JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2009. Vol. **3**(2), p. 591-596

Potential use of *Oscillatoria terebriformis,* A Marine Cyanobacterium for the Treatment of Industrial Effluents through Bioremediation

C. Arunachalam* and A. Ambika

PG and Research Department of Microbiology,
Sri Venkateshwara College of Arts and Science, Peravurani, India.

(Received: 18 April 2009; accepted: 08 June 2009)

In order to develop an efficient and integrated waste treatment and recycling process, as a preliminary step, economically important cyanobacterium, *Oscillatoria terebriformis* was tested to treat the tannery industry efficient with used in both free and immobilized conditions. Treatment of effluent by *Oscillatoria terebriformis* for immobilized polyurethane foam cubes was used. The effluent was analyzed initially for physicochemical and heavy metals such as chromium (Cr), Copper (Cu) and Zinc (Zn). Treatment of effluent by *Oscillatoria* reduced the above heavy metals constituents of the effluent to a considerable amount and brought down the pH to near neutrality. Level of physicochemical and heavy metal was found to be reduced in the effluent treated immobilized cyanobacterium when compared to free and control.

Key words: Immobilization, Oscillatoria, Bioremediation, Tannery effluent, Environmental pollution, Physico-chemical parameter, Microbial treatment.

Tanning industry is one of the oldest and major industries using large quantities of water for processing raw hides into finished goods. Leather industry in India has shown rapid progress during the last few decades, but still today tannery effluent is considered as a major source of environmental pollution. Disposal of untreated effluent possess a major water pollution problem mostly in regions situated in the vicinity of the industries. Effluent is toxic due to the presence of certain hazardous materials like carbon and chromium.

Tannery waste discharged into surface water bodies and sewers or on land creates a problem on environmental disruptions. It is there fore necessary to emphasize that this water needs adequate treatment before they are discharged. Disposal of this waste ultimately pollutes the soil, surface and groundwater in the vicinity of tanneries. Tannery effluents are complex in nature and have a high pollution potential. Liquid wastes are of great concern to environment and ecological system if they are discharged into the agricultural fields or to the streams, without any proper treatment[1].

* To whom all correspondence should be addressed.
Mob.: +91-9842699532
E-mail: agro_arun@rediffmail.com

The environment is under increasing pressure from solid and liquid wastes emanating from the leather industry. These are inevitable byproducts of the leather manufacturing process and cause significant pollution unless treated in some way prior to discharge. In some instances, liquid waste is discharged into sewage systems where it undergoes full – scale treatment before being returned to the environment via surface waters.

Leather industry is one of the oldest and fastest growing industries in our country. There are more than 3000 tanneries in our country. The annual processing capacity of Indian tanneries, as per 1995 data is about 6,00,000 tons of hides and skins per year. Leather industry is the fifth largest foreign exchange earner in India and situated mainly in states like TamilNadu, Uttar Pradesh and Rajasthan, West Bengal, Maharastra, Karnataka, Punjab, Andhra Pradesh and Bihar.

TamilNadu contributes more than 50 percent of the export of finished leather and finished leather goods from India. It is interesting to note that North Arcot Ambedkar District alone contributes more than 25 percent of Indian exports in this sector. Of the exports of these products from TamilNadu more than 60 percent is contributed by North Arcot Ambedkar District.

Tirchirappalli is one of the active centers for tannery industries in TamilNadu. Out of 13 functioning tanning industries of Tirchirappalli, 10 have established their own individual effluent treatment plant and remaining 3 tanneries are connected to CETP for waste water treatment. In recent years a number of researchers have carried out studies to characterize and to evaluate the performance of lagooning system for tannery effluent treatment in south India.

Tannery wastewater contains organic as well as inorganic pollutants in suspended or soluble form. The term "Tannin "refers to specific category of phenolic compound of high molecular weight. Tannin is a substance, which is present in number of vegetable extract, which is responsible for converting animal's skin into stable leather products.

In the tanneries the major effluents are tannic acid. The composition of tannic acid includes sulfides, pyrocaenol and chromium which is mostly hexavalent and trivalent. These tannin need to be degraded to prevent the serious problem of pollution for which microorganisms have been used. Breakdown of these substances is largely depending on the microbial population of the soil.

