), potassium was applied as per the treatment schedule. The photographs representations were given in Figs. 1 and 2.

2.2.2 Yield and Yield Attributes of Sesame

Yield attributes like number of capsules per plant, capsule length, number of seeds per capsule and hundred seed weight were recorded and the mean values obtained were expressed as per the SI system of units. Seed and stalk yield from the net plot area were recorded and expressed in kg ha^{-1}.

Fig. 1 Application of distillery spentwash to experimental field through tanker lorry

Fig. 2 Overview of experimental field of sesame at vegetative stage under irrigated condition

2.2.3 Oil Content

Oil content of the Sesame seed was estimated by Soxhlet method (Anonymous 1960). Samples from four replications in each treatment with twenty five grams of powered seed material were dried at 105 °C in a hot air oven for 16 h and then they were allowed to cool in a desiccator. A sample of 5 g was taken and transferred to an extraction thimble.

The thimble was then placed inside the soxhlet apparatus and solvent was added and heated for 6 h until 6–8 siphoning were completed. After siphoning the extraction flask was taken out and placed in a hot air oven (60 °C) to completely evaporate the ether containing oil. The oil percentage was then calculated using the Eq. 1.

$$\text{Oil content}\,(\%) = \frac{\text{Weight of oil}\,(\text{g})}{\text{Weight of sample}\,(\text{g})} \tag{1}$$

3 Results and Discussion

From the results of growth attributes for sesame such as Plant height, Number of primary branches, Number of capsules per plant, No. of seeds per capsule, Seed yield per plant, Crop residue, Seed yield (720 kg ha^{-1}) and Oil content (51.10) were recorded higher in treatment that received 75 % N through DSW and 25 % N

through inorganic source based on crop requirement. Rajannan et al. (1998) reported that fertigation of distillery spentwash at 40–50 times dilution increased the yield of sugarcane, banana, gingelly and rice. The method of application of distillery spentwash (pre-sown) did not show marked difference in the yield of sesame crop. Figures 3 and 4 represent the effect of distillery spentwash application on yield attributes in irrigated sesame crop (data given in Table 1) and the effects on oil content.

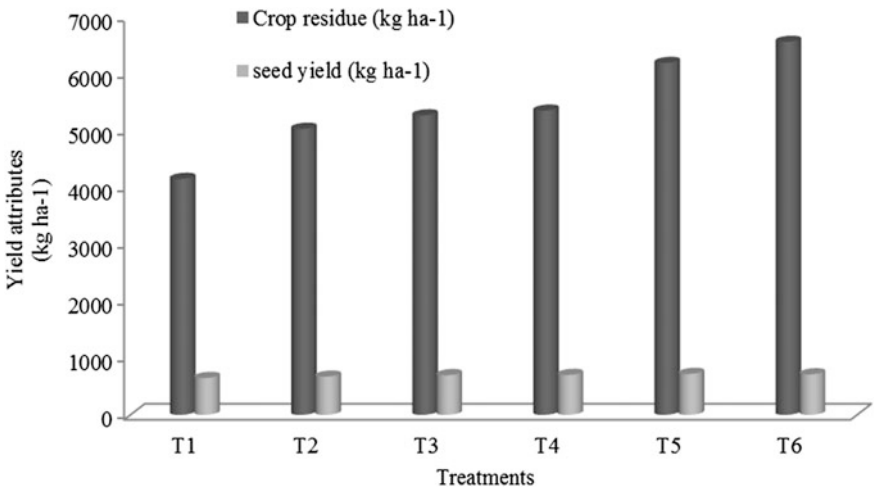

Fig. 3 Effect of distillery spentwash application on yield attributes in irrigated sesame crop

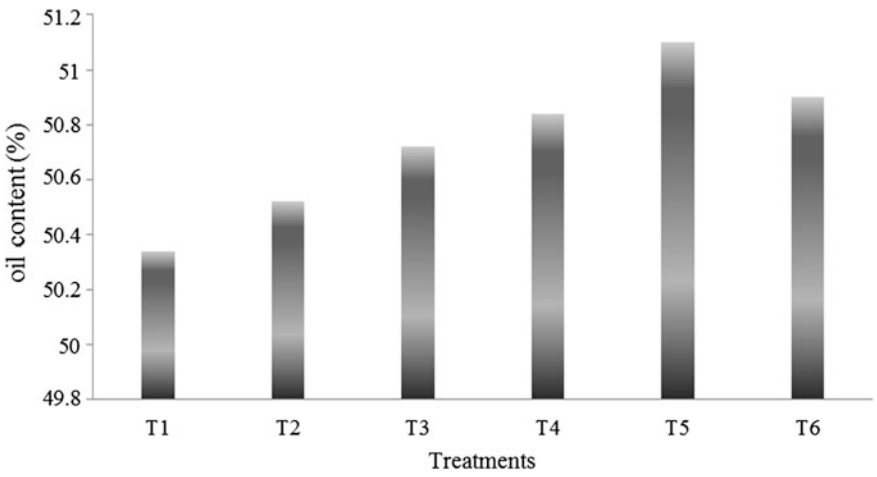

Fig. 4 Effect of distillery spentwash application on oil content in irrigated sesame crop

Table 1 Effect of DSW on the sesame yield attributes under irrigated condition

Treatments	Seed yield plant^{-1} (g)	Yield parameters		Quality parameters
		Crop residue (kg ha^{-1})	Seed yield (kg ha^{-1})	Oil content (%)
T_1	40.1	4150	648	50.34
T_2	44.6	5031	672	50.52
T_3	46.6	5267	698	50.72
T_4	50.2	5350	702	50.84
T_5	55.2	6189	720	51.10
T_6	52.0	6561	712	50.90
Mean	48.12	5424.6	692	50.73
SEd	0.84	144.5	18.4	6.17
CD (0.05)	2.24	298.7	38.8	NS

This corroborates with the findings of Bhalerao et al. (2005) who reported that the pre-sowing application and fertigation (application in the standing crop) of distillery spentwash recorded no variation in the yield of dhaincha and sugarcane. These findings add strength to the results of present study. From the results of field experiments on sesame and laboratory experiments, it is concluded that the 75 % N through DSW and 25 % N through inorganic source based on crop requirement proved to be beneficial in increasing the crop yield, yield attributes and quality of produces of sesame crop.

4 Conclusion

Based on the field experiments on sesame, it is concluded that the 75 % N through DSW and 25 % N through inorganic source based on crop requirement proved to be beneficial in increasing the crop yield and Quality parameter of the sesame crop.

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ISO 14001: Environmental Management System

R. Mayamurugan

Abstract ISO 14001:2004 specifies requirements for an environmental management system to enable an organization to develop and implement a policy and objectives which take into account legal requirements and other requirements to which the organization subscribes and information about significant environmental aspects. It applies to those environmental aspects that the organization identifies as those which it can control and those which it can influence. It does not itself state specific environmental performance criteria.

Keywords Environment · Management · Policy

1 Introduction

Environmental management system (EMS) refers to the management of an organization's environmental programs in a comprehensive, systematic, planned and documented manner. It includes the organizational structure, planning and resources for developing, implementing and maintaining policy for environmental protection.

2 ISO 14001:2004

ISO 14001:2004 sets out the criteria for an environmental management system and can be certified. It does not state requirements for environmental performance, but maps out a framework that a company or organization can follow to set up an effective environmental management system. It can be used by any organization regardless of its activity or sector. Using ISO 14001:2004 can provide assurance to

R. Mayamurugan (✉)
Department of Economics, Alagappa University, Thondi, Tamil Nadu, India
e-mail: drmaya1984@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_11

company management and employees, as well as external stakeholders that environmental impact is being measured and improved.

An ISO 14001 environmental management system (or commonly referred to as an EMS) is a structured system designed to help organisations manage their environmental impacts and improve environmental performance caused by their products, services and activities. An environmental management system provides structure to environmental management and covers areas such as training, record management, inspections, objectives and policies. The benefits of using ISO 14001:2004 can include

- Reduced cost of waste management
- Savings in consumption of energy and materials
- Lower distribution costs
- Improved corporate image among regulators, customers and the public

3 Objectives of the Study

To organization shall establish, implement and maintain documented environmental objectives and targets, at relevant functions and levels within the organization. The objectives and targets shall be measurable, where practicable, and consistent with the environmental policy, including the commitments to prevention of pollution, to compliance with applicable legal requirements and with other requirements to which the organization subscribes, and to continual improvement. When establishing and reviewing its objectives and targets, an organization shall take into account the legal requirements and other requirements to which the organization subscribes, and its significant environmental aspects. It shall also consider its technological options, its financial, operational and business requirements, and the views of interested parties.

4 Management Review

Top management shall review the organization's environmental management system, at planned intervals, to ensure its continuing suitability, adequacy and effectiveness. Reviews shall include assessing opportunities for improvement and the need for changes to the environmental management system, including the environmental policy and environmental objectives and targets. Records of the management reviews shall be retained. Input to management reviews shall include

(a) Results of internal audits and evaluations of compliance with legal requirements and with other requirements to which the organization subscribes
(b) Communication(s) from external interested parties, including complaints

(c) The environmental performance of the organization
(d) The extent to which objectives and targets have been met
(e) Status of corrective and preventive actions
(f) Follow-up actions from previous management reviews
(g) Changing circumstances, including developments in legal and other requirements related to its environmental aspects, and
(h) Recommendations for improvement.

Implementation of an environmental management system requires the following steps to be completed by an organisation:

- Development of an environmental policy that reflects its commitments
- Appointment of a person(s) responsible for its coordination
- Identification of how the organisation interacts with the environment
- Identification of actual and potential environmental impacts
- Identification of relevant legal and other requirements
- Establishment of environmental objectives, targets and programs
- Monitoring and measurement of the progress to achieve its objectives
- Reviewing the system and environmental performance, and
- Continuous improvement of the organisation's environmental performance.

By design the system and environmental performance run in a continuous improvement cycle outlined in the Fig. 1.

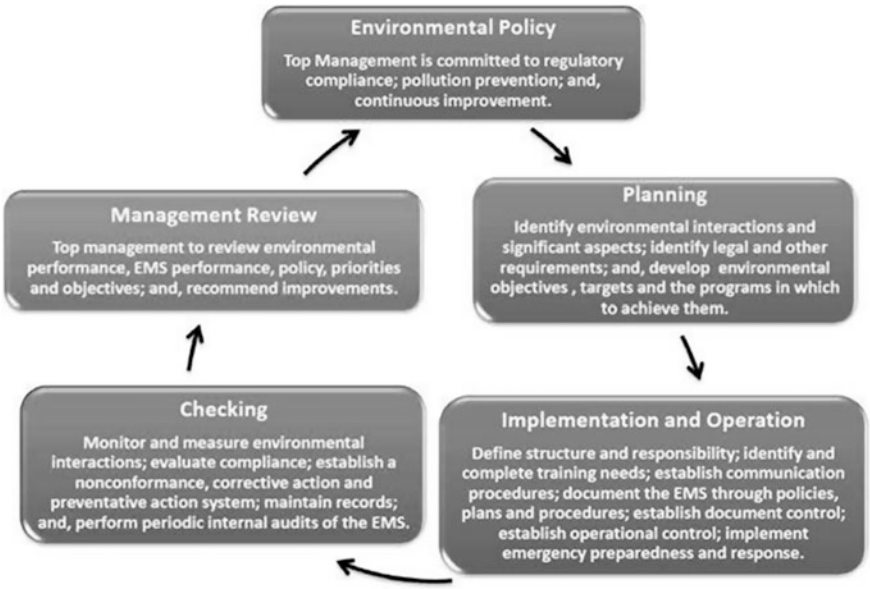

Fig. 1 System and environmental performance run

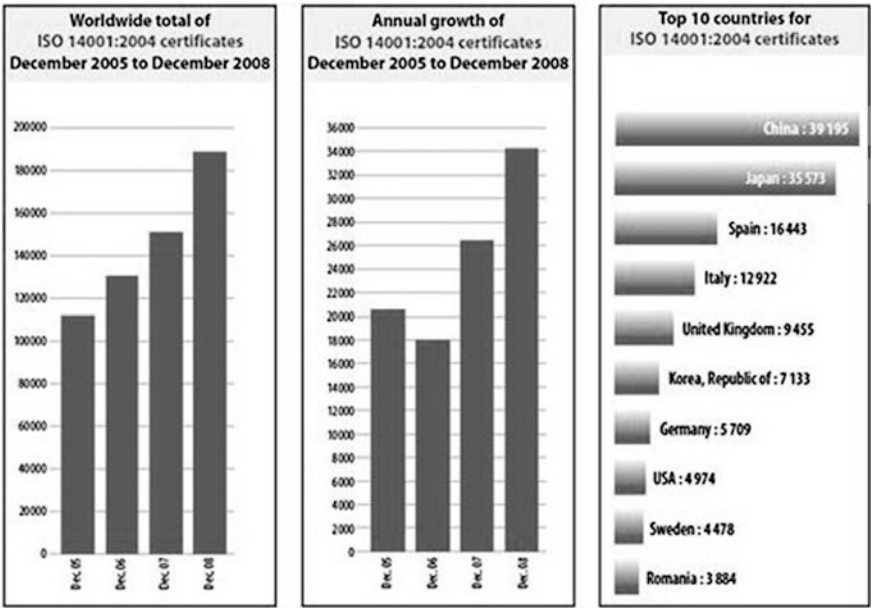

Fig. 2 ISO 2008 survey results. (*Source* http://www.iso14001.com.au/iso-14001-standard.html)

Currently in Australia there are over 2000 organisations certified to the ISO 14001 environmental management system standard. Up to the end of December 2008 there were over 188,000 organisations globally certified. A total of 155 countries had organisations participating in the scheme. Figure 2 illustrates the ISO 2008 survey results for the standard. It should be noted that there are many more organisations with environmental management systems not certified to the ISO 14001 standard.

5 Environmental Policy

The ISO 14001 standard is probably the best reference standard for the development of an environmental policy. In summary, an environmental policy must be:

- Appropriate to the organisation
- Include a commitment for continual improvement and prevention of pollution
- Include a commitment to comply to relevant legal and other requirements, and
- Provide the framework for setting and reviewing environmental objectives and targets.

Also, consideration should be given to the reader's expectations. From a customer perspective they would like to clearly know,

- What the organisation does and how it does it (e.g. Do they follow best practice and embrace cleaner production, if so how?)
- Understand whether the organisation is greener or browner than similar organisations
- Understand whether the organisation presents a direct environmental risk to their operations
- Understand whether the organisation presents a risk to public perception if they are engaged
- Understand whether the organisation present an environmental risk by not understanding and not complying with legal and other requirements; and
- Understand whether the organisation has any environmental programs to their reduce environmental impact and improve their environmental performance.

6 Conclusion

This chapter has been important in the presentation of the actual situation of ISO 14001, Overall ISO 14001 led to improvements in the organisations' performance. The motivations for adopting ISO 14001 varied amongst organisations and the perceived benefits provided justification to implement the system. The guidelines provided by ISO 14001 were easily incorporated into the organisations framework with little external guidance. This led to the identification of significant environmental aspects, which were subsequently addressed. Key difficulties experienced by organisations included gaining staff buy-in, quality of auditors and achieving targets. Performance varied across organisations because of the quality of environmental policies, setting of objectives and targets and commitment of management. Improvements in environmental performance were allegedly experienced by all organisations, however this was difficult to quantify and did not correspond to direct economic benefits.

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http://www.iso.org
http://www.environmentalmanagementsystem.com.au

Part IV
Bioremediation and Environmental Sustainability

Utilization of Medicinal Oil Effluent for Lipase Production by *Penicillium citrinum* MKF3

N. Vinod Kumar, Mary Esther Rani, R. Gunaseeli and N.D. Kannan

Abstract Lipase is an enzyme with numerous applications in industries as well as in environmental management. There are many lipolytic microorganisms and among them, fungal strains like *Aspergillus* and *Penicillium* are the prominent ones. Coastal environmental samples are good source for lipolytic fungi as they were exposed to oil spillages. Coastal soil samples were collected from Fort Kochi, Kerala and fungal isolates were isolated using serial dilution and spread plate method. Lipolytic fungal isolates were screened using phenol red based plate assay with olive oil as a substrate. Lipase assay was performed using titerometric method and a potential fungus was obtained which was identified through molecular methods as *Penicillium citrinum*. The optimization study revealed that dextrose and peptone were the best carbon and nitrogen sources. Similarly, medium pH of 7 and incubation temperature of 35 °C favoured the lipase production. Medicinal oil effluents from ayurvedic pharmaceuticals were obtained and studied for the suitability in lipase production under submerged conditions. About 20 % effluent with minimal medium could achieve maximum lipase activity of 266 U/ml using *Penicillium citrinum* MKF3. Hence, the present fungal isolate was found to be a promising lipase producer and suitable for managing oil rich effluents.

Keywords Lipase · *Penicillium citrinum* · Medicinal oil effluent · Submerged fermentation

N.V. Kumar (✉) · M.E. Rani
Research Centre, Department of Botany and Microbiology,
Lady Doak College, Madurai, Tamil Nadu, India
e-mail: nvkibt@gmail.com

R. Gunaseeli
Center for Environmental Studies, Lady Doak College,
Madurai, Tamil Nadu, India

N.D. Kannan
Department Plant Biotechnology, Madurai Kamaraj University,
Madurai, Tamil Nadu, India

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_12

1 Introduction

Lipase is an important enzyme used in the industries for numerous applications. It acts upon the triglycerides to release fatty acids and glycerol. Lipases are produced by microorganisms as well as by plants during germination of seeds. However, the microbial lipases are more attractive due to their superior efficiency and functionality. Microbial lipases are widely used in the processing of fats and oils, decreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, production of cosmetics, paper manufacture, waste management, biosensors, etc. (Rubin and Dennis 1997; Kazlauskas and Bornscheur 1998). Some lipases are also able to catalyze transesterification and enantioselective hydrolysis reactions (Brune and Gotz 1992).

Lipases produced by fungi are typically extracellular and therefore relatively easy to recover after the fermentation. As in any application that demands high enzyme quantities, such as treatment of oily wastewaters and biodiesel production, lipase utilization depends on the reduction of its cost to become economically feasible (Cammarota and Freire 2006). There are many lipolytic fungal strains identified and *Penicillium* and *Aspergillus* are the most potential strains. The lipase production using fungi is usually achieved using both submerged and solid state fermentation. However, the industrial production adopts solid state fermentation to reduce the production cost as well as to achieve a better yield.

Medicinal oil effluent is highly complex with hydrocarbons and high percentage of triglycerides from many plant sources. Due to its complexity, the effluent resulting from such industries is very difficult to be treated for its safe disposal. However, when the lipolytic fungal strains are utilized for treating the medicinal oil effluents, there is a transformation of waste effluent into a valuable product i.e., lipase enzyme. The biopharmaceutical oil waste was found to be a potential medium for lipase production using the fungal strains (Mohanasrinivasan et al. 2009). In this study, the medicinal oil effluent was utilized for the production of lipase enzyme using the fungal isolate *Penicillium citrinum* MKF3.

2 Materials and Methods

2.1 Isolation and Screening of Lipolytic Fungi

Oil spillage exposed soil sample was collected from Fort Kochi, Kerala and the serial dilution of the sample was performed up to 10^{-6} dilution. Spread plating was done onto potato dextrose agar medium and incubated at 28 ± 2 °C. Screening of lipase producing fungi was performed using PDA plates supplemented with 1 % olive oil as substrate and phenol red as an indicator. On lipase production, free fatty acid will be released into the medium which turn the pH to acidic and thereby change the plate colour from red to yellow.

2.2 Secondary Screening of Lipase Activity

Secondary screening for lipase activity was performed using standard titrimetric assay. 2.5 ml of water was added into test tube and blank test tubes followed by 1 ml of 100 mM Tris HCl buffer (pH 7.5). 3 ml of olive oil was added as the substrate and mixed well and incubated for 5 min. 1 ml of enzyme was added into test sample and incubated for 30 min at room temperature. After incubation, 1 ml of 95 % ethanol was added into the sample and performed titration against 0.1 M NaOH with phenolphthalein as the indicator. Appearance of pale pink colour was considered as the end point. Lipase activity was calculated using standard formula (Parry et al. 1966).

2.3 Molecular Identification of Lipolytic Fungi

Fungal genomic DNA was extracted using standard method by Melo et al. (2006). DNA was further amplified using DR [5′-GGTCCGTGTTTCAAGACGG-3′] and DF [5′-ACCCGCTGAACTTAAGC-3′] universal primers for amplification of LSU 28S rDNA (Kurtzman and Robnett 1997). PCR amplicon was sequenced and the sequence thus obtained was used for Blastn analysis to identify the isolate.

2.4 Optimization of Submerged Fermentation Parameters

Fungal basal medium (2.0 g KH_2PO_4, 0.3 g urea, 0.3 g $MgSO_4.7H_2O$, 0.3 g $CaCl_2$, 5 mg $FeSO_4.7H_2O$, 1.6 mg $MnSO_4.H_2O$, 1.4 mg $ZnSO_4.7H_2O$ and 1.5 mg $CoCl_2.6H_2O$ in 1000 ml distilled water) was supplemented with four different carbon sources namely dextrose, sucrose, xylose and CMC. Peptone was also included as a nitrogen source and fungal inoculum of 3 % was added into the sterilized medium and incubated on a rotatory shaker at 150 rpm at room temperature for 3 days. Enzyme activity was quantified at regular intervals to understand the enzyme production kinetics. Optimization of nitrogen source was performed by supplementing basal medium with any one of the following nitrogen sources like peptone, beef extract, sodium nitrate and ammonium nitrate. Enzyme activity was quantified as described earlier. The identified best carbon source will be further used for nitrogen source optimization. Four different pH and incubation temperature were evaluated for optimum enzyme production. Medium pH was fixed at 3, 5, 7, 9 and incubated at temperatures 25, 35, 45 and 55 °C (best pH with best temperature). Likewise, the medium was inoculated with 3 % fungal inocula and kept on a rotatory shaker at 150 rpm for 3 days (Vinod et al. 2014).

2.5 Medicinal Oil Effluent for Lipase Production

Medicinal oil effluent rich in oil and higher forms of hydrocarbon were obtained and were made into different concentrations of 15, 20 and 25 % (v/v) using phosphate buffer (pH 8.2). *Penicillium citrinum MKF3* was inoculated into the sterilized effluent and incubated under shaking condition at 120 rpm for 5–7 days at room temperature. Lipase enzyme assay was performed during 3rd, 5th and 7th day of incubation and compared.

3 Results and Discussion

3.1 Isolation and Screening of Lipolytic Fungi

Five discrete fungal colonies were obtained from the PDA plates after 3 days of incubation. The individual colonies were screened for lipolytic activity based on the phenol red supplemented olive oil containing plates. Among the 5 colonies, 3 were found to be positive for lipase activity based on the colour change of the plate from red to yellow (Fig. 1). The secondary screening was performed based on the titrimetric assay using olive oil substrate. There were 2 isolates found to be potential lipolytic strains and MKF3 was further selected for the study based on the assay values. Based on the colony morphology and microscopic observation, MKF3 was identified as *Penicillium* sp. (Fig. 2).

3.2 Molecular Identification of Potential Strain

D1/D2 region amplification of the potential isolate MKF3 was performed using genomic DNA as template and the PCR amplicon was subjected sequencing. The

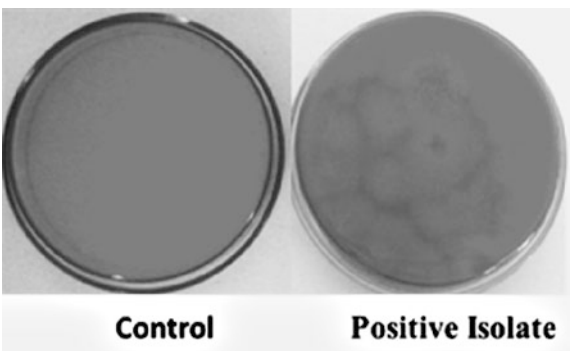

Fig. 1 Screening of lipolytic fungal strain *Penicillium citrinum* MKF3

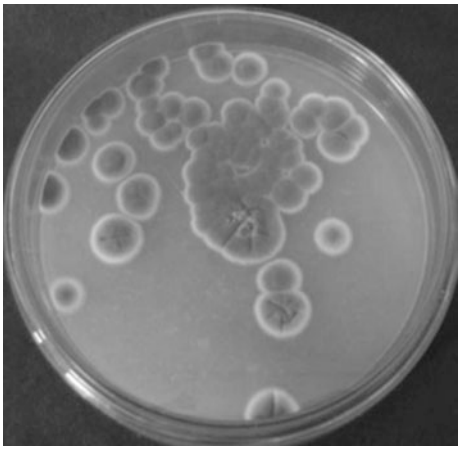

Fig. 2 Colony morphology of *Penicillium citrinum* MKF3

Blastn analysis of the sequence obtained revealed that the strain was *Penicillium citrinum*. The sequence was submitted in NCBI GenBank and obtained an accession number-KF922322.1.

3.3 Optimization for Lipase Production

Lipase production is influenced by the type and concentration of carbon and nitrogen sources, pH, growth temperature and dissolved oxygen concentration (Elibol and Ozer 2001). Various parameters were optimized for the lipase production using *P. citrinum* MKF3. Carbon, nitrogen sources, medium pH and incubation temperatures were varied. The lipase assay was performed on samples retrieved at intervals of 3rd, 5th and 7th day of incubation. In the carbon source optimization, there was an increasing trend of enzyme production in all the carbon sources tested. However, the dextrose was found to be best carbon source (Fig. 3a). In case of nitrogen source optimization, the highest production was achieved using peptone after 7th day of incubation (Fig. 3b). Other nitrogen sources showed a declining trend of lipase activity beyond the 5th day of incubation which may be due to the protease activity. Nehad and Ahmed (2008) reported the maximum lipase activity of 325 U/ml using *Aspergillus niger* with 3 % peptone and 1 % olive oil: glucose as carbon source. Medium pH of 5 and 7 exhibited a similar trend of activity up to the 5th day but a slight increase was shown on the 7th day in medium with pH 7 (Fig. 3c). Similarly, increasing lipase activity was observed in incubation temperature optimization also. Though there was a low enzyme activity observed on the 5th day for 35 °C, there was an increase in activity by the 7th day (Fig. 3d). The present results were supported by Hiol et al. (2000).

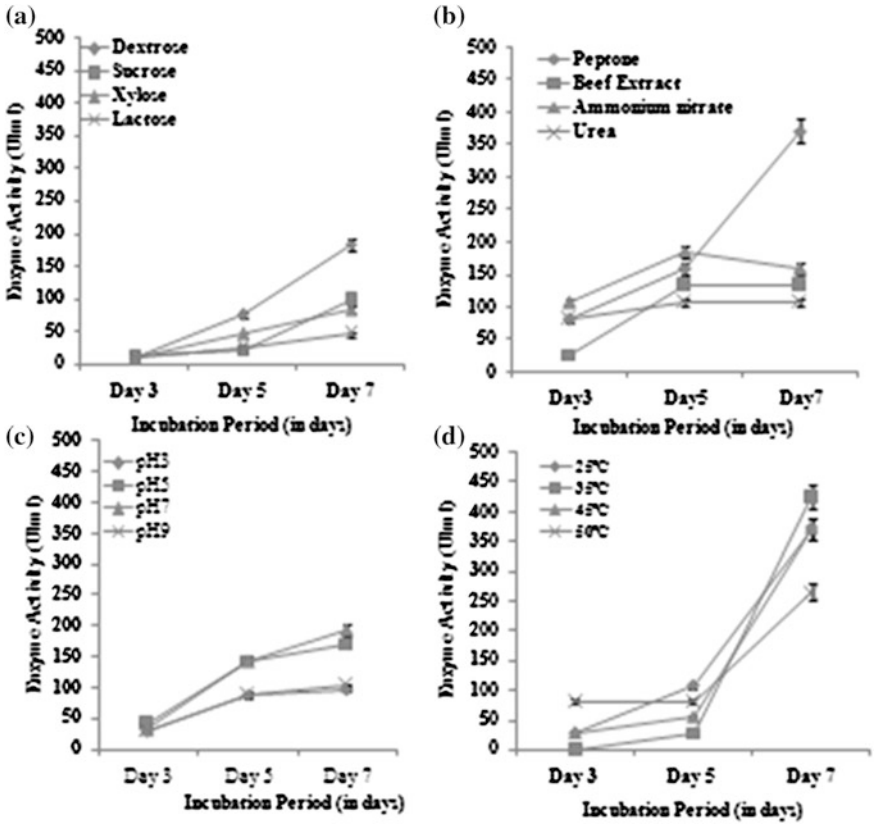

Fig. 3 Optimization of Lipase production using *Penicillium citrinum* MKF3. **a** Carbon source. **b** Nitrogen source. **c** pH. **d** temperature

3.4 Utilization of Medicinal Oil Effluents for Lipase Production

Medicinal oil effluent is a complex mixture of hydrocarbons and oils. The oil effluent as such is not suitable as a medium for microbial growth. Hence, the effluent was diluted to 15, 20 and 25 % using the phosphate buffer. The oil content can act as a sole carbon source to facilitate the fungal growth and to stimulate the lipase production. After the 7th day of incubation, there was a maximum lipase activity observed in the medium with 20 % oil effluent followed by 25 and 15 % (Fig. 4). However, there was an increased production of lipase compared to the production medium supplemented with 1 % olive oil as inducer which served as a control. Mohanasrinivasan et al. (2009) reported the use of biopharmaceutical waste in lipase production where *Aspergillus* sp. was found to be the promising strain and solid state fermentation was better for high enzyme yield. However, when

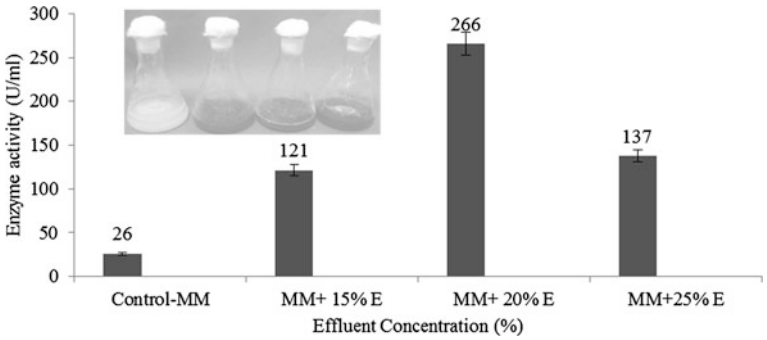

Fig. 4 Medicinal oil effluent based medium for lipase production using *Penicillium citrinum* MKF3

considering the production strategy as a bioremediational approach, the submerged state fermentation could be adopted to reduce the effluent toxicity and thereby facilitating the safe disposal of the same. Moreover, the strategy is involving the utilization of waste effluent to transform into the valuable lipase like enzyme which has got many industrial applications. Maximum lipase activity of 199.696 U/ml was achieved using *Penicillium* sp. under submerged fermentation of the oil effluents (Mohanasrinivasan et al. 2009). However, the present isolate *Penicillium citrinum* MKF3 could achieve a better activity with 20 % effluent which was remarkable achievement of this study. Lipase production is inducer dependent, the requirement of a lipid carbon source is very critical for high enzyme yield (Mahadik et al. 2002). The high yield of lipase achieved is likely to be due to this reason.

4 Conclusion

The coastal isolate *Penicillium citrinum* MKF3 identified through molecular techniques was found to be a promising lipolytic fungus. Dextrose and peptone was identified as suitable carbon and nitrogen source respectively. Similarly, medium pH 7 and incubation temperature of 35 °C was found to the optimum for lipase production. About 20 % medicinal oil effluent achieved maximum lipase activity using *P. citrinum* MKF3.

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Waste Water Sludge Treatment Using Rice Bran

P. Geetha and M. Kokila

Abstract Activated sludge process is the most commonly used aerobic wastewater treatment system. It uses a culture of microorganisms that utilizes organic matter in sewage for its sustenance, growth and synthesis of new cells. Problems like sludge bulking and foaming are the serious issues in this systems, which is formed because of excessive filamentous growth, high F/M ration or due to poor settling of waste sludge. Some methods are there, for removing the sludge bulking which are quite expensive. The efficient and cheap way of sludge removal is using rice bran, since it has enough nitrogen and phosphorous, filamentous bacteria growth is suppressed and favourable microorganism growth is enhanced. The shortcomings such as low density structure and less sedimentation rate are rectified while using rice bran which is estimated by SSVI OR SVI test. Additionally, laboratory test were conducted which reveals nitrogen and phosphorous do not dissolve completely from the rice bran.

Keywords Active sludge · Sludge bulking · F/M ratio

1 Introduction

The activated sludge process is the mainstay of aerobic wastewater treatment. The success of the process mainly depends on the efficiency of aeration system and the development of suitable condition for synthesis of new population of microorganisms and ease at which the biomass could settle in a sedimentation tank. Nearly 50 % of the activated sludge plants in India and abroad are beset by problems like sludge bulking and foaming so finally the treatment gets impaired. It puts limitations on the quantity of waste water that gets treated and also the quality of effluent is degraded.

P. Geetha (✉) · M. Kokila
Remote Sensing and Wireless Sensor Networks (CEN), Amrita Vishwa Vidyapeetham University, Coimbatore, Tamil Nadu, India
e-mail: p_geetha@cb.amrita.edu

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_13

Fig. 1 Bulking sludge

Bulking can be said to have occurred in activated sludge plants when the sludge does not settle easily and has an excessive volume. This leads to carry over from the final effluent clarifies. Main character of bulking sludge is its sedimentation rate value which is less than 0.3 m/h approximately, an SSVI or SVI of above 120 and 180 ml/g respectively and a low density structure. Polysaccharide produced by excessive filamentous growth or due to micro-organisms causes bulking. To reduce bulking some short term control measures are biocide addition, use of flocculating chemicals or increase the RAS flow. These measures are generally used for treating the symptoms but still underlying problem exists. The production of extracellular such as polysaccharide due to excessive filamentous microorganisms leads to deficiency of nutrients. This article deals with the control of activated sludge bulking based on these causes. The photography of the bulking sludge is in Fig. 1.

There are many reasons attributed to bulking and some measures are suggested to control bulking of sludge. Chlorination of the return sludge and application of hydrogen peroxide are some methods used. They are costly and can have adverse effects on the bacterial culture. The uses of coagulants like poly electrolytes are also expensive. There are indications that the control of bulking in the activated sludge plants can enhance the efficiency of the system in great deal. In this paper, discussion is made how to control the sludge in efficient and cost effective method.

1.1 Problems in Effluent Treatment Plants (ETP)

Bulking sludge is a major problem that can cause serious operational issues to the management of waste water. Basically with this condition around, it is very difficult to get a good separation of sludge and water, which leads to carry over solids to the discharge side and clog up the final polishing filter. A bulking sludge is a condition defined by solids with poor settling characteristic (which are either slow or unable to settle so that they will just float on top) and this can be observed by the high SV test result (Adachi et al. 2005). The SV test will indicate the volume of settled solids after 30 min time period. Another characteristic that we can use to refer to the bulking sludge problem is the poor compatibility of the sludge in which water or gas trapped in between the solid floc and thus leads to the sludge having a low density and it would not agglomerate well together. The above described sludge characteristics are the main reasons that will affect quality of waste water discharge.

Growth of filamentous bacteria is the main cause that leads to poor settling characteristic of the waste sludge (Adachi et al. 2005). Although presence of these microorganisms can help towards efficient removal and breakdown of organic matter, they have weak floc forming behaviour and, sludge mass containing these bacteria will be slow to settle. Large number of filamentous bacteria is generally blamed and identified as the main cause. Some secondary causes could be other microorganisms that could also lead to the same condition, which are the growth of acid-favouring fungi, which predominates due to the low nitrogen content of the feed water and acidic condition in the pond. These microorganisms do not have specific names but they are all grouped under the slime producing genera.

Lack of macronutrients and imbalance in terms of F/M (i.e. the amount of organic matter fed per day/the amount of microorganisms present in the aeration tank) ratio can also induce growth of these unwanted microorganisms and in order to solve the sludge bulking issue, the best approach is to resolve the low pH problem and ensuring the F/M ratio with in threshold value. These methods are considered as more viable and workable solutions without involving extra cost because it basically involves monitoring and ensuring the process control is correct. Sometimes depending on available time both options are applied, another costly option is bacteria seeding which can be troublesome as it involves stripping and reloading of the whole system.

On sludge compatibility issue, so far one of the main causes that lead to the sludge having low density floats to the surface is entrapment of gas coming from denitrification (Adachi et al. 2005). The sludge here is called as rising sludge and apparently it will float to the surface. Unfortunately there is not much you can do when it comes to this but generally the approach to tackle this issue is by adjusting to increase the sludge take off rate (desludging rate) and also decreasing the incoming waste water flow to the aeration pond. All in all, with the tight process control and monitoring, the sludge bulking issue can actually be prevented from happening (Fig. 2).

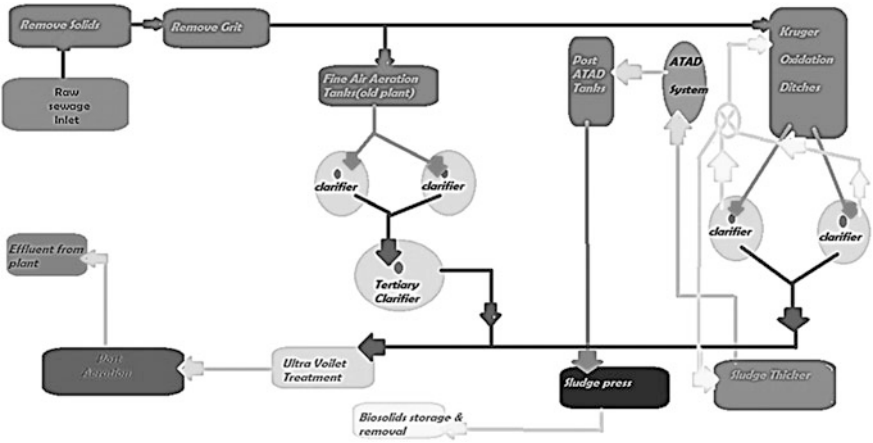

Fig. 2 Schematic diagram of ETP process

2 Methodology

Rice bran is a waste product in the process of making polished rice from brown rice and is inexpensive, costing 1/100 of a commercial synthetic polymer. In addition, the use of rice bran is an effective utilization of waste. Taken together, the findings of this study suggest that the use of rice bran in the activated sludge process is an efficient and cost-effective method to prevent bulking. It can be purchased at a local market. The composition of the rice bran is shown in Table 1.

