Biosciences, Biotechnology Research Asia Vol. **6**(1), 111-120 (2009)

Biological decolorization and removal of metal from dye industry effluent by microalgae

R. DHAMOTHARAN, S. MURUGESAN*, M.C. SRIDHARAN and M. YOGANANDAM[1]

PG and Research Department of Plant Biology and Plant Biotechnology,
Presidency College Chennai - 600 005 (India).
*PG and Research Department of Plant Biology and Plant Biotechnology, Unit of Algal Biotechnology and Nanotechnology, Pachaiyappa's College, Chennai - 600 030 (India).
[1]Department of Industrial Biotechnology, MGR University, Chennai - 95 (India).

(Received: February 02, 2009; Accepted: March 09, 2009)

ABSTRACT

The potential of microalgae as a viable biomaterial for treatment of dye industry effluent was investigated. The stability and efficacy of the microalgae in long-term repetitive operations was also examined. The results obtained from the experiments revealed that the ability of the select algal species in removing colouring agents from the medium and metals. The effect for physico-chemical parameters on decolorization was examined. The findings provide evidence for the suitability of certain microalgae as indicators of effluent status and determine its composition.

Key words: Biological decolorization, dye industry effluent, microalgae.

INTRODUCTION

Industries are major sources of pollution to all environments. Based on the type of industry, various levels of pollutants can be discharged into the environment directly or indirectly through various routes, including public sewer lines. Wastewater from industries includes sanitary waste, manufacturing waste, wash waters and relatively uncontaminated water from heating and cooling operations (Emongor *et al*., 2005). The increasing global concern on the environment demands that wastes should be properly managed in order to minimize or possibly eliminate their potential harm to public health and the environment

Bioremediation is a key area of 'white' biotechnology, because the elimination of a wide range of pollutants from water and soils is an absolute requirement for sustainable development. There are numerous processes of cleaning water, industrial effluents and solid wastes, using aerobic and anaerobic microorganisms. Some of them are quite sophisticated, while others are simple and adapted to the conditions of developing countries.

Algae have been found to be effective organisms for bioremediation. The role of algae in the removal of various kinds of inorganic and related substances has been studied by several workers during the last several years (Senegar and Sharma, 1981). Algae serve as indicators of water pollution since they respond typically to many ions and toxicants. Blue green algae are ideally suited to play a dual role of treating wastewater in the process of effective utilization of different constituents essential for growth and leading to enhanced biomass production.

Dyes are synthetic aromatic compounds, having potential applications for colouration of various products in a wide range of industries. Textile industry ranks first in dye usage for colouring fabrics. Presently, more than 9000 distinctly different dyes are listed in the colour index. Dyes are classified into various application classes (direct, acid, basic, disperse, reactive, vat etc.) and chemical classes (azo, anthraquinone, phthalocyanine, xanthene, nitrothiazine, etc.). They are sometimes fused with heavy metals on the structural interface and are considered to have relatively bad consequence on the surrounding environment due to its toxic and inhibitory nature (Venkata Mohan *et al*., 2002a, b).

Among the dyes, azo group of dyes are the largest and most versatile class of dyes. More than half of the annually produced amounts of dyes are azo dyes (Stolz, 2001). Treatment of dye based effluents is considered to be one of the challenging tasks of environmental fraternity.

In the light of stringent environmental regulations, world over the textile industry is seeking to develop a cost-effective and environmental friendly wastewater remediation technology, especially those that allow colour removal that is largely unaffected by conventional treatment methods (O'Neill *et al*., 1999). Despite the existence of a variety of physical and chemical treatment processes, phycoremediation of textile effluent is still seen as an attractive solution due to its low cost and environmental friendly nature. Biological decolourisation and degradation of dyes remain more cost effective (Banat *et al*., 1996). Limited numbers of studies are available on biological treatment by algal species in spite of their ubiquitous distribution and their central role in the fixation and turnover of carbon and other nutrient elements (Semple *et al*., 1999). It is therefore appropriate to study the role of microalgae in removing dyes because they are the natural habitants of oxidation pond of wastewater treatment plants. Algal species of *Oscillatoria acuminata* and *Pithophora polymorpha* have been selected in the present study because they are the common algae found in the oxidation ponds of wastewater treatment plants in India. The present study aims to analyse physico-chemical properties of untreated and treated dye effluent and to evolve effective and economic biological treatment method for dye effluent using micro algae.

MATERIAL AND METHODS

Dye industry effluent was collected from Kanchipuram (a suburb of Chennai). The microalgae were maintained in CFTRI medium (Singh *et al*., 2002) and Bold Basal medium following Nicholas and Bold (1965). The role of microalgae in dye industry effluent was studied as follows: i) Effluent without *Oscillatoria acuminata* and *Pithophora polymorpha* (control) and ii) Effluent treated with *Oscillatoria acuminata* and *Pithophora polymorpha.* Experiments were conducted in duplicates and repeated three times. Samples were periodically (every 6th day) analyzed for various physico-chemical parameters using standard methods (APHA, 2000). The experiments were carried out for a period of 25 days.

RESULTS AND DISCUSSION

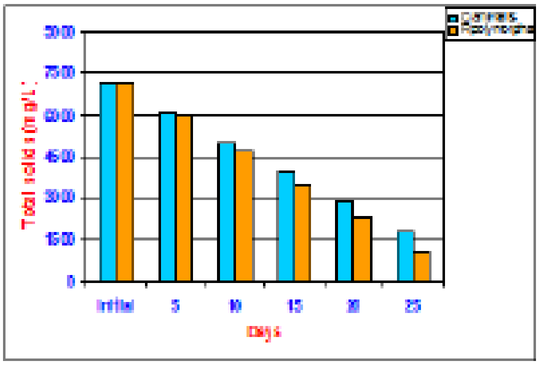
Due to rapid industrialization, large quantities of industrial wastes which contain toxic pollutants including heavy metals are being generated. Such waste finds their way into natural water bodies and continuously contaminates and deteriorates its quality.