Bioremediation is the only solution for this burning problem. The treatment of these effluents using cyanobacteria especially *Oscillatoria terebriformis* has not been studied so far. Hence in the present study an attempt has been made to study the characterization of tannery effluent and to biodegrade tannery effluents using both free and immobilized cyanobacteria.

MATERIAL AND METHODS

Study area

The samples of tannery effluent were collected from Primer Tanners and N.M Tanners, Sembattu, Trichy. TamilNadu, India. Raw samples of effluents were obtained from final discharge point wherein effluents from all the stages of processing are released together. The effluent was collected in polythene containers (2 liters capacity) and were brought to the laboratory with due care and stored at 20°C. The cyanobacterial strain, *Oscillatoria tereberiformis* was obtained from Bharathidasan University, Trichy. The cyanobacterium was made unialgal and sub cultured in BG 11 medium[2].

Immobilizations of Cyanobacteria

Immobilization of cyanobacteria in alginate beads was carried out as described below 1.1 g sodium alginate was dissolved in 30ml BG 11 medium at 80°C. After cooling, the solution was mixed with 10ml of sterile concentrated suspension of cyanobacteria. The sodium- alginate *cyanobacterium* mixture was then added drop by drop from a glass tube into a 0.1 M $CaCl_2$ solution at room temperature. The alginate beads formed were harvested, washed in growth medium, and resuspended in fresh growth medium. Free-living control cultures were also grown parallely and used. The initial inoculum of cyanobacteria was the same in all the above conditions[3].

Experimental Conditions

To study the removal of heavy metals compounds from the tannery effluent using free and immobilized cyanobacterium, the following experimental procedures were employed.

1. Raw tannery effluent (control)
2. Tannery effluent with inoculated free cells of *Oscillatoria sp.*
3. Tannery effluent with inoculated immobilized *Oscillatoria sp.*

For treating the effluent 2ml of uniform suspension of algal samples was inoculated for free treatment. Simultaneously 15 numbers of Sodium alginate foam were inoculated for the treatment of effluent under immobilized condition. The experiment was conducted in batch cultures in duplicates for a total period of 20 days under controlled conditions. The cultures were maintained at less than 14/10 LD with fluorescent tubes (13.7 W m^{-2}) at a temperature of 25 ±2° C. After 20days, the cultured *cyanobacterium* was separated from the effluents and BG 11 medium by filtration and washed repeatedly using sterile distilled water. Then the effluents were analyzed for physico- chemical analysis[4] and heavy metal compounds.[5]

RESULTS

In order to study the physicochemical characteristics and the heavy metal removal from the tannery industry effluent the *Cyanobacterium. Oscillatoria terebriformis* was used in both free and immobilized conditions. For immobilization polyurethane foam cubes were used. The color of the effluent was yellowish brown and the effluent was analyzed initially for physicochemical and heavy metals such as chromium (Cr), Copper (Cu) and Zinc (Zn) and presented in the Table 1 and Fig. 1-3.

The pH of the Tannery effluent was high (10.3) but after treatment with free and immobilized *cyanobacteria sp* it decreased to (6.5) in the range of neutral. In this analysis, P^H of the treated effluent registered a neutral pH in both *cyanobacteria* inoculated samples. Most of the parameters were significantly reduced from the initial values of effluent indicating their high amount of total suspended solids (1602mg/l). However, after treatment with free and immobilized cyanobacteria these are reduced to (1409mg/l and 982mg/l), respectively.

The total suspended solid was found to be high approaching the maximum permissible limit which may be harmful for soil characteristics in long term use for irrigation. Further the total dissolved solids (2475mg/l) were significantly high in tannery effluent, however when it was treated with free and immobilized *cyanobacteria sp* it was significantly reduced to 1902mg/l and 790mg/l respectively.

When Chloride concentration of the tannery effluent is high (98.5mg/l) it is not suitable for living things. After treatment with *cyanobacteria* the concentration of magnesium is considerably reduced (63.4 mg/l), to a moderate level. The tannery effluent contains a high amount of sulphate (20.2mg/l) which is harmful for soil characteristics in long-term use for irrigation. However, after treatment with free and immobilized *cyanobacteria sp* a considerable

Table 1. Physico-Chemical parameters of Tannery Effluent

S. No	Parameter	Raw	Treatment with free *Cyanobacterium*	Treatment with immobilized *cyanobacterium*
1.	Colour	Yellow	Colour less	Colour less
2.	Odour	Unpleasant	Odorless	Odorless
3	pH	10.3	7.5	6.5
4	Temperature	30°C	30°C	30°C
5	TSS	1602	1409	982
6	TDS	2475	1902	790
6	Sulphate	20.2	18.7	16.5
7	Chloride	98.5	84.5	63.4
8	BOD	12.8	9.7	5.8
9	COD	256	240	223

(All the chemical parameter values are expressed in mg/l)

reduction was noticed (18.7mg/l) and 16.5mg/l).