The water treatment method used in this plant is an activated sludge process. The activated sludge plant consists of an aeration tank and sedimentation tank. Raw water enters the aeration tank from the food plant, which produces etiolated seedings. The waste water then flows from the aeration tank to the sedimentation tank. Most of the fine flocs or particles settle in the sedimentation tank. Finally, treated wastewater is drained through an outlet. The plant currently treats about 180 m^3 of raw water per day (Adachi et al. 2005). The quality of raw water is shown in Table 2. The addition of rice bran to the aeration tank of the food plant is once in a day.

3 Results and Discussion

The influent and effluent pH, suspended solids (SS), COD, BOD, total nitrogen and total phosphorous were determined according to the method mentioned. The SV value is determined by settling a 1000 ml graduated cylinder. The SV30 can be

Table 1 Composition of rice bran

Constituent	Concentration (g/100 g)
Water	13.5
Protein	13.2
Lipid	18.3
Carbohydrate (glucose)	38.3
Fiber	7.8
Ash	8.9

Table 2 Water quality

Parameter	Range
pH	6.6–7.4
SS (mg/l)	12–92
COD (mg/l)	16–160
BOD (mg/l)	14–292
Total nitrogen (mg/l)	1.5–8.7
Total phosphorous (mg/l)	0.3–3.3

expressed as: SV30 = volume of sludge settled in 30 min/sample volume. The SVI is the volume in millimetres occupied by the sludge after 30 min of settling, and the volume occupied the sludge is reported as a percentage. SVI can be expressed as: SVI (mL/g) = SV × 1000/MLSS, Where mixed liquor suspended solids (mg/L). Mixed liquor suspended solids (MLSS) are the concentration of suspended solids in a 1000-ml sample of mixed liquor.

After the addition of rice bran (Comas et al. 2008), the SV30 value began to decrease gradually. The SVI is one measure employed to determine the settling qualities of sludge and is useful as a relative indicator of bulking conditions. Good sludge has an index of 50–100, whereas poor sludge, with bulking characteristics may have an index of 200 or higher. After the addition of rice bran the MLSS value began to rise gradually from 3300 to 4000 mg/l. These results show the rice bran accelerates the growth of effective bacteria to produce sludge with good settling qualities. Both COD and BOD reductions were obtained after the addition of rice bran was begun. Both COD and BOD are means of assessing the degree of pollution of waste and are employed as measure of the amount of organic materials in samples. Rice Bran accelerates the growth of effective microorganisms. Both pH and SS showed significantly less variations during the experimental period (Adachi et al. 2005). Rice bran does not affect pH and SS values. If an increase in SS were observed, the rice bran in the final tanks would spill over the weirs and the BOD of the final effluent would increase. Rice bran contains adequate nitrogen and phosphorous.

4 Conclusion

In the laboratory tests, it was confirmed that nitrogen and phosphorous does not dissolve in rice brans. Rice bran accelerated the growth of the effective microorganisms and suppressed the growth of filamentous bacteria that cause bulking (Nishikawa and Kuriyama 1974). The effects of rice bran on the growth of effective microorganism was further measured or examined on a laboratory scale using the experimental apparatus used in this study.

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A Novel Lipopeptide Bacterial Biosurfactant for Bioremediation

C. Elizabeth Rani Juneius and Jayasundari

Abstract Bioremediation is an efficient tool practiced now a days for the removal of contaminants from contaminated soil and water. In this present study, Cr(VI) contaminated water collected from leather tanning industry in Kandigai, Kanchipuram district, Tamil Nadu, was remediated by biological method in an eco-friendly manner. Amount of Cr(VI) present in raw effluent and centrifuged raw effluent was estimated by Inductively Coupled Plasma Optical Emission spectroscopy and the amount determined was 57 and 2.83 mg/l respectively. Bacteria were isolated from the effluent and oil contaminated marine soil. They were identified by phenotypic methods and they were *Citrobacter freundii* EIB 1, *Citrobacter freundii* EIB 2 and *Pseudomonas aeruginosa* EIB 3, and oil contaminated marine soil flora were identified as *B. licheniformis* OSB 1 and *Pseudomonas aeruginosa* OSB 2. Nature of biosurfactant was characterized and it was found to be lipopeptide biosurfactant. Efficiency of bioremediation process was superior (99 % Cr VI removal) by the biosurfactant produced from the bacteria isolated from oil contaminated marine soil. FTIR analysis was carried out and which revealed the presence of peptides and GC-MS showed the presence of fatty acids hexadecanoic acid methyl ester (1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine) and octadecenoic acid methyl ester and a protein derivatives such as 5-methyl enol (3,2-b) pyridine was also present in the biosurfactant produced from oil contaminated marine soil (*Pseudomonas aeruginosa* OSB 2) and which was found to be a potent candidate to effectively remove the metal contaminant (CrVI) present in the industrial effluent.

Keywords Biosurfactant · FTIR · G-MS · Bioremediation · Cr(VI)

C. Elizabeth Rani Juneius (✉) · Jayasundari
Department of Biotechnology, Hindustan College of Arts and Science, Chennai, Tamil Nadu, India
e-mail: elizabeth.juneius7@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_14

1 Introduction

There has been a tremendous growth of industries worldwide in the last few decades and the associated anthropogenic activities have often resulted in environmental pollution. Heavy metals such as As, Cr, Pb etc. are prominent components of industrial effluents which are discharged into the environment and consequently pollute the ecosystem. The presence of these heavy metals in the environment has been a subject of great concern due to their toxicity, non-biodegradable nature and the long biological half-lives for their elimination from biological tissues (Olatunji et al. 2009).

Several technologies exist for the remediation of metal-contaminated soils like subsurface barriers, immobilization, pyro-metallurgical, solidification/stabilization, vitrification, extraction, toxicity and/or mobility reduction, electrokinetic treatment, chemical and physical treatments etc. (Evanko et al. 1997). But all these treatments are very expensive and found to affect the texture of the soil. There is an increased interest in using surfactants to complex metals and remove them from waste streams and in situ by soil washing and pump and treat remediation technologies (Herman et al. 1995). Most known biosurfactants are glycolipids. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids (Desai and Banat 1997). Due to the anionic nature of rhamnolipids, they are able to remove metals from soil and ions such as arsenic, cadmium, copper, lanthanum, lead and zinc due to their complexation ability (Herman et al. 1995).

2 Materials and Methods

2.1 Collection of Industrial Effluent Contaminated with Cr(VI)

Industrial effluent was collected from the leather industry situated in Kandigai and oil contaminated marine soil was collected from the shore of Marina, Chennai. The samples were transported to the laboratory for the analysis under aseptic condition. Estimation of Cr(VI) in untreated effluent by using Perkin Elmer Optima 5300 DV ICP-OES. The untreated industrial effluent was centrifuged and then amount of Cr (VI) present in the sample was estimated by Perkin Elmer Optima 5300 DV ICP-OES (SAIF-IIT-Chennai).

2.2 Isolation of Indigenous Bacteria from the Effluent

Effluent and oil contaminated marine soil samples were serially diluted by tenfold dilution. Nutrient agar and Zobell marine agar were prepared to isolate bacteria from effluent contaminated soil and oil contaminated marine soil respectively and sterilized by autoclaving. Ten sterile petriplates were taken and labeled as 10^{-3},

10^{-4}, 10^{-5} and 10^{-6} in duplicates. One ml of serially diluted samples was transferred into a respective petriplates. Sterile nutrient agar in a molten state was poured on the plates labeled for effluent water, whereas Zobell marine agar for oil contaminated marine soil and which was mixed gently. Plates were allowed to solidify and then incubated at 37 °C for 24–48 h. Morphologically different colonies were selected and pure cultured (Zobell 1941).

2.3 Identification of Metal Tolerant Bacteria

Nutrient agar and Zobell marine agar with various concentration of Cr(III) (200–1000 ppm) were prepared and the selected isolates from effluent and oil contaminated marine soil were inoculated and incubated at 37 °C for 48 h. Metal tolerability was tabulated. There were five different isolates showed metal tolerability. The unknown bacteria were identified by phenotypic methods (Gelmi et al. 1994).

2.4 Identification of Isolated Bacteria

The unknown bacteria were identified based on Bergey's Manual of Systematic Bacteriology. Identification of bacterium was performed by morphological characterization and biochemical methods. Classification as Gram negative or Gram positive was done by Gram stain reaction. Morphological characteristics of the isolated bacterium were also performed according to the standard method. Biochemical methods were carried out according to Bergey's manual of Determinative Bacteriology (Baron et al. 1995).

2.5 Production of Biosurfactant from the Selected Strains

Mineral salt medium (100 ml in each) with 3 % glycerol was prepared in five different conical flasks and sterilized by autoclave. Selected strains were inoculated and incubated at 37 °C at 100 rpm for 24 h (Bushnell and Hass 1941).

2.6 Separation of Biosurfactant by Solvent Extraction Method

Cell free extract was collected by centrifuging the broth at 10,000 rpm for 15 min. CFE was further used for solvent extraction. Chloroform:methanol (2:1) was used as a solvent. Taken 50 ml of CFE and was transferred in solvent extraction funnel,

followed by the addition of 50 ml of solvent mixture. The mixture were agitated vigorously for 30 min and then kept it in resting position for 30 min. Organic phase was collected for further analysis.

2.7 Measurement of Biosurfactant Activity by Oil Displacement Method

Biosurfactant activity was determined by oil displacement method. A sample solubilized in 10 µl of 0.1 M Tris-HCL (pH 8) buffer was put on the centre of an oil membrane which was formed on the surface of water in a 15 cm diameter petriplate. The size of the resultant oil-displaced circle reflects the activity of a surfactant. One BS unit was defined as the amount of surfactants forming 1 cm^2 of oil displaced area (Thaniyavarn et al. 2008).

2.8 Quantification of Biosurfactant

Qualitative TLC was performed to find out the nature of biosurfactant. Silica gel was used as stationary phase and mobile phase Butanol:acetic acid:distilled water (80:20:20). Biosurfactant samples were spotted on TLC slides and were placed in a solvent chamber. It was allowed to run for 30 min. Slides were allowed to dry and ninhydrin was sprayed on the slide. Formations of pink color spots were observed and the R_f value was calculated.

2.8.1 Estimation of Phospholipids

About 200 µl of biosurfactant was evaporated in a glass tube and dissolved in 0.5 ml methanol. To that 0.5 ml of working solution was added and mixed for 30 min at room temperature. 4 ml of 95 % ethanol was added and the absorbance was taken at 355 nm against a blank in which 0.5 ml of 3 % KI was added to the sample instead of the iodine solution and also a control was used in a tube in which the sample is omitted. The molar extinction coefficient is about 27,500. The µmoles vinyl groups was calculate as ((absorbance of iodine control − absorbance of sample)/27,500) × 5000.

2.8.2 Estimation of Amino Acid by Ninhydrin Method

Standard amino acid solution (0.1–1 ml) was pipetted out into the respective labeled test tubes. Distilled water was added in all the test tubes to make up the volume to 4 ml. 4 ml of distilled water was added to the test tube labeled Blank. 1 ml of

ninhydrin reagent was added to all the test tubes including the test tubes labeled 'blank' and 'unknown'. The contents of the tubes were mixed by shaking the tubes. All the test tubes were placed in boiling water bath for 15 min. The test tubes were cooled in cold water and 1 ml of ethanol was added to each test tube and mixed well. The absorbance was taken at 570 nm of each solution using a colorimeter.

2.9 Treatment Cr(VI) Contaminated Effluent by Biosurfactant

Ten ml of effluent containing Cr(VI) was taken in five different conical flasks. 1000 µl of biosurfactant was added in each flask. It was kept under agitation for 1 h and centrifuged at 5000 rpm for 10 min. Supernatant was used for the determination of Cr(VI) by ICP-OES method.

2.10 Characterization of Biosurfactant

Fourier transform infrared spectroscopy (FTIR) is particularly useful for identifying different types of chemical bonds (functional groups) and can therefore be used to identify the components of mixtures of unknown composition. One milligram of freeze-dried crude biosurfactant was ground with 100 mg of KBr and with a pressure of 7500 kg was applied for 30 s in order to produce translucent pellets, which were then analyzed by spectrometry. All spectra were obtained from 180 scans with a resolution of 4 cm^{-1} in the range of 550–4000 cm^{-1}. A KBr pellet was used as background reference.

Biosurfactant was dissolved in methanol and mixed thoroughly. The mass spectrometric analysis of the biosurfactant was carried out in LCQ quadrupole iontrap mass spectrometer utilizing electrospray ionization (ESI). Standard solutions samples under investigation were infused into the mass spectrometer at a flow rate of 10 µl/min. In the ESI, nitrogen and auxiliary gas flows were maintained at 50 and 5 ml/min respectively and referred to arbitrary values set by the software. The heated capillary temperature was 250 °C and spray voltage was set to 5 kV.

3 Results and Discussion

3.1 Isolation of Marine Bacteria

There were three morphologically different bacterial colonies isolated from marine soil and two different colonies were chosen from oil contaminated soil. They were sub cultured and used for further analysis.

Figure 1 depicts about metal tolerability test result which revealed the ability of three isolates from effluent EIB 1, EIB 2 and EIB 3 could able to tolerate trivalent chromium up to the concentration of 200 ppm/l (Table 1). Whereas, other two isolates from oil contaminated marine soil had showed sensitivity even at the concentration of 200 ppm/l. Tripathi and Garg (2010), reported that bioremediation is being viewed as a clean technology for decontamination of pentachlorophenol and chromium from tannery effluent. This study was conducted to isolate an efficient bacterial culture from treated tannery effluent which is tolerant to pentachlorophenol and Cr(VI) and could be employed for simultaneous bioremediation of both the toxic contaminants. Tannery effluent sample was collected from Common Effluent Treatment Plant, Kanpur (India). In their study, Bacillus sp was found to be maximally tolerant to high concentration of both pentachlorophenol (500 mg l^{-1}) and chromium(VI) (200 mg l^{-1}).

Bacteria EIB 1, EIB 2, EIB 3, OSB 1 and OSB 2 were identified as *Citrobacter freundii*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *B. licheniformis* and *Pseudomonas aeruginosa*, respectively (Table 2).

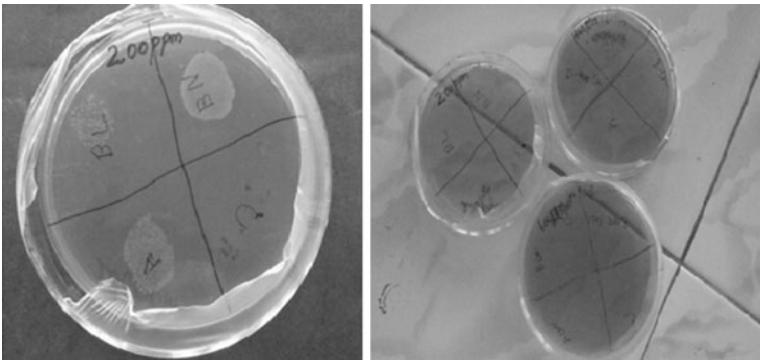

Fig. 1 Metal tolerability test

Table 1 Metal tolerability test

S. No	Isolate	Concentration of Cr(III)				
		200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm
1.	EIB 1	+ve	−ve	−ve	−ve	−ve
2.	EIB 2	+ve	−ve	−ve	−ve	−ve
3.	EIB 3	+ve	−ve	−ve	−ve	−ve
4.	OSB 1	−ve	−ve	−ve	−ve	−ve
5.	OSB 2	−ve	−ve	−ve	−ve	−ve

Table 2 Phenotypic characterization of bacterial

S. No	Name of the test	EIB 1	EIB 2	EIB 3	OSB 1	OSB 2
1	Gram staining	−	−	−	+	−
2	Motility	+	+	+	−	−
3	Macconkey agar	LF	LF	NLF	LLF	LF
4	Indole	+	+	−	−	−
5	Methyl red	+	+	−	−	−
6	Voges Proskauer test	−	−	−	+	+
7	Citrate utilization test	+	+	+	+	+
8	Triple sugar iron agar test	K/A	K/A	K/A	K/A	K/A
9	Classical tests oxidase	+	+	+	−	+
10	Reaction catalase	−	−	+	−	+
11	Reaction growth at 42 °C	−	−	+	−	+
12	Production of fluorescent pigment	−	−	+	−	+
13	API 20 NE Indol	−	−	−	−	−
14	Production on tryptophan	−	−	−	−	−
15	Glucose acidification	−	−	−	−	−
16	Agrinine dihydrolase	−	−	+	−	+
17	Urease	−	−	−	−	−
18	Esculin hydrolysis	−	−	−	−	−
19	Gelatin hydrolysis	−	−	+	−	+
20	B	−	−	−	−	−
21	Galactosidase	−	−		−	
22	D-Glucose	−	−	+	−	+
23	L-Arabinose	−	−	−	−	−
24	D-Mannose	−	−	−	−	−
25	D-Mannitol	−	−	+	−	+
26	N-Acetyl-D-glucosamine	−	−	+	−	+
27	Maltose	−	−	−	−	−
28	Gluconate	−	−	+	−	+
29	Caprate	−	−	+	−	+
30	Adipate	−	−	+	−	+
31	L-Malate	−	−	+	−	+
32	Phenylacetate	−	−	−	−	−
33	API ZYM	−	−		−	
34	Alkaline phosphatase	−	−	D	−	D
35	Esterase (C4)	−	−	+	−	+
36	Lipase (C8)	−	−		−	
37	Lipase (C14)	−	−	+	−	+
38	Leucine arylamidase	−	−	+	−	+
39	Valine arylamidase	−	−	D	−	D
40	Cystine arylamidase	−	−	−	−	−

3.2 Production of Biosurfactant and Purification

Biosurfactant was produced using all the five isolates and it was extracted using solvent extraction procedure. Figures 2 and 3 illustrate the presence of biosurfactant in solvent–aqueous interface. Chloroform:methanol (2:1) ratio had served as a suitable proportion for the extraction of biosurfactant. Similarly Banat (1995), had also used chloroform–methanol to extract biosurfactant. Biosurfactant produced by our isolates were in crystalline form. Similarly, Dubey and Juwarkar (2001) had also reported that *Pseudomonas aeruginosa* produced a crystalline biosurfactant as the secondary metabolites and its maximal production occurred after the onset of nitrogen-limiting conditions.

Oil displacement method revealed the biosurfactant activity of the crude extract collected from solvent extraction procedure. Among the five strains studied, *Bacillus licheniformis* (OSB 1) and *Pseudomonas aeruginosa* (OSB 2) showed 12 cm diameter where as other strains showed 10 cm diameter of displacement of oil (Table 3). The oil spreading method is rapid and easy to carry out, requires no specialized equipment and just a small volume of sample. It can be applied when the activity and quantity of biosurfactant is low. Plaza et al. (2006) demonstrated that the oil spreading technique is a reliable method to detect biosurfactant production by diverse microorganisms. The assay was also applied for screening by Huy et al. (1999).

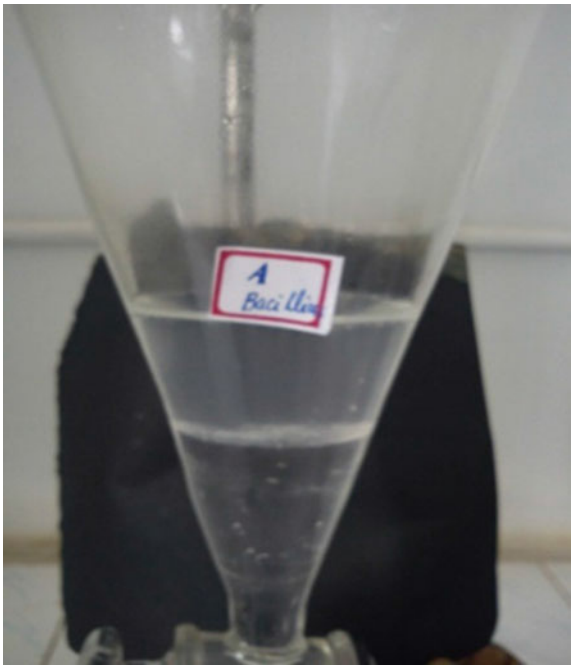

Fig. 2 Vacuum dried biosurfactant produced in the form of crystalline

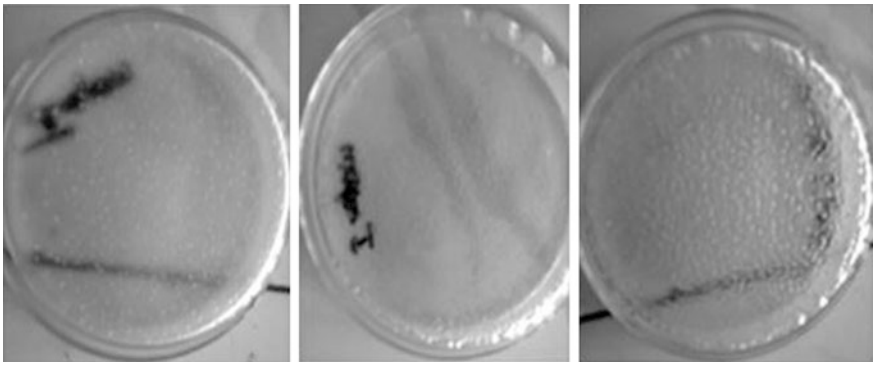

Fig. 3 Measurement of biosurfactant activity by oil displacement method

Table 3 Measurement of biosurfactant activity by oil displacement method

S. No	Name of the bacteria	Diameter of displacement (cm)
1.	*Citrobacter freundii* (EIB 1)	10
2.	*Citrobacter freundii* (EIB 2)	10
3.	*Pseudomonas aeruginosa* (EIB 3)	10
4.	*B. licheniformis* (OSB 1)	12
5.	*Pseudomonas aeruginosa* (OSB 2)	12

3.3 Estimation of Phospholipids

Amount of phospholipids present in the prepared biosurfactant was determined in all the five isolates and the concentration of phospholipids present in *Pseudomonas aeruginosa* (OSB 2) was higher than other isolates (Table 4).

3.4 Estimation of Cr(VI) in Raw and Treated Effluent

Amount of Cr(VI) present in raw effluent and centrifuged raw effluent was estimated by Inductively Couple Plasma Optical Emission spectroscopy and the amount determined was 57 and 2.83 mg/l respectively. After treating such samples with the biosurfactants produced by five different isolates, the amount of Cr(VI) present in the treated samples were determined. The result revealed that 97 % was removed by the isolates from indigenous flora were as 99.9 % removal was achieved by the isolates from oil contaminated soil (Table 5).

Table 4 Vinayl groups present in bacterial isolates

S. No	Name of the bacteria	Amount of vinyl group (μmoles/ml)
1.	*Citrobacter freundii* (EIB 1)	0.089
2.	*Citrobacter freundii* (EIB 2)	0.078
3.	*Pseudomonas aeruginosa* (EIB 3)	0.034
4.	*B. licheniformis* (OSB 1)	0.130
5.	*Pseudomonas aeruginosa* (OSB 2)	0.320

Table 5 The efficiency of biosurfactant in treated effluent

S. No	Name of the bacteria	Amount Cr(VI) (mg/L)	% reduction
1.	*Citrobacter freundii* (EIB 1)	0.066	97.6
2.	*Citrobacter freundii* (EIB 2)	0.066	97.6
3.	*Pseudomonas aeruginosa* (EIB 3)	0.068	97.5
4.	*B. licheniformis* (OSB 1)	0.002	99.9
5.	*Pseudomonas aeruginosa* (OSB 2)	0.001	99.96

3.5 Characterization of Biosurfactant

3.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

Al-Ajlani et al. (2007) revealed that the biosurfactant produced presents the main characteristic groups of a surfactin molecule, indicating the presence of aliphatic hydrocarbon, as well as a peptide fraction. The absorption band with a maximum of 3307 cm^{-1} corresponding to the N–H stretch can attributed to peptide residues. Another intense band with maxima of 2958 and 2927 cm^{-1}, corresponding to the C–H(CH_3) and (CH_2) stretch can be associated with the lipopeptide portion of the molecule. At 1726 cm^{-1}, a medium intensity band is observed that can be related to the absorption of C = O groups from lactonization. At 1651 cm^{-1}, a CO–N stretch points to the amide group. The bands at 1466 and 1388 cm^{-1} indicate aliphatic chains (–CH_3, –CH_2). These results suggest that the biosurfactant produced by *Bacillus subtilis* LAMI005 in a medium containing glycerine is a cyclic lipopeptide, mainly surfactin.

In this study, FTIR peaks were noted at 3600, 2942, 2900, 2222, 2110, 1622, 1400, 1300, 1200, 1100, 1044, 992, 921, 890 and 700 cm^{-1}. As a result of C–H stretching vibrations and N–H stretching vibrations, a broad absorbance peak at 3600 cm^{-1} was observed. This is typical of carbon-containing compounds with amino groups. Sharp absorbance peaks were also observed at 2942, 2900, 2222, and 1200 cm^{-1} and are indicative of a carboxylic group (R(C = O)O–H). Another strong band was also observed at 1400 cm^{-1} which is an indication of presence of aromatic ring. The FTIR spectrum implied the production of a lipopeptide biosurfactant. Though the compound is a lipopeptide, there is a difference in the composition (Fig. 4).

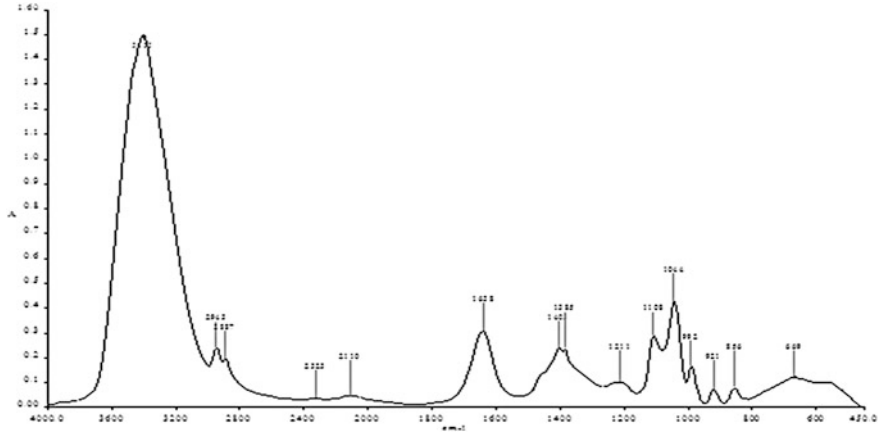

Fig. 4 FTIR analysis for the biosurfactant *B. licheniformis* (OSB 1)

3.5.2 GC-Mass Spectrometric Analysis

Presence of fatty acids and protein derivatives in the biosurfactant produced by *Bacillus licheniformis* OSB 1 were detected by GC-MS which showed the presence of hexadecanoic acid methyl ester (1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine) and octadecanoic acid methyl ester. And a protein derivatives 5-methyl enol (3,2-b) pyridine and 5-(p-aminophenyl) 4(1-naphthyl)2-thioazo alanine were also present in the biosurfactant, which are novel composition in the biosurfactant (Figs. 5, 6, 7, 8 and 9).

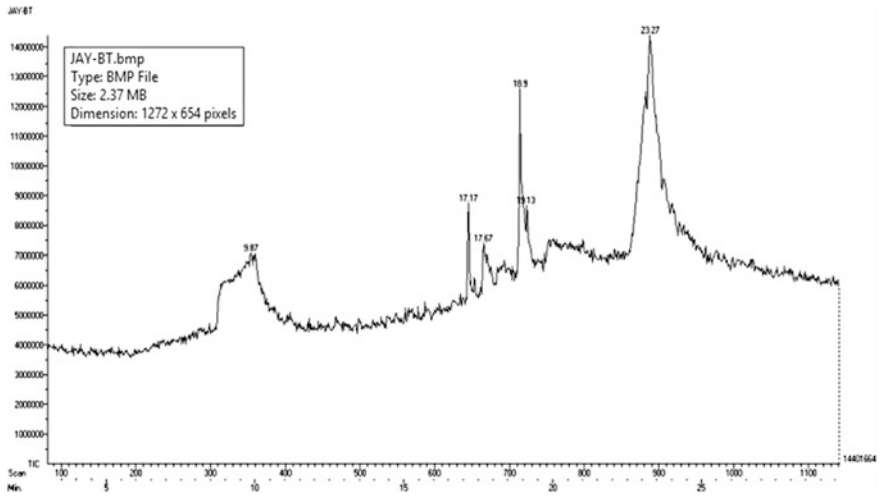

Fig. 5 GC-mass spectrometric analysis of *Bacillus licheniformis* OSB 1

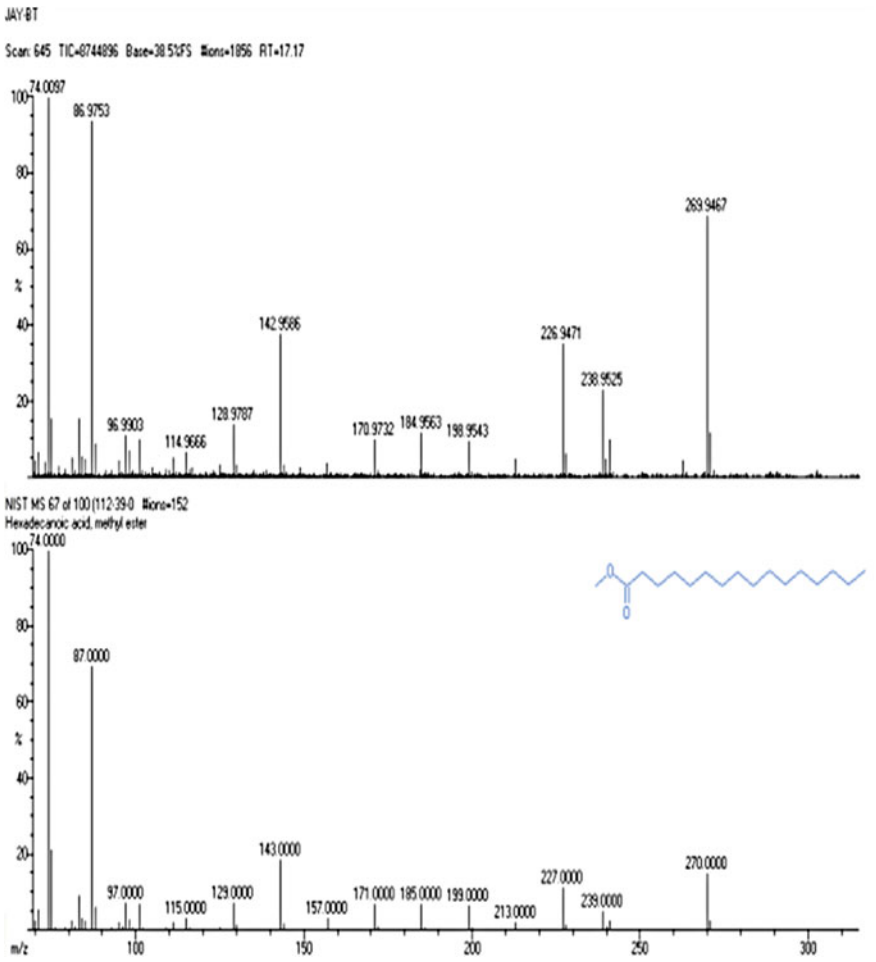

Fig. 6 GC-mass spectrometric analysis of hexadecanic acid methyl ester

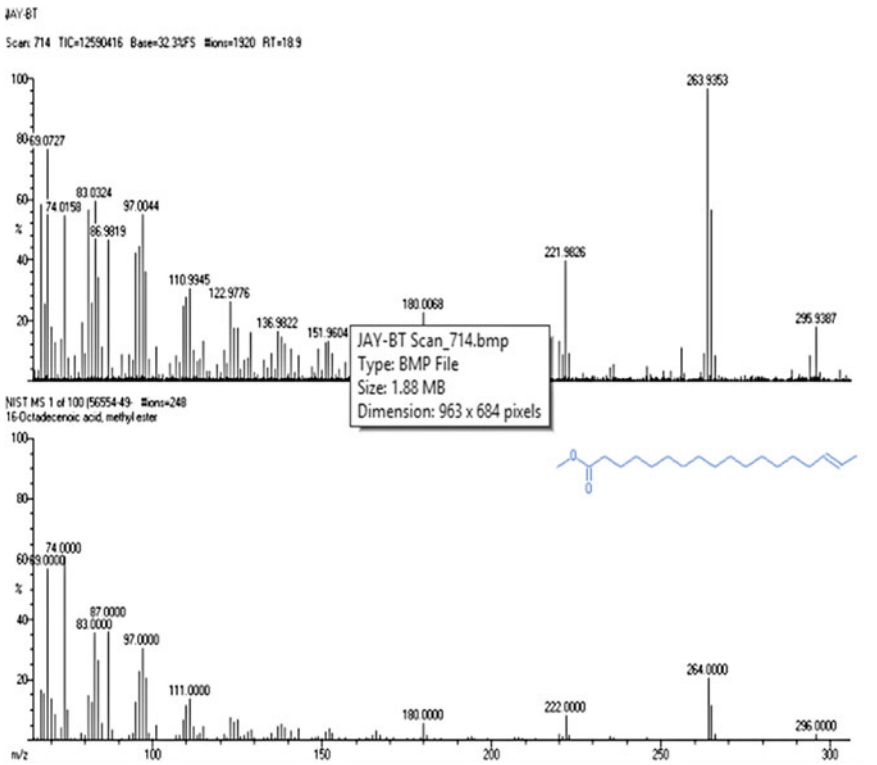

Fig. 7 GC-mass spectrometric analysis of octadecenoic acid methyl ester

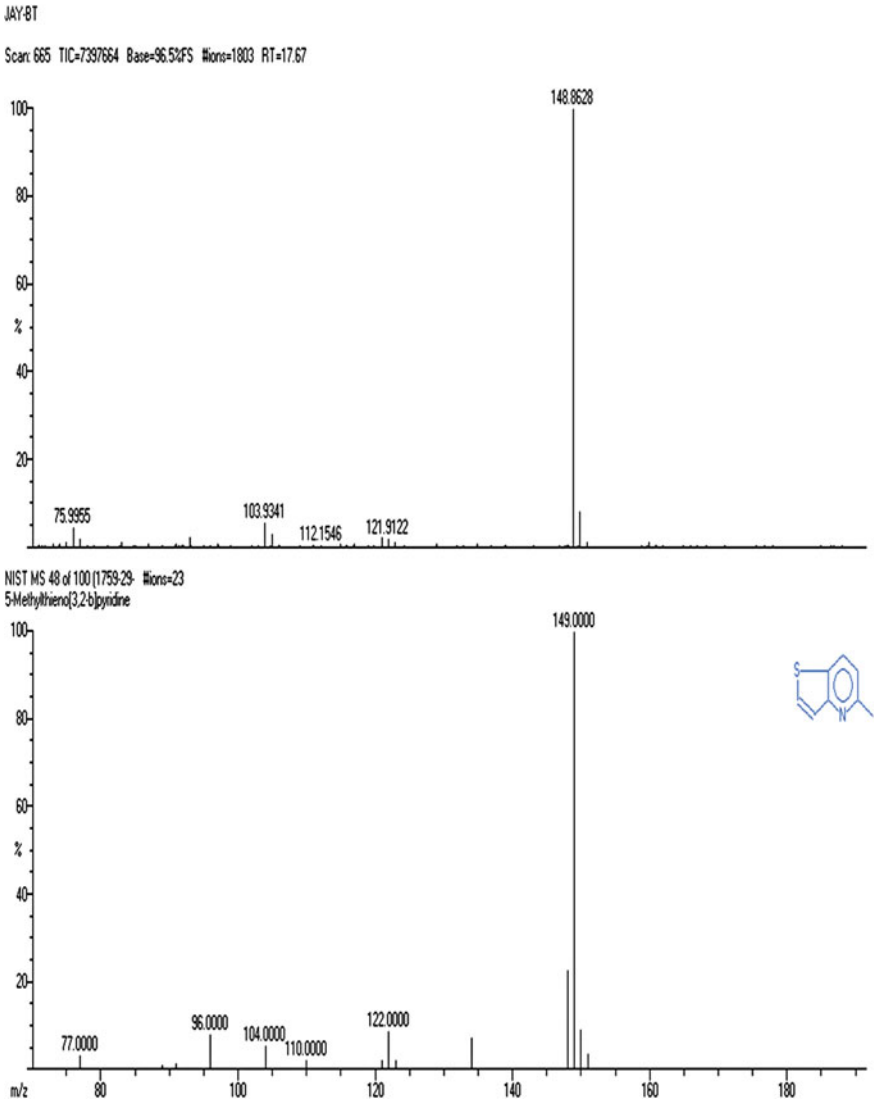

Fig. 8 GC-mass spectrometric analysis of 5-methylenol(3,2-b) pyridine

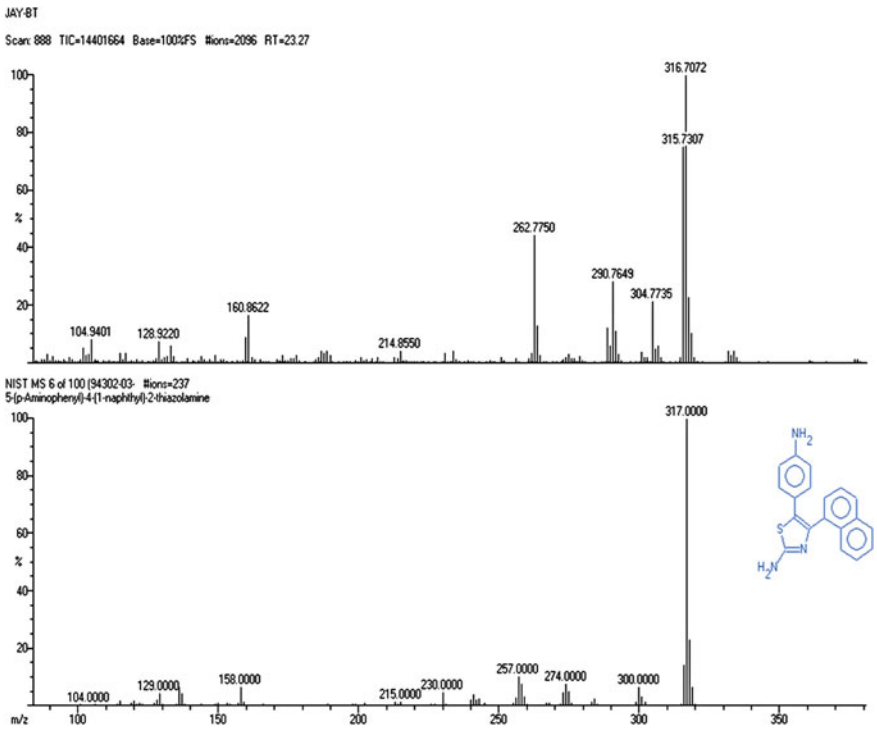

Fig. 9 GC-mass spectrometric analysis of 5-(p-Aminophenyl(-4(1-naphthyl)-2-thiazolamine

4 Conclusion

Based on the above study it can be concluded that the tannery effluent can be effectively treated to remove Cr(VI). All the five strains were able to produce biosurfactant with almost similar characteristics. Although other strains could able to produce biosurfactant, bacteria isolated from oil contaminated marine soil showed maximum activity (99.96 %). Characterization of biosurfactant revealed that they are phospholipopeptides. FTIR was used to confirm the presence of peptides and GC-MS analysis for methanolic suspension of biosurfactant revealed the presence of hexadeconic acid methyl ester and octadeconic acid methyl ester. The previous studies revealed the advantages of using biosurfactant to treat waste water because of its less sludge producing ability and greater efficiency. The present study also supports the same. It is concluded that the biosurfactant produced by the bacteria *B. licheniformis* OSB 1 is a lipopeptide with an excellent bioremediating potentials which can also be used for commercial applications.

