Cyanobacterial remediation of industrial effluents II. Paper mill effluents

Sonil Nanda [1], Prakash Kumar Sarangi [2], Jayanthi Abraham [1,*]

[1] School of Bio Sciences and Technology, VIT University, Vellore 632 014, India
[2] Department of Botany and Biotechnology, Ravenshaw University, Cuttack 735 003, India
jayanthi.abraham@gmail.com

Abstract: The disposal of paper mill effluents into the environment creates adverse effects by altering the normal physiochemical properties of soil and water. In the treatment system, *Nostoc* was employed for the bioremediation of paper mill effluents. The effluents were analysed for their physiochemical and elemental parameters. The major interests were evaluating the percent removal of biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and colour of the effluents. The results revealed a considerable decrease of 53.1% in colouration, 49.6% in BOD, 39.7% in COD and 53.0% in TDS of paper mill effluents after 4 weeks of treatment with *Nostoc*. [New York Science Journal 2010;3(12):37-41]. (ISSN: 1554-0200).

Keywords: Paper mill effluents, *Nostoc*, bioremediation

1. Introduction

Paper is manufactured from raw materials containing cellulose fibres, generally wood, recycled paper and agricultural residues. In developing countries, about 60% of cellulose fibres originate from non-wood raw materials such as sugar cane bagasse, cereal straw, bamboo, reeds, esparto grass, jute, flax and sisal. The significant environmental impacts of the paper manufacture result from the pulping and bleaching processes. In some processes, sulphur compounds and nitrogen oxides are emitted to the air, and chlorinated and organic compounds, nutrients, and metals are discharged in wastewaters.

Paper mill industries are found to be economically important in trading and fetching foreign exchange in global market. The manufacture of pulp, for paper production, involves mechanical (including thermo-mechanical), chemi-mechanical and chemical methods. In processes like pulping, most of the original lignin in the raw pulp is retained and bleached with peroxides and hydrosulphites. Oxygen, hydrogen peroxide, ozone, peracetic acid, sodium hypochlorite, chlorine dioxide, chlorine and other chemicals are used to transform lignin into an alkali-soluble form. Sodium hydroxide is necessary in the bleaching process to extract the alkali-soluble form of lignin. Release of these effluents into aquatic ecosystems alters the pH, increases the BOD and COD and gives the water intense colourations (Ajayi and Osibanjo, 1980). In general, the effluents contain several types of chemicals such as dispersants, levelling agents, acids, alkalis, carriers and various dyes, phenols, carbonates, alcohols, cyanide, heavy metals etc (Cooper, 1995). However, the toxicity of the effluents depends on the technologies and operations employed in paper manufacture. Proportionate amount of solid wastes from paper mills is of additional concern which constitutes wastewater treatment sludges. Components in these wastes that can be reused include waste paper, which can be recycled, and bark, which can be used as fuel. Lime sludge and ash may need to be disposed in an appropriate landfill.

The polluted wastewater from paper mills poses a substantial danger to health and environmental quality. Paper mill are one of such sources of effluents whose disposal into the environment creates serious adverse effects by altering the normal physiochemical properties of soil and water. In fact, the organic matter present in these effluents favours the growth of various microorganisms as ideal substrates and depletes oxygen by rapid respiration and oxidation. As a result, a major oxygen deficiency is encountered by the aquatic ecosystem which is deleterious for the native flora and fauna.

Treatment of paper mill effluents typically includes neutralization, screening, sedimentation, and floatation or hydrocycloning to remove suspended solids and biological/secondary treatment to reduce the organic content in wastewater and destroy toxic organics. Chemical precipitation is also used to remove certain cations. In this study, biological treatment system involving cyanobacteria, especially *Nostoc* was implemented for bioremediation of the paper mill effluents.

2. Material and Methods

2.1. Sample collection

Paper mill effluents were collected from a few paper manufacturing industries situated in eastern India. As per the request, the details of the industries and proprietors have been kept confidential and are not discussed anywhere in this article. The effluents were collected in sterile glass bottles, transported in cold condition to the laboratory for physiochemical and microbiological analysis. For microbiological analysis, the effluent samples were sterilized by autoclaving at 121^{o}C for 15 mins.

2.2. Physiochemical analysis

Physiochemical analyses of the paper mill effluents were performed following the standard methods by APHA (1992). The parameters analysed were colour, conductivity, pH, TDS (Valentine, 1996), BOD, COD and total nitrogen. Methods described by APHA (1980) were followed for determination of bicarbonate, carbonate, sulphite and sulphate. Gravimetric estimation for chloride was performed (Strickland and Parson, 1972) and phosphate was estimated by procedures described by Murphy and Riley (1962). Total nitrogen was estimated using Kjeldhal N-analyzer.

