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Decolourization of Paper Mill Effluent Using Hypersaline Cyanobacterium

¹A. Nagasathya and ²N. Thajuddin

¹Department of Microbiology, J.J. College of Arts and Science,
Pudukkottai-622 404, Tamil Nadu, India

²Department of Microbiology, Bharathidasan University,
Tiruchirappalli -620 024, Tamil Nadu, India

Abstract: Biological treatment of wastewaters is a growing field of active research mainly by using microalgae including cyanobacteria. Hypersaline cyanobacterium *Phormidium tenue* KMD33 isolated from the salt pans of Kattumavadi and was maintained in Mn medium with 100 ppt of salinity. *P. tenue* KMD33 isolate was inoculated in the 250 mL conical flask containing 200 mL of each effluent samples collected from different locations of Paper Mill industry and were maintained in culture room, Department of Microbiology, J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India under white fluorescent lamp. Among the physicochemical parameters studied initially and after 20 days of incubation period with *P. tenue* KMD33, considerable reduction in the levels of chloride, salinity, alkalinity, calcium, magnesium, nitrite, nitrate, inorganic phosphate, COD, BOD and sulphate and the increasing levels of pH, dissolved oxygen and electrical conductivity were observed. A significant change in the color reduction (from 60-90%) was recorded after 20 days of incubation period. This preliminary result showed that the hypersaline cyanobacterium *P. tenue* KMD33 is an efficient candidate for the removal of colour from the effluent.

Key words: Effluent, cyanobacteria, COD, BOD, salinity

INTRODUCTION

Pulp and paper rayon industries in general are one of the highly polluting industries in India. The increasing environmental concern has led many researchers to investigate the biotechnological potential of pulping in pulp manufacture to avoid the polluting effects of effluents (Hussain *et al.*, 2003). Pulp and paper industry, generated large quantity of solid and liquid waste in its operation. The waste produced from this industry is mainly organic in nature, which is hazardous to the environment (Bist *et al.*, 1994). These organic compounds are readily degraded in aqueous medium by soil and microorganisms present in the sewage. During this process, dissolved oxygen in the stream is used up. When dissolved oxygen is reduced below a certain limit, aquatic life is affected adversely. Colour in the liquid effluents also cuts the sunlight required for photosynthesis. Use of synthetic detergents in industries such as paper and pulp (containing lignin and lignosulphonates) gives rise to foam that is quite stable due to the presence of surfactant. Foams have the capacity to carry suspended solids as well as pathogenic bacteria (Bajpai and Bajpai, 1997). Pollution control measures involved are process modification as well as treatment of liquid wastes. The implant cost operating as well as capital generally decreases with the liquid waste volume but the treatment cost increases with the volume of paper produced.

Biological treatment of wastewaters is a growing field of active research and development in many countries, whether heavily industrialized or developing. Physicochemical techniques being costly, more

Corresponding Author: Nagasathya, A., Department of Microbiology, J.J. College of Arts and Science,
Pudukkottai-622 404, Tamil Nadu, India Tel: 0091 04322 267593

or less efficient or leading to secondary pollution problems, biological treatments are appealing (de la Noüe and De Pauw, 1988). While considerable work has been carried out on the pretreatment, primary treatment and biological treatment of these wastewaters using ozone (Sinder and Porter, 1974), activated carbon (Dejohn, 1977; Ghosh *et al.*, 1978), trickling filters and activated sludge processes and their modifications (Davis *et al.*, 1977; Shriver and Dague, 1977). Very little information is available on the treatment of highly coloured and organic wastewaters by methods like oxidation ponds, aerated lagoons and stabilization ponds. (Oswald and Golueke, 1965; McGarry and Tongkasame, 1971; Brockett, 1977).

In recent years algae have been widely used as the test system for evaluating the extent of pollution caused by industrial effluents (Rai and Kumar, 1976). Algae being primary producers and occurring widely in almost all aquatic habitats serve as indicator of habitat condition (Rana *et al.*, 1971; Palmer, 1980). Various pollution tolerant strains have been isolated and growth behavior of these selected strains were studied in the laboratory to evaluate the nutrient status of industrial effluents. (Rai and Kumar, 1976; Adhikary, 1987; Adhikary and Sahu, 1988). Some recent studies carried out by various researchers (Govindan and Sundaralingam, 1979; Govindan, 1982; Groves *et al.*, 1979; Kobayashi and Yoshida, 1983; Jalal and Aziz, 1986; Rahaman and Aton, 1986; Srivastava *et al.*, 1986) shown encouraging results in removing the colour from industrial waste waters by using algae.