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Bioremediation of Ethylbenzene by Soil Column Study and Bioreactor Study for Polluted Soil and Water Samples Using Optimized Bacterial Consortium

S. Ashok, V. Akila, P.M. Ayyasamy and S. Rajakumar

Abstract The bacterium designated strain, able to degrade ethylbenzene was isolated from petroleum contaminated soil at automobile workshops and petrol pumps in Tiruchirapalli, Tamil Nadu, India. Bacterial analysis of the samples revealed the presence of ethylbenzene degrading bacteria belonging to the genera *Pseudomonas*, *Enterobacteriaceae*, *Moraxella*, *Bacillus* and *Micrococcus* sp. The microbial consortium consists of SRA91 and SRA104 is having higher degradation of ethylbenzene. The biodegradation rate of ethylbenzene were relatively low in Bushnell Haas (BH) broth, but addition of carbon source had a substantial impact on the biodegradation of ethylbenzene, which suggested that carbon might provide a factor that was necessary for its ethylbenzene biodegradation. Influence of various carbon sources, incubation temperature and pH on ethylbenzene degradation from synthetic ethylbenzene-rich BH broth also studied. The results showed a rapid and efficient process of ethylbenzene degradation (99 %) from synthetic ethylbenzene-rich BH broth supplemented with glucose (1 %), inoculated by 1 % bacterial consortium (*Enterobacteriaceae* sp. SRA91 and SRA104) at incubation temperature of 45 °C at pH 7. This study suggests that isolated *Enterobacteriaceae* strains SRA91 and SRA104 may play an important role for biodegradation of ethylbenzene in the contaminated soil.

Keywords Bioremediation · Ethylbenzene · Oil pollution · Degradation

S. Ashok · V. Akila · S. Rajakumar (✉)
Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India
e-mail: kodairaj@gmail.com

P.M. Ayyasamy
Department of Microbiology, Periyar University, Salem 636011, Tamil Nadu, India

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_15

1 Introduction

In recent years, petroleum and its byproducts pollution has been increasing concern both nationally and internationally. These pollutants are removed by biotechnological process using microorganisms (Agarry et al. 2012). Bioremediation process is used to remove environmental pollutants such as organic and inorganic substances from soils, water and sediments (Pala et al. 2006). It has countless advantages when compared to other processes employed to remove pollution such as extraction with solvents addition of chemical oxidizers (Gestel et al. 2001; Gogoi et al. 2003; Nano et al. 2003; Demnerova et al. 2005; Morelli et al. 2005). The presence of hydrocarbon contamination in the environment has influenced the biodiversity of the region. The petroleum hydrocarbon degradation from environment is limited by large number of factors. An important factors in the biodegradation of contaminated soils is low bioavailability and solubility of the hydrocarbon (Lloyd and Cackette 2001; Mishra et al. 2001; Latha and Kalaivani 2012). The degradation of petroleum hydrocarbons and its byproducts using environmental microorganisms have been recognized as an efficient, versatile, economic and environmental friendly treatment. The research for effective and efficient methods of petroleum hydrocarbons from contaminated sites has intensified in recent years, because biodegradation is responsible for cleaning of the environment pollutants (Grangemard et al. 2001).

BTEX (Benzene, Toluene, Ethylbenzene and Xylene) are commonly present in crude oil and petroleum products. These monoaromatic hydrocarbons are of particular concern because of toxicity and carcinogenicity, even at low concentrations (Margesin et al. 2003). High water solubility of BTEX, compared to other hydrocarbons contributes to migrate in the subsurface and eventually pollute ground water (Blume 1990; Leiwandowski et al. 1997; Chang et al. 2001; Margesin et al. 2003). Ethylbenzene is a toxic aromatic compound found as a component of petroleum hydrocarbons. Among these compounds, ethylbenzene often enters the environment in the form of industrial discharges from petroleum refining, plastic, resin and drug manufacturing or from oil spills. Ethylbenzene can also used as a starting material for the preparation of styrene and in making rubber and plastic wrap (Parameswarappa et al. 2007). The US Environmental Protection Agency (US-EPA 1996) has reported that short-term exposure of ethylbenzene at levels above 0.7 ppm causes drowsiness, fatigue, headache, mild eye and respiratory irritation. The long-term exposures to ethylbenzene can potentially damage the liver, kidneys, central nervous system and eyes. Hence, ethylbenzene is considered as one of the priority pollutant. Also, ethylbenzene binds moderately to aquatic sediment and soils thereby leading to ground water contamination if released to land. In the light of above said reasons, there is an urgency to eliminate such pollutants from the environment (Suneetha et al. 2005). Hence biodegradation of ethylbenzene is the very important for environment. The present study focuses on biodegradation of ethylbenzene using bacterial consortium and optimization of bacteria with different carbon source, pH and temperature. Further degradation of

ethylbenzene in soil was studied by soil column and the concentration of ethylbenzene is analyzed in HPLC.

2 Materials and Methods

2.1 Isolation and Identification of Bacteria

Soil samples were collected in sterile containers from oil spilled sites like petrol pump and automobile workshop in Tiruchirapalli, Tamil Nadu. These locations were chosen as they were rich in the petroleum hydrocarbons and effective heterotrophic bacteria. Isolation and identification of bacteria were done based on Bergey's Manual of determinative bacteriology.

2.2 Preliminary Screening

One milliliter of inoculum was added to scrumcap test tubes that contained 10 ml sterile Bushnell-Hass (BH) medium and 1 % v/v of the ethylbenzene. The 0.16 mg/ml concentration of 2, 6-dichlorophenol indophenol (DCPIP) was added as a redux indicator. The tubes were kept under agitation of 120 rpm at 37 °C. Biodegradation activity of bacteria was observed during the change of blue color of DCPIP to colorless. The high efficiency of the ethylbenzene degrading bacteria was selected for secondary screening.

2.3 Secondary Screening

One milliliter of above selected bacterial culture was inoculated in presterilized 100 ml BH broth with 100 ppm ethylbenzene. The flask was kept in a shaker at 120 rpm for 10 days at 37 °C. Uninoculated medium flasks containing ethylbenzene served as control during the incubation period. After incubation, the sample was analyzed in Shimadzu HPLC (High Performance Liquid Chromatography) and the ethylbenzene degradation was calculated. The highest efficiency of the ethylbenzene degrading bacteria was selected for the preparation of microbial consortium.

2.4 Ethylbenzene Degradation by Bacterial Consortium

Bacteria were grouped for consortium such as A + B, A + C, A + D, B + C, C + D, A + B + C, A + B + D, A + C + D, B + C + D and A + B + C + D. Ten milliliter of

the above isolates were taken and mixed. The suspension was taken as seed inoculums and 1 ml of inoculum of above consortium was inoculated in BH medium with 100 ppm ethylbenzene and kept in a shaker at 37 °C for 10 days. The samples were analyzed at 10th day in the ethylbenzene concentration.

2.5 *Effect of Various Carbon Sources on Ethylbenzene Degradation in BH Medium*

The BH medium containing 100 ppm of ethylbenzene and 1 % concentrations of different carbon substrates such as glucose, starch and cellulose were prepared. One milliliter of bacterial consortium SRA 91 and SRA 104, the highly efficient consortium among tested, was inoculated and kept in a shaker (120 rpm) at 37 °C. The samples were drawn aseptically at regular intervals (2, 4, 6, 8, 10, 12, 14 and 16 days), analyzed by Shimadzu HPLC and estimated ethylbenzene concentration.

2.6 *Effect of Various Temperatures on Ethylbenzene Degradation in BH Medium*

The BH medium with 100 ppm of ethylbenzene and supplemented with 1 % glucose was prepared. One milliliter of bacterial consortium SRA 91 and SRA 104 was inoculated to the medium and kept in a shaker (120 rpm) at different temperature (25, 30, 35, 40 and 45 °C). At every 2 days intervals, the ethylbenzene concentration in the medium were determined using Shimadzu HPLC up to 16 days.

2.7 *Effect of Various pH on Ethylbenzene Degradation in BH Medium*

The BH medium was prepared at various pH (3, 5, 7, 9 and 11) and sterilized then supplemented with 100 ppm of ethylbenzene and 1 % glucose. One milliliter of bacterial consortium SRA 91 and SRA 104 was inoculated and kept in a shaker (120 rpm) at 40 °C. The samples were drawn aseptically at regular intervals (2, 4, 6, 8, 10, 12, 14 and 16 days) and the concentration of ethylbenzene in the medium was determined using Shimadzu HPLC.

2.8 Effect of Various Anions on Ethylbenzene Degradation in BH Medium

The BH Medium with 100 ppm of ethylbenzene at pH 7 was prepared and supplemented with 1 % glucose, 1 % various anions such as nitrate, phosphate and sulphate. One milliliter of bacterial consortium SRA 91 and SRA 104 was inoculated to the medium and kept in a shaker (120 rpm) at 40 °C. The samples were drawn aseptically at regular intervals. The concentration of ethylbenzene in the medium was determined using Shimadzu HPLC.

2.9 Treatment of Ethylbenzene Polluted Soil

The 1000 g of sterilized soil sample was taken and 100 ppm of ethylbenzene was artificially contaminated and subjected to microcosms study. Microcosms used in the bioremediation experiments consisted of glass column (8.5 cm diameter, 13 cm length) containing 1000 g of soil and was treated with BH Broth (pH 7) growing optimized bacterial consortium (SRA 91 and SRA 104) supplemented 1 % glucose as a carbon source and 1 % phosphate as a anions was passed through the soil using peristaltic pump for 16 days and the elution was analyzed in the HPLC.

2.10 Treatment of Ethylbenzene Polluted Water

The 1000 ml of water sample was taken and 100 ppm of ethylbenzene was artificially contaminated and subjected to microcosms study. Microcosms used in the bioremediation experiments consisted of 1 l glass bottle containing 1000 ml of water for the treated with BH Broth (pH 7) growing optimized bacterial consortium (SRA 91 and SRA 104) supplemented 1 % glucose as a carbon source and 1 % phosphate as a anions for 120 rpm agitation for 16 days and the sample was analyzed in the HPLC.

2.11 Analytical Method

The ethylbenzene concentrations of the samples were monitored by high performance liquid chromatography (HPLC) analysis of liquid-phase samples (filtered through syringe filter; 0.45 μm pore size) with a Shimadzu HPLC equipped with a C18 column and PDA detector, with the solvent system binary gradient methanol: water (70:30), flow rate is 1 ml/min and 20 μl sample used in the analysis.

3 Results

3.1 Isolation and Screening of Ethylbenzene Degrading Bacteria

Forty-three bacteria were isolated from contaminated soil from petrol pumps and automobile workshops. All the 43 isolates were able to grow well using ethylbenzene as a sole source of carbon. Among the isolates, efficient ethylbenzene degrading nine strains (SRA3, SRA8, SRA9, SRA16, SRA19, SRA21, SRA91, SRA104 and SRA37) were chosen based on the preliminary screening (DCPIP Test).

3.2 Identification of Bacteria

The selected nine bacterial isolates were subjected to morphological and biochemical character studies and identified according to Bergey's Manual of Systematic Bacteriology and identified as *Moraxella*, *Micrococcus*, *Bacillus*, *Enterobacteriaceae* and *Pseudomonas.*

3.3 Secondary Screening of Ethylbenzene Degradation

The selected nine bacterial isolates were inoculated in BH medium containing 100 ppm of ethylbenzene. Among nine bacterial isolates only four isolates showed higher degradation of ethylbenzene. The maximum of 66.05, 45.92, 45.15 and 45.05 % of ethylbenzene degradation was recorded in the isolates SRA37, SRA9, SRA91 and SRA104 respectively at 16 days of incubation and these four isolates were used for further microbial consortium experiment (Fig. 1).

3.4 Effect of Microbial Consortium on the Degradation of Ethylbenzene

Totally 11 combinations of consortium were prepared by using selected four bacterial isolates (combination of two, three and four) and inoculated in BH medium containing 100 ppm of ethylbenzene. Among different combinations used a

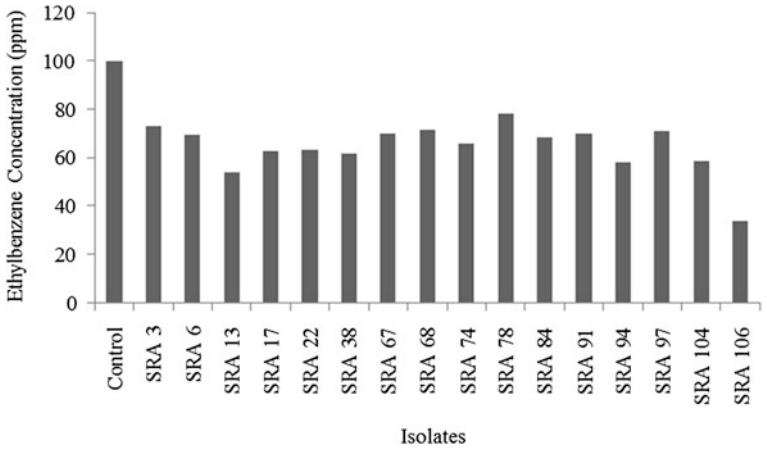

Fig. 1 Percentage of ethylbenzene degradation in secondary screening

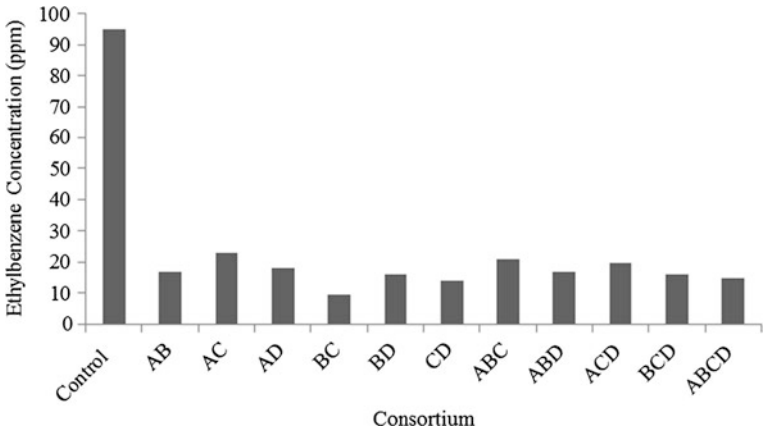

Fig. 2 Effect of microbial consortium on ethylbenzene degradation

maximum of 90.5 % ethylbenzene degradation was recorded in the combination BC (SRA91 and SRA104) at 16 days and selected for further ethylbenzene degradation studies (Fig. 2).

3.5 Effect of Various Carbon Sources on the Degradation of Ethylbenzene

Effect of various carbon sources (glucose, starch and cellulose) on the degradation of ethylbenzene were found maximum in synthetic medium supplemented with

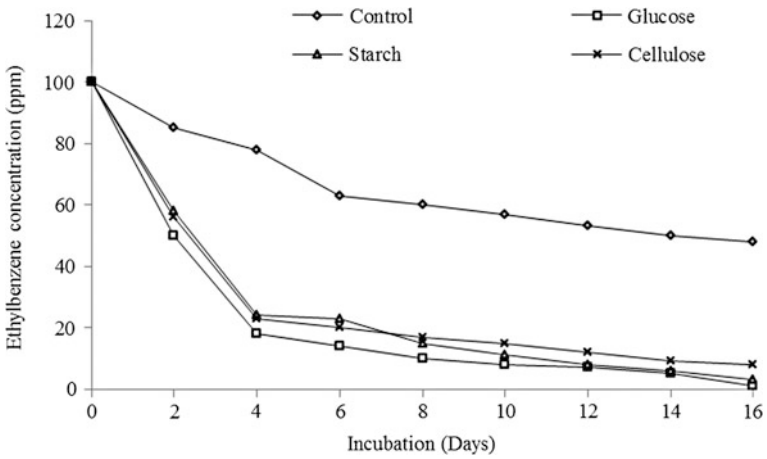

Fig. 3 Effect of various carbon sources on ethylbenzene degradation

glucose followed by cellulose and starch. The bacterial consortium SRA91 and SRA106 was degraded maximum amount of ethylbenzene from 100 to 1.00 ppm (99.00 %) in synthetic medium supplemented with glucose as a carbon source followed by cellulose (from 100 to 8.62 ppm) and starch (from 100 to 3.45 ppm) and the ethylbenzene degradation rate is very low (from 100 to 48.25 ppm) for without carbon source. The ethylbenzene degradation was very negligible and constant in the medium without carbon sources (Fig. 3).

3.6 Effect of Various Temperatures on the Degradation of Ethylbenzene

The maximum degradation of ethylbenzene was observed at 45 °C at 16 days followed by 40 °C. Whereas the degradation rate decreased when the experiment was carried out at 35 and 30 °C. At lower temperature (25 °C) the ethylbenzene degradation was only 51.79 ppm at 16 days. The bacterial consortium (SRA91 and SRA104) degraded ethylbenzene from 100 to 0.34 ppm (99.7 %) in synthetic medium amended with 1 % glucose at 45 °C at 16 days. At the temperatures of 25, 30, 35, 40 and 45 °C, the ethylbenzene degradation was 48.3, 74.35, 91.23, 98.46 and 99.66 %, respectively. At 45 °C, about 99.66 % of degradation was noticed at 16 days (Fig. 4). The temperature at 40 and 45 °C were showed higher ethylbenzene degradation but 45 °C is impossible for the applying the environment and small variation of degradation percentage are recorded in 40–45 °C. Hence, 40 °C of incubation are selected to further degradation study.

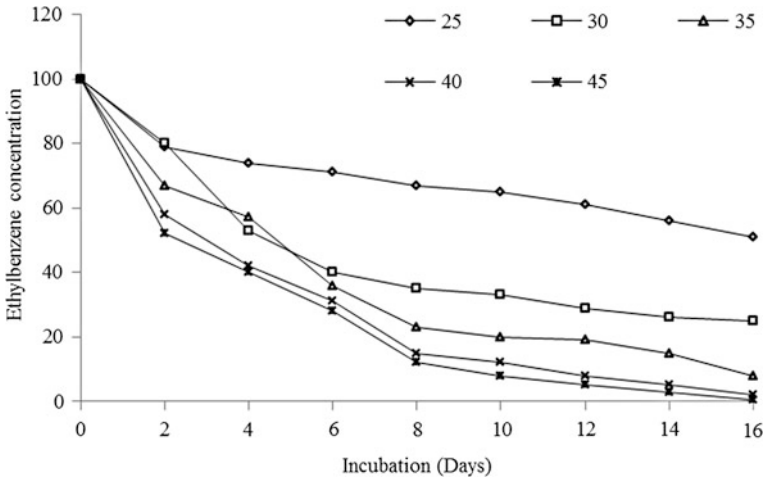

Fig. 4 Effect of various temperatures on ethylbenzene degradation

3.7 Effect of Various pH on the Ethylbenzene Degradation

The ethylbenzene degradation of bacterial consortium (SRA91 and SRA104) in synthetic medium containing 100 ppm ethylbenzene with 1 % glucose is given in Fig. 5. The maximum degradation of ethylbenzene was observed in pH 7 (from 100 to 1.25 ppm) at 16 days followed by pH 9 (from 100 to 8.18). In the case of pH 3, pH 5 and pH 11 the degradation was less. The maximum of ethylbenzene was degraded from 100 to 1.25 ppm (98.75 %) in synthetic medium amended with 1 % glucose in pH 7 at 40 °C for 16 days. At pH 3, 5 and 11 the ethylbenzene degradation was 58.3, 87 and 57.3 % respectively.

3.8 Effect of Various Anions on the Degradation of Ethylbenzene

The effect of various anions on ethylbenzene degradation was studied at 100 ppm of ethylbenzene containing synthetic medium supplemented with 1 % phosphate and 1 % cellulose. The maximum of ethylbenzene was degraded from 100 to 0.5 ppm (99.5 %) in synthetic medium amended with 1 % phosphate and 1 % cellulose in pH 7 at 40 °C for 16 days of incubation. At supplemented with anions nitrate and sulphate the degradation of ethylbenzene was 98 and 95 %, respectively (Fig. 6).

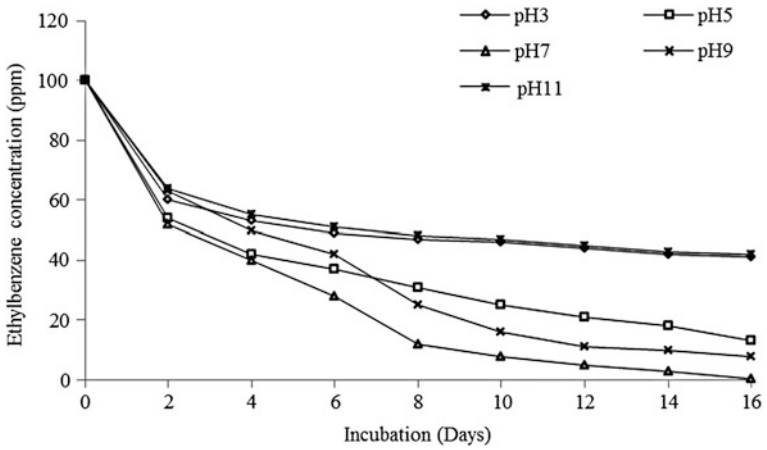

Fig. 5 Effect of various pH on ethylbenzene degradation

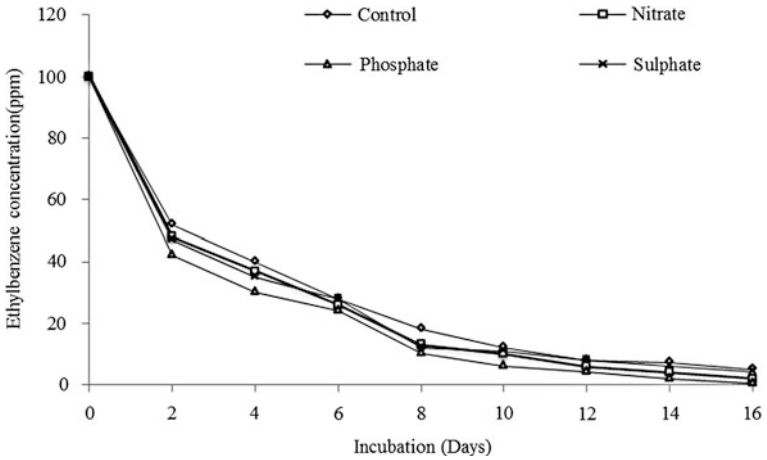

Fig. 6 Effect of various anions on ethylbenzene degradation

3.9 Biodegradation of Ethylbenzene in Soil by Column Study

The soil sample containing 100 ppm of ethylbenzene was degraded by bacterial consortium (SRA91 and SRA104) in soil column study with 1 % glucose and 1 % phosphate (Fig. 7). About 92 % of ethylbenzene (100 to 8 ppm) was degraded by SRA91 and SRA104 in soil sample contains 100 ppm ethylbenzene supplemented with 1 % glucose and 1 % phosphate at 16 days of incubation.

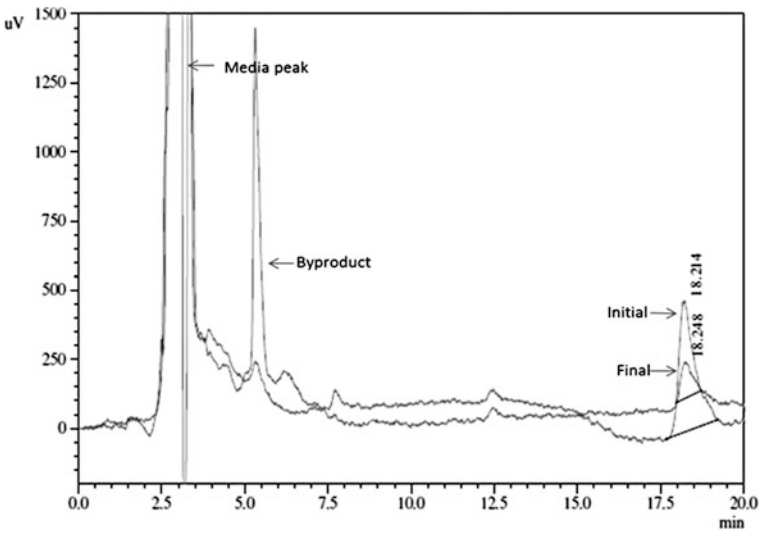

Fig. 7 HPLC analysis result for treatment of soil sample

3.10 Biodegradation of Ethylbenzene by Bioreactor Study

The water sample containing 100 ppm of ethylbenzene was degraded by bacterial consortium (SRA91 and SRA104) in bioreactor with 1 % glucose and 1 % phosphate (Fig. 8). About 90 % of ethylbenzene (100 to 10 ppm) was degraded by SRA91 and SRA104 in water sample contains 100 ppm ethylbenzene supplemented with 1 % glucose and 1 % phosphate at 16 days of incubation.

An environmental contaminant acts on the indigenous biota of the ecosystem, eliminating or selecting microorganisms in accordance sensitivity in the presence of the toxic agent. Among the microorganisms present in the contaminated site, microorganisms capable of using contaminants or just resisting their toxicity can be found (McNaughton et al. 1999). The soil, groundwater and superficial waters contain microorganisms are able to degrade the compounds and used as energy source, thereby eliminate them from polluted environments (Pedrozo et al. 2002). According to Kataoka (2001), the biodegradation of organic compounds is more efficient when the microorganisms in the inoculum are preselected and thus become potentially more adapted to target pollutants. In many studies, different bacterial strains have been capable of degrading BTEX in pure cultures (Attaway and Schmidt 2002; Lee et al. 2002; Li et al. 2006; Plaza et al. 2007) and in mixed cultures (Bielefeldt and Stensel 1999; Deeb and Cohen 1999). Only a few studies have reported that some bacterial strains were able to catalyzing the conversion of ethylbenzene as a source of carbon and energy (Cox and Goldsmith 1979; Utkin et al. 1990; Burback and Perry 1993; Lee and Gibson 1996; Parameswarappa et al. 2007).

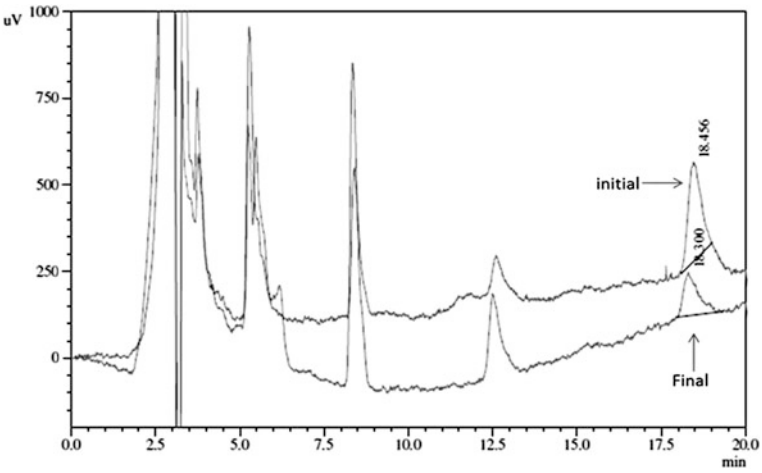

Fig. 8 HPLC analysis result for treatment of water sample

Among the BTEX compounds, the bacterial consortium has been degraded in BTX supplemented the ethylbenzene as a biggest inhibitor (Deeb and Cohen 1999; Chang et al. 2001). In present study, the bacterial consortium that degrading ethylbenzene in BH medium was identified and effects of degradation at various carbon sources, pH, temperature and anions was analyzed. The bacterial consortium (SRA91 and SRA104) degrade maximum amount of ethylbenzene which was about 99.5 % in BH medium (pH 7) supplemented with 1 % glucose as a carbon source, 1 % phosphate as a anions and revealed that the degradation rate of ethylbenzene was much higher than other reported bacterial species (Burback and Perry 1993; Kim and Lee 2011). In the microcosms study, the mixed culture of SRA91 and SRA104 had an ethylbenzene degradation rate of 92 and 90 % at 16 days of incubation for soil column and bioreactor study respectively. Further 99.5 % ethylbenzene degraded in 20 days of in soil column study and 23 days in bioreactor study. According to the World Health Organization the permissible limit of ethylbenzene in the environment is 0.7 ppm. In this study using bacterial consortium, ethylbenzene could be degraded below the permissible limit. These results showed that this consortium is more efficient for ethylbenzene degradation than other bacteria reported in other studies (Bielefeldt and Stensel 1999; Deeb and Cohen 1999; Attaway and Schmidt 2002; Lee et al. 2002; Li et al. 2006; Plaza et al. 2007).

4 Conclusion

In this study, the genera of *Pseudomonas*, *Enterobacteriaceae*, *Moraxella*, *Bacillus*, and *Micrococcus* were isolated from petroleum polluted soil samples collected from automobile workshops and petrol pumps. Among them, *Enterobacteriaceae*

sp. SRA91 and SRA104 were found to be the most efficient in terms of ethylbenzene degradation. It could be concluded that ethylbenzene degradation by SRA91 and SRA104 were influenced by various carbon sources, temperature and pH. The rate of ethylbenzene degradation were high in BH medium supplemented with 1 % glucose as the sole carbon source compared with those in the BH medium supplemented with starch and cellulose under anaerobic conditions at an optimum temperature of 40 °C and pH 7. Hence, 1 % glucose could be used as the best carbon source and concentration for ethylbenzene degradation in BH medium for the bacteria used in this study. Similarly, the temperature at 40 °C and neutral pH were optimum in the BH medium amended with 1 % glucose and 1 % phosphate. Thus, glucose and phosphate is a successive nutrient source for the ethylbenzene degradation and useful to degrade polluted soil and polluted water containing ethylbenzene. The microcosms study also degraded 92 % of ethylbenzene within 16 days and 99.5 % degraded within 20 days of incubation using bacterial consortium for the optimized condition.

Acknowledgement This work was financially supported by the Department of Science and Technology (SERB/F/2765/2011-12) through Fast Track—Young Scientist Project scheme, Government of India.

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Bio-degradation of Reactive Dyes by Indigenous Bacteria Obtained from Textile Effluent Contaminated Site

P. Muthukumaran, M. Priya, P. Vignesh and J. Aravind

Abstract This experimental work deals with the degradation of textile dyes using bacterial species obtained from the effluent spilled soil site of CETP (Common Effluent Treatment Plant) in SIPCOT (State Industries Promotion Corporation of Tamil Nadu Limited), Perundurai. Reactive Red 120, Reactive Black 5 and Direct Red 81 were used as model dyes. Bushnell and Haas medium (BHM) was used to tests textile dyes degradation, initial screenings were carried out with the isolated 10 bacterial strains and the absorbance was measured spectrophotometrically and the (%) dye removal was calculated. Among 10 bacterial isolates, 5 bacterial isolates showed good synthetic dye degradation efficiency. The bacterial isolates were identified to be *Bacillus subtilis, Bacillus cereus1, Bacillus cereus2, Bacillus megaterium* and *Pseudomonas fluorescens*. Among five bacterial isolates *Bacillus subtilis* showed higher degradation efficiency on three synthetic reactive dyes. For Reactive Red 120 (36 %), Reactive Black 5 (44 %) and Direct Red 81 (59 %) were achieved. Bacterial consortium containing these five bacterial isolates was further explored and a maximal 80 % degradation of the three synthetic dyes was reached.

Keywords Effluent · Bacteria · Synthetic dyes · Screening · Consortium · Biodegradation

1 Introduction

Environmental pollution is one of the major problems of the modern world. On one hand, industrialization is necessary to satisfy the needs of the world's overgrowing population but on the other hand, it threatens life on earth by polluting the environment. In general, textile industries consume a considerable amount of water in their manufacturing processes. Considering both the volume and the effluent composition, the textile industry is rated as the most polluting among all industrial sectors. The

P. Muthukumaran (✉) · M. Priya · P. Vignesh · J. Aravind
Department of Biotechnology, Kumaraguru College of Technology,
Coimbatore, Tamil Nadu, India
e-mail: m.kumaran005@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_16

decolorization of the textile dyes with the microbial isolates from the dye contaminated sites is widely studied. The bacterial and fungal strains isolated from the contaminated sites can easily adapt to the adverse environment and thus can be used for the decolorizing the textile dyes. One of the most important environmental problems related to dye effluents is the improper disposal of waste water from textile dyeing industry (Jayarajan et al. 2011). An azo dye reducing bacteria *Paenibacillus azoreducens* was isolated from textile industry waste water (Colette et al. 2001). The bacterial species such as *Acinetobacter, Legionella* and *Bacillus* isolated from textile industries wastewater and drains decolorized the textile dyes (Olukanni et al. 2006).

Decolorization of the textile dyes namely Orange 3R, Blue 3R, Yellow GR, Black RL and T blue was carried out with indigenous soil fungi isolated from the soil samples around textile distillery industries (Raju et al. 2007). A*spergillus* sp. isolated from the soil sample near textile industry effectively decolorized Reactive Blue and other structurally different synthetic dyes (Mohandass et al. 2007). Microbial isolates both bacterial and fungal obtained from the contaminated sites were used to decolorize Three azo dyes [Acid Navy Blue (Acid Blue 120), Fast Red A (Acid Red 88) and Acid Sulphone Blue (Acid Blue 89)] and one triarylmethane dye [Acid Magenta (Acid Violet 19)] (Prachi and Anushree 2009). The isolation of bacteria capable of aerobic decolourisation and mineralization of dyes, specially sulfonated azo dyes, has proven difficult (McMullan et al. 2001).

There are many reports regarding the use of microbial consortia for the decolorization of the textile dyes. For example, A bacterial consortium developed from the organisms isolated from the soil samples of the contaminated sites decolorized Reactive Violet 5 and 10 other dyes (Safia et al. 2005). Four different aerobic mixed consortia collected from basins of wastewater streams coming out of dying plants showed decolorization of Drimarene Orange K-GL, Drimarene Brilliant Red K-4BL, Foron Yellow SE4G and Foron Blue RDGLN dyes (Muhammad et al. 2006). The consortium-GB (*Galactomyces geotrichum* and *Bacillus* sp.) exhibited 100 % decolorization ability with the dye Brown 3REL (Jadhav et al. 2008). Microbial consortia developed with *Bacillus cereus* (KEB-7) and *Bacillus pumilus* (KEB-10) for bacteria and *Aspergillus alliaceus* (KF-3) for fungi showed 100 % decolorization of textile wastewater (Eltaief et al. 2009). The bacterial consortium consisting of five different bacterial species *Bacillus vallismortis*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* were efficient in decolorizing individual as well as mixture of dyes (Bella et al. 2009). This study will help to promote biodegradation and biotreatment of textile dye effluent eco-friendly.

2 Materials and Methods

2.1 Sample Collection

The dye effluent samples and soil sample from the dye contaminated sites were collected from CETP in SIPCOT, Perundurai, Erode District. About 3 effluent

contaminated soil sample were collected. The samples were transferred immediately to the laboratory for further analysis.

2.2 Isolation of Bacterial Colonies from Effluent and Soil Samples

Collected effluent sample was used as the parent source of inoculum in this study. For isolation of bacterial heterotrophic (TH) population of dye degrading isolates in the 3 different effluent contaminated soil samples, 1 mL of the soil suspension was aseptically added to 100 mL of enrichment medium (Bushnell and Haas medium), containing 0.1 % (w/v) glucose as carbon source. The flasks were incubated in shaker condition at 150 rpm at 37 °C for 2 days. The isolation of dye degrading bacteria from contaminated samples was performed by modifying the method as described by Akhilesh et al. (2010). Based on different colony morphology of bacterial isolates, bacterial isolates were selected and maintained on nutrient agar slants at 4 °C.

2.3 Dyes and Decolorization Studies

The decolorization studies were carried out for the selected colonies with three synthetic dyes namely Reactive Red 120, Reactive Black 5 and Direct Red 81. These dyes were prepared at a concentration of 50 mg/100 ml and the decolorization assay mixture consists of 10 % of dye. The decolorization studies were carried out for the isolated Bacterial colonies on a specialized media called Bushnell & Haas medium of the following composition (g/l): NH_4NO_3, 1; $CaCl_2$, 0.02; $FeCl_3$, 0.05; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1; Glucose, 0.1 % w/v; Yeast extract, 0.05 % w/v and pH 7. The dye removal % was calculated after 48 h and the bacterial colonies with high decolorization efficiency were selected. The decolorization activity was expressed in terms of decolorization % using the formula of Cheriaa et al. (2012). Dye removal (%) was calculated by using the Eq. 1.

$$\text{Dye\_removal}\,(\%) = \frac{A_{t0} - A_{tf}}{A_{tf}} \times 100 \tag{1}$$

where A_{t0} is initial absorbance and A_{tf} is absorbance at incubation time.