The effluent was treated with *Oscillatoria acuminata* and *Pithophora polymorpha* without dilution; it took 25 days to decolourise from reddish brown. Similar kind of observation was made in textile dye using various fungi by Kousar *et al*., (2000). Limited work has been carried out on the usage of algae as decolourising agent. Mohan and Chaska (2004) studied the biological decolourisation of two azo dye effluents (direct and reactive dye) using a commonly available green algae *Spirogyra* sp. Previously, several researches have proved that biosorption processes of algae was highly pH dependent, hence pH is the most important parameter to be considered (Aksu and Tezer, 2005; Kumar *et al*., 2005 and 2006). Khanidtha Marungruenga and Prasert Pavasant (2005) reported that the macro alga *Caulerpa lentillifera* was found to have adsorption capacity for a basic dye, Astrazon Blue FGRL. Daneshvar *et al*., (2006) reported that the potential of *Cosmarium* species, belonging to green algae, was investigated as a viable biomaterial for biological treatment of triphenylmethane dye, Malachite Green (MG).

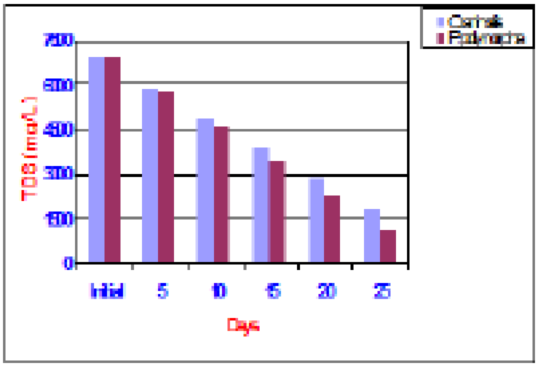
Colour removal by algae was due to three intrinsically different mechanisms

i) assimilative utilization of chromophores for production of algal biomass ii) CO_2 and H_2O transformation of coloured molecules to non-coloured molecules and iii) adsorption of chromophores on algal biomass. Algae capable of degrading azo dyes, through an induced form of an azo reductase showed good colour removal was reported by Jinqui and Houtian (1992). Several species of *Chlorella* and *Oscillotoria* were capable of degrading azo dyes to their aromatic amines and in further metabolizing the aromatic amines to simple organic compounds or CO_2. Some algae are even capable of utilizing a few azo dyes as their sole source of carbon and nitrogen. Use of such algae in stabilization ponds was proposed by Banat *et al.,* (1996) as they play an important role in aromatic amine removal.

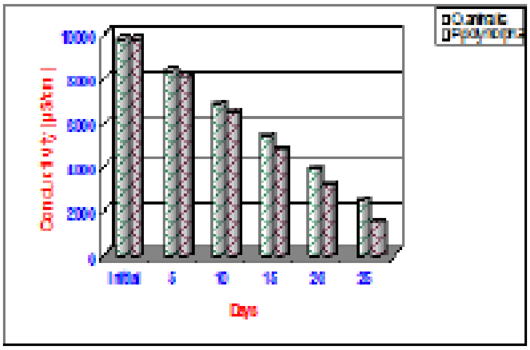
The dye colour removal could be attributed to a combination of biosorption, biodegradation and biocoagulation process as reported by Mohan and Chaska (2004) during the initial stages of the experiment. This observation is in accordance with an earlier study of Aziz and Ng (1988), that adsorption on the surface of the algal cells could be the primary mechanism of colour removal from textile wastewaters. Then, these sorbed dye molecules may permeate into the algal cells and stored in vacuole, and may participate in the metabolism leading to ingestion (bioconversion). Dilek *et al.,* (1999) has reported the mechanism of



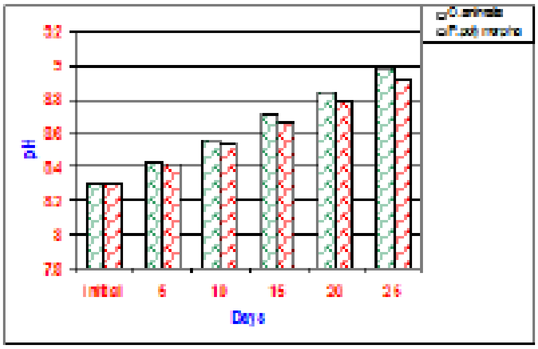
(a)



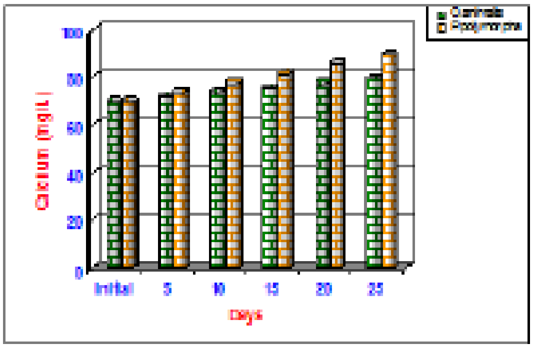
(b)