Further, chemical oxygen demand (256mg/l) is decreased (240mg/l and 223mg/l) after cyanobacterial treatment indicating the measure of oxygen consumed during the oxidation of the utilizable organic matter. BOD is the amount of oxygen required by organisms has high organic material so has high BOD value and vice versa. The initial amount of BOD is 12.8 mg/l but after treatment with *cyanobacteria* the BOD is reduced significantly (9.7mg/l and 5.8mg/l). Thus BOD is an important parameter indicating the levels of biological pollution in water providing an index of the amount of microbiologically utilizable organic matter.

Among the metals analyzed, Cr was recorded maximum (37 mg l^{-1}) followed by Zinc (0.43 mg l^{-1}) and Copper (0.15 mg l^{-1}). The amounts of heavy metals, such as Cr, Cu and Zn, were estimated in the control as well as *Oscillatoria* inoculated effluent (both free and immobilized cells) on 20th day. However, *Oscillatoria* treated effluent showed a substantial reduction of all forms of heavy metals when compared to initial level. (Fig: 1-3). Among the heavy metals, Cr was removed maximum (76%) followed by Zn (65%) and Cu (39%) as a result of immobilization of *cyanobacterium.* Where as the effluent with free cells showed 63.47 and 28% removal of Cr, Zn and Cu respectively.

The control (grown in BG11 medium) and the effluent treated *Oscillatoria* (both free and immobilized cells) were analyzed for the accumulation (absorption) of Cr, Zn, and Cu in their cells. For this, the samples were extracted by the extraction solvent and analyzed for heavy metals. The control did not show any traces of Cr, Zn, and Cu, whereas the effluent treated *Oscillatoria* recorded sufficient amount of Cr, Zn and Cu. Of the heavy metals tested, Cr incorporation in the immobilized cells was more than (97%) other heavy metals such as Zn and Cu recorded (85 and 80% accumulation respectively) whereas the free cells recorded 75 and 69% accumulation respectively. In general, immobilized *Cyanobacterium* was more efficient in removing and accumulating Cr, Zn, and Cu than free cells.

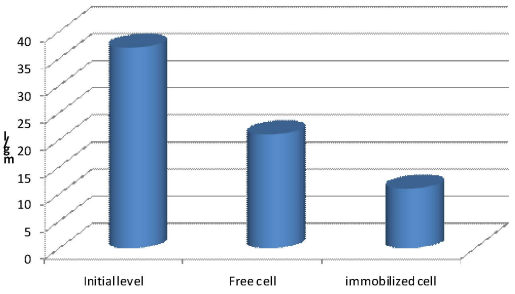

Fig. 1. Chromium level in effluent samples using free and immobilized Cyanobacteria

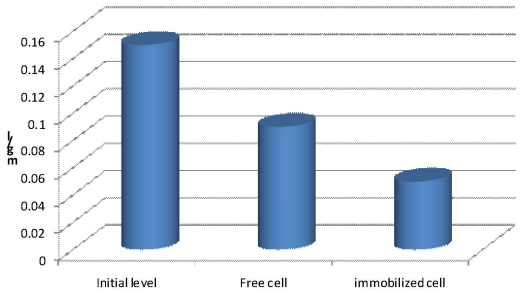

Fig. 2. Copper level in effluent samples using free and immobilized Cyanobacteria

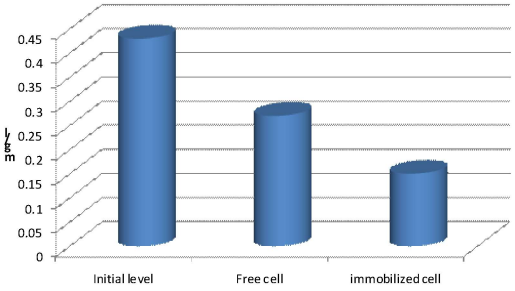

Fig. 3. Zinc level in effluent samples using free and immobilized cyanobacteria

DISCUSSION

The results of the present investigation when correlated with environmental variation, the pH of the samples was high. The extreme alkaline pH of the effluent sample renders the environment unfit for any purpose. Further the extremely high

pH may be unfit for most of the aquatic life[6] .The higher pH value may perhaps be due to the presence of considerable amount of carbonate.[7]