2.3. Elemental analysis

Metals in the effluents were determined by atomic absorption spectrophotometer following wet oxidation of the effluent sample by di-acid digestion method with a mixture of concentrated HNO_3:$HClO_4$ (3:1 v/v) (Hossner, 1996).

2.4. Source of organism and culture

Nostoc was cultured in BG11 medium in Erlenmeyer flasks at 30^{o}C and 190 rpm (Yoon *et al*., 2002) for about 21 d. The culture environment was illuminated properly to facilitate the cyanobacterial growth. The organism was obtained in mats and maintained for further analysis on the effluent samples.

2.5. Determination of biodegradability

50 ml of sterile paper mill effluent samples were supplemented to 250 ml of BG11 media inoculated with *Nostoc* in Erlenmeyer flasks and kept under illumination at 30^{o}C for 28 d under aerobic condition. For control, 50 ml of sterile effluents were added to 250 ml BG11 media without inoculation of *Nostoc*. For first 48 h of incubation, the flasks were kept in an incubator shaker at 100 rpm for the purpose of uniform mixing of the media and effluents. Periodic weekly monitoring of the samples was done for investigating the physiochemical characteristics and biodegradability of the effluents.

For determining decolouration of the effluents, the media was centrifuged at 5000 rpm for 15 mins to get cell free filtrate. The clear filtrate was analysed in a spectrophotometer for measuring its absorbance at 485 nm wavelength. Percent removal of colour, BOD, COD and TDS of the effluents by *Nostoc* was evaluated.

3. Results and Discussion

Effluent samples were analyzed for their physiochemical and elemental characteristics before and after treatment with *Nostoc*. Table 1 makes a comparison between the control and treated paper mill effluents on a weekly basis. The effluents were characterized with an initial pH of 9.0±0.1. This alkalinity is due to the rate of aerobic decomposition. The acceptable limits for discharge of wastewaters to both surface waters and sewers vary, ranging between from pH 5.5 to 10 (Bosnic *et al*., 2000). Formation of NH_3 from NH_4^+ is favoured by an alkaline pH which might result in NH_3 volatilization (Contreras-Ramos *et al*., 2004). This interaction can be related to the total N of the effluents which was 192.4±3.5 on day 28.

A decrease in electrical conductivity from 33.2±0.5dSm/l to 17.0±0.2dSm/l was found on day 28 of treatment. The high conductivity however did not affect cyanobacterial activity during bioremediation. Santamaria-Romero and Ferrera-Cerrato (2001) reported that salt concentration above 8.0dSm/1 negatively affected the microbial populations as well as biotransformation of organic matter. A subsequent fall in TDS levels from 2621.4±22.0mg/l to 1231.2±13.0mg/l was found after the cyanobacterial treatment.

A change in the effluent colour was an initial indication of biodegradation. The initial effluent colour at the time of collection was tan and finally after cyanobacterial treatment for 4 weeks it turned peach. The colour is a contribution of the raw materials, dissolved solids and minerals of vegetable origin, tannins, synthetic dyes etc.

The dyes colour the water bodies and hampers light penetration which is a very critical factor for aquatic life forms (Goncalves *et al*., 2000). However, after a due course of discharge of the effluents in the water bodies there is a marked loss in colouration between 10 to 15% (Vaidya and Datye, 1982). As the chief ingredients of BG11 medium are salts, so the supplementation of paper mill effluents into the minimal medium acted as the carbon source for the cyanobacteria to metabolize it and reduce its concentration from the medium.

Figure 1, 2, 3 and 4 illustrate the percent removal of BOD, COD, TDS and colouration of the effluent after cyanobacterial treatment for 28d. On day 28 of treatment, the percent degradation for BOD, COD, TDS and colour were 49.6%, 39.7%, 53.0% and 53.1%, respectively.

Table 1. Physiochemical and elemental characteristics of paper mill effluents before and after treatment with *Nostoc*