The main aim is to treat the effluents emanating from Hindustan Newsprint Limited, Emakulum, Kerala, India by using the hyper saline cyanobacteria *Phormidium tenue* (KMD 33).

MATERIALS AND METHODS

The effluent samples were collected from Hindustan Newsprint Limited, Kerala during the month of June 2004 from a different sampling sites such as Clarifier No.1 and 2, Cooling pond No.1 and 2, Lagoon, Final, Sanitary and Sludge which was classified as high solids. The samples were collected into a sterile borosilicate bottles. Samples were protected from sunlight during transportation. Physico-Chemical analysis of the effluent namely colour reduction, pH, chloride, salinity, total alkalinity, calcium, magnesium, sulphate, nitrate, nitrite, inorganic phosphorus, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and electrical conductivity was analysed following the methods given in APHA (1995) by analytical methods. The hypersaline cyanobacterial isolate namely, *Phormidium tenue* (KMD 33) isolated from the salt pans of Kattunavadi 10° 21' N and 79° 22' E of Palk Strait region was maintained in Mn medium with 100 ppt of salinity. The *P. tenue* (KMD 33) isolate was inoculated in the 250 mL conical flask containing 200 mL of each unsterile effluent samples collected from different locations namely Clarifier 1 and 2, Cooling pond 1 and 2, Lagoon, Sanitary and Final. The flasks were maintained in culture room Department of Microbiology, J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India under white fluorescent lamp (1400 lux): 14±10 L/D at 25±2°C. The results obtained before and after treating with *P. tenue* (KMD 33) were statistically analyzed by using paired t-test and correlation analysis (SPSS Version 10).

RESULTS

Among the physicochemical parameters studied, chloride, salinity, total alkalinity, calcium, magnesium, nitrite, nitrate, Inorganic phosphate, COD, BOD and sulphate were showed reduced concentration while pH, dissolved oxygen and electrical conductivity showed increased concentration after treating with *P. tenue* (KMD 33) (Table 1a-c). There was a drastic change in the colour reduction before and after treatment in various collecting spots, viz., clarifier 1 and 2, cooling pond 1 and 2, Lagoon, Sanitary and Final.

Table 1a: Physicochemical characteristics of paper mill effluent before and after treating with *Phormidium tenue* (KMD 33)

Physico chemical factors (mg L ⁻¹)	Clarifier No. 1		Clarifier No. 2	
	Before	After	Before	After
Colour	Brownish	Pale yellow	Brownish	Pale yellow
Optical density	0.1350±0.5 ^{ac}	0.0300±0.1 ^{ac}	0.257±0.5 ^{ac}	0.074±0.1 ^{ac}
pH	5.9000±0.18 ^{bd}	7.1000±2.85 ^{bd}	7.000±0.18 ^{bd}	7.400±2.85 ^{bd}
Chloride	1290.7±39 ^{ac}	1099.7±32 ^{ac}	404.2±39 ^{ac}	255.3±32 ^{ac}
Salinity (ppt)	32.900±8.8 ^{ac}	28.100±8.3 ^{ac}	10.32±8.8 ^{ac}	6.520±8.3 ^{ac}
Total alkalinity	60.000±0.46 ^{ad}	60.000±0.86 ^{ad}	80.00±0.46 ^{ad}	40.00±0.86 ^{ad}
Calcium	800.00±0.96 ^{ad}	400.00±0.71 ^{ad}	1200.±0.96 ^{ad}	1200.±0.71 ^{ad}
Magnesium	240.00±0.28 ^{ac}	Nil	360.0±0.28 ^{ac}	120.0±0.96 ^{ac}
Dissolved oxygen	0.3130±0.13 ^{bc}	2.1440±0.60 ^{bc}	0.256±0.13 ^{bc}	2.859±0.60 ^{bc}
Chemical oxygen demand	248.00±43.0 ^{bc}	136.00±8.5 ^{bc}	264.0±43.0 ^{bc}	140.0±8.5 ^{bc}
Biological oxygen demand	3.4000±0.78 ^{ac}	2.8000±0.78 ^{ac}	1.550±0.78 ^{ac}	0.920±0.78 ^{ac}
Sulphate	38.500±5.4 ^{bc}	3.0000±0.6 ^{bc}	47.20±5.4 ^{bc}	2.000±0.6 ^{bc}
Nitrate	3.0000±1.1 ^{bc}	NIL	1.000±1.1 ^{bc}	NIL
Nitrite	6.0000±1.6 ^{ad}	1.5000±0.55 ^{ad}	1.700±1.6 ^{ad}	0.500±0.55 ^{ad}
Inorganic phosphorus	16.500±4.7 ^{ac}	NIL	15.00±4.7 ^{ac}	2.500±0.9 ^{ac}
EC (dS m ⁻¹)	3.2900±0.98 ^{ac}	3.5900±1.0 ^{ac}	0.860±0.98 ^{ac}	1.230±1.0 ^{ac}