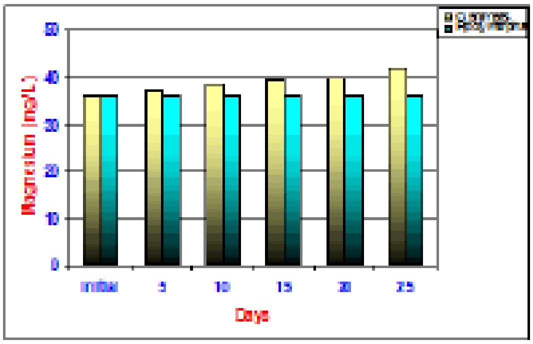
(c)



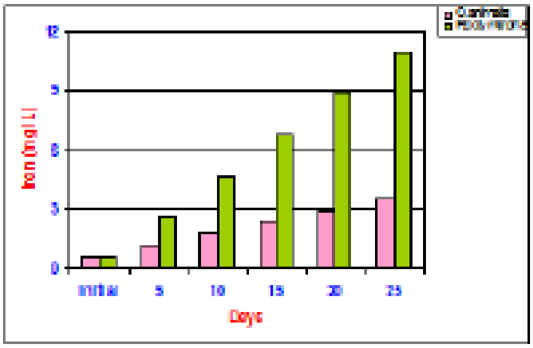
(d)



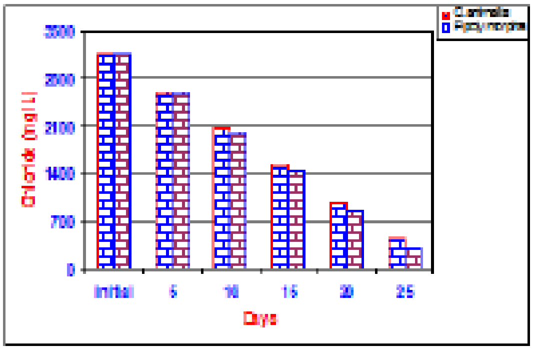
(e)



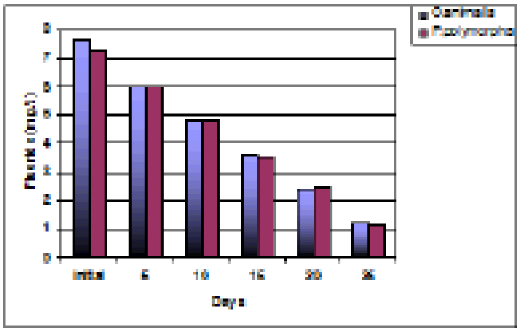
(f)



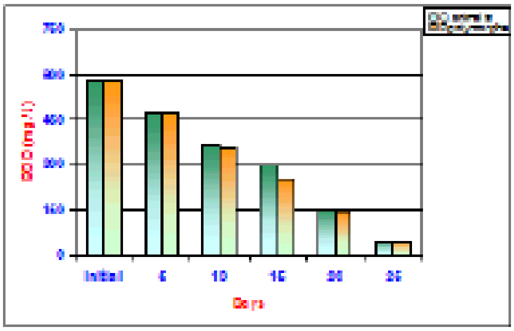
(g)



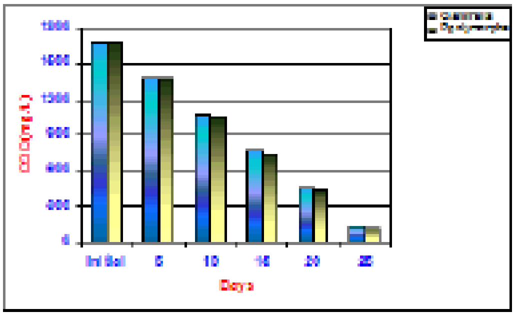
(h)



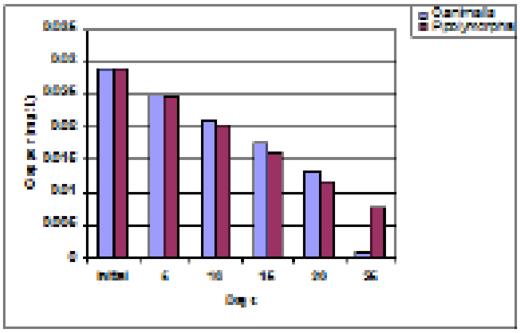
(i)



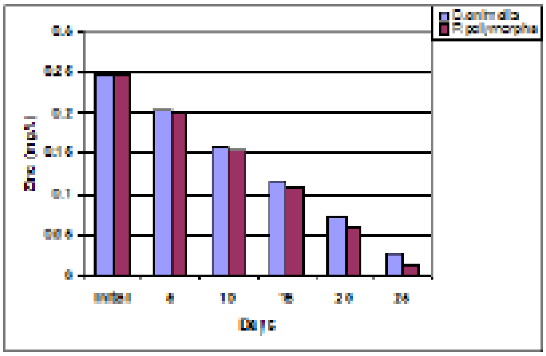
(j)



(k)



(l)



(m)

Fig.1: Changes in the concentrations of a) Total solids b) TDS c) Conductivity d) pH e) Calcium f) Magnesium g) Iron h) Chloride i) Fluoride j) BOD and k) COD k) Copper and m) Zinc

colour removal from pulping effluent by algae was mainly by metabolism. On the other hand, Lee *et al.,* (1978) reported that colour removal by algae is caused by metabolic transformation of coloured molecules to non-coloured molecules, with limited assimilation or degradation of the molecular entities. Due to metabolic activity and especially under stress algae secretes exopolymers into the environment. The released polymers possess excellent complexing capacity which may result in the removal of remaining dye molecules from the aqueous phase by chelation/complexation reactions (biocoagulation). Therefore, it could be possible that the dye removal is due to a combination of biosorption, bioconversion and biocoagulation reactions and since the removal starts with the biosorption and biodegradation, they could be the main mechanism in removing most of the dye molecules.