Parameter	Concentration				
	Day 1	Day 14		Day 28	
		Control	Treatment	Control	Treatment
Physiochemical analysis					
Colour	Tan	Tan	Peach	Tan	Peach
Odour	Rotten egg smell	Rotten egg smell	Fowl smell	Rotten egg smell	Foul smell
pH	9.0 ± 0.1	8.7 ± 0.2	7.9 ± 0.2	8.6 ± 0.2	7.3 ± 0.2
Electrical conductivity (dSm/l)	33.2 ± 0.5	32.7 ± 0.5	26.3 ± 0.4	29.9 ± 0.5	17.0 ± 0.2
Total dissolved solids (mg/l)	2621.4 ± 22.0	2610.0 ± 18.2	1852.3 ± 14.0	2561.4 ± 16.4	1231.2 ± 13.0
Biological oxygen demand (mg/l)	821.1 ± 33.1	813.4 ± 31.5	591.5 ± 30.1	789.0 ± 20.0	413.1 ± 17.0
Chemical oxygen demand (mg/l)	994.0 ± 40.1	989.7 ± 41.3	813.1 ± 19.2	973.2 ± 38.0	598.7 ± 14.5
Total nitrogen (mg/l)	562.0 ± 8.5	551.6 ± 5.5	389.2 ± 7.5	512.1 ± 5.1	192.4 ± 3.5
Bicarbonate (%)	12.1 ± 0.4	10.3 ± 0.3	8.92 ± 0.4	9.91 ± 0.3	6.23 ± 0.1
Carbonate (%)	10.7 ± 0.4	9.24 ± 0.5	7.13 ± 0.2	8.85 ± 0.1	7.41 ± 0.2
Chloride (mg/l)	75.1 ± 2.0	71.9 ± 1.1	58.1 ± 2.0	66.3 ± 2.3	41.1 ± 1.5
Phosphate (mg/l)	63.8 ± 3.5	58.3 ± 2.4	47.2 ± 1.3	53.8 ± 2.7	33.1 ± 1.0
Sulphide (mg/l)	79.4 ± 5.0	76.9 ± 4.5	56.9 ± 3.2	71.4 ± 2.1	41.5 ± 0.5
Sulphate (mg/l)	65.9 ± 1.5	63.5 ± 1.5	42.0 ± 2.4	53.5 ± 1.1	29.1 ± 1.6
Elemental analysis					
Arsenic (mg/l)	3.1 ± 0.1	3.0 ± 0.1	2.3 ± 0.1	2.7 ± 0.3	1.2 ± 0.4
Calcium (mg/l)	188.0 ± 13.5	182.0 ± 10.2	127.2 ± 9.5	156.0 ± 10.9	98.6 ± 3.5
Cadmium (mg/l)	3.0 ± 1.1	3.0 ± 0.3	2.6 ± 0.8	2.7 ± 0.2	1.9 ± 0.1
Cobalt(mg/l)	1.1 ± 0.1	1.1 ± 0.07	0.8 ± 0.03	1.0 ± 0.03	0.7 ± 0.02
Chromium (mg/l)	69.1 ± 2.2	63.1 ± 2.1	48.0 ± 1.5	54.1 ± 1.1	38.1 ± 1.0
Copper (mg/l)	4.7 ± 0.1	4.5 ± 0.2	3.2 ± 0.4	3.9 ± 0.2	2.1 ± 0.04
Iron (mg/l)	28.0 ± 4.4	26.8 ± 3.0	21.3 ± 2.4	21.1 ± 1.4	9.4 ± 1.0
Lead (mg/l)	3.2 ± 1.2	3.0 ± 0.4	2.3 ± 0.3	2.8 ± 0.4	1.7 ± 0.5
Manganese (mg/l)	79.0 ± 8.2	73.2 ± 4.5	56.2 ± 3.0	68.3 ± 0.2	39.2 ± 1.5
Sodium (mg/l)	82.3 ± 8.5	75.3 ± 7.5	69.0 ± 6.0	66.3 ± 3.1	45.0 ± 2.5
Zinc (mg/l)	8.1 ± 0.1	7.8 ± 0.1	6.5 ± 0.2	7.6 ± 0.6	4.7 ± 0.1

Note: Mean ± standard deviation (n = 5)

The high BOD often creates septic conditions, generating foul-smelling hydrogen sulphide, which in turn precipitates iron and any dissolved salts, turning the water black and highly toxic for aquatic life (Akbar and Khwaja, 2006). The biological treatment systems such as activated sludge, aerated lagoons and anaerobic fermentation are found to reduce BOD by over 99% and achieve a COD reduction of 50% to 90%. Tertiary treatment studies have shown reduction in toxicity, suspended solids, and colour of the effluents.

The elemental studies imply that the effluent is rich in calcium, sodium, manganese and chromium. Substantially higher levels of chloride, phosphate, sulphide and sulphate were also evident in the effluents. The main source of nutrients, nitrogen, and phosphorus compounds was the raw material such as wood. The use of peroxide, ozone, and other chemicals in bleaching often makes it necessary to use a complexing agent for heavy metals such as manganese. In modern paper mills, oxygen is used in the first stage of bleaching to avoid the use of any kind of chlorine chemicals and employ total chlorine-free (TCF) bleaching. Certain bacteria and fungi are often sensitive to higher levels of these salts which cause their cellular breakdown.