Values are mean±SD from ten replicates; ^a: Values followed by identical letter(s) in the same row are positively correlated; ^b: Values followed by identical letter(s) in the same row are negatively correlated; ^c: Values followed by identical letter(s) in the same row are statistically significant; ^d: Values followed by identical letter(s) in the same row are not statistically significant

Table 1b: Physicochemical characteristics of paper mill effluent before and after treating with *Phormidium tenue* (KMD 33)

Physico chemical factors (mg L ⁻¹)	Cooling pond 1		Cooling pond 2	
	Before	After	Before	After
Colour	Brownish	Pale yellow	Brownish	Pale yellow
Optical density	0.630±0.5 ^{ac}	0.065±0.1 ^{ac}	0.399±0.5 ^{ac}	0.032±0.1 ^{ac}
pH	6.300±0.18 ^{bd}	7.400±2.85 ^{bd}	6.300±0.18 ^{bd}	7.200±2.85 ^{bd}
Chloride	333.3±39 ^{ac}	219.8±32 ^{ac}	609.9±39 ^{ac}	546.0±32 ^{ac}
Salinity (ppt)	8.540±8.8 ^{ac}	5.640±8.3 ^{ac}	15.60±8.8 ^{ac}	13.90±8.3 ^{ac}
Total alkalinity	220.0±0.46 ^{ad}	160.0±0.86 ^{ad}	200.0±0.46 ^{ad}	60.00±0.86 ^{ad}
Calcium	600.0±0.96 ^{ad}	600.0±0.71 ^{ad}	600.0±0.96 ^{ad}	600.0±0.71 ^{ad}
Magnesium	Nil	Nil	Nil	Nil
Dissolved oxygen	0.634±0.13 ^{bc}	2.430±0.60 ^{bc}	0.450±0.13 ^{bc}	3.573±0.60 ^{bc}
Chemical oxygen demand	256.0±43.0 ^{bc}	160.0±8.5 ^{bc}	196.0±43.0 ^{bc}	144.0±8.5 ^{bc}
Biological oxygen demand	1.440±0.78 ^{ac}	0.860±0.78 ^{ac}	2.400±0.78 ^{ac}	1.780±0.78 ^{ac}
Sulphate	36.50±5.4 ^{bc}	3.500±0.6 ^{bc}	46.00±5.40 ^{bc}	3.000±0.6 ^{bc}
Nitrate	NIL	1.000±0.37	NIL	NIL
Nitrite	1.000±1.6 ^{ad}	0.500±0.55 ^{ad}	1.900±1.6 ^{ad}	1.000±0.55 ^{ad}
Inorganic phosphorus	10.00±4.7 ^{ac}	3.000±0.9 ^{ac}	15.00±4.7 ^{ac}	2.500±0.9 ^{ac}
EC (dS m ⁻¹)	0.520±0.98 ^{ac}	0.350±1.0 ^{ac}	2.150±0.98 ^{ac}	2.320±1.0 ^{ac}

Values are mean±SD from ten replicates; ^a: Values followed by identical letter(s) in the same row are positively correlated; ^b: Values followed by identical letter(s) in the same row are negatively correlated; ^c: Values followed by identical letter(s) in the same row are statistically significant; ^d: Values followed by identical letter(s) in the same row are not statistically significant

The statistical analysis was carried out to know significant difference before and after treating the effluent. The colour, chloride, salinity, total alkalinity, calcium, magnesium, nitrite, inorganic phosphate, biological oxygen demand and electrical conductivity were showing positive correlation and pH, dissolved oxygen, chemical oxygen demand, sulphate and nitrate were showing negative correlation. In paired t-test, the calculated value was higher than the tabulated value in the parameters namely, colour, chloride, salinity, magnesium, inorganic phosphate, biological oxygen demand, electrical conductivity, dissolved oxygen, chemical oxygen demand, Sulphate and nitrate. Hence the null hypothesis was rejected i.e., it has been inferred that the above parameters showing significant difference between before and after treatment. The rest of the parameters like pH,