In tannery effluents, the total dissolved and suspended solids exceeded the recommended values. Further, Sewage waters and other industrial effluents also reveal their high amount of TS and TDS.[8] The total hardness level was comparatively higher in match industry (2000 mg/l), plate making (1200 mg/l) and sugar mill (1850 mg/l) effluents, than the permissible limit (300 mg/l). The sodium and chloride content was also high in all the effluents. Increased sodium and chloride content observed in these effluents may be responsible for the high salinity. As the total suspended solid was above permissible limit[9] , the high Total suspended solid may decrease the soil porosity due to salt deposition[10], has also studied the effect of wastewater and observed the higher suspended matter in waste water that cause the clogging agents of soil. Due to the higher concentration of solids in the effluent the germinated seeds may get low amount of oxygen in the dissolved form.

The biodegradation of metals from industrial effluents may be achieved by many microbial species such as bacteria, fungi, yeast, and algae which are known and capable of adsorbing heavy metals on their surface and accumulating within their structure[11,12,13,14]. Physical adsorption or ion exchange either at the living or non – living cell surface is very rapid and occur rapidly after the microorganisms come into contact with heavy metal ions. Accumulation occurs in living cells which is slow but related to metabolic activity[15,16,17]. Although there are a number of studies on removal of heavy metals, knowledge of application of biosorbents in the environment and industry has not been adequately studied.

Cyanobacteria possess an affinity to metal absorption capacity with very high multiplication rate. Such desirable characteristics of cyanobacteria have been employed for the application of this microbial biomass in detoxification of effluents[18] and have an edge over conventional wastewater treatment facilities[19]. Moreover, cyanobacteria being photosynthetic in nature provide a favourable environment because of their interior pH is almost two units higher than surrounding liquid. Several inorganic ion removals have also been tested by using free living and immobilized cyanobacteria[20,21].

From the above, it is evident that both free and immobilized cyanobacterium significantly remove the heavy metals such as Cr, Zn and Cu as evident from the present study. Moreover, metal accumulation appears to be high where immobilized cells are more efficient in metal removal than the free cells as evident from the present observation confirming the observations[22]

The utility of algae to reduce the heavy metal pollution load in river water has been attempted. Further, mixed algal culture removes the heavy metals such as Fe. Zn and Cu completely on 30th day as reported.[23] However, in the present investigation it is evident that more than 60 – 70% removal of Cr was observed with in 20days by both free and immobilized *cyanobacterium.*

In the present investigation the removal of heavy metals such as Cr, Zn and Cu from the effluent employing free and immobilized *Oscillatoria* has been recorded. Among the heavy metals 76% of Cr was removed by immobilized cells treated effluent, whereas, free cells of *Oscillatroria* only identified the presence of Cr from the effluents. The reason for removal of Cr from the effluent would be the oxidation state of the metal as pointed out, where he has stated that hexavalent chromium reduced the uptake rate as well as removal from the effluent.

The *cyanobacterium* cells have developed natural methods of responding to metals such as copper, lead, and cadmium through passive accumulation of cells and through surface binding to various functional groups. They have also been reported to be effective in the removal of harmful metals from the environment[24].

Form the foregoing it is concluded that, *Osicillatoria terebriformis* removes the heavy metals such as Cr, Zn and Cu from the chemical industries effluents effectively by immobilized status. Further, nearly 70 percent accumulation of heavy metals, removal from the effluent, has also been observed. Based on these, it is suggested that the tannery effluents may be successfully treated with both free and immobilized conditions of cyanobacteria.

ACKNOWLEDGEMENTS

The Authors are thankful to Dr. V. Ramaiyan, Principal, Sri Venkateshwara College of Arts and Science, Peravurani for their encouragement and various helps.