2.4 Development of a Bacterial Consortium and Evaluation of Its Dye Decolorizing Efficiency

A bacterial consortium was developed by using five bacterial isolates which were capable of decolorizing the synthetic dyes. For consortia preparation, loop full of

the selected bacterial isolates was individually inoculated for 24 h at 37 °C to form a consortium (Tony et al. 2009). The bacterial consortium composed of bacterial isolates B1, B2, B3, B4 and B5. The decolorization efficiency of this consortium was evaluated on three commercial dyes Reactive Red 120, Reactive Black 5, and Direct Red 81.

3 Results and Discussion

The decolorization and degradation of commercially available reactive dyes was carried out using a microbial consortium which was developed from the combination of microbial isolates were isolated from the effluent contaminated soil samples. Bacterial consortium was developed with isolated bacterial isolates and degradation studies were performed with these consortia.

3.1 Isolation of Bacterial Isolates from the Effluent Contaminated Soil Samples

Totally, 10 different bacterial isolates were obtained from the soil samples. Similarly, Nishant et al. (2006) reported the di-azo dye direct red 81 degrading bacterial isolates were isolated from dye contaminated effluent samples. In this study, bacterial isolates were named based on the source from which it was isolated. The culture name and the source of the bacterial isolates are provided in Table 1. Selected bacterial isolates were further purified and sub cultured. The pure cultures were identified based on their biochemical activity and by Bergy's Manual of determinative Bacteriology (Holt et al. 1994).

3.2 Decolorization of Studies

3.2.1 Decolorization of the Synthetic Dyes Using the Selected Bacterial Isolates

All the 10 selected isolates were screened for their ability to decolorize three different reactive dye Direct Red 81.

Table 1 Name and source of the bacterial colonies isolated from CETP samples

Source of the organism	Culture name
CETPE1[a]	CETPB1, CETPB2, CETPB3, CETPB4
CETPE2[a]	CETPB5, CETPB6, CETPB7
CETPE3[a]	CETPB8, CETPB9, CETPB10

[a]CETPE1—CETP Effluent contaminates soil sample 1, CETP Effluent contaminates soil sample 2, CETPE3—CETP Effluent contaminates soil sample 3

The synthetic dye decolorization studies were made for 10 bacterial isolates. Similar kind of observation was made by Saranraj et al. (2010). The dye removal % was calculated for all the bacterial isolates and the isolates with high decolorization efficiency were then selected. The following Fig. 1 depicts the dye removal % for the selected 10 different bacterial isolates.

Based on the decolorization studies, totally 5 bacterial colonies were selected for further studies based on their high dye removal %. Of these 10 bacterial isolates from the CETP sample, CETPB1 (53 %), CETPB2 (72 %), CETPB3 (54 %), CETPB7(52 %) and CETPB10 (56 %) were screened based on decolorizing efficiency and tested for their ability to decolorize the remaining 2 different synthetic reactive dyes namely Reactive Red 121 and Reactive Black 5. Figures 2, 3 and 4 shows dye removal percentage.

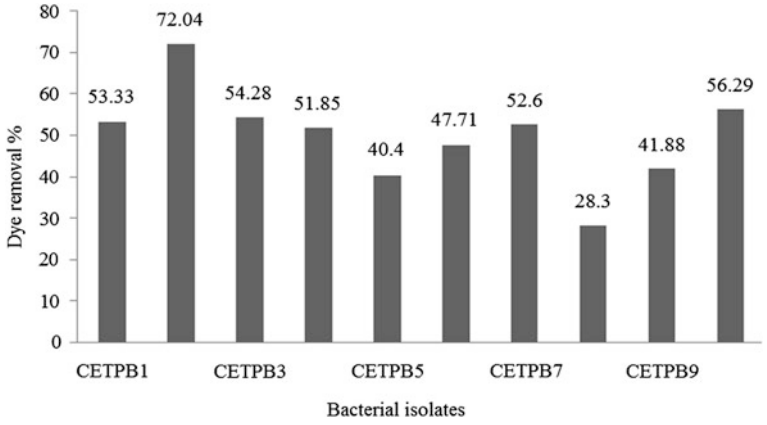

Fig. 1 Dye removal (%) of direct red 81 by bacterial isolates from CETP samples

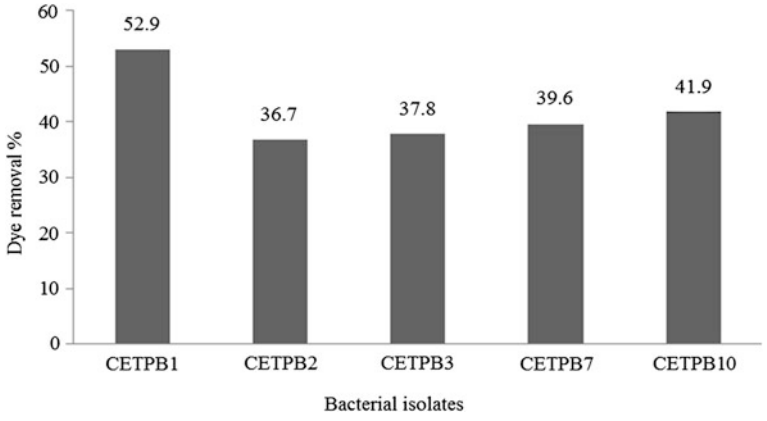

Fig. 2 Reactive red 121 removal % of screened bacterial isolates

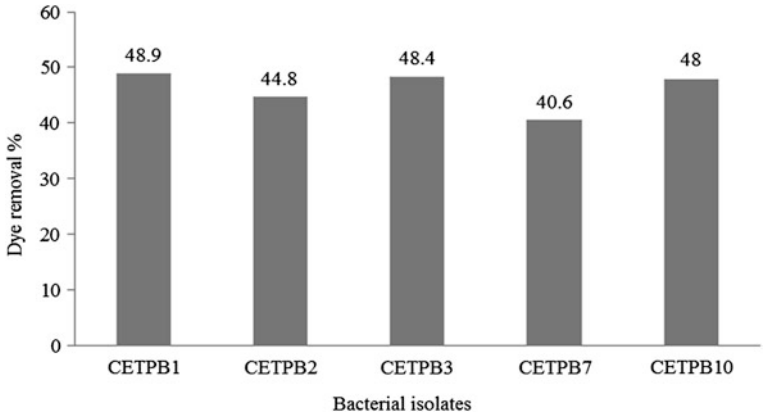

Fig. 3 Reactive black 5 removal % of screened bacterial isolates

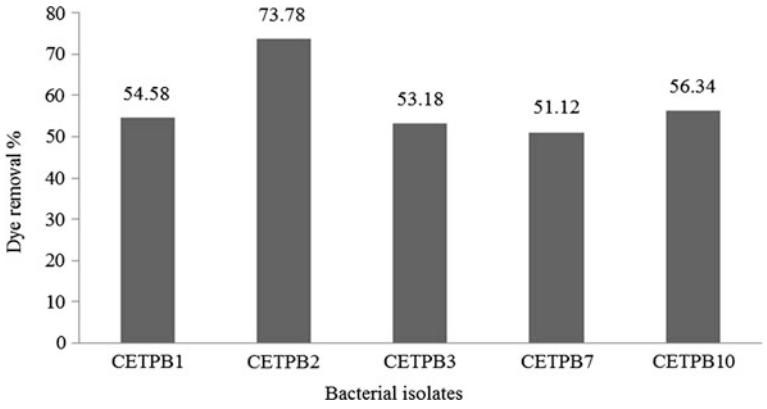

Fig. 4 Direct red 81 removal % of screened bacterial isolates

Five different bacterial cultures were selected for the development of the consortia on the basis of their high dye removal efficiency of the three synthetic reactive dyes. All these 5 bacterial isolates were tentatively identified by Bergy's Manual of determinative bacteriology (Holt et al. 1994). Table 2 shows, 5 different bacterial isolates that were selected and were renamed accordingly.

Table 2 Biochemical characterization and identification of selected bacterial isolates

Culture name	Bacterial isolates (tentatively identified as)
CETPB1	*Bacillus subtilis*
CETPB2	*Bacillus cereus*
CETPB3	*Bacillus cereus*
CETPB7	*Bacillus megaterium*
CETPB10	*Pseudomonas fluorescens*

Table 3 Synthetic dye removal of bacterial consortium

Bacterial consortium	Dye removal (%)					
	Reactive red 121 (520 nm)		Reactive black 5 (574 nm)		Direct red 81 (510 nm)	
	24 h	48 h	24 h	48 h	24 h	48 h
BC	13.26	23.25	31.12	53.23	45.38	78.56

3.3 Development of the Microbial Consortia and Determination of the Decolorization Efficiency

The microbial consortia were developed by using mixed culture of the microorganisms. One bacterial consortium was developed and their decolorization efficiency of the commercially available reactive dyes was determined.

3.3.1 Development of a Bacterial Consortium

A bacterial consortium was developed by using five bacterial isolates which were capable of decolorizing the commercial dyes. The bacterial consortium was named BC and it composed of bacterial isolates *Bacillus subtilis, Bacillus cereus*, *Bacillus megaterium* and *Pseudomonas fluorescens.* Table 3 gives the percentage dye removal by the bacterial consortium BC.

The consortium development in the literature shows that all the researchers developed consortium from single source, whereas Mao et al. (2012) reported that heterogeneity of the bacterial community; in this way consortium gave better results in degradation/detoxification of dyes. Han et al. (2012) also isolated the bacteria for decolorization of textile dyes from the different sources. Thus, the bacterial consortium showed high degradation of Direct Red 81 dye (78 %) while it showed moderate removal of Reactive black 5 (53 %) and very low removal of Reactive Red 121 (23 %). The decolorization of the synthetic dyes by the microbial consortia can be due to adsorption or degradation. These samples were centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved in the Bushneel and Haas medium and the absorbance reading was measured spectrophotometrically. It was found that there was no dye in the pellet obtained from the bacterial samples. Thus, there is degradation of the synthetic dyes.

4 Conclusion

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrial progress has now assumed serious proportions. Textile dyes are one of the most prevalent chemicals in use today. With increasing usage of

the wide variety of dyes in the industries, pollution from the effluents has become increasingly alarming. Microbial decolorization and degradation is and environmentally friendly and cost—effective process. The bacterial isolates were isolated from the effluent contaminated soil samples. Isolated bacterial culture were subjected to tests for their ability to decolorize three synthetic dyes Reactive Red 120, Reactive Black 5 and Direct Red 81 and then final screening was done based on these studies. After final screening, 5 bacterial isolates were identified to be *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Pseudomonas fluorescens.*

Finally, bacterial consortium was developed with the selected bacterial isolates. This consortium was tested for the ability to decolorize the synthetic dye and it was found that they showed about 53–78 % dye removal. It was concluded that bacterial consortium obtained by grouping the 5 different bacterial isolates isolated from the samples of the dye contaminated sites showed higher synthetic dye removal % than the individual bacterial isolates. Thus, the microbial consortia can be used for the decolorization and degradation of textile dyes efficiently.

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Hydrolytic Enzyme Profiling of *Bacillus Subtilis* COM6B and Its Application in the Bioremediation of Groundnut Oil Mill Effluent

P. Kanmani, J. Aravind and K. Kumaresan

Abstract Industrial effluents rich in oil and grease pose hindrance to the functioning of wastewater treatment units and also affect the quality of receiving water bodies. Pretreatment of such wastewaters to bring about lipid hydrolysis makes them more amenable to conventional biological treatment and hydrolytic enzymes, especially lipases, find promising applications in this sector. In our study, the bacterial strain *Bacillus subtilis* COM6B isolated from groundnut mill effluent was cultivated in minimal media based on residual oil waste from the extraction process, in which it produced lipase and other extracellular hydrolytic enzymes such as protease and amylase. Applying response surface methodology led to a 1.8-fold increase in oil waste removal by the isolate. As a further study, the effluent discharged from the oil mill was treated in batch mode using pure cultures of the isolate and the effects of incubation time, inoculum size and effluent dilution on the treatment process were investigated. A maximum of 95, 93 and 98 % reduction in biochemical oxygen demand, chemical oxygen demand and oil and grease respectively, were achieved after treatment with COM6B. Hence, the isolate could serve as a potential candidate for remediating the fat and oil contaminants and reducing the organic load of wastewaters.

Keywords Biochemical oxygen demand · Hydrolytic enzymes · Oil and grease · Oil mill waste · Response surface methodology

1 Introduction

Wastewaters with high oil and grease load are discharged from dairies, oil mills, food processing units, restaurants, slaughter houses, etc. The oil and grease contents impede the wastewater treatment process by decreasing the cell-aqueous phase

P. Kanmani · J. Aravind (✉) · K. Kumaresan
Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India
e-mail: dr.j.aravind@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_17

transfer rate, formation of bulking sludge, loss of biomass from the reactor, clogging and unpleasant odors (Vidal et al. 2000). Moreover, lipid hydrolysis occurring during the treatment process can release glycerol and long chain fatty acids that are inhibitory to microorganisms in the biological treatment process. Such wastewaters are subjected to pretreatment using grease traps and dissolved air floatation before release into the main biological treatment unit. However, these measures are often ineffective in removing emulsified oil and the cost is also prohibitive (Willey 2001). Hence, an alternative enzymatic hydrolysis strategy could be applied in pretreatment, which brings about lipid hydrolysis and improves the biological treatment efficiency. Lipase-producing microbial strains find promising applications in this arena.

Lipases (triacylglycerol acylhydrolases, EC. 3.1.1.3) are serine hydrolases that catalyze the hydrolysis of esters formed from glycerol and long-chain fatty acids releasing di- and mono-acyl glycerols, fatty acids, and glycerol. This ester hydrolysis is catalyzed in the aqueous environment. In non-aqueous and micro-aqueous environments, the reverse reactions of esterification, interesterification and transesterification occur. Lipases possess chemo-, regio- and enantio-selectivity. The authors find widespread application in leather, paper, pharmaceutical, food, detergent and textile industries (Hasan et al. 2006). The enzyme's environmental relevance, such as in the bioremediation of oil and grease contents has also been explored (Cammarota and Freire 2006).

In the present study, *Bacillus subtilis* strain COM6B was investigated for production key extracellular hydrolytic enzymes such as lipase, protease and amylase in a medium based on oil mill waste. Statistical optimization of oil waste hydrolysis was performed using response surface methodology (RSM). The oil mill effluent was characterized and treated with pure cultures of the isolate in batch mode to bring about a reduction in oil and grease content and overall organic load.

2 Materials and Methods

p-Nitrophenyl palmitate (p-NPP) and casein for the lipase and protease assays were procured from Sigma Chemicals, USA. All other reagents and chemicals used were of analytical grade and purchased from standard sources in India. Wasted oil residue after the extraction process (referred to as oil mill waste—OMW) was obtained from a groundnut oil mill located in Coimbatore District of Tamil Nadu, India and used to supplement the culture medium. The effluent discharged from the same mill was used for characterization and treatment studies. Bacterial strains were isolated from soil sample in the vicinity of the oil mill by serial dilution and plating in mineral salts agar supplemented with 2 % groundnut oil. They were screened for lipase activity in tributyrin agar plates. Lipase activity was quantified using spectrophotometric assay with *p*-NPP as the substrate (Winkler and Stuckmann 1979). The absorbance was measured at 410 nm, against an enzyme-free blank. Molar

extinction coefficient of 0.0146 μM^{-1} cm^{-1} was used. One unit of lipase activity was defined as μM of *p*-nitrophenol released per minute under the assay conditions.

Genotypic identification was carried out for the isolate showing the best lipase activity. It was cultivated in 100 mL of mineral salts medium supplemented with different concentrations of OMW (5.0–30 g/L). The culture medium pH was adjusted to 7.0 ± 0.1, autoclaved and inoculated with overnight grown culture of the bacterial isolate (cell density of 53×10^6 CFU/mL). The culture was incubated at 35 °C for 48 h and turbidity was measured by reading the absorbance at 600 nm in a UV-Visible Spectrophotometer. It was then centrifuged and the pellet was used to estimate biomass dry weight. Based on the results, the maximum concentration of OMW that permitted good bacterial growth was established. At high concentrations, the oil layer could interfere with oxygen transfer and hamper cell survival and growth. For the isolate to be effectively used for bioremediation of oil mill effluent, it should not only produce lipase for oil biodegradation but also other important hydrolytic enzymes, so as to bring about an overall reduction in the effluent organic load. The culture supernatant was hence used to assay lipase, protease and amylase activities, following the standard protocols of Winkler and Stuckmann (1979), Folin and Ciocalteu (1929) and Bernfeld (1955) respectively. The residual oil content in the medium after bacterial growth and metabolism was extracted and estimated using the partition gravimetric method in order to know the extent of removal (Greenberg et al. 1992).

Box Behnken Design of RSM was used to optimize the process of oil hydrolysis and the Design Expert Software 8.0 (Stat Ease Inc. Minneapolis, U.S.A, trial version) was used for this purpose. OMW (A), dextrose (B) and peptone (C) were the independent variables chosen and studied at three different levels (−1, 0, +1). A matrix encompassing 17 experiments was generated and production was carried out as per the design. At the end of the incubation period, residual oil was extracted from the cell-free supernatant and percent reduction in the oil content was recorded as the response. These data were fed into the software and analyzed. Standard analysis of variance (ANOVA) and contour plots were generated. A quadratic polynomial regression model was assumed for the predicted response. For a three-factor system, the model Eq. 1.

$$Y = \beta 0 + \beta 1 * A + \beta 2 * B + \beta 3 * C + \beta 11 * A2 + \beta 22 * B2 + \beta 33 * C2 + \beta 12 * AB + \beta 13 * AC + \beta 23 * BC \quad (1)$$

where, Y is the predicted response, β_0 is the intercept, β_1, β_2, β_3, are the linear coefficients, β_{11}, β_{22}, β_{33}, are the squared coefficients, and β_{12}, β_{13}, β_{23}, are the interaction coefficients.

The oil mill effluent was subjected to physicochemical and biological characterization. It was then treated with pure culture of the bacterial isolate in batch mode to bring about a reduction in the pollution parameters. A 250 mL Erlenmeyer flask containing 100 mL effluent (diluted 1:1 using lipid minimal medium—LMM) was

inoculated (2 % v/v) with overnight grown culture and incubated at 35 °C in a rotary shaker set at 120 rpm. Samples were withdrawn every 12 h and checked for percent reduction in biochemical oxygen demand (BOB), chemical oxygen demand (COD) and oil and grease (O&G) content. The inoculum percentage and effluent dilution were then varied and their effects on the organic load removal were checked. All effluent parameters before and after treatment were determined as per the protocols of standard methods for the examination of water and wastewater (Greenberg et al. 1992).

3 Results and Discussion

During the isolation and screening process, 21 bacterial strains were isolated from the soil sample, out of which 12 showed good zone of clearance in tributyrin agar screening. In *p*-NPP assay, 7 of the isolates displayed appreciable lipase activity >10 U/mL. The strain COM6B showing the highest activity of 15.25 U/mL was identified as *B. subtilis* based on 16S rRNA gene sequencing. The NCBI GenBank Accession No. for the sequence is KJ886969 and its phylogenetic tree is presented in Fig. 1.

Nucleotide Blast was used to perform sequence similarity search in the NCBI database and distance tree was constructed by the fast minimum evolution method. The strain COM6B was cultivated in mineral salts broth supplemented with OMW. Bacterial growth was enhanced due to the availability of the oil waste as a carbon

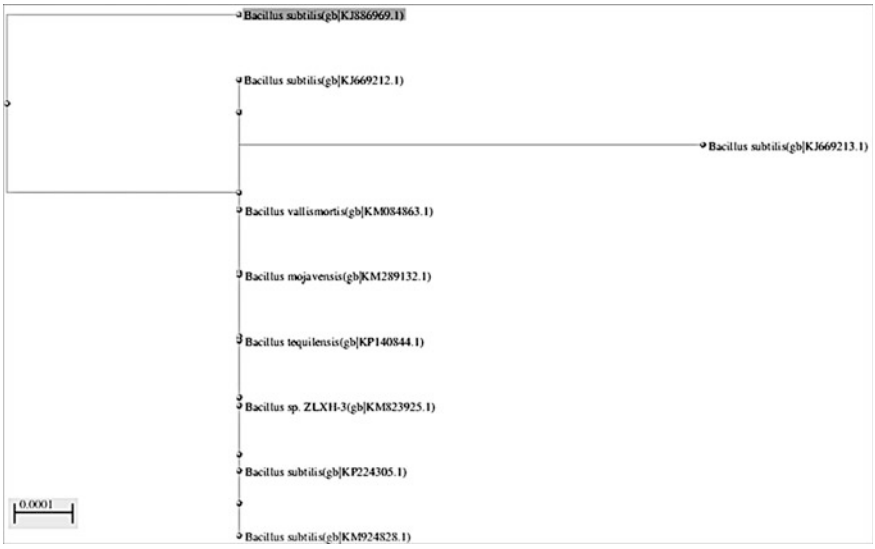

Fig. 1 Phylogenetic tree of *Bacillus subtilis* COM6B

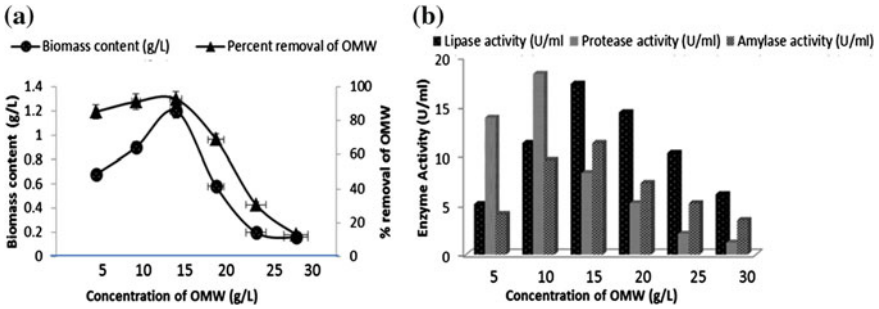

Fig. 2 Bacterial growth and oil removal (**a**) and extracellular enzymatic activities (**b**)

source for lipolytic microorganisms. However, beyond an optimal level (15 g/L), further increase in the concentration of OMW had adverse effect on bacterial growth, as reflected in a decreased biomass production. At a concentration of 25 g/L, it had a severe inhibitory effect and only negligible amount of biomass was produced (Fig. 2a). From these observations, it was concluded that 15 g/L is an ideal concentration of OMW to spur the growth of COM6B.

Oil removal by the isolate also gave agreeable results, with the removal efficiency touching 92.7 % for an initial OMW concentration of 15 % (Fig. 2a). This came down to 68.8 % for 20 g/L initial concentration, with only scanty removal being achieved at higher concentrations, as the growth and biomass production itself was minimal. Similar results have been documented in literature for lipid degradation in slaughter house wastewater (Prasad and Manjunath 2011).

The isolate COM6B was shown to produce all the three important hydrolytic enzymes that were investigated in the study (Fig. 2b). When the enzymatic activities were explored in media containing different concentrations of the oil waste, highest lipase activity of 17.25 U/mL was observed at 15 g/L OMW. Peak amylase activity of 11.32 U/mL was also recorded in this medium. A maximum protease activity of 18.76 U/mL was witnessed at a lower OMW concentration of 10 g/L. Combined hydrolytic activities in *S. xylosus* strain MHB32, which was able to produce an array of hydrolytic enzymes including protease, L-asparginase, lipase, xylanase and cellulase have been reported by other researchers. However, waste materials were not used as substrates to culture the organism (Bhosale et al. 2013). Mineral salts medium containing (g/L) $(NH_4)_2SO_4$—2.5, $CaCl_2$—1.0, $NaNO_3$—0.5, K_2HPO_4—0.5 and $MnSO_4$—0.05 was supplemented with varying concentrations of OMW ranging from 5.0 to 30 g/L.

Response surface methodology has been widely used for process optimization, including lipase production by bacterial strains. For example, lipase production by *Geobacillus thermoleovorans* (Sanchez-Otero et al. 2011), *Stenotrophomonas maltophilia* CCR11 (Hasan-Beikdashti et al. 2012), *Burkholderia* sp. C20 (Liu et al. 2012) and *Burkholderia multivorans* (Gupta et al. 2007) has been optimized using statistical design. However, the production media did not employ cheap substrates

Table 1 Box Behnken experimental design and responses

Run	Factor A	Factor B	Factor C	Response
1	1.75	17.5	10	85
2	1.75	17.5	10	85
3	1.75	17.5	10	85
4	3	30	10	98
5	0.5	17.5	5	88
6	3	17.5	15	94
7	0.5	17.5	15	89
8	3	5	10	91
9	0.5	30	10	87
10	1.75	5	15	86
11	1.75	30	5	92
12	1.75	17.5	10	85
13	3	17.5	5	90
14	1.75	30	15	94
15	1.75	17.5	10	85
16	1.75	5	5	86
17	0.5	5	10	82

Factor A—OMW (%), B—Dextrose (g/L), C—Peptone (g/L), Response—% Oil reduction

and moreover, percent reduction in oil as a result of the lipolytic activity has not been optimized. Table 1 represents the experimental design and its despondence.

The medium was inoculated with overnight grown culture of COM6B, incubated for 48 h at 35 °C and residual oil was extracted using the partition gravimetric method. ANOVA for the response surface quadratic model showed model F-value of 9.13 which implies that the model is significant. There is only 0.01 % chance that F-value this high could occur due to noise. Model p-value of 0.00405 also indicates that the model is significant. A, B, A^2, B^2 and C^2 were significant model terms with low p-value and high F value. p-values greater than 0.1000 indicate that the model terms are not significant. In general, larger magnitudes of t, F and smaller p values indicate that the corresponding coefficient terms are significant.

The R^2 value gives a measure of how much variability in the observed response can be explained by the experimental parameters and their interactions. The closer the value of R^2 is to 1, the better the correlation between the experimental and predicted values and the model predicts the response well. The R^2 value obtained for the quadratic model was 0.8915. Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. The obtained value of 32.41 indicates an adequate signal and the model can be used to navigate the design space.

The Eq. 2 can be used to make predictions about the responses, for given levels of each of the factors. They are also useful for identifying the relative impacts of the factors by comparing the factor coefficients.

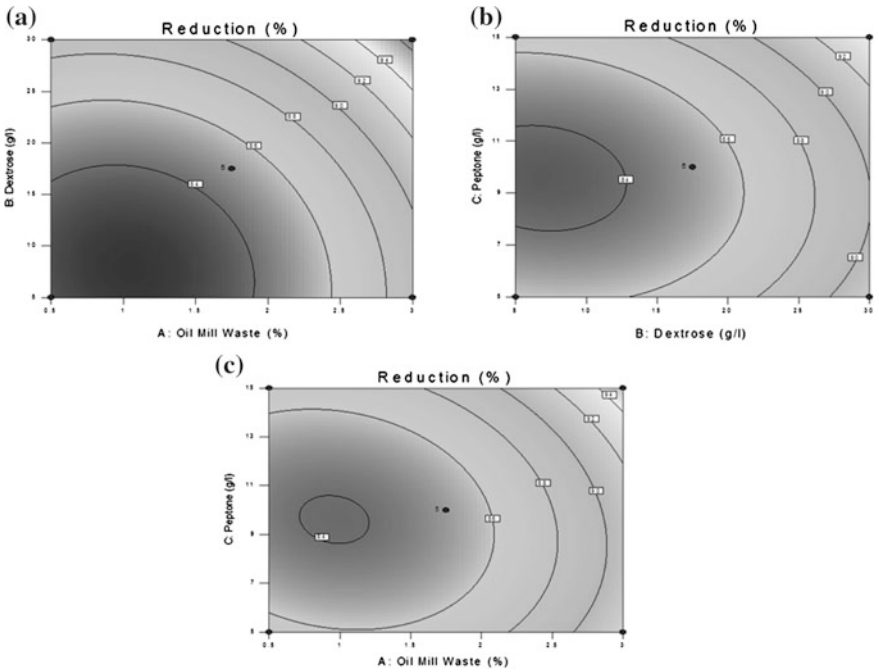

Fig. 3 Contour plots depicting interactions between OMW and dextrose (**a**), dextrose and peptone (**b**), OMW and peptone (**c**) and their effects on the response

$$Y = +15.40 + 7.81 * A + 4.03 * B + 1.56 * C + 2.85 * AB + 0.73 * AC - 1.50 * BC + 1.46 * A2 + 0.39 * B2 + 0.41 * C2 \quad (2)$$

The contour plots are a way of expressing the regression equation graphically. They depict the interactions among the variables and are used to determine the optimum concentrations of each factor for good response. The contour plots for the interactions between OMW and dextrose (A-B), dextrose and peptone (B-C), and OMW and peptone (A-C) are depicted in Fig. 3.

Physicochemical and biological characterization of the oil mill effluent was performed in order to know its pollution load before proceeding with treatment. The results are furnished in Table 2, from which it is seen that the effluent has high TDS, BOD and COD levels. Initially, the effluent was diluted 1:1 with lipid minimal medium, inoculated with the bacterial strain and the change in pollution parameters over a period of 92 h was monitored. A maximum of 89, 92, and 98 % reductions in COD, BOD and O&G contents respectively, were obtained due to the enzymatic activities of the isolate (Fig. 4). From the figure, it could also be inferred that considerable stabilization of the parameters had occurred within 72 h and only slight changes were noticed beyond that.

Table 2 Physicochemical and biological characterization of the oil mill effluent

Parameters	Value
pH	9.20
TDS (mg/L)	7955
TSS (mg/L)	87
BOD (mg/L)	1560
COD (mg/L)	3782
Ammoniacal nitrogen (mg/L)	4.5
Nitrate nitrogen (mg/L)	0.85
Phenol (mg/L)	–
Phosphate (mg/L)	0.30
Oil and grease (mg/L)	12.50
Total microbial load	
Bacteria (CFU × 10^3/mL)	13
Fungi (CFU × 10^2/mL)	–
Actinomycetes (CFU × 10^2/mL)	–

TDS—Total dissolved solids, *TSS*—Total suspended solids, *BOD*—Biochemical oxygen demand, *COD*—Chemical oxygen demand

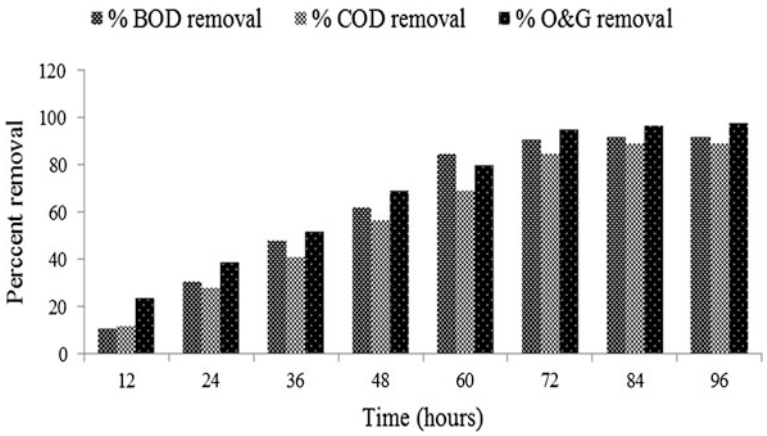

Fig. 4 Effect of incubation time on BOD, COD and O&G removal by the isolate

In the subsequent study, inoculum percentage was varied from 1 to 5 %. Increasing the inoculum percentage enhanced the biodegradation process, resulting in better organic load removal (Fig. 5). It could be seen that the removal efficiency is low at 1 and 2 % inoculum, with good removal occurring at a concentration of 3 % and above. A maximum of 93, 95 and 98 % COD, BOD and O&G removal were achieved with 5 % inoculum. However, it was observed that increasing the inoculum percentage from 4 to 5 % led to just a marginal increase in removal efficiency.

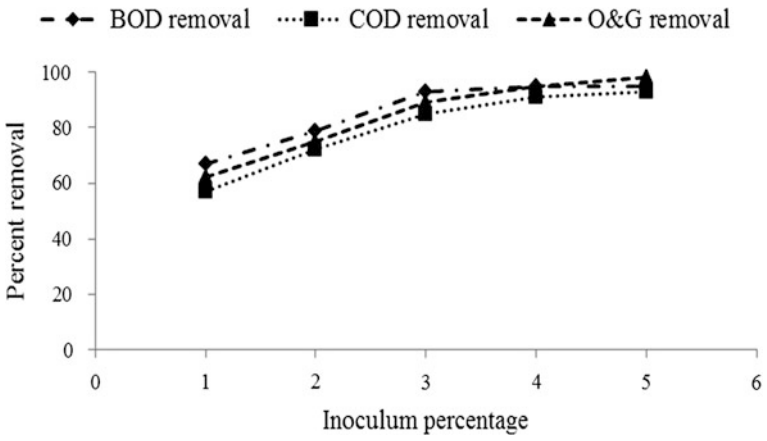

Fig. 5 Effect of inoculum percentage on BOD, COD and O&G removal by the isolate

Effluent dilution also seems to be an important factor influencing the biodegradation rate. At 1:1 ratio of effluent and LMM, maximum removal efficiencies of 90, 95 and 97 % for COD, BOD and O&G were observed (Fig. 6). Reducing the effluent dilution hampered the biodegradation process and at an effluent and medium ratio of 5:1, around 40 % reduction in removal efficiency was observed as the microorganisms could not tackle the high pollution load in the concentrated effluent and were also deprived of nutrients to support their growth and activity.

The dilutions were made using LMM, 3 % inoculum was added and incubated for 72 h at 35 °C. From these preliminary batch experiments it could be concluded that a contact time of 72 h, inoculum level of 4 % and 1:1 dilution of the effluent are

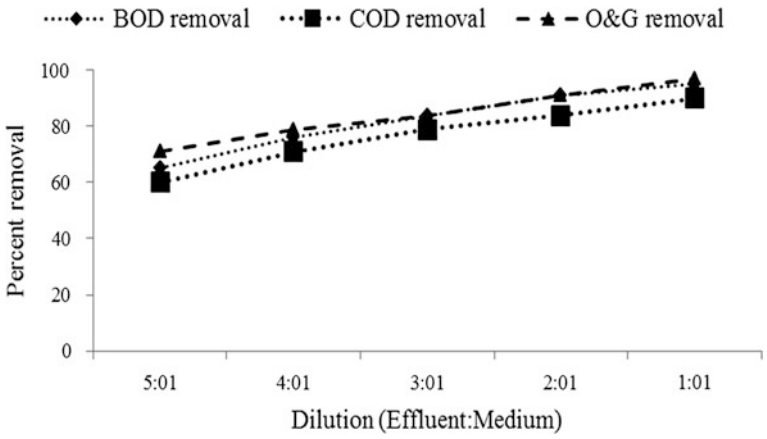

Fig. 6 Effect of effluent dilution on BOD, COD and O&G removal by the isolate

conducive to bring about the desired reduction in the pollution parameters. Validating these shake-flask results in a bioreactor, process scale up and use of immobilized cell systems are to be taken up as further studies.

Research in olive mill and palm oil mill wastewaters are more rampant and groundnut mill wastewaters have not been well represented in literature. In a study involving the biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic thermophilic conditions using *Bacillus thermoleovorans* IHI-91, a continuously operated stirred-tank reactor was used to degrade olive oil to more than 90 % at a residence time of 2 h (Becker et al. 1955). In another study on aerobic biodegradation and detoxification of wastewaters from the olive oil industry, average COD removals were 55.0, 52.5 and 62.8 % in wastewaters fermented with *Geotrichum* sp., *Aspergillus* sp. and *Candida tropicalis*, respectively (Fadil et al. 2003). In another study, central composite design of response surface methodology was applied for the advanced treatment of olive oil processing wastewater using Fenton's peroxidation. Quadratic models were predicted for the response and the maximum model-predicted removals were 56, 100, 33 and 32 % for COD, total phenolics, color and aromatics, respectively (Ahmadi et al. 2005).

4 Conclusion

These research findings suggest that *B. subtilis* strain COM6B is promising and further research to exploit its potential for environmental clean-up needs to be undertaken. The results are suggestive of the isolate's desirable features that could be favorably exploited for the treatment of oil mill wastewaters. The strain's extracellular hydrolytic enzyme activities of lipase, protease and amylase have aided in the biodegradation process and facilitated a reduction in not only the O&G contents, but also the overall organic load of such wastewaters. The oil removal process optimization that formed a part of this study could help in carefully selecting the variables and their levels, thereby enhancing the strain's performance in bioremediation. Pretreatment of high lipid content wastewaters using hydrolytic enzymes is a proven strategy that makes them more amenable to anaerobic digestion and the isolate and its enzymes could find promising application in this area.

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Management of Grey Water with Bio-beds Using Phytotechnology

N.D. Shrinithivihahshini, R. Chithra Devi, R. Viji, D. Mahamuni and M. Sheela Mary

Abstract The waste water treatment trial has been carried out at Department of Environmental Management, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The aim of the trial was to evolve a suitable user friendly treatment system, for handling the domestic liquid waste at the point of generation; subsequent reuse in flushing the toilets, irrigating gardens, etc. To achieve these objectives, four different bio beds were designed and filled with pea gravel, sand, clay, red soil and charcoal in different ratios. All the bio-beds (excluding bio-bed 4), were planted with plant species viz., *Lipia nodiflora* (bio-beds 1 and 2) and *Vettiver Zizanoides* (bio-bed 3). Physico-chemical properties of the water at both before and after the treatment were compared and reductions percentages were calculated for major parameters. The test results gave concrete evidence for a reduction in the biological oxygen demand (89 %), chemical oxygen demand (81 %), carbonate (100 %), sulphate (77 %), sodium (77 %) and potassium (74 %). It is found that continuous vertical flow based constructed wetland system can be used for decentralized management of grey water in institutions. This filter bed method is natural, simple and cost-effective.

