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Role of Cyanobacteria in the Removal of Lignin from the Paper Mill Waste Waters

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Water pollution is a global problem. The pulp and paper mills produce toxic wastes which contain large amounts of modified lignin and lignin derivatives. Pulp and paper mill wastes have changed the river ecosystem to a considerable extent. Lignin is chemically a complex wood polymer, resistant to the action of many microorganisms. Lignin is a major component of perennial plants, and is common name for heterogenous group of phenolic polymers that together with (hemicellulose and pectin) fill the spaces between cellulose fibrils in the woody cell tissue. Such wastes may be partially treated in conventional treatment plants (Hosetti 1987). Currently most of the mills in India do not opt for recovery plants, as they add considerable tonnage of lignin waste to the neighboring dilution streams. The rivers and streams receiving pulp and paper mill wastes have lost their original color and acquired a blackish or coffee color with considerable foaming on the surface. However, fungi belonging to the Basidiomycetes Ascomycetes, and few species of bacteria are claimed to show degradation activity towards lignin (Roy 1985). There are no reports on the cyanobacteria on lignin degradation. Hence the present investigation on lignin degradation by two species of cyanobacteria is undertaken.

MATERIALS AND METHODS

The pulp and paper mill waste waters were collected from the effluent channels of Mysore Paper Mills, Bhadravati and West Coast Paper Mills, Dandeli, Karnataka State, India in the month of August 1988. Samples were collected in a clean 5-L polyethelene can by grab method. The collected sample was transported to laboratory in Ice box and stored in refrigerator at 4°C until the next day.

The raw waste and its serial dilutions 25%, 50%, 75%, with sterile tap water were tested for the growth of Phormidium

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ambiguum and Chroococcus minutus. The 25% waste with sterile tap water (1:3 ratio by volume) was selected for further studies as it gave growth of test organisms. Chroococcus minutus and Phormidium ambiguum were collected from the fresh water pond near Karnatak University campus and isolated by using the technique of Stein (1973). Both these algae were maintained in Gorham's media (Nirmala Kumari 1982).

The raw waste has coffee brown color and contains 0.4-mg/L oxygen and large amounts of lignins. 2-L of growth medium (25% waste with tap water) was taken in 2.5-L culture flasks plugged with cotton and autoclaved in 15 lb pressure for 15 min. The flasks in duplicate in case of West Coast Paper Mills and triplicate in case of Mysore Paper Mills were inoculated separately with 0.800-g/L, 0.930-g/L of 10-d old cultures of Chroococcus minutus and 0.196-g/L, 0.207-g/L of Phormidium ambiguum to Mysore Paper Mill waste and West Coast Paper Mill Waste under aseptic condition at 30.00 ± 0.20°C under day light conditions. This experiment was run for 30-d. About 200-L of sample was drawn at 5-d intervals from each flask and analysed for lignin, dissolved oxygen, biochemical oxygen demand, inorganic phosphorus, sulfates and nitrate nitrogen. The catalase activity was measured according to the procedure of Euler and Josephson and as adopted by Sridhar and Pillai (1974). Photocolorimetric estimation of lignin by using tannin-lignin reagent and carbonate-tartarate reagent at 700 nm was made. Dissolved oxygen was estimated by following the Azide modification method. Biochemical oxygen demand was determined as 5-d BOD by incubating the sample at 20°C in BOD incubator. Inorganic-phosphorus was analysed by stannous chloride method, and sulfate estimation by turbidimetric method (APHA 1980). Nitrate nitrogen estimation was made by following the phenoldisulphonic acid method (Allen 1974). Fresh weight of algae was estimated by centrifugation technique. The pH was measured by pH meter. Correlation coefficient values were calculated to find out the relationship between algal fresh weight and physico-chemical variables (Rao 1983).

RESULTS AND DISCUSSION

The effect of two algae on lignin, dissolved oxygen and nutrient levels of the pulp and paper mill wastes are presented in Figs 1 and 2. It is interesting to note that maximum growth of algae was observed on 5-d and growth was gradual thereafter (Table 1).

The biomass of Chroococcus minutus was 13.58-g/L 14.66-g/L in Mysore Paper Mill and West Coast Paper Mill. The biomass of Phormidium ambiguum was 91.00-g/L and 24.00-g/L in MPM and WCP Mill. The concentration of oxygen on 5-d was 10.2-mg/L and 12.2-mg/L in the case of Chroococcus minutus and 11.0-mg/L and 12.08-mg/L in the case of Phormidium ambiguum in MPM and WCPM waste waters. It is obvious that they were most active on the said date. The maximum amount of lignin

Table 1. Correlation coefficient values of physico-chemical parameters and algal fresh weight.

