IJASRM
International Journal of Advanced Scientific Research and Management, Volume 3 Issue 6, June 2018
www.ijasrm.com
ISSN 2455-6378

Bioremediation of Polymer Effluent using Bacteria and its Reuse for the Growth of Ornamental Plant

R. Harinee[1], U. Irfana Fathima[2], S. Kalaiselvi[3], A. Raziya[4], B. Sowmiya[5] and C. M. Noorjahan*[6]

1-5, PG & Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai – 600018, Tamil Nadu, India.

*[6]Assistant Professor, PG & Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai – 600018, Tamil Nadu, India.

Abstract

Polymer effluent is one of the most important water polluters of the environment. It contains large quantity of toxic substances. Discharge of such waste water containing toxic substances into the environment cause hazards to the living organisms. Hence the present study was carried out to analyse Physico-chemical parameters like colour, odour, pH, EC, TSS, TDS, BOD and COD of untreated Polymer effluent and degraded using bacteria. The results of the analysis of the physico chemical parameters revealed that untreated Polymer effluent was yellowish in colour with pungent odour. pH was alkaline with high organic load such as EC, TSS, TDS, BOD and COD which were higher than the permissible limits of CPCB (1995) for its disposal indicating high pollution potential of the waste. Since the effluent had high organic load, microbes (bacteria) present within the effluent were analysed. The results of Gram staining and biochemical tests for bacterial identification revealed the occurrence of 2 bacterial species namely, Gram positive, *Staphylococcus aureus* and Gram negative, *Enterobacter aerogenes*. Since the untreated polymer effluent has high organic load, disposal of such waste water into the environment without proper treatment pose hazards to environment. As *Staphylococcus aureus* is a native bacteria of polymer effluent, it was used to degrade the untreated polymer effluent for a period of 96 hrs. Results of analysis of degradation of the effluent revealed that colour and odour of 100% untreated sample has changed to almost colourless and odourless nature. pH has changed from alkaline to neutral and also showed marked reduction (11.5% - 99.5%) of parameters in biotreated sample using native *Staphlococcus aureus*. The biotreated sample was reused for the growth of ornamental plant, *Impatiens balsamina.* The results of the study revealed that growth rate is increased in biotreated sample than in untreated sample.

Keywords: *Polymer effluent, Physico-chemical parameters, bacterial identification, Staphylococcus aureus, biodegradation, reuse - Impatiens balsamina.*

1. Introduction

Environmental Pollution is the disposal of contaminants in to the natural environment that causes adverse environmental changes to the environment. This form of environmental degradation occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds. Water pollution is the contamination of water bodies (lakes, rivers, oceans, aquifers and ground water) due to the discharge of waste water without treatment from different industries (Goudar and Subramanian, 1996).

1.1 Polymer and polymer effluent

Among the major different industries, the polymer industry is a notorious polluting industry of the environment. The polymer industry manufactures various plastic products. The polymer effluent is the waste water generated after the production of polymer by the industry which pollutes soil and ground water when it is discharged without treatment. Polymer generate significant amount of effluent which has to be treated before being discharged into water stream.

1.2 Impact of polymer effluent on the environment

Polymer effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and environment. It affects the resources, health and livelihood of thousands of people and also causes decline of trees. Some of the plastic products cause human health problems because they mimic human hormone. Hence polymer effluent with high pollution load should be treated before its disposal (Hall and John, 2009).

1.3 Methods for treatment

Though there are physical and chemical treatment methods of waste water but the biological treatment method using microbes has gained considerable attention as it is ecologically sound and economical, relative to other technologies and it has been used successfully in many countries. Microbes in the environment plays an important role in cycling and destroying toxic pollutants through biodegradation (Noorjahan, 2017).

1.4 Recycling/reutilization of degraded polymer water

It is well known that water of good quality and free of pollutants are primary requirements for agricultural practice. Hence after degradation, the treated water could be used for crop cultivation, irrigation and aquaculture purpose. Taking into consideration of all the above said investigations carried out by many researchers, research pertaining to degradation of polymer effluent using microbes especially bacteria was meagre. Hence an attempt has been made to degrade the untreated polymer effluent using native bacteria *Staphylococcus aureus* and to reuse the biotreated water for the germination and growth of ornamental plant, *Impatiens balsamina*.