Keywords Bio-bed · Grey water · Decentralized management · Plant species · Bioremediation

1 Introduction

Water scarcity in the world is increasing day by day. Globally 90 % of wastewater is untreated and let into the sewage. It is essential to reuse, recover the waste water from various activities. In domestic activities there are three categories of water

N.D. Shrinithivihahshini · R. Chithra Devi (✉) · R. Viji
D. Mahamuni · M. Sheela Mary
Department of Environmental Management, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
e-mail: cdevi16@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_18

required to reuse, they are rainwater, grey water and black water. Wastewater is basically water that has been used as the input for the solution system. It is generally a mixture of pollutants. Household wastewater can be divided grey water and black water. Black water, released from toilets and soiled diapers, contamination of fecal bacteria along with generally high organic matter (Welton et al. 2005). The Grey water originates from untreated household wastewater, which infected like toilet waste water. It consists of water from bathtubs, showers, hand basins, laundry, floor wastes, kitchen sinks, dishwashers and washing machines (Pathan et al. 2011). Several organizations have taken up the initiative of treating wastewater. Grey water recycling is now accepted solution to increase in the fresh water demands, water shortages and for environmental protection. Grey water is generated from household activities such as laundry, dish washing and bathing without including toilet (Jefferson et al. 1999; Otterpohl et al. 1999; Eriksson et al. 2003; Ottoson and Stenstrom 2003). One such initiative taken was by coca cola. In urban slums, the grey-water accounts for 65–90 % of the domestic wastewater production (Katukiza et al. 2014). The main problem is treating the grey water in a sustainable way. Grey water is a worldwide concern and has a biological diversity of aquatic ecosystems, disrupting the essential reliability of our life is extensive from city development, food disposals and industry emissions. Practically all human activity results in the production of grey water, with the rising global population and financial activities, the amount of grey water will also increase. The domestic water demand in industrialized countries varies between 100 and 180 liters/capita/day (L/c/d) (Maimon et al. 2010), of which 70 % is transformed into grey water as the rest is black water (i.e., water from flushing the toilet) (Friedler et al. 2005). Control of pollution sewage and industrial wastewater is a major challenge for almost all the parts in India, as 16 % of fresh water resources compared Indian households total of 2.5 % of the total world. The total usage of water is 91 % for domestic purposes and municipal, industrial and maintains purpose for about 9 % (Brown and Matlock 2011). Grey water generates from bathing 50–60 %, cloth washing 25–35 % and kitchen 10 % (NEERI 2007).

The water requirement for each person was expected as 100 L/day for domestic purposes. It was estimated in a study in 1991 by the Department of Agriculture. The basic domestic activities such as food preparation, drinking and washing can be met by smaller quantity 94.5 L. Additional up to date (Falkenmark and Widstrand 1992; Gleick 1996) give estimates of basic water necessity between 50 and 100 L/c/d. There are many ongoing research projects in this topic, since grey water is the most important resource for the whole mankind and living being for their survival. The majority strict criteria for grey water require a biochemical oxygen demand (BOD) lesser amount of 10 mg/L, (USEPA 2004; Tajima 2005). There are various methods applied for the effective treatment of the grey water, which includes physico-chemical, biological methods, etc. Traditional methods are naturally adopted by altering the physico-chemical parameter naturally by infiltration and by sand bed methods. The latest methods are membrane technology, ORCA (physical, chemical treatment), rotating biological contractors, sequencing batch rotators, ion exchange rexin magnetic (MIEX) and a small number of other methods are used for recycling.

Experiences of treating grey water by physico-chemical treatment systems have not been widely reported. In this study the pollutants of grey water are treated and reduced by the biofilter bed method. The reuse grey water eco-friendly treatment would be useful for recycling the grey water for various domestic purposes. Grey water reduces the fresh water usage and solves scarcity; it could be very helpful for country's health and development.

2 Materials and Methods

2.1 Grey Water Sample Collection

Waste water was collected from the hostel on a daily basis for about 35 days (300 × 35 = 10,500 L). Around 300 L of liquid wastes were treated in the biobed designed for wastewater treatment. The waste water before it was passed through bio-beds, was initially assessed for 20 parameters (Table 1).

Table 1 Parameters and methods employed in the physico-chemical examination of water samples

S. no	Parameter		Method
1	pH	pH	pH meter (henna)
2	EC, µS/cm	Electrical conductivity	Electrical conductivity meter (henna)
3	TDS, ppt	Total dissolved solids	Total dissolved solids meter (henna)
4	TA, mg/L	Total alkalinity	Volumetric method
5	CO_3^{2-}, mg/L	Carbonate	Volumetric method
6	HCO_3^-, mg/L	Bicarbonate	Volumetric method
7	TH as $CaCO_3$, mg/L	Total hardness	Volumetric method
8	CaH as $CaCO_3$, mg/L	Calcium hardness	Volumetric method
9	Ca, mg/L	Calcium	Volumetric method
10	Mg, mg/L	Magnesium	Volumetric method
11	Cl^-, mg/L	Chloride	Argentometric method
12	NO_3^-, mg/L	Nitrate	Colorimetric method
13	PO_4^{3-}, mg/L	Phosphate	Colorimetric method
14	SO_4^{2-}, mg/L	Sulphate	Colorimetric method
15	Na, mg/L	Sodium	Flame photometric method
16	K, mg/L	Potassium	Flame photometric method
17	DO, mg/L	Dissolved oxygen	Do meter (lutron, do-5509)
18	BOD_3, mg/L	Biochemical oxygen demand	Winkler's mayer method
19	COD, mg/L	Chemical oxygen demand	Volumetric method

2.2 Design of the Biobeds

Totally four biobeds were made for treating the waste water.

Bio-bed 1: Material Used: Rectangular tank was made using Kadappa slabs with following dimension 55 cm (l) × 39.5 cm (b) x 23 cm (h). The tanks of specified dimension were filled vertically with gravel and sand in an alternate columns each at a width of 17 and 10.5 cm respectively. Totally there were 2 gravel and 2 sand columns in an alternate manner. The beds were initially washed with tap water to release trapped air packs and void spaces. Then the surface of the bed was planted with *Lipia nodiflora* as vegetative cover. The vegetative cover was purposely introduced in the system to retain the alignment of alternate columns of sand and gravel in addition to trap the suspended impurities of the influent. Since the suspended solids are filtered by both the plant roots and alternate gravel and sand columns, instead of mere physical removal of suspended impurities, they are also biodegraded and this would help the system to rejuvenate on its own without getting choked up with filtered materials. The specifications of the bio-bed 1 are tank dimension: 55 cm (l) × 39.5 cm (b) × 23 cm (h); height of the biobed: 20 cm; the remaining 3 cm was unfilled to allow the water samples entering into the structure. Size of the gravel: 6–8 mm; sand: 2 mm; number of gravel and sand column: 2 each; plant component: *Lipia nodiflora.* The tank was kept just below the sewage tank outlet exactly so that the waste water will run through columns of gravel, sand gravel and sand. The idea behind passing the waste water in this manner will facilitate effective filtration of suspended impurities and will facilitate oxygen diffusion in the waste water flowing through gravel and sand columns. Roots of the vegetative cover will find sufficient air and space for respiration and rooting.

Biobed 2: Rectangular tank was made using Kadappa slabs with following dimension 55 cm (l) × 39.5 cm (b) × 23 cm (h). The tank was filled horizontally with sand (7 cm), coir waste (6 cm), sand (7 cm) and leaving 3 cm area unfilled to capture water entering into the structure. The surface of the bed (over sand layer) was planted with *Lipia nodiflora* as vegetative cover (for similar reason as mentioned earlier). The specifications of the bio-bed 2 are tank dimension: 55 cm (l) × 39.5 cm (b) × 23 cm (h); height of the biobed: 20 cm; the remaining 3 cm was unfilled to allow the water samples entering into the structure. Size of the Sand: 2 mm; number of sand and coir layer: coir sandwiched between 2 layers of sand; plant component: *Lipia nodiflora*.

Bio-bed 3: Rectangular tank was made using Kadappa slabs with following dimension 55 cm (l) × 39.5 cm (b) × 23 cm (h). The tank was filled horizontally with gravel (2 cm), clay (0.5 cm), red soil (14.5 cm), humus (3 cm) and leaving (3 cm) area unfilled to accommodate water entering into the structure. Tank bottom up to 2 cm height was filled with gravel, followed by clay 0.5 cm (collected from pond), red soil 14.5 cm and humus (partially degraded plant debris) for about 3 cm. The tank was finally planted, with vetiver grass (60 tillers of *Vetiveria zizanioides)* at 15 cm depth. The specifications of the bio-bed 3 are tank dimension: 55 cm (l) × 39.5 cm (b) × 23 cm (h); height of the biobed: 20 cm; the remaining 3 cm was unfilled to allow the water samples entering into the structure. Size of the gravel:

approximately 6 mm; number of gravel, clay, red soil and humus layer: each layer; plant component: Vettiver (*Vetiveria zizanioides).*

Filter-bed 4: Rectangular tank was made using Kadappa slabs with following dimension 55 cm (l) × 39.5 cm (b) × 23 cm (h). The tank was filled up to (8 cm) from the bottom with sand, followed by charcoal (7 cm), sand (5 cm) and leaving 3 cm area unfilled to provide space for water entering into the structure. The specifications of filter-bed 4 are tank dimension: 55 cm (l) × 39.5 cm (b) × 23 cm (h); height of the biobed: 20 cm; the remaining 3 cm was unfilled to allow the water samples entering into the structure. Size of the sand: 2 mm, charcoal powder: finely powdered and passed through sieve (0.500 mm); number of sand and charcoal layer: charcoal sandwiched between 2 layers of sand; biobeds and filter bed were washed initially and irrigated subsequently with tap water for one month for allowing the plants for establishment. The graphical representation of treatment beds were illustrated in Fig. 1.

After the establishment of plants, treatment beds were used for grey water treatment. Around 300 mL of grey water was treated on daily basis (300 × 35 = 10,500 L) for 35 days; for which the grey water was collected from the common wastewater collection facility available at student's hostel. Flow rate of the water was adjusted to 3.4 mL/s/day. The wastewater both before and after treatment was analysed for pH, electrical conductivity, total dissolved solids, total alkalinity, carbonate, bi-carbonate, total hardness, calcium hardness, calcium, magnesium, chloride, nitrate, phosphate, sulphate sodium, potassium, dissolved oxygen, biological oxygen demand, chemical oxygen demand and total organic carbon.

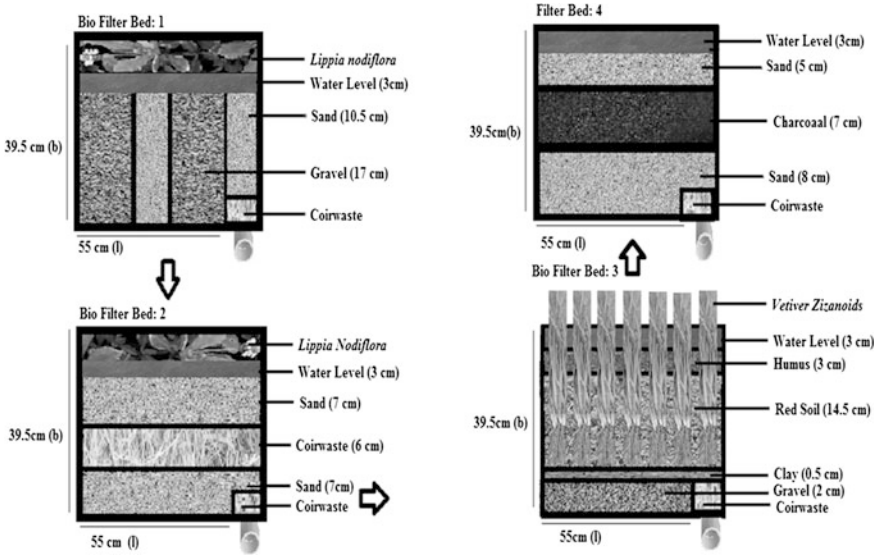

Fig. 1 Design of treatment beds

2.3 Analysis of Samples

Changes in pH, electrical conductivity, total dissolved solids, total alkalinity, carbonate, bi-carbonara, total hardness, calcium hardness, calcium, magnesium, chloride, nitrate, phosphate, sulphate sodium, potassium, dissolved oxygen, biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon were monitored at the end of completion of the treatment (8 h). pH meter, TDS (ppt) meter and EC (µS/cm) meter (Henna) and DO meter (Lutron, DO-5509) (mg/L) used for the analyses were, calibrated every one hour to maintain the accuracy. Parameters such as total alkalinity, carbonate, bi-carbonate, total hardness, calcium hardness, calcium, magnesium, chloride, nitrate, phosphate, sulphate sodium, potassium, BOD, COD and total organic carbon were estimated (Table 1) by American public health association procedure (APHA 2005).

A total of 10,500 L of grey water was treated through treatment system over a period of 35 days. The reason for keeping the standard 8 h running period is, in real time situation, grey water generation at homes is taking place on an average for 8 h (morning 6–10 a.m. and evening 6–10 p.m.). Additionally, the fundamental reason for designing a bio-bed is to aid the residents in managing their wastewater at grassroot level without seeking assistance from municipal corporation for sewerage system. Moreover the implementation of the described facility will also facilitate blue water conservation by reusing such treated water for other purposes like flushing the toilets, car washing, recharging borewells and backyard gardening.

3 Results and Discussion

The present study has used natural materials, sand, pea gravel, coir pith, humus, red soil, clay and charcoal as bio filter for the gray water along with plants like *Lippia nodiflora* and *Vetiveria zizanioides* growth and were able to ensure higher level of water purification in a natural and economic way. All physico-chemical parameters of untreated grey water and treated water was calculated for 35 days. The grey water present in untreated and filter bed treated water shown positive effect on the pH level (Fig. 2). The average level of pH reduced from 8.3 to 7.9 (Table 2). The deviation in pH was observed in each filter bed because each filter bed had different capacity of adsorption of ions.

The average EC values of treated water reduced from 1781 reduced 709 µS/cm after filtration (Table 2). The EC removal rate treated water are presented in Fig. 3.

The TDS values untreated and treated were reduced from 1.11 to 0.45 ppt (Table 2). The TDS removal values are presented in Fig. 4. The total dissolved solid enhances electrical conductivity. Dissolved solids in the grey water are not significant unlike suspended solids and total solids and would clog when water is used for irrigation purpose. The TDS reduction was the effect of sedimentation, filtration bacterial decomposition and adsorption (Arivoli and Mohanraj 2013).

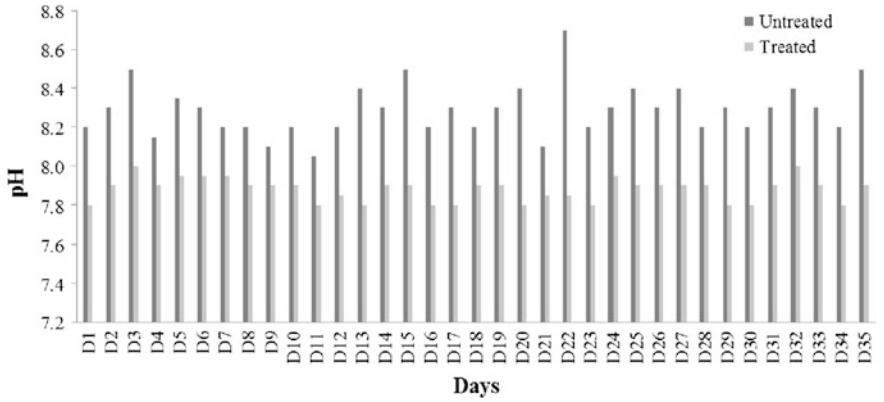

Fig. 2 pH of untreated grey water and treated water

Table 2 Physico-chemical parameters of untreated grey water and treated water

Parameters	Untreated grey water	Treated water
Physical parameters		
Colour	Greyish white	Colourless
Odour	Rotten egg smell	Odourless
Transparency	Cloudy	Transparent
Chemical parameters		
pH	8.3 ± 0.1	7.9 ± 0.1
EC, µS/cm	1781 ± 96	709 ± 56
TDS, ppt	1.11 ± 0.06	0.45 ± 0.04
TA, mg/L	252 ± 40	128 ± 14
CO_3^{2-}, mg/L	21 ± 12	0
HCO_3^-, mg/L	281 ± 48	156 ± 17
TH as $CaCO_3$, mg/L	382 ± 23	145 ± 14
Calcium hardness, mg/L	205 ± 12	80 ± 7
Ca, mg/L	82 ± 5	32 ± 3
Mg, mg/L	43 ± 3	16 ± 3
Cl, mg/L	277 ± 17	100 ± 8
NO_3^-, mg/L	17 ± 3	6 ± 1
PO_4^{3-}, mg/L	3.6 ± 0.2	1.2 ± 0.2
SO_4^{2-}, mg/L	44 ± 2	10 ± 2
Na, mg/L	132 ± 9	30 ± 4
K, mg/L	19 ± 5	5 ± 1
DO, mg/L	0.3 ± 0.2	3.7 ± 0.4
BOD_3, mg/L	133 ± 11	15 ± 3
COD, mg/L	357 ± 27	68 ± 7

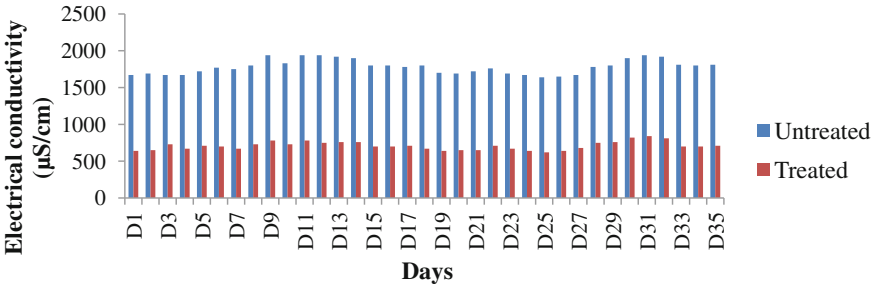

Fig. 3 Electrical conductivity of untreated grey water and treated water

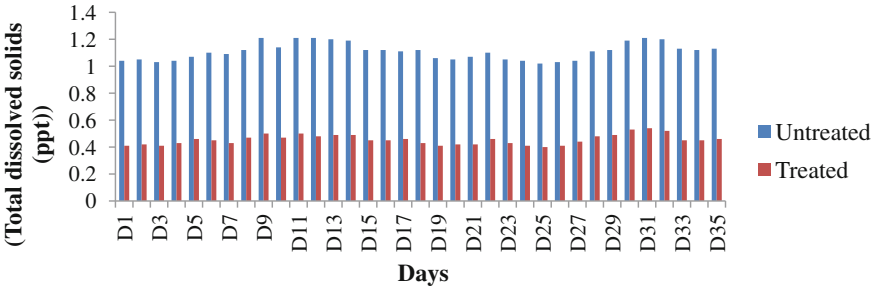

Fig. 4 Total dissolved solids of untreated grey water and treated water

Alkalinity (bi-carbonate and carbonate) would improve the acid neutralizing capability and ensure pH lesser than 4.3. The average of total Alkalinity untreated and treated reduced from 252 to 128 mg/L. The average of bi-carbonate measure untreated and treated reduced from 281 to 156 mg/L, average of carbonate measurement indicated untreated and treated reduced from 21 to 0 mg/L (Table 2). The removal rate of total alkalinity, carbonate and bi-carbonate in untreated and treated water were presented in Figs. 5, 6 and 7.

Most hardness is due to water containing dissolved calcium or magnesium compounds expressed in milligrams of calcium carbonate equivalent per liter. The average of total hardness untreated and treated was found to get reduced from 382 to 145 mg/L. The average calcium hardness removal rate of untreated and treated reduced from 205 to 80 mg/L (Table 2). The removal rate of treatment was presented in Figs. 8 and 9.

The average removal rate of untreated and treated reduced from calcium, magnesium, chloride of sodium, sodium and potassium values are presented in 82,

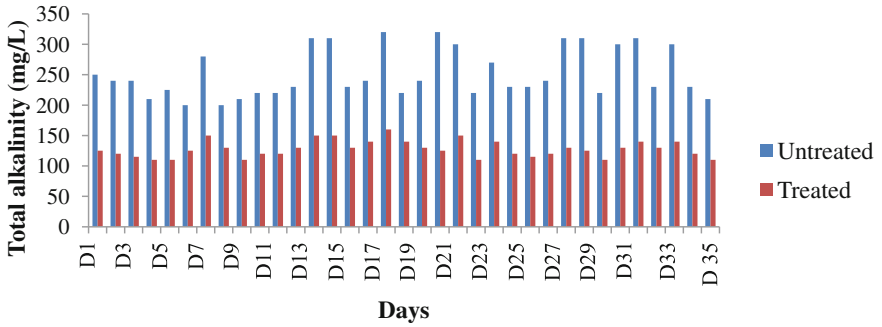

Fig. 5 Total alkalinity of untreated grey water and treated water

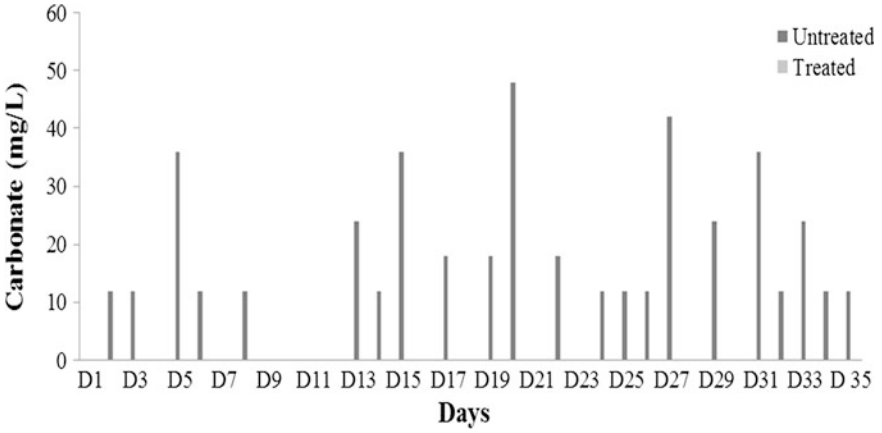

Fig. 6 Carbonate level of untreated grey water and treated water

43, 277, 132 and 19 mg/L were able to get reduced to 32, 16, 100, 30 and 5 mg/L respectively (Table 2). The removal rate of untreated and treated was presented in Figs. 10, 11, 12, 13 and 14.

Sodium chloride present in grey water was utilized by microorganisms during waste treatment. The sodium level of most shampoos and conditioners was low, although the long term effects of some of the shampoos would need to be investigated further as low sodium content in grey water can still cause long-term problems when the levels of other captions such as calcium and magnesium are low (Graaff and Patterson 2001). This also applies to the body washes.

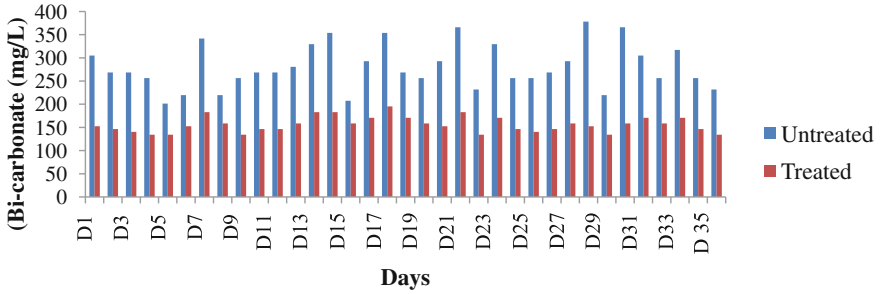

Fig. 7 Bi-carbonate level of untreated grey water and treated water

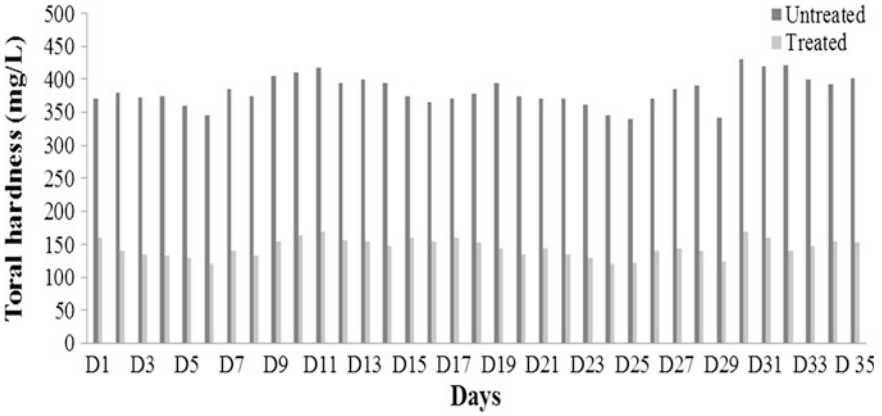

Fig. 8 Total hardness of untreated grey water and treated water

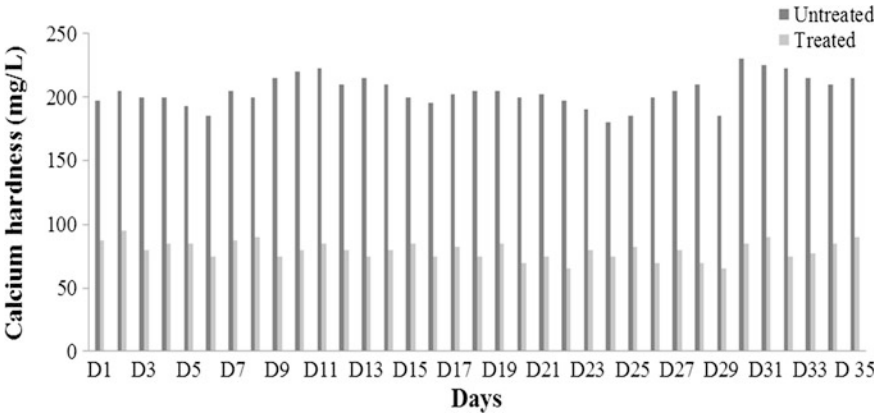

Fig. 9 Calcium hardness of untreated grey water and treated water

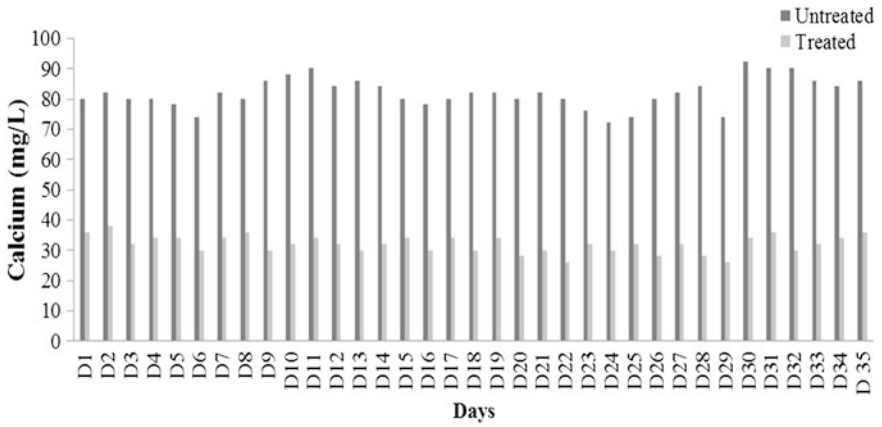

Fig. 10 Calcium level of untreated grey water and treated water

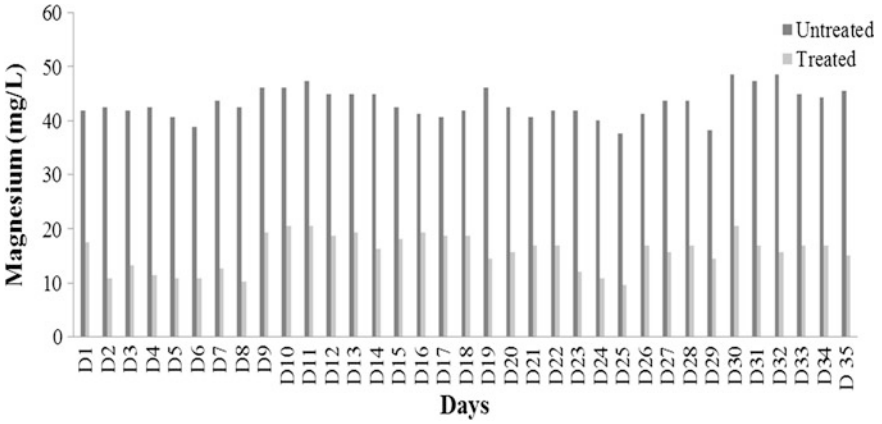

Fig. 11 Magnesium level of untreated grey water and treated water

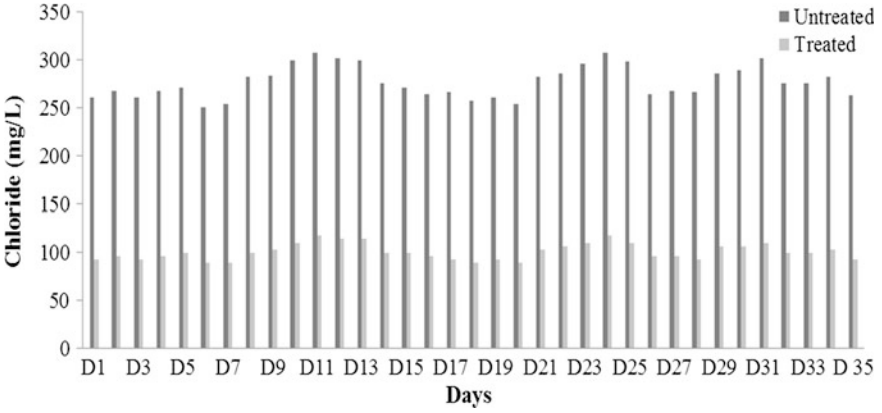

Fig. 12 Chloride level of untreated grey water and treated water

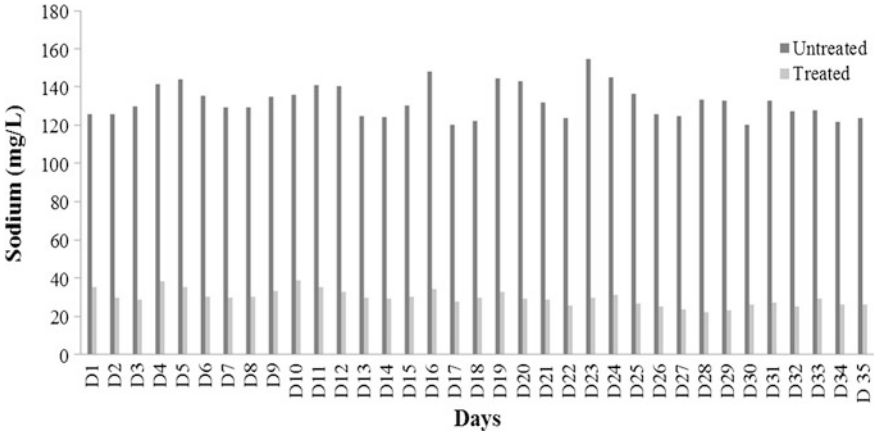

Fig. 13 Sodium level of untreated grey water and treated water

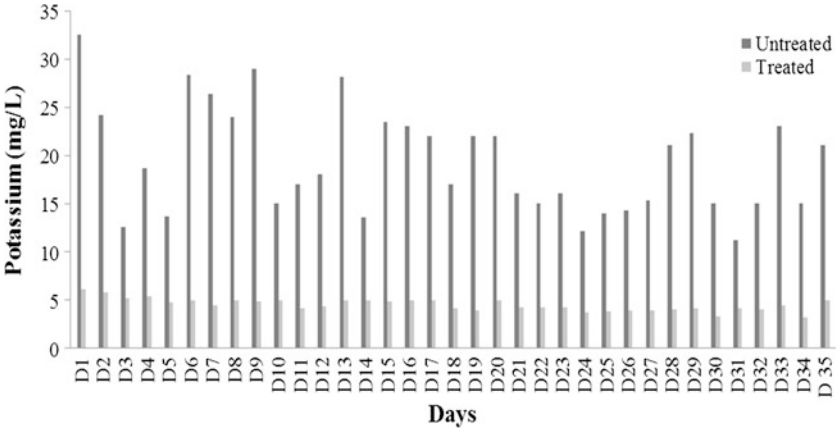

Fig. 14 Potassium level of untreated grey water and treated water

The average phosphate untreated and treated reduced from 3.7 to 1.2 mg/L. The critical nutrients in artificial detergents turned as degraded form of protein (Table 2). Thanh et al. (2006) reported that phosphate free detergents could significantly reduce negative impact on the environment and public health. Li et al. (2009) reported that kitchen grey water has a balanced COD: N: P; which is safe for irrigation. The phosphate removal rate of untreated and treated was presented. The Phosphorus removal in Constructed wetland was because of precipitation and adsorption (Prochaska and Zouboulis 2006). The Nitrates and sulphate untreated

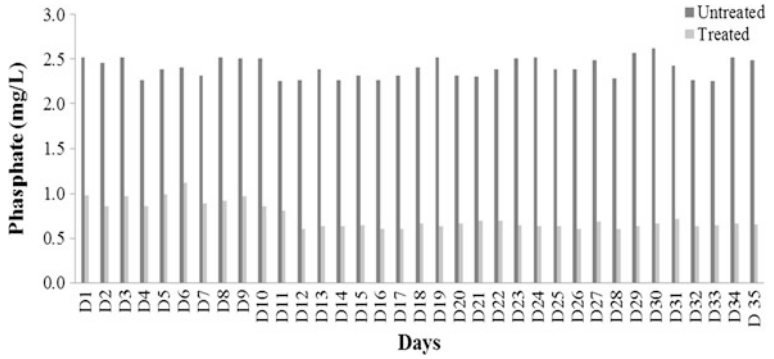

Fig. 15 Phasphate level of untreated grey water and treated water

and treated from 17 and 44 mg/L reduced to 6 and 10 mg/L respectively (Table 2). The phosphate, nitrates and sulphate removal rate in untreated and treated are presented in Figs. 15, 16 and 17.

The average dissolved oxygen untreated and treated enhanced from below 0.3 mg/L to increased 3.7 mg/L. The average BOD untreated and treated reduced from 133 to 15 mg/L. The average COD level untreated and treated reduced from 357 to 68 mg/L (Table 2). The decrease of organic matter due to decomposition was achieved by biological communities comprising aerobic, anaerobic and facultative, algae and protozoans attached to the filter medium (Ling et al. 2009). The DO, BOD and COD removal rate of untreated and treated was presented in Figs. 18, 19 and 20.

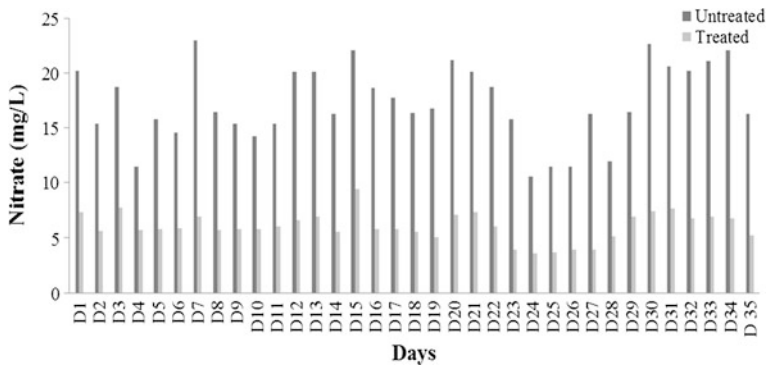

Fig. 16 Nitrate level of untreated grey water and treated water

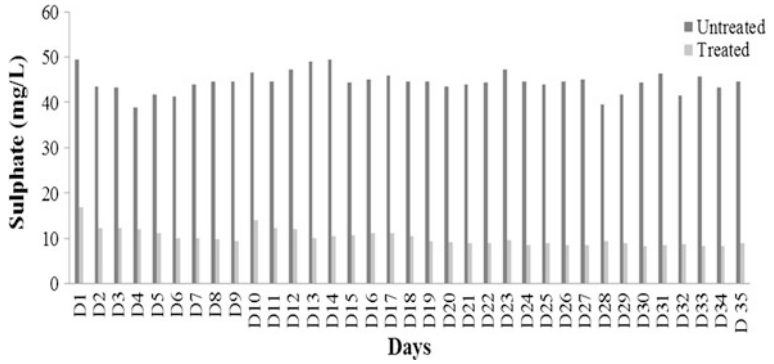

Fig. 17 Sulphate level of untreated grey water and treated water

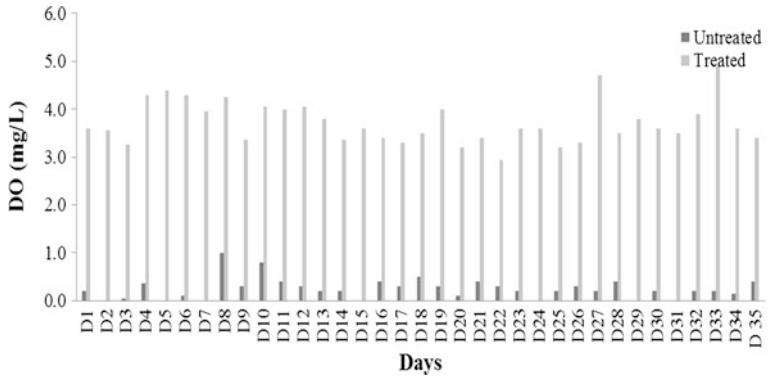

Fig. 18 Dissolved oxygen level of untreated grey water and treated water

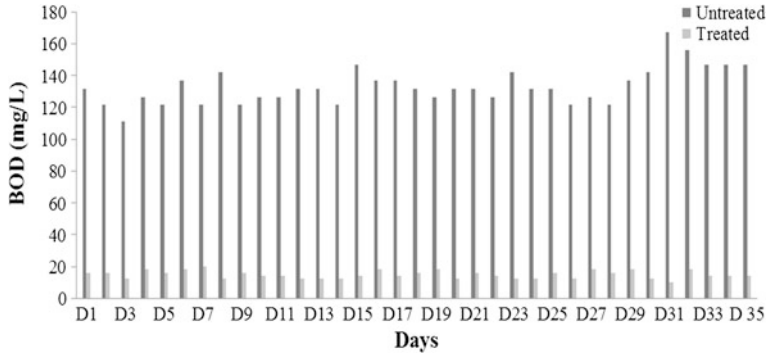

Fig. 19 Biological oxygen demand of untreated grey water and treated water

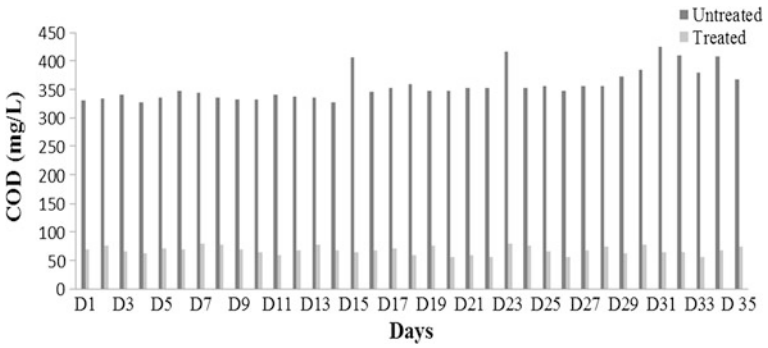

Fig. 20 Chemical oxygen demand of untreated grey water and treated water

4 Conclusion

The study was able to conclude that vertical flow constructed wetland can be utilized effectively for water utilization through reuse of waste water after treatment to purposes like landscape irrigation, gardening, plant growths and toilet flushing. The designed low-cost technology for grey water has high potential for water usage efficiency and would percent removal in biological oxygen demand, chemical oxygen demand, chloride, total alkalinity, carbonate, nitrate, phosphate and bicarbonate. There is a need to assess if the treatment and disinfection is effective against other microorganisms like viruses that are usually present in grey water and would be the scope for further improvisation of the study. The consumable materials used in this system such as flocculants and disinfectants are reasonable and locally available materials, sand, gravel, clay, red soil, humus, coir waste, charcoal *Lippia nodiflora* and *Vetiveria zizanioides*were economical and also acts as effective purifier. However the absolute efficiency of reusing treated water in flushing and gardening activity shall be established through further studies.