Total solids in the present study were reduced to 75.09 percent when the effluent was treated with *Oscillatoria acuminata* and 84.96 percent with *Pithophora polymorpha* (Fig.1a). The study confirmed that due to reduction of total solids; the effluent is suitable for safe disposal on land for irrigation. The untreated effluent contains considerable amount of suspended and total dissolved solids. Kotteswari *et al*., (2007) reported 24.82 percent reduction of total solids when the dairy effluent was treated with *Spirulina platensis.* Similarly, Dhamotharan *et al*., (2008) reported 56.89 percent reduction of total solids when the sewage was treated with cyanobacteria.

Total dissolved solids in the effluent were found to be reduced to 74.16 percent when the effluent was treated with *Oscillatoria acuminata* and 84.44 percent by *Pithophora polymorpha* (Fig.1b). Similar kind of work was reported by (Kannan *et al*., 2004). The study confirmed that due to reduction of total dissolved solids the effluent is suitable for safe disposal on land through irrigation. Similarly, Veeralakshmi *et al*., (2007) reported 19.16 percent reduction of TDS when the petroleum effluent was treated with *Oscillatoria* sp.

Presence of salts and contamination with ionic moieties increase conductivity. Electrical conductivity of the treated effluent was reduced to 74.43 percent by *Oscillatoria acuminata* and 84.47 percent by *Pithophora polymorpha* (Fig.1c). A high level of conductivity is due to increased concentration of salts. Increase in electrical conductivity of soil by distillery effluent has already been reported (Kaul *et al.,*1995).

In the present study, pH was found to increase in dye industry effluent when treated with algae, whereas there was no change in pH in the control. Interestingly, the pH of the dye effluent increased from 8. 29 to 8.98 with *Oscillatoria acuminata* and to 8.91 by *Pithophora polymorpha* (Fig.1d). Along with the limited nutrients from wastewater for their improvement over growth metabolism, they produce oxygen. The byproduct oxygen released during algal metabolism is utilized by the aerobic bacteria for biological oxidation of dissolved organics in effluent. These bacteria oxidize the effluent into simpler compounds which can serve as carbon source for algal species. Vijayakumar *et al.,* (2005) reported increase of pH in dye effluent, when treated with *Oscillatoria* sp. Murugesan *et al*., (2007) reported increase of pH in oil refinery effluent treated with cyanobacterium.

Industrial effluent showed higher values of hardness. Wastewater treatment processes generally have little effect on the hardness of wastewater. Hardness of water is due to carbonates of calcium, magnesium, silicate and phosphate. Hardness in effluent would make it unsuitable for industrial purpose as it may cause scaling of equipments (Goel, 2000). Saravanan *et al*., (1998) stated that the total hardness was due to Na, K, Ca etc, will affect the wastewater treatment plants. Total hardness was increased to 18.75 percent by *Oscillatoria acuminata* and 71.42 percent by *Pithophora polymorpha*. The reported values are similar to that of Ramasubramanian *et al*., (2006).

In the present study calcium was increased to 22.20 percent by *Oscillatoria acuminata* and 12.50 percent by *Pithophora polymorpha* (Fig.1e). Similarly magnesium was reduced to 14.28 percent by *Oscillatoria acuminata* and no change in *Pithophora polymorpha* proving efficient uptake of both species (Fig.1f). Vijayakumar *et al.,* (2005) reported 90 percent reduction of calcium and magnesium in dye effluent, when treated with

Oscillatoria sp. Veeralakshmi *et al*., (2007) reported 53.33 percent of calcium and 52.94 percent magnesium reduction when the petroleum effluent was treated with *Oscillatoria* sp.

In the present study, 85.02 percent of iron was increased when the effluent was treated with *Oscillatoria acuminata* and 95.12 percent by *Pithophora polymorpha*. (Fig.1g). Similar observation was reported by Senthil Kumar (2001) and Kannan *et al*., (2004). Ting *et al*., (1989) reported that the uptake of metal ion can be divided into two stages; rapid and slow stage. Most of algal forms occurring in the polluted fields have well defined sheath. Only the ensheathed forms of blue-green algae were found tolerating high concentrations of industrial effluents in laboratory culture (Adhikary, 1985; Adhikary and Sahu, 1988). Thus it is fairly convincing that the outermost surface structures play an important role for making ensheathed forms of blue green algae to thrive in adverse conditions.

Chloride is generally considered as one of the major pollutant in the effluents which are difficult to be removed by conventional biological treatment methods. In the present study, 85.97 percent of chloride was removed when the effluent was treated with *Oscillatoria acuminata* and 90.09 percent by *Pithophora polymorpha* (Fig.1g). In the present study amount of chloride, lowers the remediation activities of microorganisms. Vijayakumar *et al.,* (2005) reported 40 percent reduction of chloride in dye effluent, when treated with *Oscillatoria* sp. Kotteswari *et al*., (2007) reported 25.98 percent reduction of chloride when the dairy effluent was treated with *Spirulina platensis.*