2. AIMS AND OBJECTIVES

- ❖ To analyse the Physico chemical parameters of untreated Polymer effluent
- ❖ To identify and isolate the microbes (bacteria) present in the untreated polymer effluent
- ❖ To degrade 100% untreated Polymer effluent in laboratory scale study (96 hours) using native bacteria – *Staphylococcus aureus* and
- ❖ To reuse the biotreated water for the germination and growth of ornamental plant, *Impatiens balsamina* for a period of 15 days.

3. MATERIALS AND METHODS

3.1 Collection of untreated polymer effluent

Untreated polymer effluent was used as the material in this study. The untreated sample was collected in polythene containers (10 litres capacity) from the point wherein all the effluent were discharged together from polymer company situated in Chennai, Tamil Nadu, India. They were brought to the laboratory with due care and stored at 25°C for further analysis. The physico chemical parameters such as colour, odour, pH, EC, TSS, TDS, BOD and COD of untreated polymer effluent were determined by following the Standard Methods outlined by APHA, 1995.

3.2 Untreated polymer effluent for isolation of microbes (Bacteria)

Polymer effluent of about 1 litre was collected in sterile bottles and brought to the laboratory. Analysis of microbes (bacteria) was carried out on the same day by following the procedure of Sundararaj, 1997.

3.3 Native Bacteria, *Staphylococcus aureus* for degradation of untreated polymer effluent

Staphylococcus aureus is a native bacteria of untreated polymer effluent. It was used for the degradation of the effluent. *Staphylococcus aureus* was isolated from untreated polymer effluent by using pour plate method in nutrient agar medium and subcultured on Nutrient Agar medium for 24 to 48 hours. Then the culture was maintained in nutrient broth for substantial growth (Sundararaj, 1997).

3.4 Biodegradation of 100% untreated polymer effluent using native bacteria *Staphylococcus aureus*

Population of 10^{-1} cells/ml *Staphylococcus aureus* was transferred to 100% untreated polymer effluent in a conical flask. Conical flask with effluent and bacteria, *Staphylococcus aureus* (experimental) was incubated separately at 30 $\pm$ 0.5°C for 96 hours on rotary shaker at 2000 rpm.

After incubation, the samples were centrifuged at 5000 rpm for 20 minutes. Control (conical flask with untreated polymer effluent without bacteria) was also run simultaneously. The procedure for degradation process was carried out by following the procedure of Durgadevi, 2012.The supernatant were analyzed for physico-chemical parameters like pH, EC, Total Suspended Solids, Total Dissolved Solids, Biological Oxygen Demand and Chemical Oxygen Demand. Physico chemical parameters of the effluent were analyzed before biotreatment (control) and after biotreatment by following the Standard procedure of APHA, 1995.

3.5 Procurement of seeds of ornamental plant, Balsam – *Impatiens balsamina* for growth in 100% untreated and biotreated water

The seeds of Balsam - *Impatiens balsamina* was procured from a local nursery located in Chennai for the germination and growth in polymer effluent and biotreated water Mehaytaab, 2008.

The seeds of ornamental plant, *Impatiens balsamina* were washed with mercuric chloride solution for 2 minutes and then thoroughly washed in distilled water. Each earthen pot filled with farm yard manure was sown with 10 seeds allowed to germinate by irrigating with equal volume of untreated polymer effluent and biotreated water separately. One set was irrigated with tap water as control. Duplicates were maintained for each concentration. The vegetative features (i.e) shoot length, root length, number of leaves and number of roots of the above plant was recorded on 15th day of plant growth.

3.6 Statistical Analysis

The data obtained from the experiments was analysed and expressed as mean, Standard Deviation and Percentage change.