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Optimization of *Bacillus* sp. (KTSMBNL-16) for Decolourization of Dye AV90 Using Batch Studies

P. Prakash, M. Dexilin, B. Gowri Manogri and K. Thamaraiselvi

Abstract Microbial community present in contaminated sites are able to tolerate any adverse conditions, due to their adaptation to extreme circumstances. Hence, in this study the bacterium was isolated from tannery effluent for the purpose of AV90 dye decolourization. Based on 16S rRNA sequence analysis the isolate was identified as *Bacillus* species (KTSMBNL-16). Decolourization process by KTSMBNL-16 was optimized for different parameters such as pH and temperature under shaking condition. Maximum decolourization (82 %) of AV90 dye was observed at an initial pH 6.0 and temperature 40 °C using *Bacillus* species. To disclose the role of functional groups in dye decolourization, FTIR spectrum (4000–400 cm^{-1}) was carried out for control and the treated sample. The spectrum reveals the significant changes in the position of the peaks, when compared to the control and decolourization products. Hence, *Bacillus* sp. KTSMBNL-16 could be used a potential candidate for the treatment of textile dyes and industrial effluents. The finding in the present study can serve as an important base for the development of an economical as well as a simplified biological treatment system for dye decolouration.

Keywords Decolourization · AV 90 · *Bacillus* species · FTIR

1 Introduction

Rapid growth in industrialization, urbanization and man's urge for color has caused the increased usage of dyestuff day by day. Even though the use of dyes makes the world more beautiful, it represents a serious pollution problem worldwide. Dyes have been widely used in textile, dyeing, cosmetics, paper, leather, color photography,

P. Prakash · M. Dexilin · B. Gowri Manogri · K. Thamaraiselvi (✉)
Laboratory of Molecular Bioremediation and Nanobiotechnology, Department of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India
e-mail: kthamaraiselvi@hotmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_19

pharmaceutical, food and other industries because of their ease and cost effectiveness in synthesis, firmness and variety in color (Sathiya Moorthi et al. 2007).

More than commercially 100,000 dyes are available with over 7×10^5 of tons of dyestuff produced annually (Robinson et al. 2001; Wang et al. 2009). In addition to that, solitary dyeing operation can utilize numerous dyes from diverse chemical classes resulting in a complex wastewater (Correia et al. 1994). In 1978, it was predicted that 2 %, (or 9000 tones) of the 4,50,000 tones of dye manufactured worldwide is discharged as effluent from manufacturing operations (Brown 1987).

Azo dyes comprise the largest chemical class of synthetic dyes which are more versatile. They play a significant role in almost every type of application and are widely used as colorants (Wong and Yuen 1996). More than 60–70 % of 10,000 dyes predominately used in the textile industry are azo dyes (Carliell et al. 1995). In case of acid dyes, azo group is the most important chromophore. It has been estimated that almost one million metric tones of dyes are produced annually in the world, of these azo dyes represent about 70 % by weight (Dos Santos et al. 2003).

Most of the dyes are visible in water and the concentrations are as low as 1 mg l^{-1}. The release of colored effluents into the environment is undesirable; not only because of their color, since the dyes from wastewater and their breakdown products are toxic and/or mutagenic to life (Weisburger 2002). Textile-processing wastewaters contain dye concentration in the range of 10–200 mg l^{-1} and are usually regarded as highly colored. Though dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environments. The ecotoxic nature of the dye affects the ecosystem through the food chain. Moreover, dyes also serve as one of the major sources of heavy metals (Wagner 1993) in water and soil (Zehra et al. 2009) and their persistent nature causes misbalance in the ecosystem. The toxic nature of textile dyes causes environmental pollution in developing nations, since the effluents from textiles are often untreated and discharged into river and open fields (Bakshi et al. 1999).

Biological treatment is the most popular and efficient method of industrial effluent treatment methods. Microorganisms capable of decolorizing azo dyes include gram-positive, gram-negative bacteria (Moosvi et al. 2005) and fungi (Verma and Madamwar 2005). Even though azo dyes are aromatic compounds, their substituents contain mainly nitro and sulfonic groups, and are quite recalcitrant to aerobic bacterial degradation (Claus et al. 2002). The non-specific action of anaerobic bacteria facilitates the removal of wide range of textile dyes. Mostly the decoloration of reactive azo dye under anaerobic conditions is a co-metabolic reaction (Stolz 2001).

The present study is focused on the identification of bacterial species to decolorize azo dye C.I. Acid Violet 90 based on 16S rRNA analysis and also to optimize the culture conditions namely pH, temperature, time and initial dye concentration. The degraded products were analysed using TLC and FTIR.

2 Materials and Methods

2.1 Sampling

Tannery effluent samples were collected from Common Effluent Treatment Plant (CETP), Chrompet, Chennai. Grab samples of the tannery effluent were collected at the point of outlet discharge from CETP. The effluent sample was collected in sterile, dry and stoppered polypropylene bottles.

2.2 Dyes and Chemicals

The dye, namely C.I. Acid Violet (AV 90), a reddish violet 1:2 metal complex was used as a model dyes in this study. The dye was obtained from Rajasthan Dye Chemicals Ltd., Chennai, India. Majority of chemical compounds and media components were purchased from Himedia Labs, Mumbai, India. All chemicals used were of the highest purity available and of analytical grade.

2.3 Dye Stock Solution

Stock solution (1000 mg l^{-1}) of C.I Acid Violet 90 was prepared and sterilized by filtration (Millipore, Millex-GS 0.22 μm filter unit) because they are unstable in moist-heat sterilization. Solutions of the desired concentrations were obtained by successive dilution.

2.4 Identification of Bacteria Based on 16 S rRNA Sequence Analysis

The extraction of bacterial DNA, agarose gel electrophoresis, PCR amplification for 16S rRNA, purification of the amplified product and phylogenetic tree analysis of 16S rRNA were followed according to the procedure of Dexilin et al. (2013).

2.5 Decolorization Experiments

2.5.1 Effect of pH on the Decolorization of AV 90 by *Bacillus* sp.

The effect of pH was studied in Minimal Basal Medium (MBM) amended with 50 mg l^{-1} of the dye (AV 90). The pH was varied (2, 4, 6, 8, 10 and 12) using dilute

HCl or NaOH. The culture was inoculated and incubated under static condition at 35 °C. Samples were separated by centrifugation at 10,000 rpm for 15 min and dye decolorization was determined spectrophotometrically.

2.5.2 Effect of Temperature on the Decolorization of AV 90 by *Bacillus* sp.

The effect of temperature on decolorization of dyes were carried out in a series of 250 ml conical flasks containing MBM amended with 50 mg l^{-1} of AV 90. The temperature was varied from 25 to 50 °C. The pH of the medium was adjusted to 7.0 as determined from the above experiment and each flask was inoculated with precultured cells (OD 0.1 at 600 nm). Samples were separated by centrifugation at 10,000 rpm for 15 min and the dye decolorization was noted.

2.5.3 Spectrophotometric Analysis of Dye Concentration and Bacterial Growth

Decolorization was monitored by UV-VIS spectrophotometer (UV-1700 Shimadzu, Japan). The absorbance peak (λ_{max}) of AV 90 were determined and it was found to be at 526 nm. The percentage of decolorization was calculated from the difference between initial and final absorbance values.

Growth of the bacterial culture at different time intervals during decolorization of dye was monitored spectrophotometrically. Upon centrifugation (6000 rpm for 15 min) of 5 ml culture, the cell pellet obtained was suspended in 5 ml of distilled water and its absorbance was read at 600 nm.

2.5.4 Extraction of the Metabolites and Characterization

Decolorized broth was centrifuged at 10,000 rpm for 10 min. The metabolites produced during the biodegradation of Acid violet 90 were extracted with the equal volume of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of HPLC grade methanol and the same sample was used for UV-visible spectral, FTIR and TLC analyses.

UV-visible spectral analysis of before and after decolorization of dye was carried in the visible range (400–800 nm). FTIR analysis was carried out using Perkin Elmer make-model spectrum RXI (range 4000–400 cm). TLC analysis was carried out on silica gel using mobile phase solvent system of n-propanol, methanol, ethyl

acetate, water and glacial acetic acid (3:2:2:1:0.5) and the spots were developed using iodine chamber.

3 Results and Discussion

3.1 Identification of Dye Decolorizing Bacteria

The genomic DNA was extracted from the decolorizing bacteria and it was run on 0.8 % agarose gel electrophoresis. The molecular weight of genomic DNA was determined as ~1500 bp (Fig. 1).

A distinct PCR product of the ~1400 bp size was produced when target DNA from isolated strain was used. From this analysis, it was determined that 25 cycles and 0.1 ng of genomic DNA is required for successful amplification.

Partial sequencing of the 16S rRNA resulted in 1224 nucleotide length. The separation of the isolated KTSMBNL17 from other *Bacillus* sp. was supported by boot strap value of 51 %. Phylogenetic analysis of tree building using other methods also supported this grouping method with high boot strap values. The boot strap and values higher than 51 % were indicated on the tree (Fig. 2).

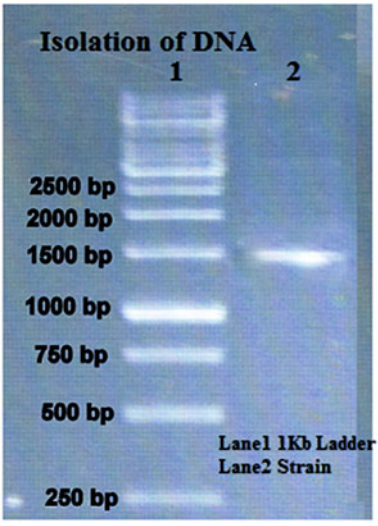


Fig. 1 Agarose gel electrophoresis of extracted DNA

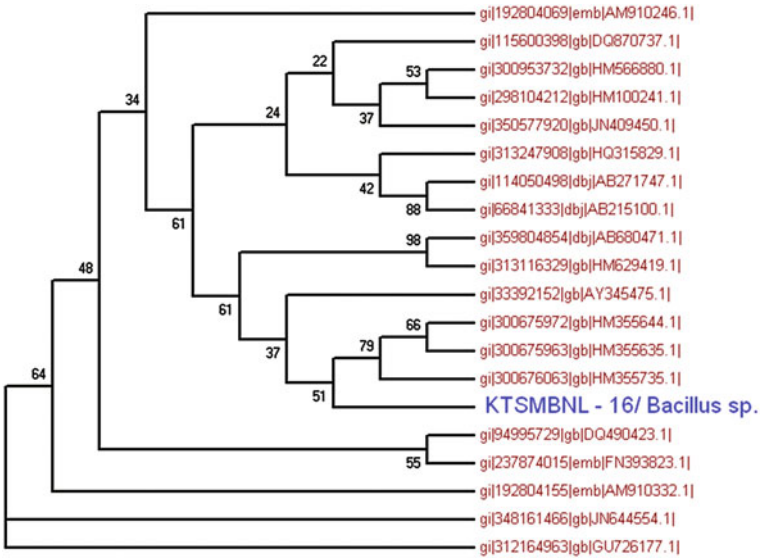


Fig. 2 Phylogenetic tree for AV 90 decolorizing bacteria

3.2 Decolorization Experiments

3.2.1 Optimization of pH

The effect of initial pH (2, 4, 6, 8, 10 and 12) of MBM on the biodegradation efficiency for AV 90 was analyzed and is presented in Fig. 3. The increase of pH from 2 to 12 led to a threefold increase in decolorization rate which reached the maximum value of 75 % at pH 6.

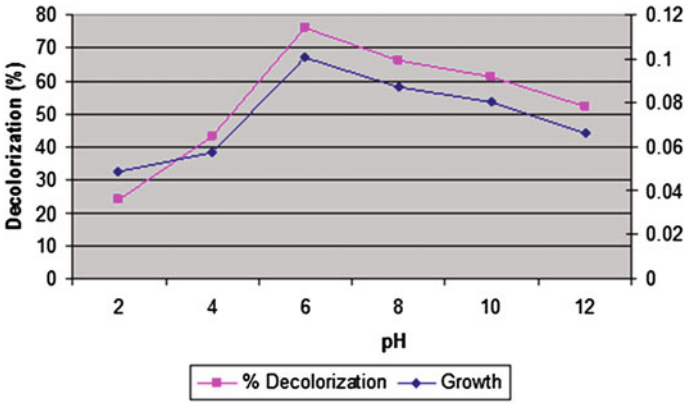


Fig. 3 Optimization of pH

3.2.2 Optimization of Temperature

The increase of temperature from 25 to 50 °C led to a threefold increase in decolorization rate which reached the maximum value of 75 % at 40 °C. The results mainly showed no thermal inactivation of decolorization activity under operational temperature, indicating that *Bacillus* sp. are active at a broad range of temperature which leads to their use for the treatment of dyeing wastewater (Fig. 4).

3.2.3 Characterization of Decolorized Dye

To disclose the possible mechanism of the dye decolorization, we have analyzed the biodegraded products of AV90 by TLC, UV-VIS (400–800 nm). Pecks observed at 540 nm (0 h) decreased without any shift in λ_{max} upto complete decolorization of the dye (24 h) and it was evidence in the removal of dye with the absorbance at λ_{max} being virtually zero after 48 h incubation (Fig. 5). TLC analysis showed the appearance of two spots in the sample containing the extracted metabolites of complete decolorized medium (48 h). The R_f value of AV90 was noted as 0.90, confirming the biodegradation of AV90 by *Bacillus* sp. (Fig. 6).

3.2.4 FTIR Analysis

FTIR spectroscopy is a useful tool for quantifying secondary structure of the compound—protein interaction by the absorption of Infra Red (IR) radiation through resonance of non-centro symmetric (IR active) modes of vibration. Analyzing the highly complex IR spectra, certain characteristic peaks can be assigned to the main functional groups. Remarkable variations in the fingerprint region between the FTIR (4000–400 cm^{-1}) spectrum of control (dye extract) and

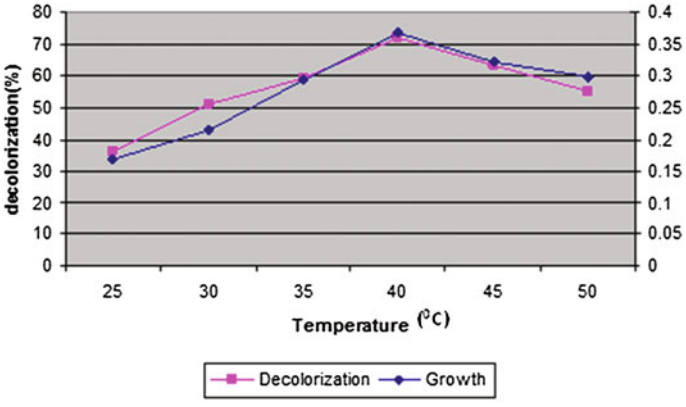
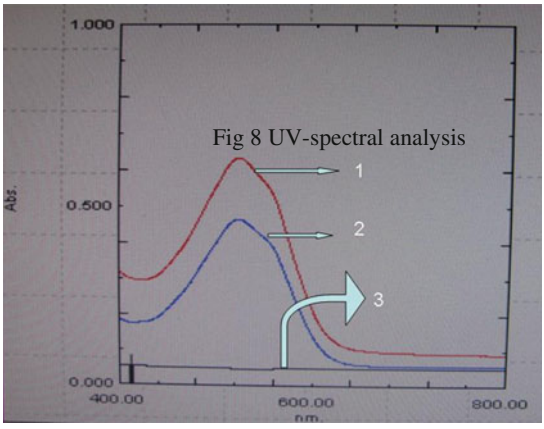


Fig. 4 Optimization of temperature

Lane 1- Decolorized dye

Lane 2- Dye

Fig. 5 TLC analysis



Lane 1 -48 h

Lane 2 -24 h

Lane 3-Before decolorization

Fig. 6 UV-spectral analysis

sample (extracted metabolites) demonstrated the dye biodegradation. The FTIR spectrum of the control is shown in Fig. 7 displayed a peak at 3410 cm^{-1} for primary amines, the stretching vibration between C=O was reported at 1711 cm^{-1}, whereas a peak at 1127 cm^{-1} represented C–O stretching of alkyl substituted ether. The peak at 2115 cm^{-1} showed alkynes group. The FTIR spectrum of the degraded product showed significant change in the position of the peaks, when compared to the control. The FTIR peaks of the degraded dye is shown in Fig. 8. The peaks at 2776 cm^{-1} and 2246 cm^{-1} corresponds to the stretching vibrations of methylamino

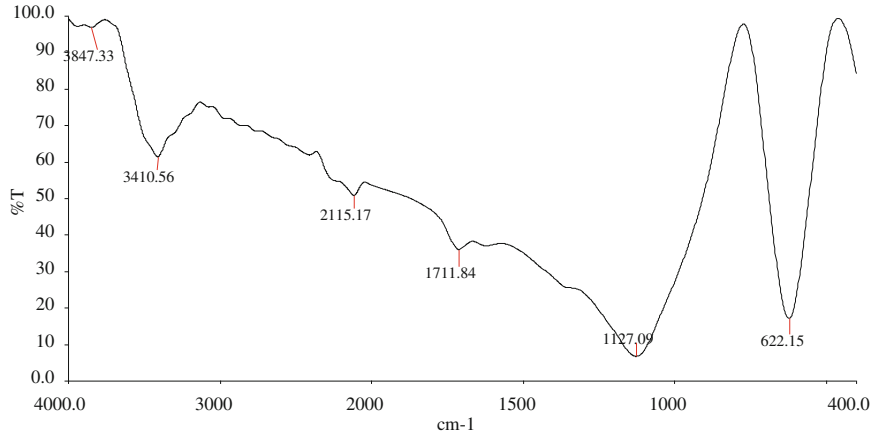


Fig. 7 FTIR spectrum of dye AV 90

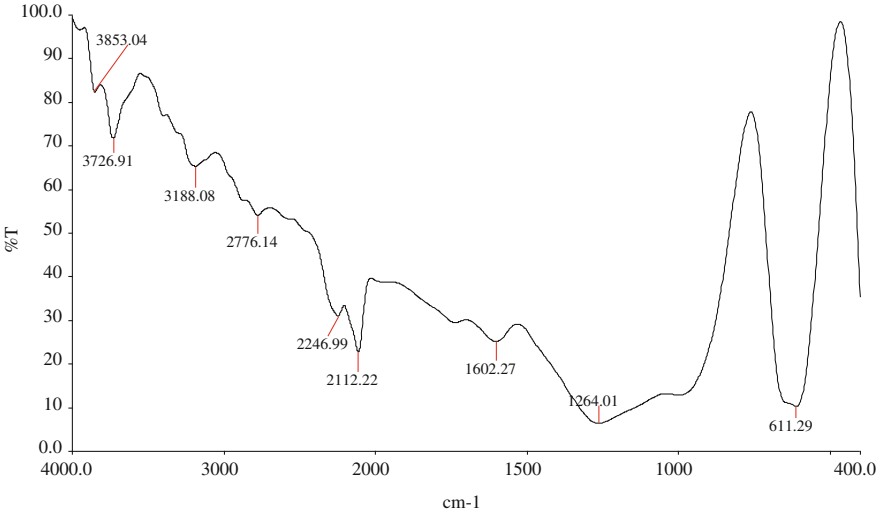


Fig. 8 FTIR spectrum dye AV 90 after degradation

and nitrite group while their bending vibrations were seen at 2112 cm^{-1} and the peak of 1602 cm^{-1} corresponds to an assigned functional group of alkynes and alkenes. The peaks at 1264 and 611 cm^{-1} corresponds to the stretching vibrations of carboxylic acids derivatives and disulfides.

4 Conclusion

A novel bacteria capable of decolorizing textile effluent was isolated from soil samples of the tannery effluent site by an enrichment culture technique. In the phylogenetic position the strain KTSMBNL 16 was in the branch of *Bacillus* genus and showed 51 % sequence similarity with other related *Bacillus* sp. The ability of the strain to tolerate, decolorize and degrade azo dyes at high concentration gives it an advantage for treatment of textile industry wastewaters. This would increase the applicability of using the strain in practical dye waste water treatment methods. In order to enhance process efficiency the search for cheaper supplementary carbon and nitrogen sources would be essential in future work. It could be suggested in future that the immobilization of this bacterium on various carriers can be used in a continuous process for the decolorization of sulfonated azo dyes in industrial effluents.

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Part V
Implications of Waste on Human Health and Community Participation in Waste Management

Role of NGO's in Protecting Environment and Health

M. Loganathan and Jothi Narendiran

Abstract The past five decades have witnessed the difficult problems encountered in providing health care services to poor people, the majority of who live in more than half-a-million villages and in the proliferating slums of our cities. Charitable and voluntary organizations since time immemorial have been contributing significantly towards the health care of the community. With the passage of time, Non-Governmental Organizations (NGOs) have equipped themselves adequately and come up enthusiastically in providing services like relief to the blind, the disabled and disadvantaged and helping the government in mother and child health care, including family planning programmes. As a result, all concerned have realized the potential of NGOs and their considerable merit compared to the public/private health sectors because of their staff's motivation, dedication and sympathy for the deprived sections of our society and their personalized approach towards the solution of problems. The National Population Policy (NPP) 2000 and National Health Policy (NHP) 2002, states that there should be greater involvement of NGOs in the implementation of different health and family welfare programmes in the country. In recognition of the crucial role played by them, Government of India started granting financial aids to NGOs for various schemes. The important role played by the various national and regional level NGOs is briefly documented in the Encyclopedia of Social Work in India, 1968 where special mention has been made of such organizations like All India Blind Relief Society, Family Planning Association of India (FPAI), Indian Medical Association, Indian Red Cross Society, National Society for the Prevention of Blindness, Sent Parmanand Blind Relief Mission, T.B. Association of India, Bombay Mothers and Children Welfare Society; to name a few. A greater role for the NGOs was seen to ensure Health for All through the primary health care approach. Their role was also considered as most crucial to translate the concept of 'People's Health in People's Hands' into action.

M. Loganathan (✉) · J. Narendiran
Department of Advanced Zoology and Biotechnology, Government Arts College for Men, Nandanam, Chennai, Tamil Nadu, India
e-mail: aswinloga@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_20

Keywords Non governmental organization · National health policy · National pollution policy · Encyclopedia · Family planning association of india and bombay mothers

1 Introduction

NGOs embrace a wide array of agencies within and across different countries of the world. At their broadest NGOs are simply agencies or groups, which are different from government bodies. However, NGOs are distinctive in containing a voluntary component and also because they do not operate for profit. Over the past quarter of a century and especially during the past few decades there has been a rapid growth in the numbers of NGOs involved in the development, the number of people working for NGOs and in the amount of money that flows into these voluntary agencies working in the activities such as disaster management, relief, development, public health, rehabilitation, environment protection, etc. However, this paper focuses on the role played by NGOs particularly in the protection of environment (John et al. 1992). The main cause for environment degradation is lack of effective enforcement of various laws (Bindeshwar 1989). The importance of public awareness and NGOs involvement in environmental protection is acknowledged worldwide. They can also contribute significantly by undertaking research and publication on environment and development related issues. It is necessary to support and encourage genuine, small and local level NGOs in different parts of the country which can provide much needed institutional support specific to the local needs.

2 Aims and Objectives of Environmental NGOs

- Conducting education and citizen awareness programmes in the field of environment
- Fact finding and analysis
- Filing public interest litigations
- Innovation and experimenting in areas which are difficult for government agencies to make changes
- Providing expertise and policy analysis
- Providing factual and reliable information with a network of professional expert staff
- Remaining independent while passing relevant information to the public and governmental bodies
- Solidarity and support to environmental defenders

- Working in collaboration with the government for capacity building and promotion of community participation in environmental awareness and protection, and
- Working out at the grassroots level and reaching far—flung areas with or without the government invitation.

Following are some of the environmental NGOs in India that have been successful and achieved much in the field of environment protection, conservation and sustainable development (Helmut and Volker 1997).

3 Citizens' Waterways Monitoring Programme (WAMP)

This programme was started with the sole purpose of developing clean and pollution free water ways in cities and for creating a healthy living environment for all city dwellers, to stop pollution of waterways and to maintain the waterways of the city cleanly.

4 Community Sanitation Improvement Projects

Inadequate sanitation facilities are a major problem to human health, especially in the neglected low-income areas and slum settlements. NGO's concept of self-help is best displayed by the community sanitation improvement projects in these areas. Two of the most successful projects have been at the Narikkurava (Gypsy) colony in Indira Nagar, Chennai and at Giriappa Road in T. Nagar, Chennai.

5 Tree Planting

The Civic Exnoras in the city have been instrumental in planting trees for the purpose of beautification of roads, parks, playgrounds, burial grounds, etc., with the larger perspective of environmental protection. The organization is closely working with the Tamil Nadu Horticulture and Agriculture Departments on this project.

6 Rain Harvesting

NGO's have propagated the system of rain harvesting in several residential areas in the city with the aim of exploiting one or another important water source, viz., and rainwater. Many cities suffer from perennial water problems every summer and

therefore it is important that all avenues of water source be tapped. A Water Conservation Committee constituted in Chennai by Metro Water Supply and Sewerage Board Exnora is a core member.

7 AIDS Awareness

NGOs are working diligently to educate the public about the prevention of AIDS and provide support and counseling for HIV-infected persons. The additional dangers of placing value judgments on how individuals became HIV-positive is one of the toughest issues facing by NGOs that work with people who are HIV positive. One key in the prevention of AIDS is to eliminate negative images of HIV-infected persons as projected by the media.

8 WWF (India)

It is engaged in a multitude of activities for protection and conservation of the environment in the Indian context. Climate change and energy conservation are among the chief areas of concern for the organization. The forest and biodiversity conservation division strives to promote and enhance conservation of forest ecosystems in the country through a participatory approach involving key stakeholders. Intimately involved in the conservation of tigers in India since late 1960s, WWF's significant efforts culminated in the launch of Project Tiger in 1973. It seeks to conserve and protect the biodiversity of maritime life and resources by sensitizing the people at large. The pollution of the river waters and the imminent threat to aquatic life is a cause of great concern. WWF (India) has stepped in on a number of occasions to launch campaigns for securing their habitat, like 'Save the River Dolphin' project. WWF believes that if you secure an animal's habitat, you secure its right to live (Helmut and Volker 1997; Gemmill et al. 2002).

9 CLEAN-India

Deeply concerned with the deteriorating environmental situation in the country, Development Alternatives initiated the CLEAN-India (Community Led Environment Action Network) programme with five schools in the national capital in 1996. Today, CLEAN Delhi has about forty schools regularly involved in monitoring water and air quality in over 150 locations spread across Delhi. Over 2000 children have been directly trained on environmental assessment and improvement activities. They keep vigil, assess environmental quality, plead, cajole and lead the community in monitoring environment. Action programmes like solid

waste management, plantation drives, energy conservation, paper recycling, etc. to improve local environmental conditions have also been initiated by schools, resident welfare associations, business and industrial associations as well as individual households. Campaigns against the use of poly bags, firecrackers during Diwali and toxic (chemical based) colours during Holi and for saving the city's 'Green Treasure' are also carried out.

10 TERI (India)

Tata Energy Research Institute (TERI) was formally established in 1974 with the purpose of tackling and dealing with the immense and acute problems that mankind is likely to be faced with in the years ahead,

- On account of the gradual depletion of the earth's finite energy resources which are largely non-renewable and
- On account of the existing methods of their use which are polluting

TERI has launched a major project, the first phase of which is completing near completion. This project called growth with resource enhancement of Environment and nature (GREEN INDIA-2047) has vigorously estimated the reduction in India's key natural resources during the period 1947–97, and has completed economic values of consequent loses, which in some cases are alarmingly high. On the basis of past experience and a careful analysis of the cost behind the degradation is that it has taken place in the past strategies for the future have been developed, whereby a fresh and creative approach can be taken in the next 50 years. TERI has now established the World Sustainable Development Forum (WSDF).

11 The National Institute of Health and Family

Accordingly, the Environmental Training Institute (ETI) was established in the year 1994. ETI is a common platform that offers training to pollution control board staff, industries, the urban sector as well as NGOs. Over the years the institute has conducted 124 technical programmes, involving over 1965 participants and 36 special environmental awareness programmes have been conducted for NGOs, Govt. officials, professionals, Universities and educational institutions. There are many similar ETI that have been established by the government in various states.

12 Limitations in the Performance of Environmental NGOs in India

- Shortage of trained personnel in the field of environment protection
- Lack of research and development facilities
- Financial constraints
- Lack of cooperation from the governmental agencies
- Difficulties in the mobility on account of lack of transport facilities
- Environmental NGOs are facing a credibility crisis with a number of cases of embezzlement and scandals involving some of them coming to the fore.

Table 1 listed out the comparative study of prominent environmental NGOs in India.

Table 1 ENGOs working in India

Name	Focus	Activities/impact
Greenpeace India	Climate change, toxic waste, nuclear safety, overfishing protection, environment degradation	Pushed the Indian Govt. for unlimited liability of supplier which led to increase in liability of suppliers from 5 billion to 15 billion in the nuclear liability bill; in 2008, Greenpeace organized the meeting of major electronic manufacture on e-waste which leads to the ball rolling for a formal law governing e-waste; exposed presence and sale of genetically engineered food in the country leading to investigation by director general of foreign trade
CERE India	Environment, education, awareness and advocacy	CERE is commissioned by organizations such as Tata power, TCS, Hindustan Lever, IndusInd Bank map carbon footprint and help cap carbon emissions. Conducted lecturers and workshops in many orgs such as ISB, Oberoi Hotel, Danik Jagran, Aga Khan Society (http://www.cere-india.org/carbon-map–cap-initiative.html)
EXNORA International	Preserving nature, degradation, waste management	Formed community based organization across India. There are 5000 civic exnoras in India targeting 30,000 streets and settlements to clean the streets and environment (http://www.exnora.org/aboutus.php)
AWAAZ foundation	Air pollution, toxic heavy metal, protection of tress, noise pollution	Compelled policy change in state of Maharashtra regarding sand mining. Sensitive areas, facilitated infrastructure using alt. technology use of natural and recycled (http://www.awaaz.org/Awaaz_Foundation/About_Awaaz_Foundation.html)

(continued)

Table 1 (continued)

Name	Focus	Activities/impact
Foundation for ecological security	Conserve ecologically sensitive area, land and water resources	Work with 2208 villages' institution across 27 districts across 6 states in protecting 1,30,000 ha. of revenue wasteland, degraded grazing lands forest lands (http://fes.org.in/)
Goa Foundation	Environment, education, soil waste management, statutory responsibility	Filed 80 PILs in high court and supreme court on environmental reports, launched a periodical curry and rice updating citizens' environment report; instrumental in removing plastic litter from Goan Environment; part of Goa state coastal zone mgmt. authority and supreme court monitoring committee on hazardous wastes
CSE India	Environment awareness, propose sustainable solution, climate change, air pollution, water management, education and training, food safety and toxins	In 2003, brought forth the issue of higher pesticide residue content in soft drink by cola giants Pepsi and Coke and also in bottled water. This led to government forming joint parliament committee on health and safety; engaged with bureau of Indian standards to put in place a methodology for testing pesticides. Involved in providing green rating award to industries on the basis of their environmental performance. Successful green awards
WWF India	Conservation, wasteful consumption of resource, minimizing water pollution, reduction of toxic waste and management of toxic waste	Working actively to conserve critical regions such as Bharatpur, sunderbans, tiger reserves; established centre for environment law (CEL)—involved in policy analysis, campaigning, legal intervention in environmental laws
CONSERVE India	Waste management, up cycling	Trained hundreds of people in Delhi's disadvantage to clear streets of plastic and bad waste. This is up cycling to create designer handbags, wallets, shoes and belts for high fashion
Winrock International India	Natural resource management, energy and environment, climate change	Become member on expert committee on clean development mechanism, constituted by ministry of new and renewable energy, GoI. Involved in CDM Project Facilitation and capacity building, involved in facilitation numerous projects such as 10.2 MW wind farm project under CDM with UNFCCC, involved in integrated rain water harvesting in Almora district. Created the Nilgiri declaration on hill water resource management

13 Conclusion

Environmental non-governmental organizations, in recent years, have grown in size and in number as a result of governmental negligence towards the environmental crisis. NGOs have grown in importance to a point where the act as key arbitrating agents within the field of environmental policy. By interrelating global and local concerns, NGOs find themselves able to not only emphasize important ecological issues, but also raise consciousness about the environment. It can be assessed by the above discussion that the very existence of NGOs and the role played by them in the protection of the environment is not only important but also necessary. Because not only government alone with any amount of laws and acts can achieve the objectives of environment protection, individual and public participation is necessary. This can be achieved only through a network of motivated and dedicated voluntary organizations like the NGOs.

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A Study on Self-sacrificing Role Played by Garbage Pickers in Cleaning up the Environment

J. Jacob Stanley Inbaraj and M. Elangovan

Abstract Disposing of waste is an unavailable by-product of every human being living in this world. Various indicators of economic development and improved living conditions tend to increase the level and complexity of garbage waste. These wastes in come of time get accumulated; stagnated, cause environmental degradation, exhaustion of natural resources which may at times affects the human health. Cleaning of these waste are becoming a major challenge for any society living under these menace conditions. Garbage pickers play a significant role, usually unrecognized in recycling the waste disposals lying on the streets. These people collect garbage items especially which can be sold to scrap merchant or other dealers. The livelihood of their garbage pickers depends on what the other people have chosen to throw away. This paper tries to make a vulnerability study on the socio-economic conditions and living style of the garbage pickers straying in Tirupattur town of Vellore district in Tamil Nadu. After identifying the garbage pickers in the study area, data were collected from them through a well-structured questionnaire with open ended questions and information were collected relevant to the study. After compiling the data, with the help of appropriate software package applying with suitable statistical methods results were obtained and interpreted accurately. This study would reveal the real living conditions of garbage pickers and their deprivation, prejudice and the servitude state which might induce their prompt inclusion in the society.

Keywords Garbage pickers · Collection of wastes · Recycling · Dumping of garbage

J.J.S. Inbaraj (✉) · M. Elangovan
Department of Economics, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, India
e-mail: jacinstan@rediffmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_21

1 Introduction

Over 15 million people make their living by collecting, sorting, recycling and selling materials that are thrown away by someone else. Vital actors in the informal economy, waste pickers provide widespread benefits to their communities, their municipalities, and the environment on the whole. However, they often face low social status, deplorable living and working conditions, and little support from people and the local governments. Waste pickers, known for their independence and individualism are increasingly motivated to organize and fight for recognition and a place within formal solid waste management systems. They are organizing, in many different ways, co-operatives, associations, companies, unions and micro-enterprises. Some are even forming "women only" organizations in order to better confront gender inequalities. By organizing benefits, waste pickers accomplish the following: raising social status and self-esteem, improving members, income and quality of life, in part by circumventing middlemen; improving working conditions and contributing to better health quality, facilitating the development of networks, providing institutional frameworks for hiring of waste pickers for local bodies/firms, preventing harassment and violence, and eliminating child labour in waste picking.

2 Need of the Study

This study would bring forth the unrecognized role played by the garbage pickers in cleaning the environment in a sustaining manner. It is necessary to study the life style of these people and to recognize their efforts in the society. If proper need and care are given to these garbage pickers India would see much a cleaner and greener environment in the days to come. Some of the problems faced by these people are unaddressed and this study would spot light on those factors and try to find the ways and means to solve the existing problems to the maximum.

3 Statement of the Problem

There is a need to explore the people behind the work and the circumstances or reasons why they have chosen to become waste pickers. Gaining insight into the lives of the waste pickers will have broad social and economic advantages for Tirupattur. Governments, policy makers, communities and social researchers need to understand the role that waste pickers play, not only in waste management and public health in Tirupattur, but also in society at large.

4 Literature Review

Nguyen et al. (2003) in the study on "Health and social needs of waste pickers in Vietnam" Hanoi, the capital city of Vietnam, and Ho Chi Minh City, have said that Vietnam's economic capital, are characterized by extensive waste recovery and recycling. The primary work in waste recovery and recycling is done by under-privileged individuals on streets and at garbage dumps. The centrality of waste recycling in the lives of the poor in Vietnam is profound. Media reports on waste recovery focus on waste pickers as the ultimate symbols of poverty and degradation (Furedy 1984). Much is unknown about their lives, their reasons for working or the health issues that confront this population. Few research studies have focused on this population and few people know about the activities of waste pickers in Vietnam. This 10 week study brings attention to the pressing health and social dilemmas faced by those at the lowest level of the waste economy, those who have few alternatives for their livelihoods. Forty one subjects at Vietnam's Dong Thanh Landfill were surveyed and general profiles were created to tell the story of the typical waste picker. Hopefully, the overview provided by this project will be of use to city planners, community organizations and social researchers. Ideally, this project will prompt discussion that will improve the health and welfare of waste pickers through effective and humane coordination of city ordinances, basic health services, community development, welfare programs and public policy.