Physico-Chemical Parameters	W.C.P.M		M.P.M	
	C.minutus r	P.ambiguum r	C.minutus r	P.ambiguum r
pH	0.198	0.360	0.122	0.297
D.O	0.724*	0.59*	0.843*	0.540*
Lignin	-0.372	0.32	0.396	-0.225
Sulfates	0.072	-0.16	-0.039	0.363
Nitrates	0.411	0.00	0.227	0.245
i-Phosphorus	0.442*	0.02	0.424*	-0.635*
Catalase	-0.193	0.09	-	-
B.O.D	0.730*	0.77*	-	-
C.O.D	0.027	0.22	-	-

* Significant at 5% level W.C.P.M = West Coast Paper Mills. M.P.M = Mysore Paper Mills.

was removed on 5-d. Lignin level dropped from 93.0-mg to 29.00-mg/L by Chroococcus minutus and 25.5-mg/L by Phormidium ambiguum in Mysore Paper Mill waste waters. In West Coast Paper Mill waste water, its level dropped from 11.0-mg/L to 8.8-mg/L by Chroococcus minutus and 6.5-mg/L by Phormidium ambiguum. The rate of removal of lignin after 5-d was gradual by Chroococcus minutus in both wastes which stopped abruptly on 20-d by Phormidium ambiguum in MPM waste waters. At the closure of experiment on 30-d the lignin level was 6.6-mg/L and 3.0-mg/L in case of Chroococcus minutus and 10.0-mg/L and 4.0-mg/L in case of Phormidium ambiguum in MPM and WCPM waste waters respectively. The pH values increased with the increase in fresh weight and subsequent utilization of nutrients and production of dissolved oxygen in the medium.

The two algae treated individually reduced the lignin, sulfates, phosphates and nitrate levels from the pulp and paper mill wastes diluted with the tap water 1:3 by volume. Earlier reports reveal that members of Phycomycetes, Ascomycetes (Molds, Yeasts) and Schizomycetes (Bacteria) can affect lignins, especially carbohydrate free lignosulfonates. In the present study it is believed that algae might be the cause for biodegradation of selectively degradable lignins. The most efficient biodegraders are Basidiomycetes and Pseudomonadanaceae bacteria play a secondary role in biodegradation of lignin. No reports are available on cyanobacteria that can decompose the lignin. In the present study on 5-d lignin removal rate by Phormidium ambiguum was much faster than Chroococcus minutus. Along with the reduction of lignin, the nutrients like phosphates, nitrates and sulfates are also utilized in the process of algal proliferation (Hosetti and Patil 1987). The

production of large amount of dissolved oxygen and subsequent increase in pH during active algal growth are well established (Rodgi 1974). Mara and Pearson (1987) reported that various specific chemical complexes are stablized in ponds through the process involving buffering and chelating actions and microbial interactions. Biochemical oxygen demand and dissolved oxygen have shown significant negative and positive influences on physico-chemical complexes and algal fresh weight. However, algal fresh weight is negatively related with lignin concentrations. It shows that the lignin removal and algal growth in waste water are influenced by interactions of physico-chemical variables.

The lignin removal from the wastes may be attributed to secretion of extracellular enzymes like phenol oxidases (both peroxidases and laccases) by the growing algal biomass. On the basis of present experiments, it is clear that the extracellular enzymes produced by the algae and the combined action of other chemical substances in the presence of large amount of oxygen made lignin level decrease. From the present investigation, it is concluded that the cyanobacteria have a definite role in the biodegradation of polymeric lignins.

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REFERENCES

APHA AWWA WPCF (1980) Standard methods for examination of water and wastewaters. 15th ed APHA, New York USA

Hosetti BB, Patil HS (1987) Performance of waste stabilization ponds at different depths. Water Air Soil Pollution 33:191-198

Hosetti BB (1987) Studies on the use of some important groups of microbes in the treatment of waste waters. Ph.D. thesis, Karnatak University, Dharwad, India

Kudesia (1980) Water pollution. Pragati Publications, Meerut, India

Mara DD, Pearson H (1986) Artificial freshwater environment waste Stabilization ponds. In: Rehm HJ Reid G (ed) Biotechnology Vol 8 VCH Verlag Chemie Weinheim Deefield Beach Florida-Basel

Rodgi SS (1974) Ecological factors in water reclamation methods. Ph.D. thesis, Karnatak University, Dharwad, India

Roy A (1987) Lignin biodegradation present status and future. Curr Sci 56:350-353

Sridhar MKC, Pillai SC (1974) An enzymatic approach to the study of sewage and sludges. Environ Pollut 6:81-87

Stein JR (1973) Isolation and purification. In: JR Stein (ed) Handbook of phycological methods, culture methods and growth measurement. Cambridge University Press, London

Rao GN (1983) Statistics for Agricultural Sciences. Oxford & IBH. Publishing Co, New Delhi
Nirmala Kumari J (1984) Studies on certain biochemical aspects of hydrobiology. Ph.D. thesis Osmania University, Hyderabad, India

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