(1). **S.D.** $= \sqrt{\frac{1}{N-1}\sum x^2 - \frac{\sum x^2}{N}}$

Where N = Number of individual observation
Σx^2= Sum of square of individual observation
(Σx^2)= Square of the total individual observation

(2) **Percentage change**

$$= \frac{\text{Control} - \text{Experimental}}{\text{Control}} \times 100$$

4. Results and Discussion

4.1 Analysis of Physico-chemical parameters of untreated polymer effluent

Environmental pollution is caused by synthetic polymer, such as wastes of plastic and water-soluble synthetic polymers in wastewater. Two general mechanisms were usually considered for degradable plastics, namely photodegradation and biodegradation. Hence a preliminary investigation has been carried out to analyse the physiochemical parameters of untreated polymer effluent such as colour, odour, pH, EC, TSS, TDS, BOD and COD.

The results of the analysis of physico-chemical parameters of untreated polymer effluent (Table – 1) revealed that the colour of the untreated polymer effluent was yellowish in colour with pungent odour. The pH of the polymer effluent was found to be alkaline. Discharge of such effluent with alkaline pH into ponds, rivers etc for irrigation may be detrimental to aquatic biota such as ornamental plants. Untreated polymer effluent showed higher level of Electrical conductivity (164 µmhos/cm) which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Krishnapriya, 2010).

Level of suspended solids was found to be higher in polymer effluent (10,528 mg/l) when compared to the permissible limit (100 mg/l) prescribed by CPCB, 1995 for effluent discharge. High amounts of suspended particles has detrimental effects on aquatic flora and fauna and reduce the diversity of life in aquatic system and promote depletion of oxygen and sliting in ponds during rainy season (Karthikeyan, 2009). The composition of solids present in a natural body of water depends on the nature of the area and the presence of industries nearby. High levels of TDS (186 mg/l) may be due to high salt content and also renders it unsuitable for irrigation hence further treatment or dilution would be required (Goel, 1997).

Determination of BOD is one of the important parameters used in water pollution to evaluate the impact of wastewaters on receiving water bodies. The present study revealed high levels of BOD (160 mg/l) in the polymer effluent due to the presence of considerable amount of organic matter. Increase in BOD which is a reflection of microbial oxygen demand leads to depletion of DO which may cause hypoxia conditions with consequent adverse effects on aquatic biota (Sukumaran, 2008). COD test is the best method for organic matter estimation and rapid test for the determination of total oxygen demand by organic matter present in the sample. The present investigation revealed high levels of COD (599 mg/l) which suppressed the standards limit for COD (250 mg/l) prescribed by CPCB, 1995 for effluent discharge into inland surface waters.

Thus the analysis of physico chemical parameters of untreated polymer effluent confirms that the wastewater released from the polymer industry has higher concentration of EC, BOD, COD, TSS and TDS, which surpassed the permissible limits prescribed by CPCB, 1995 for discharge of industrial effluent into inland surface water as well as on the land for irrigation. Alkaline pH, high TSS, TDS, BOD and COD of the polymer effluent reveals that the polymer effluent is highly polluted and it has to be treated before disposal. Hence it is imperative to adopt technologies to reduce or degrade the polymer effluent using microbes. Microbes in the environment play an important role in the cycling and fate of organic chemicals and can destroy them through the biodegradation. Microbes especially bacteria act as bio indicator of high polluted effluents as reported by Noorjahan, 2017, which prompted to analyse the native bacterial population present in polymer effluent and to use it for biodegradation.

4.2 Isolation of Microbes (Bacteria)

Hence in the present study, the bacterial species present in 100% untreated polymer effluent were isolated and identified. The results (Table – 2a and 2b) of the study showed the presence of 2 bacterial species namely Gram positive - *Staphylococcus aureus* and Gram negative - *Entrobacter aerogenes* which were confirmed by carrying out gram's staining and biochemical tests. Polymer effluent is rich in organic and inorganic nutrients which would have supported the growth of bacterial population. The result of the microbial study is in accordance with the work of Sukumaran, 2008 who has reported the occurrence of 8 bacterial species in polymer effluent. Further as pointed out by Radha, 1995, the presence of native microbes in polymer effluent would be successfully exploited to remove the pollutants, a technique which is more economically and industrially effective. Based on the above suggestions, biodegradation of polymer effluent was planned and executed using native bacteria, *Staphylococcus aureus* to biodegrade the polymer effluent.