Fluoride content in the present study was reduced to 83.56 percent when the effluent was treated with *Oscillatoria acuminata* and 84.93 percent *by Pithophora polymorpha* (Fig.1h). In general cyanobacteria are highly sensitive to fluoride, whereas green algae appear almost resistant to fluoride concentrations up to 10mM (Bhatnagar, 1997). Resistance to fluoride toxicity in micro algae has been correlated to be due to a lack of fluoride uptake. Fluoride is a classical inhibitor of algal photosynthesis. The threshold concentration at which toxicity is manifested varies between green algae and cyanobateria and is strongly pH dependent. The rate of fluoride removal was rapid initially and then slowed down gradually until it attained equilibrium beyond which there was no significant increase in the rate of removal. Uptake of fluoride ions that indicates the utilization of all active sites over the sorbent surface and attainment of the saturation or equilibrium phase. The initial rapid adsorption was perhaps due to the participation of specific functional groups and active surface sites (Venkata Mohan and Karthikeyan, 1997) in the adsorptive removal of the fluoride molecule. A large fraction of fluoride ion was removed within 15 days (equilibrium time) of the treatment. Fluoride also enters aquatic systems as a result of effluent discharge. Biosorption process is dependent on the aqueous phase pH and the functional groups on the algal cell walls and their ionic states (at particular pH) determine the extent of biosorption (Venkata Mohan *et al*., 2003). The algal cell wall contains a high amount of polysaccharide and some of them are associated with proteins and other components (Ilhami *et al*., 2005). These biomacromolecules on the algal cell surfaces have several functional groups (such as, amino, carboxyl, thiol, sulfydryl and phosphate groups) and biosorption phenomena depends on the protonation or unprotonation of these functional groups on the surface of the cell wall (Ilhami *et al*., 2005). The ionic form of fluoride in solution and the electrical charge of the algal cell wall components (i.e., functional groups carrying polysaccharides and proteins) depend on the solution pH. The presence of calcium and chloride reduce the toxicity of fluoride to fish (CCREM, 1987). Because of the limited effluent concentration data available for fluoride, it is recommended that it can be included in effluent surveys to expand the database, hence allowing for assessment of how significant a potential substance it may be. Dhamotharan *et al*., (2008) reported 48.64 percent reduction of fluoride when the sewage was treated with cyanobacteria

BOD and COD values were also considered as an index for the survival of living organisms in industrial effluent or waste. Mishra and Saksena (1989) have reported that BOD and COD are widely recognized as important parameters for measurement of organic load of wastewater. BOD indicates the quality of pollutant present in the waste that can be decomposed by bacteria under aerobic condition; hence BOD value increases with increase in organic content. Singh and Srivastava (1988) found higher values of BOD and COD, which may be due to high organic pollution that reduces flow of water. The COD on the other hand measures the oxygen requirement for chemical oxidation of matter. The high content of organic matter results in high value of COD of wastewater because chemical oxygen demand measures the recalcitrant (non-biodegradable) organic matter in biologically treated industrial effluents (Malviya *et al*., 2001). The BOD values in the effluent were found to be reduced by 92.24 percent when treated with *Oscillatoria acuminata* and 93.10 percent by *Pithophora polymorpha* (Fig.1i). The COD values in dye industry effluent were found to be reduced by 91.18 percent when treated with *Oscillatoria acuminata* and 93.33 percent by *Pithophora polymorpha* (Fig.1j). Kotteswari *et al*., (2007) reported 47.14 percent of BOD and 24.69 percent COD reduction when the dairy effluent was treated with *Spirulina platensis.* Similarly, Veeralakshmi *et al*., (2007) reported 50.00 percent of BOD and 11.54 percent COD reduction when the petroleum effluent was treated with *Oscillatoria* sp. It thus becomes evident that the reduction in COD was less as compared to reduction of BOD thus it is obvious that the degradation sought was through biological activity and not through a chemical agent. These compounds have different biodegradability. Depending on their ratios the waste stream at various outlets, the amount of BOD have varied widely compared to the COD values. That could be the reason that in some of the values where BOD has not increased to that extent as compared to the increase in the CODs observed. It was due to the presence of the compounds which is mostly non biodegradable in nature. These process accelerate the over blooming of phytoplankton in the presence of sunlight and oxygen. Reduction of BOD and COD levels might occur due to the removal of dissolved organic compounds and derivatives to some extent from the effluent during the treatment process. (Verma *et al*., 1988). This observation was in conformity with Baruah *et al* (1996); Amudha and Mahalingam (1999) and Kumar *et al* (2001) in different industrial effluent. COD measures the equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. It is an important parameter for industrial waste studies and control of waste treatment plants. Currently algae were found to be used to treat wood–based pulp and paper industry wastewater to remove up to 58% of COD (Tarlan *et al*., 2002).

Copper exposure in the environment is inevitable. The effectiveness of copper uptake by microalgae grown in the dye effluent was studied. The results showed that a high percentage of copper removal (66.70%) was achieved by *Oscillatoria acuminata* and 73.61 present by *Pithophora polymorpha* reared in the dye effluent (Fig.1k). Similar results were also obtained by Chan *et al*., (1981) in mixture of electroplating and sewage effluent. Wundram *et al*., (1996) in his study concluded that 72% of the variability in species richness could be attributed to increases in copper. A phytotoxicity test, evaluating the inhibition of photosynthesis in the green alga *Chlamydomonas* by heavy metals (Hg, Cu, Pb, and Cd) leaching from salt mine wastes, showed that inhibition varied from about 100% (Hg) and 80% (Cd) to almost 0% for Pb. Dhamotharan *et al*., (2008) reported 97.84 percent reduction of copper when the sewage was treated with cyanobacteria