Sarkar and Papiya (2003) in his study on "solid waste management in Delhi"—a social valuable study has analyzed the management of burgeoning solid wastes which has become a critical issue for almost all the major cities in India. Although the responsibility of solid waste management remains primarily with the municipal bodies, several other stakeholder groups play significant roles in the process. In the Indian scenario the so-called waste pickers, who come from highly vulnerable social backgrounds, play a unique role. Waste pickers, scavengers or rag pickers as they are commonly called eke out a living by collecting and selling recyclable materials out of municipal solid wastes. In the process they make a significant contribution to the environmental management in different metropolitans over and above rendering a great service to the local community.

Ramos et al. (2013) have made an attempt to study "Profile survey of waste pickers in Brazil" and analyzed requirements for the development of a collection vehicle and optimized routing this study presents information collected from waste pickers in the southern, south-eastern and north-eastern regions of Brazil to guide the development of a collection vehicle and a support system for the definition of collecting routes. The study had three objectives: (1) To specify the profile of waste pickers of recyclable materials in the three surveyed regions (2) To diagnose the working conditions of individuals linked to associations and cooperatives of waste pickers (3) To identify the physical and operational structure of the waste picker organizations. To reach these objectives questionnaires were issued to waste pickers linked to associations and cooperatives that collect recyclables using human- or animal-powered vehicles and to the waste picker organizations themselves. Based

on the results of this study, the authors able to provide the requirements for the development of the collection vehicle, to draw a profile of the waste pickers in three areas of study and have better understanding of the working and physical conditions and the organizational structure of waste picker entities. It can be concluded that waste pickers suffer several forms of deprivation, resulting in the marginalization, prejudice and exclusion of individuals who employ themselves in this work, making it essential to promote actions that contribute to the social inclusion of waste pickers in their productive segment.

Devi et al. (2014) in their study on "The Solid Waste Collection by Rag Pickers at Greater Hyderabad Municipal Corporation, India" have analyzed the unavoidable byproduct of human activities. Economic development, urbanization and improved living standards in cities augment the quantity and complexity of generated solid waste. If accumulated, it leads to degradation of urban environment, stresses natural resources and leads to health problems. Solid waste management has become a major environmental issue in India. Waste management, however, remains a major challenge for any society, since all natural processes generate waste. Rag pickers play an important, but usually unrecognized role in the waste management system in Indian cities. They collect garbage in search of recyclable items that can be sold to scrap merchant like paper, plastic, tin etc. This activity requires no skills and is a source of income for a growing number of urban poor people. The present paper intends to present a vulnerability study of the rag pickers of Greater Hyderabad Municipal Corporation (GHMC) with focus on the socio-economic and occupational health aspects. The paper makes use of a database, parenting to the socio-economic profile of the rag pickers including the working conditions, their health problems and expectations.

Rakib et al. (2014) in the study on "An Emerging City: Solid Waste Generation and Recycling Approach" have analyzed to understand solid waste generation rate and its consecutive management approach using qualitative technique in Rangpur city corporation area of Bangladesh. The city corporation area of Rangpur is 203.19 km with a population of around 1 million. The solid waste generation rate is gradually increasing owing to population growth. From this study it was found that, solid waste generation rate is around 23.94 ton d1 in the city corporation area. A number of social components like income level, education and age limit showed significant positive correlation with waste segregation and recycling behavior. Unregulated waste generation was negatively impacted on environmental and human health. Results also showed a thematic future trend of hazards where it will certainly imply on environmental disaster.

5 Objectives

- To evaluate the socio-economic status of the garbage pickers.
- To determine the types of recyclable items obtained from the disposal site.

- To identify the problems and give suggestion to improve the living conditions of garbage pickers.

6 Methodology

This study is focused on the present scenario of waste generation and its proper handling technique with third party involvement. However, it is identified as a significant technique to evaluate the environmental condition while it would be freed from pollution. The study sample consisted of both males and females in all age ranges who worked as waste pickers. The working environment was observed directly to obtain information on the occupational health issues. The researcher collected descriptive information about waste picker demographics, personal opinions and perceived health status. The working environment, work-related health problems and various other details entailed to this type of work was discussed followed by various character sketches of waste pickers according to age group.

The study was conducted in Tirupattur town of Vellore district taking around 40 samples by applying convenience sampling method. The data were collected through direct personal interview with the help of open ended questionnaire. Percentage method is used to analyzing the data by using SPSS and interpretations are given accordingly.

7 Analysis and Interpretation

The noble roles played by the garbage pickers are un-noted in different parts of the world. Their contribution towards sustaining the environment and conservation of resources are remarkable. In this study an attempt is made to study the lifestyle of the garbage pickers in Tiruapttur town of Vellore district in Tamil Nadu. A sample of 40 garbage pickers were identified consisting of both male and female of different age group and information were collected in accordance to the study and interpretations are given in the following section.

The greatest concentration of 50 % was in the age group between 31 and 40 years. About 22.5 % of the respondents were under 30 years with the youngest being only 20 years (Table 1). It is important to note that, the majority of garbage pickers are in the age group which is generally perceived as an economically active period and yet, many may consider that the activity they are engaged in is unproductive, compared to the economic structures of society.

Out of the total respondents nearly 45 % have revealed that they had no formal schooling, while 35 % have had only primary education. It was not expected to find pickers with higher education levels, viz. secondary and tertiary. The poor level of

Table 1 Age of the respondents

Age group	Frequency	Percentage
20–30	9	22.5
31–40	20	50.0
41–50	11	27.5
Total	40	100.0

Source: Primary data

Table 2 Educational status of the respondents

Educational status	Frequency	Percentage
Illiterate	18	45.0
Primary	14	35.0
Secondary	8	20.0
Total	40	100.0

Source: Primary data

education probably explains the poor income earnings; opportunities for employment are limited and so are their knowledge of resale rates of recovered items. The details are listed in Table 2.

It is clear from Table 3, that waste pickers received their income, based on the collection of waste and efficiency in collecting them. Maximum number of waste pickers i.e. 40 % of the respondents is earning 700–800 per week. This is mainly from collecting wastes such as plastic, bottles, paper etc.

It is clear from the Table 4, that 50 % of the respondents are collecting only plastics and 20 % of the respondents are collecting only paper plastic is ever to collect because, it is found thrown everywhere.

Table 5 shows that 92.5 % of the respondents are collecting wastes in *ganny* bags and only 7.5 % of the respondents are collecting wastes in buckets. Since, carrying *ganny* bag is much easier than any other container.

Table 3 Income range of the respondents

Income range (per week)	Frequency	Percentage
500–600	4	10.0
600–700	11	27.5
700–800	9	22.5
800–900	9	22.5
Total	40	100.0

Source: Primary data

Table 4 Type of waste

Type of waste	Frequency	Percentage
Plastic	20	50.0
Bottles	12	30.0
Paper	8	20.0
Total	40	100.0

Source: Primary data

Table 5 Material used for collecting Wastes

Material used	Frequency	Percentage
Sack bag	37	92.5
Bucket	3	7.5
Total	40	100.0

Source: Primary data

Table 6 Place of selling the wastes

Place of selling	Frequency	Percentage
Waste paper merchant	23	57.5
Petty shop	10	25.0
Recycling unit	7	17.5
Total	40	100.0

Source: Primary data

Table 7 Reasons for choosing this job

Reasons	Frequency	Percentage
Lack of employment	13	32.5
Following parents' foot steps	12	30.0
Family situation	9	22.5
Low level of education or lack of skills	6	15.0
Total	40	100.0

Source: Primary data

From Table 6 it is clear that 57.5 % of the respondents are selling the wastes to the waste paper merchant and only 17.5 % of the respondents are selling the waste to recycling units. It is concluded that majority of the respondents are selling wastes to the waste paper merchant because they get a little higher income from waste paper merchants.

Table 7 shows that the various reasons for choosing rag picking as a job. The following responses were obtained when the respondents were asked why they chose garbage picking. 32.5 % of the respondents were unable to set a proper employment, and 30 % of the respondents have followed the parent's footsteps. It must be noted well over that 32.5 % of the respondents have no formal jobs thereby highlighting the poverty scenario.

Table 8 Problems encountered during work

Problems during work	Frequency	Percentage
Climate condition	10	25.0
Dogs barking	8	20.0
Not allowing	6	15.0
Illness	16	40.0
Total	40	100.0

Source: Primary data

Table 9 Suggestions for betterment

Suggestions	Frequency	Percentage
To give high prices for waste	14	35.0
Provide clean drinking water and sanitation facilities	7	17.5
Prohibit the entry of children	6	15.0
Make dust bins as mandatory	9	22.5
Govt. should safeguard the Environment from waste	4	10.0
Total	40	100.0

Source: Primary data

It is clear from Table 8 that 40 % of the respondents are facing problem of various diseases, such as fever, headache etc. while picking up wastes.

From Table 9, it is clear that 35 % of the respondents suggested that give high prices should give for waste, and 10 % of the respondents suggested that government should safeguard the environment from various wastes in order to keep the environment clean and to protect the environmental conditions.

8 Limitations of the Study

- This study is confined only to garbage pickers in the study area and the results obtained may not be suitable for other areas.
- The major limitation is the size of the sample which is very much limited only to the particular area.
- The study mostly focuses on the point of the view of the waste pickers.

9 Conclusion

This study paints a picture of people who are forced to rummage through waste for their livelihood because of financial need. Young or old, the decision of men, women and children to become waste pickers is often their only choice. The waste pickers in Tirupattur town are uneducated and have no opportunities to break away from this occupation. Their days are characterized by an endless cycle of sleep and work. Although the income accrued through waste picking is stable, this is countered by an unhealthy nocturnal lifestyle accompanied by many physical dangers and risks to their health. If circumstances are more conducive and be much within their reach, public could see a healthier environment in future.

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Impact of Dairy Effluent on Environment—A Review

B.V. Raghunath, A. Punnagaiarasi, G. Rajarajan, A. Irshad, A. Elango and G. Mahesh kumar

Abstract Dairy industry is among the most polluting of the food industries in regard to its large water consumption. Dairy is one of the major industries causing water pollution. Considering the increased milk demand, the dairy industry in India is expected to grow rapidly and have the waste generation and related environmental problems are also assumed increased importance. Poorly treated wastewater with high level of pollutants caused by poor design, operation or treatment systems creates major environmental problems when discharged to the surface land or water. Various operations in a dairy industry may include pasteurization, cream, cheese, milk powder, etc. The dairy industry handles large volumes of milk and the major waste material from processing is the water. The water removed from the milk can contain considerable amounts of organic milk products and minerals. In addition cleaning of plant, results in caustic wastewater. This review article discusses the impact of wastewater released in the environment, methods to minimise the amount of both the organic and inorganicmaterial in the wastewater and waste water treatment.

Keywords Dairy effluent · Pollution · Impact

1 Introduction

Waste water generated in a dairy contains highly putrescible organic constituents. This necessitates prompt and adequate treatment of the waste water before its disposal to the environment. Almost all the organic constituents of dairy waste are easily biodegradable. Hence, the wastewater is amenable to biological treatment, either aerobic or anaerobic. Rapid growth of industries has not only enhanced the

B.V. Raghunath · A. Punnagaiarasi · G. Rajarajan (✉) · A. Irshad · A. Elango
G. Mahesh kumar
Department of Livestock Products Technology (Dairy Science),
Veterinary College and Research Institute, Namakkal, Tamil Nadu, India
e-mail: rajarajanvet@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_22

productivity but also resulted in the production and release of toxic substances into the environment, creating health hazards and affected normal operations, flora and fauna. These wastes are potential pollutants when they produce harmful effects on the environment and generally released in the form of solids, liquid effluents and slurries containing a spectrum of organic and inorganic chemicals. Effluent treatment in industries to meet the discharge standards mentioned by pollution control board has always been a great problem for the industrialists. Before discharging the treated effluent on to the land or any surface water body the industries should meet the effluent discharge standard norms. In order to have proper processes in the effluent treatment plant, Characterization of waste water, treatability studies and planning of proper units and processes for effluent treatment is very much necessary (Barnett et al. 2010).

2 Wastewater and Their Sources

Wastes from milk product manufacture contain milk solids due to varying concentration and in dilute condition. These solids enter the waste from almost all of the operations. In general, the wastes generated from dairy industry are as follows,

- The washing and cleaning out of product remaining in the tank, trucks, cans, piping, tanks and other equipment is performed routinely after every processing cycle.
- Spillage is produced by leaks, overflow, freezing-on, boiling over and careless handling.
- Processing losses include, sludge discharge from settling tank, discharges from bottles and washers, splashing and container breakage in automatic packaging equipment.
- Detergents and other compounds are used in the washing and sanitizing solution that are discharged as wastes.

3 Characteristics of the Effluent

The characteristics of a dairy effluent contain temperature, color, pH (6.5–8.0), BOD, COD, dissolved solids, suspended solids, chlorides, sulphate, oil and grease. It depends largely on the quantity of milk processed and type of product manufactured. The waste water of dairy contains large quantities of milk constituents such as casein, inorganic salts, besides detergents and sanitizers used for washing. It has high sodium content from the use of caustic soda for cleaning (Ferguson 1976). Typical characteristics of dairy industry wastewaters reported by various authors are given in Table 1.

Table 1 Characteristics of dairy industry wastewaters (composition in mg/l, except pH)

Waste type	COD (mg/l)	BOD (mg/l)	pH	TSS (mg/l)	TS (mg/l)
Milk and dairy products factory	10251.2	4840.6	8.34	5802.6	–
Dairy effluent	1900–2700	1200–1800	7.2–8.8	500–740	900–1350
Dairy waste water	2500–3000	1300–1600	7.2–7.5	72,000–80,000	8000–10,000
Dairy effluent (CPCB 1993)	1120–3360	320–1750	5.6–8	28–1900	–
Whey	71,526	20,000	4.1	22,050	56,782
Cheese whey pressed	80,000–90,000	120,000–135,000	6	8000–11,000	1
Aavin dairy industry washwater	2500–3300	–	6.4–7.1	630–730	1300–1400
Dairy industry wastewater	2100	1040	7–8	1200	2500

4 Effects of Effluents

4.1 Effects on Environment

The dairy industry is one of the most polluting of industries, not only in terms of the volume of effluent generated, but also in terms of its characteristics as well. It generates about 0.2–10 liters of effluent per liter of processed milk with an average generation of about 2.5 L of wastewater per liter of the milk processed. Dairy processing effluents are generated in an intermittent way and the flow rates of these effluents change significantly. The volume, concentration, and composition of the effluents arising in dairy industry are dependent on the type of product being processed, the production program, operating methods, design of the processing plant, the degree of water management being applied, and subsequently the amount of water being conserved. The sweet whey form the most polluting effluent by its biochemical composition rich in organic matter (lactose, protein, phosphorus, nitrates, nitrogen) and is from 60 to 80 times more polluting than domestic sewage.

The waste water of dairy contains large quantities of milk constituents such as casein, inorganic salts, besides detergents and sanitizers used for washing. All these components contribute largely towards their high biological oxygen demand (BOD) and chemical oxygen demand (COD). Which is much higher than the specified limits of Indian standard institute (ISI), now Bureau of Indian standard (BIS), for the discharge of industrial effluents; As these wastes are generally released to the nearby stream or land without any prior treatment, it is reported to cause serious pollution problems.

Dairy effluents decompose rapidly and deplete the dissolved oxygen level of the receiving streams immediately resulting in anaerobic conditions and release of

strong foul odors due to nuisance conditions. The receiving water becomes breeding place for flies and mosquitoes carrying malaria and other dangerous diseases like dengue fever, yellow fever, chikungunya. It is also reported that higher concentration of dairy wastes are toxic to certain varieties of fish and algae. The casein precipitation from waste which decomposes further into a highly odorous black sludge at certain dilutions the dairy waste is found to be toxic to fish also. Dairy effluent contains soluble organics, suspended solids, trace organics. They decrease do, promote release of gases, cause taste and odor, impart color or turbidity, promote eutrophication.

The main environmental problems related to milk production affect the pollution of water, air and biodiversity. They often cause a growth of algae and bacteria that consume oxygen in the water and eventually suffocate the rivers leading to the gradual disappearance of fish. Hence, the need to treat dairy effluents by various processes is necessary (Deshpande et al. 2012).

4.2 Effects on Water

4.2.1 The Organic Components

The organic components of the wastewater from dairy processing operations can be classified as proteins, lactose and fat. These will affect the environment in different ways depending on their biodegradability and their solubility.

4.2.2 River Oxygen Levels and BOD

Fully aerated rivers at temperatures of 15–25 °C contain oxygen concentrations of at least 8 g/m^3. It is therefore essential that discharges to rivers maintain an oxygen concentration of at least 6 g/m^3. In order for this, the discharge to the river must not increase the river BOD by more than about 3 g m^{-3} (depending on the reaeration characteristics of the river). The organic components in dairy processing wastewater are highly biodegradable. In waterways, bacteria will consume the organic components of the waste. The process of biodegradation in waterways consumes oxygen according to Eq. 1.

$$\text{Organic Material} + O_2 \rightarrow CO_2 + H_2O + \text{Bacteria} \quad (1)$$

4.2.3 Sewage Fungus

Low molecular weight organic compounds promote the growth of certain filamentous slimes in waterways. These bacterial colonies are collectively known as

sewage fungus. The most common bacterial species in this category is *Sphaerotilus natans*. One of the major constituents of dairy factory wastewaters is lactose, a low molecular weight sugar that is known to promote sewage fungus growth. Sewage fungus growth has been related to lactose concentrations in rivers by Eq. 2 and this can be used to predict the extent of sewage fungus growth in a receiving waterway.

$$\text{Growth/g/m}^2 = 0.333 + 2.479\ \text{m(lactose)/g/m}^3 \quad (2)$$

4.2.4 Color and Turbidity

Wastewaters that are highly colored are likely to alter the color of receiving water. Dairy factory wastes probably contain little soluble color, although after various forms of treatment true color may result. Colloidal and particulate components in the waste reflect light back to an observer. This is known as apparent color. The concept of turbidity is closely related to this phenomenon. Milk wastes contain significant quantities of material that will result in turbidity of discharges.

4.2.5 The Inorganic Components (Mainly Nitrogen and Phosphorus)

One of the main aims of industry is to recover the protein (organic nitrogen component) of the waste and convert it to saleable products. Nitrogen is, therefore, a very important component of the dairy factory wastewaters. Some protein will be lost to the waste streams. Bacteria convert the nitrogen in proteins to the inorganic forms including ammonia, and the ammonium, nitrite and nitrate ions. Each of these inorganic forms of nitrogen has different environmental effects. Nitrate ions are toxic in high concentrations to both humans and livestock. In young infants, nitrate can be converted to the nitrite form, absorbed into the bloodstream and convert haemoglobin to methaemoglobin. Methaemoglobin cannot transport oxygen. The condition of methaemoglobinaemia affects infants less than six months in age because they lack the necessary enzyme to reconvert the methaemoglobin back to haemoglobin. To protect humans the usual limit placed on drinking water supplies is 10 g m^{-3} of nitrate-nitrogen.

Livestock can also suffer from methaemoglobinaemia. Since ruminants have a more neutral stomach pH and rumen bacteria that reduce nitrates to nitrite, deaths from methaemoglobinaemia can occur. This usually results from the consumption of nitrate rich feed; although a limit of 30 g m^{-3} nitrate-nitrogen on drinking water for stock has been suggested. Inorganic forms of nitrogen (nitrate, nitrite and ammonium ions) and inorganic phosphates act as plant nutrients in waterways. To protect receiving waters from undesirable growths it has been suggested that total inorganic nitrogen concentrations in receiving waters are limited to less than about 30–100 mg m^{-3} or that dissolved reactive phosphorus (inorganic phosphorus) concentrations are less than about 15–30 mg m^{-3}.

4.3 Effects on Land

Wastewater application to soils is a common method of waste treatment in the dairy industry.

4.3.1 Nutrients (Nitrogen and Phosphorus)

The major mechanisms for nutrient removal in soil based treatment systems are,

- Plant uptake and incorporation in animal products
- Adsorption and immobilization in the soil
- Losses to the atmosphere
- Losses to groundwater (leaching).

Plant uptake of nitrogen amounts to up to 500 kg/ha/year. For phosphorus, the amount is about 30 kg of phosphorus. If animals subsequently consume the pasture, up to 90 % of the nitrogen and phosphorus is recycled to the pasture. Losses of nitrogen to the atmosphere occur through volatilization of ammonia from urine and dung patches, and through the process of denitrification. Denitrification is the process where microorganisms reduce nitrate to either nitrous oxide or dinitrogen gas. This occurs under anoxic conditions (i.e. a lack of oxygen) and when a suitable organic carbon supply is available for energy. Denitrification rates can be quite high at wastewater irrigation sites. Losses of nitrogen (principally in the nitrate form) to groundwater can occur at some irrigation sites depending on the amounts of nitrogen removed by other means. The factor usually limiting the disposal of nitrogen containing wastes to soils is nitrate contamination of groundwaters that are subsequently used as water supplies for humans or stock. It is usual to apply normal drinking water guidelines under these circumstances. Phosphorus does not usually cause a problem by leaching to groundwater because of the high retention and immobilization of phosphates in soils.

4.3.2 Sodium and Other Minerals

Sodium, potassium, calcium and magnesium are all immobilized by soils and occupy cation exchange sites on soil colloids and clays.

4.4 Effects on the Atmosphere

4.4.1 Gaseous Emissions

Manufacturing operations can result in a number of emissions to the atmosphere. Boiler stacks result in emissions of carbon dioxide, sulphur oxides and nitrogen

oxides to the atmosphere. Methane may be emitted from anaerobic waste treatment systems and nitrous oxide (N_2O) is emitted from the soil at wastewater irrigation sites. Carbon dioxide, methane and nitrous oxide are very important greenhouse gases, and it is likely that the consequences of these emissions will need to be considered in the future.

4.4.2 Dust/Odors

Particulate materials can be emitted from boiler stacks, powder driers, etc. Losses of particulate material may also occur from other factory processes. If particulate emissions are high then surrounding buildings are coated with dust and powder which, as well as being undesirable, can also be corrosive. Smoke and steam plumes from factories may also be regarded as a form of visual pollution. The emission of objectionable odors must be considered at industrial processing sites. Many waste treatment plants can produce undesirable odors (Onet 2010).

5 Wastewater Treatment

5.1 Need to Treat the Wastewater

Wastewater from dairies and cheese industries contain mainly organic and biodegradable materials that can disrupt aquatic and terrestrial ecosystems. Due to the high pollution load of dairy wastewater, the milk-processing industries discharging untreated/partially treated wastewater cause serious environmental problems. Hence, it is important to carry out a whey treatment as a starting point, in order to optimize a simple and economic method to treat the whole dairy effluent. Moreover, the Indian government has imposed very strict rules and regulations for the effluent discharge to protect the environment. The wastewater treatment which does not give any monetary benefit to dairy industry owners they release it directly to nearby water streams or on land (i.e. in nature) by giving only some of the primary treatment, due to lack of awareness in this regard and lack of funds.

As described previously, dairy processing wastewaters contain substantial quantities of organic matter, nitrogen and phosphorus. If excessive concentrations of these enter waterways, oxygen depletion and plant growth in the waterways may reach nuisance proportions. The manufacturing dairy industry uses two main methods of treating wastewater includes biological treatment in extended aeration systems and by spray irrigation to pasture (APHA 2005; Jaiprakash et al. 2011).

5.2 Pretreatment

Pretreatment in the dairy industry for many years meant some form of dampening flow, pH or organic load variations and a rudimentary fat/ solids tank. This is changing with the industry now installing pretreatment systems to reduce loadings on wastewater treatment systems and also to allow some factories to continue to discharge to municipal systems. Pretreatment systems are now being maximised to remove solid material using air flotation principles coupled with neutralisation of the wastewater and the addition of flocculants and polyelectrolytes. These systems, while removing solids and nutrients, are limited in their ability to reduce the organic loading in the wastewater because the main source of BODs in dairy plant wastewater is lactose which is soluble and hence cannot be removed by physical/ chemical means. The disposal of the recovered material can be of concern as environmental pressures increase and the solid material cannot safely be placed in landfills as it is still biologically active. Biologically active solids can be composted and utilized as a fertilizer. Work is being undertaken in New Zealand whereby the solid material is heated, the fat used in other processing industries and the remaining material, mainly protein, is being composted.

5.3 Land Treatment

Land treatment systems are used extensively in the New Zealand dairy industry. They use the soil as a biological medium to treat the components of the applied wastewater and hence they need to be designed to the appropriate criteria to ensure efficient operation.

5.4 Organic Loading

When wastewater is applied to pasture, soil microorganisms convert the organic matter present to carbon dioxide and water. During this process, biological slimes and additional bacteria are produced. On fine textured soils the production of slimes *etc*. can inhibit the movement of liquid through soil pores and lead to undesirable effects such as ponding. Dairy factory wastewaters can contain high concentrations of BOD primarily due to their lactose, fat and protein content. In the soil matrix, the normally soluble lactose is converted to bacteria. Some reports have shows an organic load of 2000 kg BOD per hectare is utilized over 16–20 days. Higher loadings can be used on some free draining soils. This application rate represents a design load of about 250 m^3 per hectare on each irrigation occasion.

The dairy industry uses aerobic or anaerobic treatment, or a combination of both, to treat the wastewater. Aerobic systems require an energy source to provide the

oxygen required to assimilate the organic matter in the wastewater and hence are more suited to low to moderate strength wastewaters, since the higher the organic content the greater the oxygen demand and the greater the costs. Anaerobic systems have been developed for their ability to treat high strength wastes and the utilization of the methane gas. *Aerobic systems*: In aerobic treatment systems, bacteria, in the presence of oxygen, convert the organic components of the waste to carbon dioxide, water and bacterial biomass. All aerobic treatment systems have the potential to cause odors if operated incorrectly. The industry worldwide has tried many forms of aerobic treatment. These have included trickling filters, rotating biological contactors and various forms of mechanically aerated lagoon systems. In New Zealand only extended aeration activated sludge systems are used. Typical treatment parameters for an activated sludge plant treating dairy plant wastewater are 94 % COD, 99 % BOD5 70 % TKN and 50 % total phosphorus removal (Rana et al. 2011).

5.5 Anaerobic Treatment

Considerable experimental work has been undertaken on the anaerobic digestion of whey from casein and cheese plants. Various forms of high rate anaerobic digestion systems have been investigated with whey. However, few anaerobic systems treating whey have been installed, despite such systems being operationally viable and the value of methane produced from these systems as the industry values the components of the whey more highly. In an anaerobic digester, anaerobic bacteria, acting in the absence of oxygen, convert the organic components in the wastewater to methane, carbon dioxide and water (Javed et al. 2011). Organic forms of nitrogen are converted to the ammonium nitrogen form. Anaerobic digestion may be carried out in low rate lagoon systems or in high rate reactors. The more recent anaerobic digesters which have been installed in the dairy industry have been high rate digesters, usually with two stages to obtain better control of the anaerobic processes (Kolhe et al. 2009; Deshannavar et al. 2012). The advantages of anaerobic digestion are,

- Produce a valuable byproduct (methane), that can be recovered and utilized as a fuel
- Remove substantial quantities of BOD and COD without the input of mechanical energy for aeration
- Produce less sludge than aerobic systems.

5.6 Nutrient Removal

Dairy factory wastewaters contain substantial quantities of the plant nutrients nitrogen and phosphorus. If excessive concentrations of these enter waterways then

they will promote the growth of plants in the waterways. Eventually these may grow to nuisance proportions. Wastewaters from dairy manufacturing are usually treated in either extended aeration activated sludge plants and discharged to suitable waterways, or are irrigated onto land after primary treatment. Activated sludge systems can remove some of the nitrogen and phosphorus in the waste sludge because these same nutrients are also required for bacterial growth. However, overall removals will, in some cases, be insufficient to meet environmental demands. Under these circumstances an alternative form of treatment or an add-on to the existing treatment will be required to meet discharge requirements (Tawfika et al. 2008).

6 Reduction of Air Emissions

The main emissions from boiler stacks are nitrogen oxides, sulphur oxides and particulates (ash and small quantities of solid fuel). Driers are extensively used by the dairy industry to dry a wide variety of milk powder products (Van-Oostrom and Cooper 1988). The main methods used to reduce atmospheric emissions in the dairy industry are,

- Cyclones and Multicyclones–Cyclones impart a swirl to combustion gasses, and separate heavier particles from the outside portion of the gas stream. These units are effective for larger particles.
- Baghouse Filters Bag–filters separate fine particles. Large surface areas are required.
- Electrostatic Precipitators–Strong electrostatic fields result in particles acquiring electric charges, and being attracted to, and precipitated on, large plate electrodes.
- Wet Scrubbers–Flue gas passes upwards through a chamber while water (with or without various additives) is sprayed down through the chamber, absorbing contaminants.

7 Conclusion

Dairy farms require some form of effluent management system. A range of site specific factors, such as herd size, proximity to creeks, gullies and underground aquifers, climate, soil type and availability of labour, should be considered when selecting the most appropriate system for a particular farm. In most situations, pond systems are more desirable than continuous application systems. However, well designed and managed continuous application systems may be quite acceptable and even more suitable than pond systems in some situations. They are generally better

able to protect the environment, and enable farmers to make the most effective use of the nutrient and water value of the effluent.

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Treatment of Waste Water from Meat Industry

A. Irshad, S. Sureshkumar, B.V. Raghunath, G. Rajarajan and G. Mahesh Kumar

Abstract Slaughter houses and meat processing industry produce large amounts of waste water with high fat, grease and protein content with BOD level of 1500–2000 mg/L. Because of the possible pollution of water sources, the efficient disposal of effluent from meat plants is important. In India, most of the slaughter houses do not have the technology to effectively collect blood, separation of manure or effluent treatment methods. Thus extremely complex effluents are discharged into land or water. Abattoir effluent treatment process mainly includes three steps such as preliminary/primary treatment, secondary treatment and tertiary treatment. Preliminary treatment is based on the physical removal of solids present in the effluent and it works based on three principles like usage of screens, air floatation methods and physico-chemical treatment. Secondary treatment is carried out using biological treatment systems, which involves mixing of culture of microorganisms. These organisms utilize the continuous supply of organic matter present in the effluent to synthesize new cells. Selection of the most suitable secondary system depends on costs, BOD level required, land area available, odour level, etc. Anaerobic process is carried out in totally enclosed systems to prevent the entry of air. It will result in a fast reduction of organic material by a two-stage fermentation process with the production of biogas. The main aerobic treatments include activated sludge process, trickling filters, lagoons, evaporation and irrigation. Combination of anaerobic and aerobic methods is the most suitable effluent treatment for meat industries. Final treatment provides the final removal of contaminants and discharged into the environment without much of risk. Several options are available for discharging effluent in soil. After secondary treatment, two types of material will be available, sludge and treated water. Liquid is treated with chorine or other suitable disinfectant like QAC and discharged into natural bodies and sludge is used for landfill purpose, composting, manure, soil conditioning, etc.

A. Irshad (✉) · S. Sureshkumar · B.V. Raghunath · G. Rajarajan · G. Mahesh Kumar
Department of Livestock Products Technology (Meat Science),
Veterinary College and Research Institute, Namakkal, Tamil Nadu, India
e-mail: irshad2k6@gmail.com

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_23

Keywords Abattoir effluent · Air floatation methods · Physico-chemical treatment · Trickling filters · Anaerobic and aerobic effluent treatment

1 Introduction

Effluent is an out flowing of water or gas from a natural body of water, or from a human made structure. Slaughter houses produce large amounts of wastewater with high fat, grease, and protein content. In most of the slaughterhouses in India, effective collection of blood, separation of manure or effluent treatment methods are not practiced and extremely complex effluents are discharged into land or water (CPCB 1992). Surface and ground water pollution in addition to the odour, fly and mosquito nuisances are posed by these practices. The slaughterhouse wastewater is well suited for anaerobic treatment because of the presence of high concentration of biodegradable organics, alkalinity and adequate phosphorous. It does not include toxic compounds and has sufficiently warm (around 30 °C) temperature. Anaerobic digestion provides high organic removal while producing recoverable source of energy in the form of methane. It generates low quantity of sludge which does not require aeration. The experimental evaluation of biodegradation of slaughterhouse effluent under anaerobic and aerobic conditions suggested the need for an anaerobic treatment stage prior to aerobic treatment (Del et al. 2003).

The meat industry uses large quantities of water especially for cleaning and processing purpose (USEPA 2004). Efficient disposal of effluent is important because the waste can act as a potent pollution of water. In meat industry effluents are mainly produced from lairage, slaughter and bleeding, dressing area, paunch handling area, rendering unit and processing and cleaning section. Characteristics of effluent are (Gracey et al. 1999),

1. Effluent contains readily biodegradable organic matter (measured in term of BODs)
2. Also it contains large amount of grease, fat and oil tend to coat system.
3. Organic matter present in the effluent reduces oxygen transfer from atmosphere to water or effluent.
4. Nitrogen present in effluent in three form, organic N_2, ammonia salt, dissolved ammonia gas.
5. Both aerobic and anaerobic bacteria are present in the effluent which may be pathogenic or non-pathogenic.
6. Comparatively the temperature of the effluent from the slaughterhouse having high temperature as compared to the water present in the natural resources.

7. It is turbid in nature and most of the time off color also.
8. Dissolved gases like methane, SO_2, etc. are usually present in the effluent from slaughter house.

Effluents can be divided into four categories,

1. Non-toxic and not directly pollutant but liable to disturb the physical nature of the receiving water, e.g. house hold effluent
2. Non-toxic and pollutant due to organic matter content of high oxygen demand, e.g. slaughter House effluent.
3. Toxic containing highly poisonous materials, e.g. industrial effluent, factory waste etc.
4. Toxic and pollutant due to organic matter of high oxygen demand and toxic in addition, e.g. effluent from chemical fertilizer factory (Gracey et al. 1999).

2 Important of Effluent Treatment

The efficient disposal of effluent from meat plants and meat-processing works is important because of the possible pollution of water sources. Hence an effluent treatment plant (ETP) is necessary in all modern abattoirs/meat plants. The meat industry uses large quantities of water, which is a significant processing cost factor. Its treatment in the plant and eventual disposal to sewers adds further overheads thus making it essential for the minimum volume of water to be used (Ranken 2000). The eventual waste load should be less than 7.5 kg per 500 kg live weight kill, the wastewater usage less than 5000 L per 500 kg live weight kill and the BOD in the region of 1500 mg/L (Hui et al. 2001).

Many different systems exist for the treatment of meat plant effluents. Onsite treatment is always necessary without final treatment in a local authority sewage plant. The processing of carcasses and the resultant by-products give rise to large amounts of highly polluting wastewater, semi-solids and solids, which must be separated and treated before being discharged into the environment. The objective of effluent treatment is to produce a product that can be safely discharged into a waterway or sewer in compliance with the recommended limits for discharge. The first priorities are to lessen the quantity of material requiring treatment, to separate solids from fluid and to lessen the amount of treatment required. This can be achieved by the use of grills over drains, fat traps and other preliminary treatments, along with continuous dry cleaning or ‘clean as you go’ during the operation of the plant and at breaks in production.

3 Pollution Parameters

Biochemical oxygen demand (*BOD*) is a measure of the readily biodegradable material in an effluent. It is obtained by measuring the oxygen consumed by aerobic organisms, when a known volume of the effluent is added to a known volume of oxygen-saturated water and incubated at 20 °C for 5 days. It is generally used to determine the concentration of pollutant remaining after treatment and prior to discharge. *Chemical oxygen demand* (*COD*) is a measure of the oxygen required for the oxidation of all organic matter in a known volume of effluent, using a standard technique. The COD is often used as a cheaper and more accurate means of determining the oxygen requirements of an effluent before treatment. *Chloride* (*Cl*) is a measure of salinity.

Dry matter (*DM*) *or total solids* (*TS*) is the final weight of a known amount of effluent that has been dried to a constant weight at 105 °C over 24 h. It is measured in g/litre or mg/litre. *Grease, fat and oil* are a group of substances having common properties of immiscibility with water and a lower specific gravity, which cause them to float. Concentrations are measured by the amount of solvent required for the effluent to become soluble. Some water authorities in the United Kingdom will accept a level of 100 mg/L. The substances tend to coat treatment systems, clogging pipes, pumping systems and screens. They reduce oxygen transfer and can seriously reduce the efficiency of aerobic treatment systems. *pH* is a measure of the acidity or alkalinity of an aqueous solution. Pure water has a pH value of 7.0.

Nitrogen (*N*) occurs in three forms in effluents: organic nitrogen, ammonium salts and dissolved ammonia gas, and as nitrates which are found in aerobically treated effluents. Ammonia in solution is toxic to aquatic life; the maximum discharge to sewers is 40 mg/L. High nitrate concentrations in natural waters encourage algae and other plant growth, thus blocking watercourses. The maximum level in potable water is 0.5 mg/L. *Pathogenic bacteria:* Potable water should not contain any coliform organisms. *Suspended solids* (*SS*) refers to matter, which is insoluble and is suspended in the water. It consists of both organic and inorganic components. The organic material will eventually be degraded and add to BOD. *Temperature* should not be more than a few degrees above the temperature of the receiving water in order not to disturb the natural bio-cycle. *Turbidity and colour:* Effluent should be clear and colourless *Volatile solids* are used as a measure of biogas production. The average values for BOD of some food processing operations are given in Table 1.

Table 1 Average BOD values for food processing operations

Source	BOD mg/l
Poultry meat plant	1000–1200
Pig meat plant	1500–2000
Cattle/sheep meat plant	1400–3200
Fish processing	1000–3000
Dairy (washings)	600–1300

4 Types of Treatment

Abattoir effluent treatment process includes,

1. Preliminary/Primary treatment
2. Secondary treatment
3. Tertiary treatment

4.1 Primary/Preliminary Treatment

Mainly it works by three methods like usage of screens, air floatation methods and physico-chemical treatment.