4.3 Biodegradation of 100% untreated polymer effluent using native bacteria, *Staphylococcus aureus*

Thus the results of the biodegradation of untreated polymer effluent using *S. aureus* (Table – 3) revealed that the colour and odour of untreated polymer effluent was yellow with pungent odour which may be due to presence of large quantity of organic and inorganic pollutants Singh *et al.*, 1998 or may also be due to microbial activities (Nagarajan and Shasikumar, 2005). But after biodegradation of the effluent for 96 hrs, the colour and odour has changed to almost colourless and odourless condition using native bacteria, *Staphylococcus aureus.* This may be due to the action of bacteria, *S. aureus*, which decomposed the toxic pollutants present in the effluent and changed the colour and odour of the effluent. This is supported by the work of Sukumaran, 2008. pH of untreated polymer effluent changed to almost neutral pH and EC was also reduced with a maximum reduction of 78.48 % using *S. aureus* thereby indicating the efficiency of the microbes to biodegrade the effluent. This is in agreement with the reports of Noorjahan *et al.*, 2004.

TSS of polymer effluent before biodegradation was very much higher than permissible limit of CPCB, 1995 (100 mg/l) but TSS of polymer effluent was reduced to a maximum percentage of 99.56% using native *Staphylococcus aureus.* TDS of polymer effluent was higher before biodegradation than the permissible limit of CPCB, 1995 which may be due to presence of large amounts of salts in the effluent but after biodegradation of effluent for 96 hrs, maximum reduction of TDS (77.05%) using *S. aureus* was recorded. This is supported by the work of Noorjahan, 2017. Since TSS and TDS are the major pollutants, the above biodegradation results are encouraging and scale up studies for continuous treatment of wastewater at pilot scale is required. The information generated would help to scale up the process and assess the economic feasibility of the technology. BOD and COD of untreated effluent before biodegradation was much higher than CPCB, 1995 permissible limit (30 mg/l). But on biodegradation for 96 hrs, the results does not show much reduction of BOD and COD level using native, *Staphylococcus aureus.* This is in agreement with the work of Noorjahan, 2017.

Thus from the foregoing discussion, it is very clear that microbes play an important role in the biodegradation of organic and inorganic matter. During biodegradation, the key element is the micro-organisms. They have enzymes that allow them to use environmental contaminants as food and hence make them ideal for biodegradation. From the results of the present study it can be inferred that, native bacteria, *Staphylococcus aureus* showed efficient degrading capabilities by degrading the contaminants as they use it for their growth and reproduction. Organic compounds are a source of carbon which forms one of the basic building blocks of new cell contaminants. In addition to the carbon source, they require nitrogen and phosphorus as primary nutrient and traces of inorganic salts through a series of complex enzymatically catalysed reaction, the toxic organic contaminant is converted to innocuous chemical compound, obtain energy by catalysing energy producing chemical reactions and this energy is used in the production of new cells (Goudar and Subramanian, 1996) finally resulting in carbon-di-oxide and water. Waste treatment is the most vital aspect in any wastewater management programme. The results of this study has shown that the biological treatment almost satisfied the irrigation water guidelines.

4.4 Reutilization of degraded water for germination and growth of *Impatiens balsamina*

It is well known fact that water of good quality and free of pollutants are primary requirements for agricultural practice. After degradation, the treated water could be used for crop cultivation, irrigation and aquaculture purpose. Hence after degradation of 100% untreated polymer effluent, the treated water was used for germination and growth of *Impatiens balsamina* for a period of 15 days using 100% untreated and biotreated samples. Seeds of *Impatiens balsamina* when treated with 100% untreated and biotreated water showed interesting results. Germination and growth of seeds - shoot length, root length, no. of leaves and no. of roots of *Impatiens balsamina* (Table – 4) in 100% untreated sample for 15 days showed decreased rate of germination as well as the growth. Whereas maximum germination and growth of plants were recorded in the plants exposed to 100% biotreated (native, *Staphylococcus aureus)* sample. The presence of toxic substances in waste water has decreased the growth of *Impatiens balsamina* exposed to 100% untreated sample (Mehaytaab, 2008). Whereas increased rate of germination and growth of ornamental plant, *Impatiens balsamina* in 100% biotreated (native, *Staphylococcus aureus)* water is due to the maximum removal of toxic substances (Noorjahan *et al.,* 2004). Hence from the results of the overall study the native bacteria, *S. aureus* degraded the toxic substances by showing maximum reduction of toxic substances ranged from 11.5 - 99.56%. This biotreated water can be reutilized for the growth of ornamental plant as evidenced in the present study.