In the present study the zinc reduction occurs up to 88.25 percent when the effluent treated with *Oscillatoria acuminata* and 94.33 percent was reduced by *Pithophora polymorpha* (Fig.1m). Zinc is an essential element for many enzymatic activities (Cheblowski and Coleman, 1976) in plants. However, zinc at toxic concentration affects the growth and metabolism of green plants (Shrotri *et al*., 1981). Falchuck *et al*., (1975a, b) described the role of zinc in cell size and morphology of *Euglena garcilis*. Since heavy metals often form the major constituents of complex industrial and energy process wastes, they may cause cellular abnormalities that affect the ability of cells to adjust to polluted conditions. The effect of heavy metals on growth on different organisms i.e. *Nostoc muscurum, Anabaena azollae, Hapalosiphon stuhlmani, Chlorogloea fritschii, Synechocystis* sps were studied by Pandey and Pandey (1994); Reddy *et al.,* (1997) and Padma *et al*., (1999). The surfaces of algae contain a number of functional groups with high affinity for metal ions and carry a net negative charge mainly due to carboxylic, sulfhydryl and phosphatic groups (Crist *et al*., 1990; 1994). Dhamotharan *et al*., (2008) reported 38.41 percent reduction of zinc when the sewage was treated with cyanobacteria

CONCLUSION

Sustainable growth of the dye industry effluent requires profitability, economic development and waste management. Waste management decisions must be made on an individual basis due to site characteristics on the farm and within the watershed. It is obvious that most industrial wastewater is too complex to have its toxic effect evaluated by chemical analysis. Combinations of algal test and the nitrification test or algal test and the biotox test are useful indicators of the toxicity of wastewater. If the effect of a treated wastewater on the recipient is of interest, then the algal test would be a relevant choice.

Thus there is a necessity to develop better treatment technologies to remove colour from industrial effluents. An important element in guiding the direction and development of decolourization technology should logically depend upon a sound scientific knowledge, which undoubtedly warrants further research. In view of the need for a technically and economically satisfying treatment technology, a flurry of emerging technologies are being proposed and tested at different stages of commercialization. Broader validation of these new technologies and integration of different methods in the current treatment schemes will most likely in the near future, render these both efficient and economically viable. Recycling of the treated wastewater by overland flow can alleviate the problem of water shortage. Complete recycling, however, may cause salt accumulation in the water. Indecent release of the water especially during rainy seasons is recommended in order to solve the salt problem. Nevertheless, the results of the present study suggest that microalgae has a potential capacity for dye colour removal and may be further investigated, for the development of oxidation (Waste Stabilization) pond treatment of wastewater for treatment of dye based effluents.

In the present study, microalgae have been used for purification and recycling of dye effluent. Further more, the better performance in field conditions gave positive indication of their usefulness in treatment of dye effluent. This system when standardized would not only be economical but also be ecofriendly and sustainable.

REFERENCES

1. Adhikary, S.P., Occurrence of ensheathed blue green algae in the sponge Iron Factory effluent polluted area. *J. O. Bot. Soc*. **7**: 18-23 (1985).
2. Adhikary, S.P. and J. Sahu, Ecophysiological studies on ensheathed blue-green algae in a distillery effluent polluted area. *Env. Ecol*. **6**: 915-918 (1988).
3. Aksu, Z., Tezer, S., Biosorption of reactive dyes on the green alga *Chlorella vulgaris*. *Process Biochem*. **40**: 1347-1361 (2005).
4. Amudha, P. and S. Mahalingam., Studies on the effect of dairy effluent on survival feeding energetics of *Cyprinus carpio*. *J Environ.Biol*. **20**(3): 275-278 (1999).
5. APHA. American Public Health Association., Standard methods for the examination of water and wastewater, Washington, D. C, USA 21st edition (2000).
6. Aziz, M.A. and Ng, W.J., 'Algae pond treatment of industrial wastewaters', *Proc. 2nd International Association on Water Pollution Research and Control Asian Conference on Water Pollution Control*, 519-525 (1988).
7. Banat IM, McMullan G, Meehan C, Kirby N, Nigam P, Smyth WF & Marchant R., Microbial decolouration of textile dyes present in textile industries effluents. In: Proceedings of the Industrial Waste Technical conference, Indianapolis, USA. 1-16 (1996)
8. Banat, I.M., Nigam, P., Singh, D. and Marchant, R., 'Microbial decolorization of textile dye containing effluents: A review', *Biores. Technol*., **58**: 217-227 (1996).
9. Bustard, M., McMullan, G. and McHale, A.P., 'Biosorption of textile dyes by biomass derived from *Kluyveromyces marxianus* IMB3', *Bioprocess Eng*., **19**: 427-430 (1998).
10. Baruah,B.K.D. Baruah and M. Das., Sources and characteristics of paper mill effluent. *Environment and Ecology*. **14**(3): 686-689

(1996).