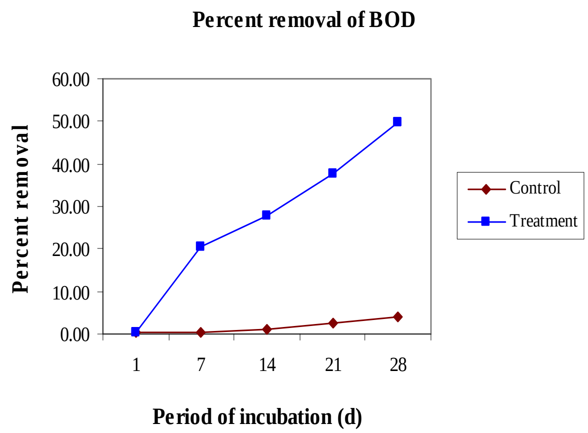

Figure 1. Percent removal of BOD of paper mill effluents under aerobic condition

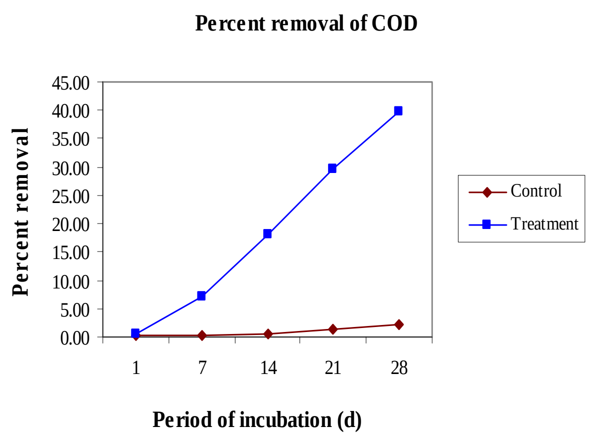

Figure 2. Percent removal of COD of paper mill effluents under aerobic condition

On the other hand, negligible effect of salts is found over cyanobacteria as it prefers to grow in salt mediums. With increasing heavy metal concentrations, cyanobacteria are indispensable tools for their bioremediation (Nriagu and Pacyna, 1988). An acceptable decrease in the heavy metals concentration was evident on day 28 of cyanobacterial treatment. However, slight reduction of the heavy metals in the control flasks was due to precipitation of their salts in aqueous solution.

Removal of heavy metals, especially cadmium by cyanobacteria has been extensively studied (Matsunaga *et al.*, 1999; Les and Walker, 1984). The pH enhanced the heavy metal bioremediation by *Nostoc*. The pH of the effluents varied between 7.3 and 9.0 during cell growth, which is similar to the natural variations in seawater, thus indicating no significant precipitation of heavy metals by alkalinization (Matsunaga *et al.*, 1999). During alkaline pH the solution was rich in sulphides.

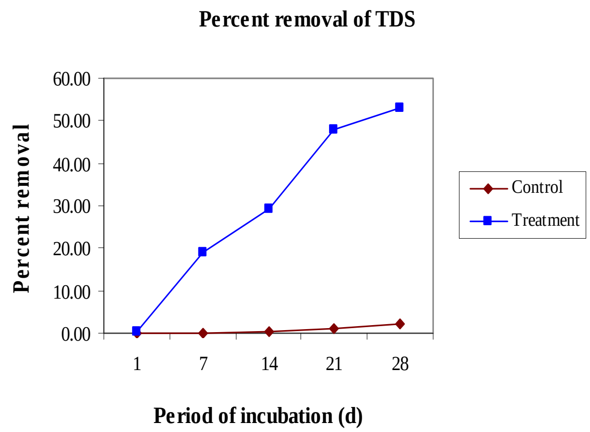

Figure 3. Percent removal of TDS of paper mill effluents under aerobic condition

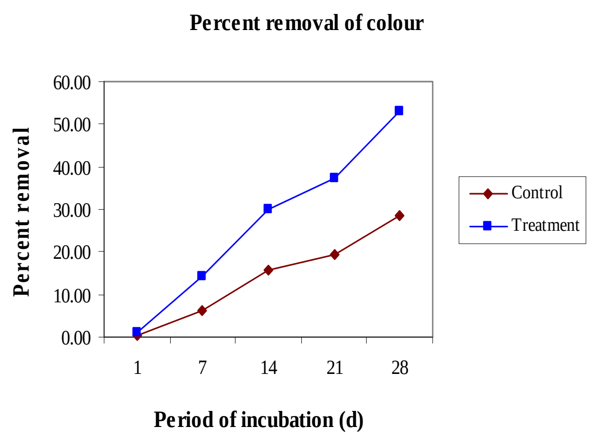

Figure 4. Percent removal of colour of paper mill effluents under aerobic condition