REFERENCES

1. Robinson, T., McMullan, G., Marchant, R., Nigam, P., Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol*. 2001; **77**: 247-255.
2. Rippka, R., Derulles, J., Waterbury, J.B., Hardman, M and Stanier, R.Y., Genetic assignmenta strain histories and properties of pure cultures of cyanobacteria, *J. Gen. Microbial.,* 1979; **111**: 1-61.
3. Kierstan, M. and Buckec., The immobilization of microbial cells, subcelllular orgnells and enzymes in calcium alginate gels. *Biotechnol Bioeng.,* 1977; : 387-1412.
4. APHA, AWWA and WPCF. Standarad methods for the examination of water and waste water, 15th edition American Public Health Association, Washington, D.C 1989.
5. Ben – Bassat, D., and Mayer, A.M., Volatilization of mercury by algae, *Physiol.Pl.,* 1975; **33**: 120-132.
6. Manivasakan, N., Physico-chemical examination of water, sewage and industrial effluents. *Appl. Microbial. Biotechnol*. 1985; **57**: 342-347.
7. Gautam, T and Bisnoi, S, Effect of dairy effluent on wheat, *J. Eco.Biol.,* 1995; 4111-115.
8. Pandit, B.R., Prasannakumar., P.G and Maheswari, R., Effect of dairy effluent on seed germination, seedling growth and pigment content of pennisetum typhoides. *J. Poll. Res*., 1996; **15**: 103-106.
9. Goel, P.K and K.P. Sharma., Quality criteria and standards for irrigation waters in Environmental guidelines and standards in India. *Techno science publications*, 1996; **34**(3): 254-259.
10. Ravina,I., Control of emitter clogging in drip irrigation with reclaimed waste water. *Irrig. Sci*., 1992; **13**: 129-139.
11. Campbell, R., and Martin, M.H., Continuous flow fermentation to purify wastewater by the removal cadmium. *Water, Air and soil Pollution,* 1990; **50**: 397-408.
12. Luef, E., Prey, T and Kubicek, C.P., Biosorption of zinc by fungal mycelial wastes, *Applied Microbiology and Biotechnology,* 1991; **34**: 688-692.
13. Mitani, T and Misic, D.M., Copper Accumulation by *Penicillium sp.* Isolated from soil, *Soil, plant nurt.* 1991; **37**(2): 347-349.
14. Vinita, V.P., and Radhanath, P.D., Biorecovery of zinc from industrial effluent using native microflora. *Inter. J Enviromental Studies,* 1992; **44**: 251-257.
15. Gadd, G.M., Heavy metal accumulation by bacteria and other microorganisms. *Experientia,* Vol. Birhauser Verlag, CH-4010, Basel Switzerland 1990.
16. Gadd, G.M., Metals and microorganisms: a problem of definition. *FEMS Microbiology Letters,* 1992; **100**: 197-204.
17. Noubakhsh, Nurbas Macit; Kilicarslan, Serpil; Iihan, Semra and Ozdag, Huseyin, Biosorption of $Cr6^{+}$, $Pb2^{+}$, and $Cu2^{+}$ ions in industrial waste water on *Bacillus sp. Chemical Engineering Journal,* 2002; **85**: 351-355.
18. Darnall, D.W., Greene, B., Benzyl, M.T., Hosea, J.M., Mc Pherson, R.A., Sneddon, J and Alexander, M.D., *Environ, Sci. Technol.,* 1986; **20**: 206.
19. Modak, J.M., and Natarajan, K.A., Miner. Metall. Process, 1995; 189.
20. Dharmanathan, R., Role of *Oscillatoria terebriformis* Ag. Ex. Gomont. In tannery effluent with reference to heavy metal removal. M.Phil Dissertation, Bharathidasan University, Tiruchirapalli, India 2003.
21. Manoharan, C and Subramanian, G., Interaction between paper mill effluent and the cyanobacteria *Osillatoria pseudogeminate* var. nigranulata, *Poll. Rea.*, 1992; **11**(2): 73-84.
22. Prakasham, R.S and Ramakrishna, S.V., The role of cyanobacteria in effluent treament, *J. Scient. Indl. Res.,* 1998; **57**: 258-265.
23. Senger, R.M.S., Sharma, K.D and Mittal, S., In vitro studies for the publication of river water by algal treatment, *Geobios.,* 1990; **17**: 77-81.
24. Slotton, D.G., C.R. Goldman, and A. Frank, Commercially Grown *Spirulina* Found to contain Low Leves of Mercury and Lead, *Nutrition Reports International,* 1989; **40**(2): 1165-1172.