11. Bhatnagar, M., Fluoride tolerance in micro algae and its ecological implications (Dissertation). Indian Agric. Res. Inst.New Delhi (1997).
12. CCREM (Canadian Council of Resource and Environment Ministers). Canadian Water Quality Guidelines (1987).
13. Chan K. Y., Wong K. H., and Wong P. K., Nitrogen and removal from sewage effluents with high Salinity of C*hlorella salina*, *Environ.Pollu*., **18**: 139-146 (1977).
14. Cheblowshi, J and Coleman, J.E., Zinc and its role in enzymes. In: Metal ions in biological systems (Biological action of metal ions) Ed: Sigel, H, New York and Basal Marcel Dekker. 61-140 (1976).
15. CPCB., Pollution control: acts, rules and modifications issued there under central pollution control board, New Delhi (1995).
16. Crist RH, Martin JR, Guptill P, Eslinger J, Crist DR., Interaction of metals and protons with algae. 2. Ion-exchange in adsorption and metal displacement by protons. *Environ Sci Technol.,* **24**: 337-342 (1990).
17. Dhamotharan, R., Murugesan. S and M. Yoganandam, Bioremediation of tannery effluent using *Cyanobacterium. Biosci*, *Biotech. Res. Asia*. **4**(1): 201 -206 (2008).
18. Dhaneshvar N, M. Ayazloo, 1, A.R. Khataee, M. Pourhassan., Biological decolorization of dye solution containing Malachite Green by micro algae *Cosmarium* sp. Science Direct (Bioresource Technology). Online (2006).
19. Dilek, F.B., Taplamacioglu, H.M. and Tarlan, E., 'Colour and AOX removal from pulping effluents by algae', *Appl. Microbiol. Biotechnol.* **52**: 585-591 (1999).
20. Emongor V, Nkegbe E, Kealotswe B, Koorapetse I, Sankwasa S, Keikanetswe S., Pollution Indicators in Gaborone Industrial Effluent. *J. Appl. Sci*. **5**(1): 147-150 (2005).
21. Goel, PK and Kulkarni, S.M., Effects of sugar factory waste germination of gram (*C. aritinum*, L). *J. Enviorn and Pollution* (2000).
22. Ilhami, T. B. Gulay, Y. Emine, B. Gokben., Equilibrium and kinetic studies on Biosorption of Hg (II), Cd (II) and Pb (II) ions onto micro algae *Chlamydomonas reinhardtii*, *J. Environ. Manag*. 77: 85-92 (2005).
23. Jinqui L & Houtian L., Degradation of azo dyes by algae. *Environ. Poll.* **75**: 273-278 (1992).
24. Kannan, N., Karthikeyan, G., Vallinayagam and TamilInselvan. N., A study on assessment of pollution load of sugar industry effluent. *ISEP* **24**(1): 256-262 (2004).
25. Kaul, S. N., T. Nandy, and R.K. Trivedy., Pollution control in distilleries. Enviro-media, Karad (1995).
26. Khanidtha Marungruenga, Prasert Pavasantb., Removal of basic dye (Astrazon Blue FGRL) using macro alga *Caulerpa lentillifera*. *Jr. Environ. Manag.* 1-7. (2005).
27. Kotteswari, M, Murugesan, S, Kamaleswari, J and Veeralakshmi, M., Biomanagement of Dairy effluent by using Cyanobacterium. *Indian Hydrobiol.* **10** (1): 109-116 (2007).
28. Kousar, N. Seshikala, D and Singara Charya, M.A., Decolourization of Textile Dyes. *Ind. J. Microbiol*., **40**: 1991-197 (2000).
29. Kumar, K.V., Ramamurthi, V., Sivanesan, S., Dyes and pigments. Biosorption of Malachite a green cationic dye onto *Pithophora* sp., freshwater algae. *Dyes Pigments* **69**: 74-79 (2006).
30. Kumar, K.V., Sivanesan, S., Ramamurthi, V., Adsorption of malachite green onto *Pithophora* sp., fresh water algae: equilibrium and kinetic modeling. *Process Biochem*., **40**: 2865-2872 (2005).
31. Kumar Senthil, R.D. R. Narayana Swamy and K. Ramakrishnan., Pollution studies of sugar mill effluent. Physico-chemical characteristics and toxic metals. *Poll Res*. **20**(1): 93-97 (2001).
32. Lee, E.G.H., Mueller, J.C. and Walden, C.C., 'Decolourisation of bleached kraft mill effluents by algae', *TAPPI*, **61**(7): 59-62 (1978).
33. Malaviya Piyush and V.S. Rathore., A correlation study on some physico chemical quality parameters of pulp and paper mill effluents. *Poll Res*. **20**(3): 465-470 (2001).
34. Manoharan, C and G. Subramanian., Feasibility studies on using cyanobacteria in ossein effluent treatment. *Indian J. Env. Hlth*. **35**(2): 88-96 (1993).
35. Manoharan, C. and G. Subramanaian., Interaction between paper mill effluent and the cyanophyacterium *Oscillatoria pseudogeminata.* var. *Unigranulata*. *Poll. Res.* **11**(2): 73-84 (1992a).
36. Manoharan C. and G. Subramanian, Sewage cyanobacteria interaction. A case study. *Indian J. Environ pro*. **12**(4): 251-258 (1992b).
37. Marwaha, S.S. Kennedy, J.F. Tewari. H.K. and Rhedu. A., Development of non-conventional technology employing immobilized yeast cell for the treatment of dairy industry effluents In. *Ind Biotechnol*. **8**: 17-23 (1998).
38. Mishra, S.R and Saxena, D.N., Industrial effluent pollution of Birna Nagar, Gwalior. *Poll Res*. **8**(2): 77-86 (1989).
39. Mohan, S.V and Chaska, Y.V., Biological