Bosnic *et al.* (2000) suggested that with a fall in pH of the effluent below 9.5 there was liberation of hydrogen sulphide from the effluent which was characterised by a smell of rotten eggs. Another factor for odour may be the ammonia and urea released from the raw materials. Since the effluents were rich in organic matter and were distinguished with a high BOD, aerobic decomposition by microorganisms produced hydrogen sulphide to impart the characteristic pungent odour. The odour of the effluent did not vary much through out the treatment process.

4. Conclusions

This study indicated that cyanobacterial treatment method is a feasible technique for bioremediation of paper mill effluents. There was a considerable decrease of 53.1% in colouration, 49.6% in BOD, 39.7% in COD and 53.0% in TDS of the effluents after 4 weeks of treatment with *Nostoc*. The mass culture of the cyanobacteria in form of mats enhanced the bioremediation process in laboratory set-up as in natural conditions.

Corresponding Author:

Dr. Jayanthi Abraham
Assistant Professor (Senior)
School of Bio Sciences and Technology
VIT University
Vellore 632 014, Tamil Nadu, India
Email: jayanthi.abraham@gmail.com

References

1. Ajayi, S.O., Osibanjo, O. The state of environment in Nig. Pollution studies of textile industries in Nigeria. *Monogra*, 1980; 1: 76-86.
2. Akbar, N.M., Khwaja, M.A. Study on Effluents from Selected Sugar Mills in Pakistan: Potential Environmental, Health, and Economic Consequences of an Excessive Pollution Load. Sustainable Development Policy Institute (SDPI) Islamabad, Pakistan, 2006; 1-41.
3. APHA. Standard methods for the examination of water and wastewater including bottom sediments and sludges, American Public Health Association, Washington DC, 1980.
4. APHA. Standard methods for the examination of water and wastewater, 18th Edn. American public health association. American water works association. Water environmental federation, Washington DC, 1992.
5. Bosnic, M., Buljan, J., Daniels, R.P. Pollutants in tannery effluents. United Nations Industrial Development Organization 2000; 1-26.
6. Contreras-Ramos, S.M., Alvarez-Bernal, D., Trujillo-Tapia, N., Dendooven, L. Composting of tannery effluent with cow manure and wheat straw. *Bioresource Technology*, 2004; 94: 223-228.
7. Cooper, P. Colour in dyehouse effluent. Society of dyers and colourists, The Alden Press, Oxford, 1995.
8. Goncalves, I.M.C., Gomes, A., Bras, R., Ferra, M.I.A., Amorin, M.T.P., Porter, R.S. Biological treatment of effluent containing textile dyes. *Coloration Technology*, 2000; 116 (12): 393-397.
9. Hossner, L.R. Dissolution for total elemental analysis. In: Methods of Soil Analysis, Part 3- Chemical Methods. Soil Science Society of America, Madison, WI, USA, 1996.
10. Les, A., Walker, R.W. Toxicity and binding of copper, zinc and Cd by the blue-green alga, *Chroococcus paris*. *Water, Air and Soil Pollution*, 1984; 23: 129-139.
11. Matsunaga, T., Takeyama, H., Nakao, T., Yamazawa, A. Screening of marine microalgae for bioremediation of cadmium-polluted seawater. *Journal of Biotechnology*, 1999; 70: 33-38.
12. Murphy, J., Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal Chem Acta*, 1962; 26: 31-36.
13. Nriagu, J.O., Pacyna, J.M. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, 1988; 333, 134-139.
14. Santamaria-Romero, S., Ferrera-Cerrato, R. Dynamics and relationships among microorganisms, C-organic and N-total during composting and vermicomposting. *Agrociencia*, 2001; 35: 377-383.
15. Strickland, J.O.H., Parson, T.R. A practical handbook of sea water analysis. *Bulletin of Fish Research Board of Canada*, 1972; 167: 130.
16. Vaidya, A.A., Datye, K.V. Environmental pollution during chemical processing of synthetic fibers. *Colourage*, 1982; 14: 3-10.
17. Valentine P. Laboratory Analysis. *Common Effluent Plant* 1996; 1-18.
18. Yoon, J.H., Sim, S.H., Kim, M.-S., Park, T.H. High cell density culture of *Anabaena variabilis* using repeated injections of carbon dioxide for the production of hydrogen. *International Journal of Hydrogen Energy*, 2002; 27: 1265-1270.

08/09/2010