5. Conclusion

Hence from the overall results of the above study it can be concluded that native bacteria, *Staphylococcus aureus* played a key role in the degradation of untreated polymer effluent and the treated water can be utilized for growth of ornamental plant as evidenced in the present work. It can be recommended that the *Staphylococcus aureus* can be used for treatment of any industrial waste water. Since they have several advantages such as low energy consumption, low operation cost, fast growth rate and simple growth requirements. More over the treated water can be recycled for washing the industries, cooling towers etc. and also reused for agricultural and aquacultural purposes.

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Table - 1: Physico chemical parameters of untreated polymer effluent

S.No	Parameters	CPCB (1995)	Sep' 2017
1	Colour	Colourless	Yellow
2	Odour	Odourless	Pungent smell
3	pH	5.5-9.0	8.9
4	Electrical Conductivity (EC µmhos/cm)	400	164
5	Total Suspended Solids (TSS mg/l)	100	10,528
6	Total Dissolved Solids (TDS mg/l)	2100	186
7	Biochemical Oxygen Demand (BOD mg/l)	30	160
8	Chemical Oxygen Demand (COD mg/l)	250	599

Table – 2a: Gram's Staining and Biochemical Tests for isolation and identification of Bacterial species from untreated polymer effluent

S.No	Sample	Bacterial species	Gram's Staining	Indole Test	Methyl Red	Voges-Proskauer Test	Citrate Utilisation Test	Special test – Coagulase test
1	Untreated Polymer effluent	*Staphylococcus aureus*	Gram positive coccus	Positive	Positive	Negative	Negative	Positive
2		*Enterobacter aerogenes*	Gram negative bacillus	Negative	Negative	Negative	Positive	Not Applicable

IJASRM
International Journal of Advanced Scientific Research and Management, Volume 3 Issue 6, June 2018
www.ijasrm.com
ISSN 2455-6378

Table – 2b: Isolation and Identification of bacteria species from untreated polymer effluent

S.No	Culture Medium	Bacteria
1	Nutrient Agar	*Staphylococcus aureus* (Gram positive)
2		*Enterobacter aerogenes* (Gram negative)

Table – 3: Analysis of physico-chemical parameters of 100% untreated Polymer effluent before (control) and after degradation using *Staphylococcus aureus* (96 hours)

S.No	Parameters	CPCB (1995)	Control (Untreated effluent)	Biotreated water (Native, *Staphylococcus aurues*)
1	Colour	Colourless	Yellowish	Colourless
2	Odour	Odourless	Pungent	Odourless
3	pH	5.5-9.0	8.9±0.115	7.3±0.0577 -17.97%
4	Electrical Conductivity(µmhos/cm)	400	16,450±1.154	3,510±53.702 -78.48%
5	Total Suspended Solids(mg/l)	100	10,528±0.577	45±0.577 -99.56%
6	Total Dissolved Solids(mg/l)	2100	10,068±0.577	2,312±1.154 -77.05%
7	Biochemical Oxygen Demand(mg/l)	30	160±0.577	340.667±2.081 (-112.5%)
8	Chemical Oxygen Demand(mg/l)	250	599±0.577	1290.33±2.516 (-115.35%)

Table – 4: Germination and Growth (Shoot length, Root length, No. of Roots and No. of Leaves) of *Impatiens balsamina* (Balsam) exposed to control (tap water), untreated and biotreated (*Staphylococcus aureus*) of polymer effluent

S. No.	Duration	Parameters	Control	Untreated sample	Biotreated*(Staphylococcus aureus)* sample
1	15^{th} Day	Shoot length	9.7±0.1	6.6±0.057	8.6±0.057
2		Root length	1.5±0.152	1.0±0.152	1.3±0.152
3		No. of Roots	5±0.2	7±0.208	4±0.305
4		No. of Leaves	2±0.11	2±0.152	2±0.251