decolourisation of simulated azo dye inaqueous phase by algae *Spirogyra* sp. *Int. J. Environ. & Poll.,* **21**(30): 211-222 (2004).
40. Murugesan, S, Dhamotharan, R and Kamaleswari, J., Phycoremediation of oil refinery effluent using cyanobacterium. *Eco. Env & Conv*. **13**(4): 35-40 (2007).
41. Nicholas, H.W and Bold, H.C., *T. polymorpha* genet sp. *F. Phycol.* **1**: 34-8 (1965).
42. O'Neill, C., Hawkes, F.R., Hawkes, D.L., Lourenco, N.D., Pinheiro, H.M. and Delee, W., 'Colour in textile effluents-sources, measurements, discharge consents and simulation: a review', *J. Chem. Technol. Biotechnol.,* **74**: 1009-1018 (1999.
43. Padma, N, Reddy, M.N and Ragothman, G., Recent trends in algal research Ed: Subbarangiah Andhra Uni.Waltair (1997).
44. Pandey Usha and Pandey, J., Effect of cadmium on growth, photosyn. and N_2 fixation of *N. muscorum* and Cyanophage N-1 resistant mutant. *Phykos* **33**(1-2): 19-23 (1994).
45. Ramasubramanian, A., Jeyaprakash, R and Ramasamy, N., Assessment of physico-chemical parameters in three industrial effluents. *ISEP* **26**(12) 2: 1090-1092 (2006).
46. Reddy, M.N. Ragothaman, G and Padma, N., Effect of cadmium on the growth of *C. fritschii* and *Synechocystis* sp. *J. Sea Weed Res. Utilizn*. **19**(1-2): 81-94 (1997).
47. Rice, C.P. and Sikka, H.C., 'Fate of dieldrin in selected species of marine algae', *Bull. Environ. Contam. Toxicol*., **9**: 116-123 (1973).
48. Saravanan, S. Saravanan, A. Elangovan, N and Kalichelvan, P.T., Decolourization of tannery effluent of *Favobacterium* sp. EK. *Indian. J. Environ. Prot*. **19**(1): 19-24 (1998).
49. Semple, K.T., Cain, R.B. and Schmidt, S., 'Biodegradation of aromatic compounds by microalagae', *FEMS Microbiol. Letters*., **176**(2): 291-301 (1999).
50. Senegar, R.M.S., K.D. Sharma and S, Mittal., Studies for the publication of river water by algal treatment. *Geobios*. **17**: 77-81 (1981).
51. Senthilkumar, R.D., Narayanaswamy, R and Ramakrishnan, K., Pollution studies on sugar mill effluent physiological characteristics and toxic metals. *Poll. Res*. **20**(1): 93-97 (2001).
52. Shrotri, C.K. Rathore, V.S. and Mohanty, P., Studies on photosynthetic electron transport, phosphorylation and CO_2 fixation in Zn deficient leaf cells of *Zea mays*. *J. Plant. Natr*. **3**: 353-954 (1981).
53. Singh, P.K., Dhar DW, Pabbi, S. Prasanna. Arora, A., BGA. *Azolla* Bertilizers. A manual for the production. *Evaluation & Ulitilization*. Venus Printers and publishers, New Delhi (2002).
54. Singh, Shree Ram and Vinod Kumar Srivastava., Variations in water quality of Ganga river between Buxar and Bull. *Poll. Res*. **3-4**: 85-92 (1988).
55. Stolz, A., Basic and applied aspects in the microbial degradation of azo dyes. Appl. *Miocrobiol. Biotechnol*. **56**: 69-81 (2001).
56. Tarlan, E., Dilek, E.B. and Yetis. U., Effectiveness of algae in the treatment of a wood based pulp and paper industry wastewater. *Biores. Techn*. **84**: 1-5 (2002).
57. Ting, Y.P, Lawson, F., Prince, I.G., Uptake of cadmium andzinc by the alga *Chlorella vulgaris*: part 1. Individual ion species. *Biotechnol. & Bioengn*. **34**: 990 (1989).
58. Tikoo, V., Scragg, A.H. and Shales, W., 'Degradation of pentachlorophenol by microalgae', *J. Chem. Tech. Biotechnol*., **68**: 425-431 (1997).
59. Veeralakshmi, M. Kamaleswari.J, Murugesan, S and Kotteswari, M., Phycoremediation of Petrochemical effluent by Cyanobacterium. *Indian Hydrobiology.* **10**(1): 101-108 (2007).
60. Venkata Mohan, S. Y. Vijaya Bhaskar, J. Karthikeyan, Biological decolorization of simulated azo dye in aq. phase by algae *Spirogyra* species, *Int. J. Environ Pollut.* **21**(3): 211-222 (2003).
61. Venkata Mohan, S., Chandrasekhar Rao, N., Karthikeyan, J., Adsorption removal of direct azo dye from aqueous phase onto coal based sorbents: a kinetic and mechanistic study. *J. Hazard. Mater*. **90**(2): 189-204 (2002a).
62. Venkata Mohan, S., Chandrasekhar Rao, N., Krishna Prasad, K., Treatment of simulated reactive yellow 22 (Azo) dye effluents using *Spirogyra. Waste Mange*. **22**: 575-582 (2002b).
63. Venkata Mohan, S., Karthikeyan, J., Removal of Lignin and tannin aqueous solution by adsorption onto activated charcoal. *Environ. Poll*. **1-2**: 183-197 (1997).
64. Verma, A.M. V.K. Dudani, B. Kumari and A.N. Kargupta., Algal population in paper mill wastewater. *Indian. J. Environ. Hlth*. **30**: 388-390 (1988).
65. Vijayakumar S., Thajuddin N., and C. Manoharan., Role of cyanobacteria in the treatment of dye industry effluent. *Poll. Res.* **24**(1): 69-74 (2005).
66. Wundram, M., Selmar, D. & Bahadir, M., The *Chlamydomonas* test: a new phytotoxicity test based on the inhibition of algal photosynthesis enables the assessment of hazardous leachates from waste disposals in salt mines. *Chemosphere* **32**: 1623-31 (1996).