4.1.1 By Using Screens

Preliminary treatment is based on the removal of solids and this is best done by letting all water pass through one or more screens. These screens should be non-clogging and self-cleaning, adaptable to different water flows, easy to clean automatically or manually (when required) and noiseless. After the removal of coarse solids, the effluent stream still contains finely suspended solids, fats and grease. For small quantities of low-grade material, a simple fat trap is all that is required. This is in the form of a minimum-turbulence, flow-through tank. Settable solids can remain long enough to settle out on the bottom of the tank, while grease and fine solids rise to the surface. Continuous sludge removal and skimming of the surface to remove scum are essential. This primary treatment is capable of reducing up to 90 % of the fats, 65 % of the solids and the BOD by 35 %.

4.1.2 Dissolved Air Flotation

It is a successful method of removing suspended solids, fats and grease and is particularly useful when disposal is to a sewer. It causes a physical separation of suspended matter, fats and grease by the production of micro-bubbles of air that attach themselves to the suspended material, lifting it to the surface to form scum, which is removed, while the supernatant liquor is discharged continuously either to a sewer or for further biological treatment. The addition of chemicals that aid flocculation makes the process easier to control automatically and assists in the production of a more consistent effluent.

A large range of flocculants is available, e.g. ferric chloride/sulphate, ferrous sulphate, aluminium sulphate (alum), sodium carbonate (soda ash), calcium carbonate (lime), polyelectrolytes and others. The pH of the effluent has to be maintained and varies depending on the flocculants used. The addition of caustic soda or

hydrochloric acid controls this. Balancing tanks may be required when strict control of hourly and daily flow rates is required or when production is cyclic throughout a 24-h period. Main advantages of dissolve air flotation techniques are

(a) It works faster and produces a drier sludge
(b) Low capital cost
(c) Less ground area requirements
(d) Less operator time
(e) Flexibility of operations in respect of recovery of oil and proteins

4.1.3 Physico-Chemical Treatment

Use of cationic (Fe^{3+} and Al^{3+} salts) and anionic coagulants (Na hexametaphosphate, lignosulfonate and Na alginate) with pH adjustment precipitate and agglomerate protein and other organic materials into larger particles (flocs) that can be recovered by a physical process such as DAF or settling. Fe^{3+} and Al^{3+} salts also precipitate out much of the phosphorus from waste water. Anionic coagulants are used to remove hemoglobin, which can make up a large proportion of the soluble organic load in wastewater from meat processing.

4.2 Secondary Treatment/Biological Treatment

Selection of the most suitable secondary system depends on costs, BOD level required, land area available, odour level, etc. Anaerobic processes may be used in which the reduction of BOD is performed by bacteria in the absence of oxygen. Ponds 4.5 m deep and loaded to 7.5 BOD per 5000 L pond volume will give a BOD reduction of 60–80 % especially at temperatures of 32.5 °C. Secondary treatment is carried out using biological treatment systems, which involve maintaining under controlled conditions a mixed culture of microorganisms, which utilize the continuous supply of organic matter present in the effluent to synthesize new cells.

4.2.1 Anaerobic Treatment

This process is carried out in totally enclosed systems to prevent the entry of air. It will result in a fast reduction of organic material with the production of biogas. With a BOD higher than 2000 mg/L it becomes advantageous. The system operates as a two-stage fermentation process in which the stages occur simultaneously within the digester. During the first stage, bacteria break down complex organic substances into simpler compounds, the most important being volatile fatty acids (VFA). In the second stage, methanogenic organisms utilize the VFA to yield methane and carbon dioxide or Bio gas (Sindhu and Meera 2012). The two-stage fermentation process is graphically illustrated in Fig. 1 and the composition of biogas produced in anaerobic treatment is given in Table 2.

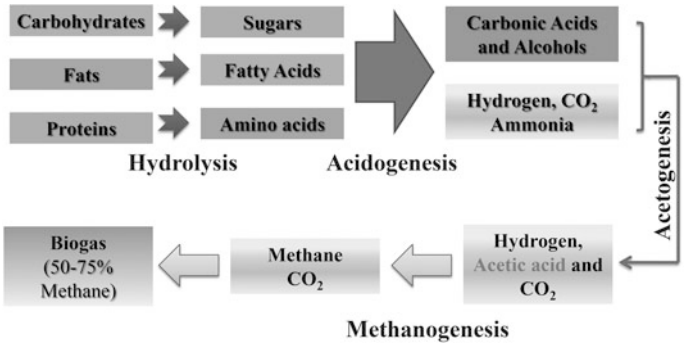

Fig. 1 Two-stage fermentation process

Table 2 Composition of biogas

Matter	%
Methane (CH_4)	50–75
Carbon dioxide (CO_2)	25–50
Nitrogen (N_2)	0–10
Hydrogen (H_2)	0–1
Hydrogen sulfide (H_2S)	0–3
Oxygen (O_2)	0–2

This is very much a 'living' process and the addition of a balanced effluent is essential. Too much protein can destroy the process and therefore blood must not be introduced. There is a high capital cost, the operatives require extensive training and the surplus treated effluent requires further aerobic treatment before it can be discharged into watercourses. Maintaining the pH at around 7.0–7.2 is very important. Overproduction of VFA will lower the pH and stop the process, which can be difficult to restart (Masse and Masse 2005).

4.2.2 Aerobic Treatment

In presence of air bacteria utilizes organic matter for their own cell synthesis. Organic carbon converted into CO_2, nitrogen or nitrate ions. Before being anaerobically treated, wastewater is discharged to waterways, it is treated aerobically to remove most residual BOD and suspended solids, and to oxidize NH_3 and H_2S to less harmful nitrate and sulphate.

The main treatments are

1. Activated sludge process
2. Trickling filters
3. Lagoons
4. Evaporation and irrigation

Activated Sludge Process

The activated sludge process involves utilizing biologically active sludge in small amounts mixed with screened, pre-settled effluent and then agitated in the presence of an ample supply of air in an aeration tank. Aerobic digestion is less sensitive to shock loading; the retention time is shorter and therefore the tanks are smaller and cheaper. Air can be forced in through compressed-air systems or surface aerators. The factors, which affect aeration of the reactor, are concentration of dissolved oxygen, the hydraulic retention time and substrate-loading rate, pH, temperature and toxic substances. The dissolved organic matter, colloidal residues and fine solids are oxidised to carbon dioxide and water. Proteins are broken down into nitrates and sulphates by a mixed culture of microorganisms in the reactor.

The major product of the process is large number of new cells (biomass). The biomass, together with material, which has resisted biodegradation, is separated out from the treated effluent in settling tanks (clarifiers). The supernatant liquor from the clarifier is discharged over a weir for disposal or further treatment, if required. A proportion of sludge, which settles out at the base of the clarifier, is returned to the reactor vessel to maintain the critical concentration of biomass. The remainder is drawn off to be concentrated and may require further treatment before disposal. Where sludges are to be applied to land, which is fallow or is to be seedbed for arable crops, the application is unlikely to become a problem unless the land is close to urban development, when odours may cause a nuisance.

Channel aeration (Pasveer) process is a modification of the activated sludge process, treatment-taking place in a continuous channel 0.9–1.8 m deep. Although large areas of land are required the system has the advantages of high BOD reduction (over 95 % depending on retention time.), low capital cost and ability to accept load fluctuations. Biological filtration processes may take the form of percolating filters or plastic- packed filters. Percolating or trickling filters consist of 1.8–2.4 m beds of stones 50–100 mm in diameter (Alvarez and Liden 2008). Purification is accomplished by the action of a film of microorganisms covering the stones on the organic matter. At loadings of 75–87 kg BOD per 5000 L of packing per day a BOD reduction of 40 % is possible. There is, however, a tendency to block and the system requires high capital cost and large areas of land. Plastic packed filters were developed in order to overcome the disadvantages of percolating filters. Higher loading of 115–125 kg BOD per 5000 L of packing will be a BOD reduction of 75–95 % depending on the number of stages of filtration and sedimentation (Torkian et al. 2003). Before any proposal to treat effluent is undertaken, data on flow rate, BOD levels fat and suspended solids levels should be determined over a period of time during which the minimum use of water is made and as few solids as possible allowed in the effluent.

Tickling Filter

In this method 3–10 m deep bed of porous media like bed of stone, slag etc. are used and wastewater is applied to the surface of the bed and trickles downwards through the media, to which microorganisms are attached. In these techniques 90 % reduction of BOD and removal of suspended solids take place.

Lagoons

Lagoons are scientifically constructed pond having 3–5 ft deep. Here sunlight, bacteria, algae and oxygen interact together. This method is most suitable in warm, clean, sunny weather. Lagoons are mainly two types.

(a) Aerobic type, which takes 2–6 days for complete treatment, consists of series of ponds. With this technique we can reduce the BOD up to 90 %.
(b) Anaerobic type also used in cold weather, which take 6–10 days for complete treatment. In this method BOD reduces up to 70–80 %.

Evaporation and Irrigation

In favorable climate, waste can be disposed of by evaporation in large shallow evaporation pond. The size of the pond may vary which depend on the availability of land and climatic conditions. The bottom of the pond is lined by impervious material like lime to prevent seepage.

4.2.3 Advantages of Anaerobic Treatment Over Aerobic Treatment

(a) Production of useful end products such as methane and digested sludge
(b) Low nutrient requirement in case of treatment of nutritionally unbalanced wastes
(c) No energy required for aeration
(d) Allows rapid dewatering of sludge which can subsequently be handled easily
(e) High loading rates can be achieved as compared to aerobic treatment

4.2.4 Combination of Anaerobic-Aerobic Method

This is the most suitable effluent treatment for meat industries. Anaerobic-aerobic lagoon system for packing house wastes provided an overall BOD removal of 99 %, suspended solids removal of 98 % and grease removal of 98 % (Bielefeldt 2009). Combined system of anaerobic lagoons followed by trickling filters for meat packing wastes remove BOD, COD and grease 74, 73 and 69 %, respectively (Masse and Masse 2000).

4.3 Final Treatment

Final treatment provides the final removal of contaminants and distributes the effluent for dispersal back into the environment. Several options are available for distributing effluent in soil. After secondary treatment, both sludge and treated water obtained.

(a) Liquid is treated with chorine or other suitable disinfectant like QAC and discharged into natural bodies.
(b) Sludge is used for landfill purpose, composting, as manure, soil conditioner, etc.

The overall process is illustrated in Fig. 2. For direct discharge to surface water, the effluent should be colorless/clear, temperature should not be more than few degrees and some other characteristics are given in Table 3 and the recommended standards of effluents are given in Table 4.

The overall treatment process is illustrated in Fig. 3.

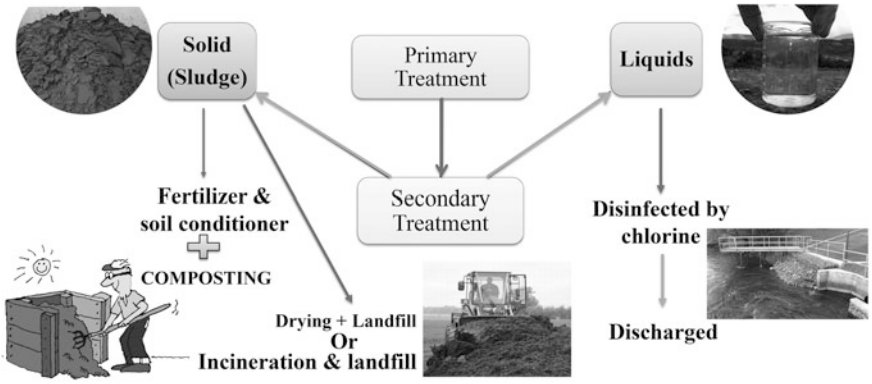

Fig. 2 Overall effluent treatment process

Table 3 Characteristics of waste water for direct discharge

Characters	Range
pH	6–9
BOD5 (mg/l)	50
COD (mg/l)	250
Total suspended solids (mg/ml)	50
Oil and Grease (mg/ml)	10
Nitrogen (total ppm)	10
Total phosphorus (ppm)	5

Table 4 Recommended effluent standards

Type	BOD (mg/L)	Faecal coliform per 100 mL	Alga (per mL)
Effluent to be discharged in to surface water	<25	<5000	<100,000
Used for restricted for irrigation	–	<5000	–
Used for unrestricted irrigation	–	<100	–

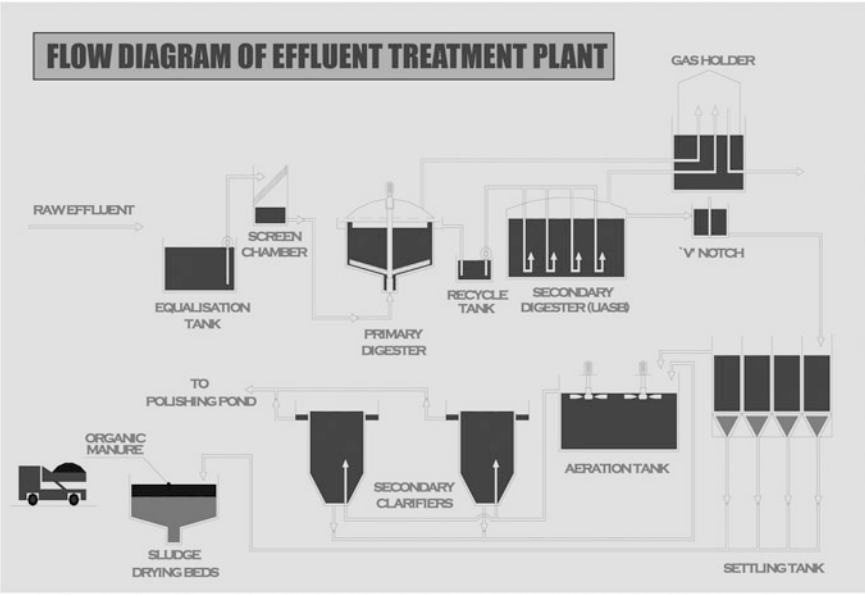

Fig. 3 Wastewater treatment process

5 Novel Methods of Treatment of Slaughterhouse Wastewater

5.1 Electrocoagulation

Electrocoagulation (EC), also known as radio frequency diathermy or short wave electrolysis, is a technique used for wash water treatment, wastewater treatment, industrial processed water and medical treatment. Electricity based electrocoagulation technology removes contaminants that are impossible to remove by filtration or chemical treatment systems, such as emulsified oil, total petroleum hydrocarbons, suspended solids and heavy metals. A fully automated modular system has no filters to clean or replace and does not require the use of chemicals. This enables shortened

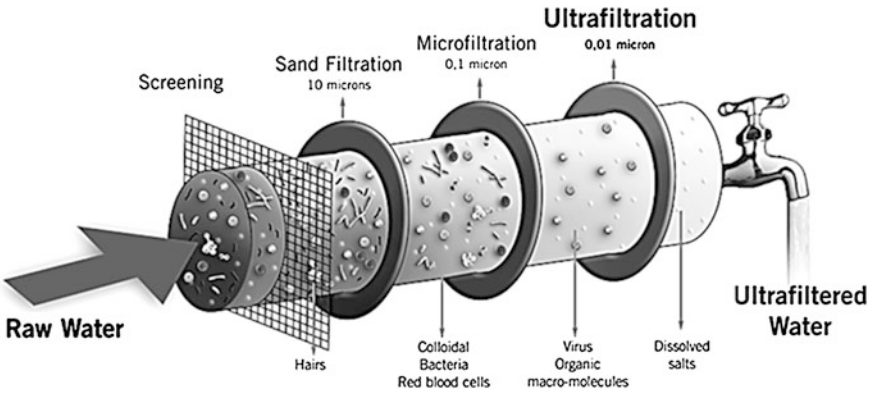

Fig. 4 Membrane treatment

reactive retention period and amount of precipitate or sludge which sediments rapidly.

5.2 Membrane Separation

There are three methods used in membrane separation. Ultrafiltration is used for separation of fats, oils or greases, reverse osmosis and nano-filtration used for water purification, desalination and disinfection and microfiltration is used in recovery/removal or to concentrate particulate materials from liquids or slurries. The schematic diagram is illustrated in Fig. 4.

6 Conclusion

Slaughterhouse wastewater contains various and high amounts of organic matter (e.g. proteins, blood, fat and lard). Untreated slaughterhouses waste entering into a municipal sewage purification system may create severe problems, due to the very high BOD and COD. Therefore treating of slaughterhouse wastewater is very important for prevention of high organic loading to municipal wastewater treatment plants. The most common methods used for treating slaughterhouse wastewaters are fine screening, sedimentation, coagulation, flocculation, trickling filters and activated sludge processes.

The treatment of slaughterhouse wastewater done by various methods such as aerobic and anaerobic biological systems and hybrid systems have been used widely. Aerobic treatment processes are limited by their high energy consumption needed for aeration and high sludge production. Also, the anaerobic treatment of

slaughterhouse wastewater is often slowed or impaired due to the accumulation of suspended solids and floating fats in the reactor which lead to a reduction in the methanogenic activity and biomass washout. In addition, it is also reported that anaerobic treatment is sensitive to high organic loading rates, as a serious disadvantage. Even though biological processes are effective and economical, both biological processes require long hydraulic retention time and large reactor volumes, high biomass concentration and controlling of sludge loss, to avoid the wash-out of the sludge. Among physico-chemical processes, DAF and coagulation–flocculation units are widely used for the removal of TSS, colloids, and fats from slaughterhouse wastewaters.

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Waste Management in Food Packaging Industry

G. Mahesh Kumar, A. Irshad, B.V. Raghunath and G. Rajarajan

Abstract The increasing amount of food packaging waste is perceived as a problem in urgent need of solution in all industrialized countries. According to the environment protection act, waste is any substance which constitutes scrap material, an effluent, unwanted surplus substance, article which requires disposing of as being broken, worn out, contaminated or otherwise spoiled. Waste leads to the production of significant greenhouse gas, methane which is over 20 times more potent than carbon dioxide. Source reduction, reuse and recycle are the most powerful and effective thing we can do to manage waste. Plastic Waste Management has assumed great significance in view of the urbanization activities. Plastic waste generated by the polymer manufacturers at the production, extrusion, quality control and laboratory testing etc., stages, as well as, by the consumers require urgent disposal and recycling to avoid health hazards. Various strategies are being devised to mitigate the impact of plastic waste in India.

Keywords Food packaging industry · Waste management · Plastic recycling · Glass recycling · Aluminum recycling

1 Introduction

Food packaging material is expected to provide optimum protective properties so that the product it encloses remains in satisfactory condition for its anticipated shelf-life. The packaging technique, in conjunction with the choice of a packaging material endowed with appropriate gas and water barrier properties, aims to prevent

G. Mahesh Kumar (✉)
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal 637 002, India
e-mail: maheshvet1989@gmail.com

A. Irshad · B.V. Raghunath · G. Rajarajan
Department of Licestock Products Technology, Veterinary College and Research Institute, Namakkal 637 002, India

M. Prashanthi and R. Sundaram (eds.), *Integrated Waste Management in India*, Environmental Science and Engineering, DOI 10.1007/978-3-319-27228-3_24

destruction of food by microbial and insect attack. According to Paine and Paine (1992) food packaging is complex, dynamic, scientific, artistic and controversial segment of business. One of the major targets of society is to satisfy its population demand for goods of every kind. The increased consumer demand for high quality, long-shelf-life, ready-to-eat foods has initiated the development of mildly preserved products that keep their natural and fresh appearance as long as possible (Baldwin et al. 1995; Guilbert et al. 1996). However, this would be impossible without suitable packaging.

The materials and designs employed for each packaging operation depend on the product itself reflecting the role that it was designed to perform. Packaging is used for meeting the following requirements (Waite 1995; Krochta et al. 1997),

- Protection of product from mechanical damage, contamination and deterioration
- Promotion and advertisement of the product
- Information disclosure to the consumer regarding the content, composition and instructions for safe use
- Improvement of distribution and reduction of storage and transportation costs
- Convenience
- Safety function and prevention of inappropriate use.

Packaging, despite the convenience it provides to the consumer, is subject to many debates concerning environmental issues. It has been considered a constant source of environmental waste due to its volume, since it occupies close to two-thirds of trash can volume. Furthermore, the constant increase in the use of plastics makes their disposal a major environmental issue. Packaging represents approximately 30 % weight of municipal solid waste (MSW), but appears much more significant because it occupies close to 65 % of waste volume due to its bulkiness (Krochta et al. 1997).

2 Waste

According to the environment protection act waste is any substance which constitutes scrap material, an effluent, unwanted surplus substance, article which requires disposing of as being broken, worn out, contaminated or otherwise spoiled. The main approaches to waste management are,

- Recycling
- Combustion for energy recovery
- Combustion for volume reduction
- Landfill
- Save money
- Help the environment

3 Purpose of Food Waste Management

3.1 Save Money

Waste reduction allows you to save money on commodities, labor, energy and disposal costs. Consider that if 4–10 % of the food you purchase will become pre-consumer waste before ever reaching a guest, it becomes clear that waste reduction should be one of the first and easiest ways to control costs (and hedge food cost inflation).

3.2 Help the Environment

Waste leads to significant carbon emissions. In the case of food waste, farm inputs, transportation and storage each require petroleum inputs. And landfill disposal often leads to production of methane gas, a greenhouse gas which is over 20 times more potent than carbon dioxide. By reducing foodservice waste, can make a real environmental difference.

3.3 Community Engagement

Engage staff, guests and community members by showing that waste reduction is achievable and makes a positive difference for all.

4 Waste Management Hierarchy in Food Service

Most foodservice operators are familiar with the phrase "Reduce, Reuse, Recycle" which has been used for many years to describe waste control options other than straight disposal, what many people do not realize is that this phrase represents a hierarchy of activities, starting with the most beneficial and moving to the least attractive (Fig. 1).

Reduce: Source reduction is the most powerful and effective thing that could manage waste. By designing systems and policies to prevent, minimize, or avoid waste in the first place, human have an opportunity to save food and labor dollars while making the largest positive impact on the environment. When preventing waste, money is not spending on raw materials that would otherwise go in the garbage. At the same time, money will be saved on labor costs associated with handling or processing these materials. It is also avoided, hauling and landfill fees

Fig. 1 The waste management hierarchy in food service

(and carbon emissions) associated with recycling, composting or disposing of the waste.

Reuse: Reuse is next best option after source reduction. With reuse, it could find a secondary way to obtain value from an item that would otherwise be wasted. In food service, the most common reuse opportunities involve: (1) redeploying overproduced food elsewhere on the menu and (2) donating to a food recovery program that will provide it to those in need. In certain jurisdictions, food can also be donated to feed animals provided it is handled and treated correctly.
Recycle/Compost: It is the final good option prior to disposal. By recycling or composting, can divert the waste from the landfill or elsewhere in the solid waste stream and ensure ongoing value when the item is converted into some-thing useful, such as a soil amendment with composting. Some of the common wastes in food packaging industry were discussed in this chapter.

4.1 Plastic Use in Packaging Application

Plastic is the major source used in packaging of foods. The thermoplastic materials used for packaging purpose are qualified based on the code as 1–7 (Fig. 2) and the various applications of the different quality of the plastic materials are given in Table 1.

Fig. 2 Codes of thermoplastic

Table 1 Thermoplastics and their used in food packaging

S. no.	Thermoplastic materials	Packaging application
1	Polyethylene terephthalate (PET)	Drinking bottles Microwavable packaging Soft-drink bottles Food jar for butter Jelly and Plastic films
2	Polypropylene (PP)	Drinking bottles Bottles for milk and juice
3	Poly vinyl acetate (PVA)	Common food packaging
4	Poly vinyl chloride (PVC)	Plastic bags Frozen foods stretch films Container lid
5	Polystyrene (PS)	Food container Bottle caps Medicine bottles Straws
6	Low density polyethylene	Disposal cups Glasses Plates Spoon
7	High density polyethylene	Custom packaging

4.1.1 Methods of Plastic Recycling

Steps involved in the recycling process are (Pappa et al. 2001)

- *selection*: The recyclers/reprocessors have to select the waste/scrap which are suitable for recycling/reprocessing
- *segregation*: The plastics waste shall be segregated as per the codes 1-7 mentioned in the BIS guidelines and
- *processing*: After selection and segregation of the pre-consumer waste (factory waste) shall be directly recycled. The post-consumer waste (used plastic waste) shall be washed, shredded, agglomerated and extruded.

Mechanical Recycling

Mechanical recycling is the material reprocessing of waste plastics by physical means into plastics products. The sorted plastics are cleaned and processed directly into end products or into flakes or pellets of consistent quality acceptable to manufactures. The steps taken to recycle post-consumer plastics may vary from operation to operation, but typically involve inspection for removal of contaminants or further sorting, grinding, washing and drying and conversion into either flakes or pellets. Pellets are made by melting down the dry plastic flakes and then extruding it

into thin strands that are chopped into small, uniform pieces. The molten plastic is forced through a fine screen (filter) to remove any contaminants that may have eluded the washing cycle. The strands are cooled, chopped into pellets and stored for sale and shipment. Different plastics may also under different reforming conditions such as different processing temperatures, the use of vacuum stripping, or other procedures that could influence contaminant levels. During the grinding or melting phases, the reprocessed material may be blended with virgin polymer or compounded with additives. Mechanical recycling is the preferred recovery route for homogeneous and relatively clean plastics waste streams, provided end markets exist for the resultant recyclate. This technique is also well suited for developing countries since it is less cost-intensive compared to the others (Dodbiba et al. 2005).

Feedstock or Chemical Recycling

Chemical recycling or feedstock recycling means a polymeric product broken down into its individual components (monomers for plastics or hydrocarbon feedstock synthesis gas) and these components is then fed back as raw material to reproduce the original product or others. Feedstock recycling include chemical depolymerisation (glycolysis, methanolysis, hydrolysis, ammonolysis etc.), gasification and partial oxidation, thermal degradation (thermal cracking, pyrolisis, steam cracking, etc.), catalytic cracking and reforming, and hydrogenation. Besides conventional treatments (pyrolisis, gasification), new technological approaches for the degradation of plastics, such as conversion under supercritical conditions and co processing with coal are being tested (Santos et al. 2005). This technique of recycling is however not suitable for developing countries. This is because it requires a lot of expertise, capital intensive and is quite cumbersome. Even in the developed countries, it is still under development and is being practiced by only a few companies. A number of companies have successfully developed and demonstrated technologies many of which can process mixed plastics streams. There has been some renewed interest in other areas of feedstock recycling, such as the depolymerisation of PET or treatment of PVC to make chemicals which can then be used in the production of new plastics.

Environmentally Sound Manner

Recycling of plastics should be carried in such a manner to enhance the efficiency of the process and conserve the energy. Plastic recycling technologies have been historically divided into four general types-primary, secondary, tertiary and quaternary. Primary recycling involves processing of a waste/scrap into a product with characteristics similar to those of original product. Secondary recycling involves processing of waste/scrap plastics into materials that have characteristics different from those of original plastics product. Tertiary recycling involves the production of basic chemicals and fuels from plastics waste/scrap as part of the municipal

waste stream or as a segregated waste. Quaternary recycling retrieves the energy content of waste/scrap plastics by burning/incineration.

Plastics Waste Disposal Through Plasma Pyrolysis Technology (PPT)

Plastic waste disposal through plasma pyrolysis is a state of the art, technology, which integrates the thermo-chemical properties of plasma with the pyrolysis process (Kaminsky 1995). In plasma pyrolysis, firstly the plastics waste is fed into the primary chamber at 850 °C through a feeder. The waste material dissociates into carbon monoxide, hydrogen, methane, higher hydrocarbons etc. Induced draft fan drains the pyrolysis gases as well as plastics waste into the secondary chamber, where these gases are combusted in the presence of excess air. The inflammable gases are ignited with high voltage spark. The secondary chamber temperature is maintained at around 1050 °C. The hydrocarbon, carbon monoxide and hydrogen are combusted into safe carbon dioxide and water. The process conditions are maintained so that it eliminates the possibility of formation of toxic dioxins and furans molecules (in case of chlorinated waste). The conversion of organic waste into nontoxic gases (CO_2, H_2O) is more than 99 %.

Some Alternative Method of Waste Disposal

Landfill defined as the disposal, compression and embankment fill of the waste at the appropriate site (Read 1999). (Anaerobic degradation) Landfill is the easy, adjustable with lower cost than other rest of disposal methods. Important factor is that selection of the correct disposal site. In anaerobic degradation or digestion, microorganisms slowly break down solid waste primarily organic based materials such as wood and paper (in the absence of oxygen) into primarily carbon dioxide, methane and ammonia. Anaerobic degradation is mostly used to treat bio solids (sewage sludge) and organic waste contaminants. More research is necessary to realize the full potential of anaerobic degradation in the management of solid waste.

Incineration is the process of combustion to convert the waste material into CO_2 and water. Reduction of waste volume through incineration achieved 80–90 %. *Pyrolysis* is best method for high molecular waste substances. It is the thermal degradation of macromolecule. Pyrolysis products consist 34 % ethylene, 9 % propane, 39 % oil (mainly aromatic compound) Pyrolysis are mainly used for plastics. *Composting* refers to self-heating, aerobic process of organic waste and other industrial organic compound in order to convert them to a mature and plant compatible substrate. The final product of composting is rich in organic matter. Generally it takes 3 months in optimal degradation condition and 1–2 years in normal condition.

4.1.2 Energy Recovery

Plastics are almost all derived from oil and plastic wastes is a waste with a high calorific value. Energy recovered from plastic waste can make a major contribution to energy production. Plastics can be co-incinerated with other wastes or used as alternative fuel (e.g. coal) in several industry processes (cement kilns). The energy content of plastic waste can be recovered in other thermal and chemical processes such as pyrolysis. As plastic waste is continuously being recycled, they lose their physical and chemical properties at their end of life cycle. Continuous recycling could lead to substandard and low quality products. Hence it would no longer be economically profitable to recycle further. Incineration with energy recovery would be the economically preferred option at this stage.

Conversion of Plastics Waste into Liquid Fuel

A research-cum-demonstration plant was set up at Nagpur, Maharashtra for conversion of waste plastics into liquid fuel. The process adopted is based on random de-polymerization of waste plastics into liquid fuel in presence of a catalyst. The entire process is undertaken in closed reactor vessel followed by condensation, if required. Waste plastics while heating up to 2700–3000 °C convert into liquid vapor state, which is collected in condensation chamber in the form of liquid fuel while the tarry liquid waste is topped-down from the heating reactor vessel. The organic gas is generated which is vented due to lack of storage facility. However, the gas can be used in dual fuel diesel-generator set for generation of electricity.

4.2 Glass Recycling

Glass has an extremely long history in food packaging; the 1st glass objects for holding food are believed to have appeared around 3000 BC (Waite 1995). However, during the last hundred years, mechanized glass blowing techniques have revolutionized the production of glass containers, allowing bottles to be produced quickly and cheaper (Vogas 1995; Pearson 1996). The production of glass containers involves heating a mixture of silica (the glass former), sodium carbonate (the melting agent), and limestone/calcium carbonate and alumina (stabilizers) to high temperatures until the materials melt into a thick liquid mass that is then poured into molds. Recycled broken glass (cullet) is also used in glass manufacture and may account for as much as 60 % of all raw materials. The process is illustrated in Fig. 3 and Table 2 give the recycling rates.

The recycling process of glass depends entirely on the type of glass that will be produced (Stotzel 1997). Main advantage is energy saving. Glass cullet requires

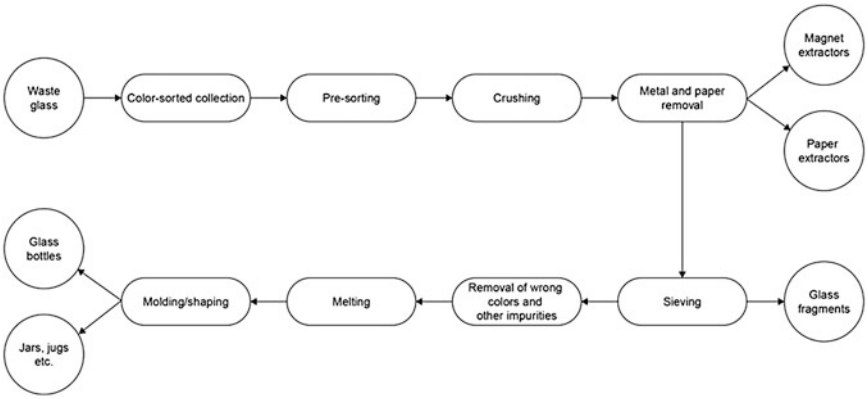

Fig. 3 Glass recycling process (*source* Arvanitoyannis and Bosnea 2001)

Table 2 Glass recycling rates around the world (%)

Country	1991	1993	1994	1995	1996	1997
Germany	55	71	75	82	85	89
France		46	48	50	50	
Italy		52	54		53	
UK	21	29	28	27	26	26
Spain		29	31		35	
Holland		73	77	80	81	
Belgium		55	67	67		
Austria		68	76			
Denmark		64	67			
Sweden		54	56		72	
Portugal		29	32			
Greece		27	29	29		
Norway			72		75	
Finland			50			
Ireland		29	31			
Switzerland		78	84		89	
Australia					89	
USA	25				33	
Japan					60	

Source Arvanitoyannis and Bosnea (2001)

less temperature for melting than raw material. Saved energy = 0.25 × % of recycled glass cullet used. There are some basic rules to be followed during glass recycling, so glass should be free from metal tops, ceramics and stones and be

sorted according to colour. There should be a thorough removal of foreign materials; otherwise the produced glass might be defective. The quantity of ceramics left on the cullet should not be more than 25 g per tonne, while the metal particles should be less than 5 g per tonne. Therefore, the basic container glass recycling process steps are (Fig. 3):

- Initial rinsing, cap and lid removal
- Color separation
- Volume reduction by breaking or crushing
- Packaging and shipping
- Final treatment.

4.3 Aluminum Recycling

In contrast to many other materials, in the recycling of metal there are no quality losses. Compared to primary metal extraction, a 95 % savings in energy can be achieved with recycling. The economic value of aluminum has always been the main reason for bringing the material into the loop of metal extraction, processing, use and recovery. Aluminum has been recycled since the days it was first commercially produced and today recycled aluminum accounts for one-third of global aluminum consumption worldwide (Fig. 4).

4.4 Paper/Carton Recycling

In spite of synthetic packaging materials and electronic media, internationally paper and board consumption is increasing steadily. While in 1950, about 50 million tonnes of paper were produced worldwide, in 2010, approximately 400 million tonnes were produced. To make this increase in paper production possible and for saving resources at the same time, paper recycling has been intensified steadily in the last decades and has now reached a high technical level. Most of the products made of paper only have a life span of a few days (e.g. newspapers) or a few weeks (e.g. packaging). The increase of recovered paper use in industrialized countries is determined by problems of disposal. Thus, recovered paper is today the most important raw material for the production of paper, paperboard and corrugated board (Fig. 5).

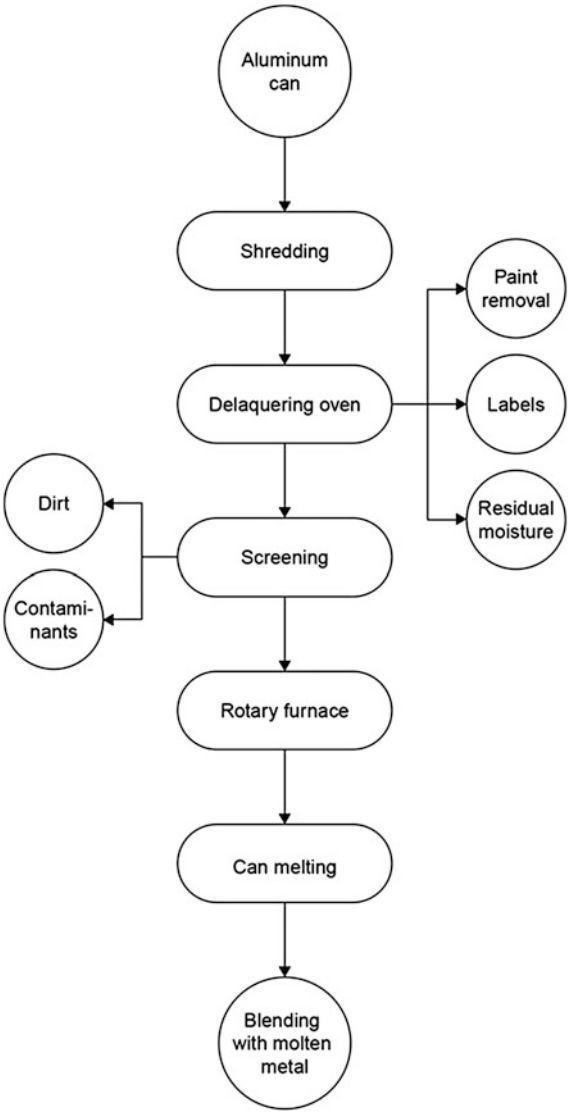

Fig. 4 Flow diagram for metal recycling (*source* Onusseit 2006)

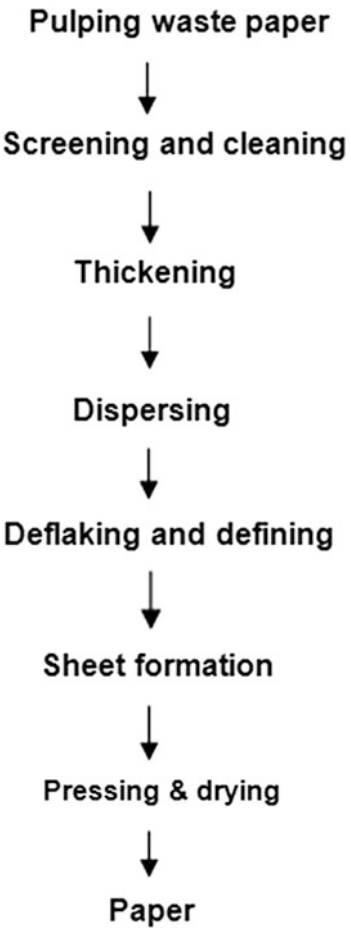

Fig. 5 Process of waste paper recycling

5 Conclusion

Packaging, despite the convenience it provides to the consumer, is subject to many debates concerning environmental issues. It has been considered a constant source of environmental waste due to its volume, since it occupies close to two-thirds of trash can volume. Plastic waste management has assumed great significance in view of the urbanization activities. Plastic waste generated by the polymer manufacturers at the production, extrusion, quality control and laboratory testing etc. it is urgent that disposal and recycling should be done to avoid health hazards. Various strategies are being devised to mitigate the impact of plastic waste in India.

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