Table 1c: Physicochemical characteristics of paper mill effluent before and after treating with *Phormidium tenue* (KMD 33)

Physico chemical factors (mg L ⁻¹)	Lagoon		Sanitary		Final	
	Before	After	Before	After	Before	After
Colour	Brownish	Transparent	Transparent	Transparent	Light brown	Transparent
Optical density	0.348±0.5 ^{ac}	0.004±0.1 ^{ac}	0.091±0.5 ^{ac}	0.009±0.1 ^{ac}	0.240±0.5 ^{ac}	0.005±0.1 ^{ac}
pH	7.200±0.18 ^{ad}	7.200±2.85 ^{bd}	6.800±0.18 ^{bd}	7.700±2.85 ^{bd}	7.400±0.18 ^{bd}	7.400±2.85 ^{bd}
Chloride	709.2±39 ^{ac}	439.7±32 ^{ac}	234.0±39 ^{ac}	120.5±32 ^{ac}	624.0±39 ^{ac}	354.6±32 ^{ac}
Salinity (ppt)	18.13±8.8 ^{ac}	11.26±8.3 ^{ac}	6.000±8.8 ^{ac}	3.100±8.3 ^{ac}	15.90±8.8 ^{ac}	9.080±8.3 ^{ac}
Total alkalinity	200.0±0.46 ^{ad}	40.00±0.86 ^{ad}	40.00±0.46 ^{ad}	40.00±0.86 ^{ad}	40.00±0.46 ^{ad}	40.00±0.86 ^{ad}
Calcium	600.0±0.96 ^{ad}	600.0±0.71 ^{ad}	1000±0.96 ^{ad}	1000±0.71 ^{ad}	1000±0.96 ^{ad}	1000±0.71 ^{ad}
Magnesium	360.0±0.28 ^{ac}	240.0±0.96 ^{ac}	480.0±0.28 ^{ac}	120.0±0.96 ^{ac}	600.0±0.28 ^{ac}	240.0±0.96 ^{ac}
Dissolved oxygen	0.441±0.13 ^{bc}	1.858±0.60 ^{bc}	0.491±0.13 ^{bc}	1.715±0.60 ^{bc}	0.314±0.13 ^{bc}	2.573±0.60 ^{bc}
Chemical oxygen demand	164.0±43.0 ^{bc}	144.0±8.5 ^{bc}	164.0±43.0 ^{bc}	156.0±8.5 ^{bc}	194.0±43.0 ^{bc}	148.0±8.5 ^{bc}
Biological oxygen demand	2.000±0.78 ^{ac}	1.320±0.78 ^{ac}	2.000±0.78 ^{ac}	1.250±0.78 ^{ac}	0.970±0.78 ^{ac}	0.350±0.78 ^{ac}
Sulphate	38.50±5.4 ^{bc}	3.500±0.6 ^{bc}	32.20±5.4 ^{bc}	3.000±0.6 ^{bc}	35.00±5.4 ^{bc}	2.000±0.6 ^{bc}
Nitrate	NIL	NIL	NIL	NIL	NIL	NIL
Nitrite	1.700±1.6 ^{ad}	1.000±0.55 ^{ad}	2.000±1.6 ^{ad}	NIL	1.500±1.6 ^{ad}	1.500±0.55 ^{ad}
Inorganic phosphorus	5.000±4.7 ^{ac}	2.500±0.9 ^{ac}	5.000±4.7 ^{ac}	2.500±0.9 ^{ac}	10.00±4.7 ^{ac}	2.500±0.9 ^{ac}
EC (dS m ⁻¹)	1.000±0.98 ^{ac}	1.250±1.0 ^{ac}	1.520±0.98 ^{ac}	1.840±1.0 ^{ac}	0.730±0.98 ^{ac}	0.970±1.0 ^{ac}

Values are mean±SD from ten replicates; ^a: Values followed by identical letter(s) in the same row are positively correlated; ^b: Values followed by identical letter(s) in the same row are negatively correlated; ^c: Values followed by identical letter(s) in the same row are statistically significant; ^d: Values followed by identical letter(s) in the same row are not statistically significant

total alkalinity, calcium and nitrate were having lower t-value than that of the tabulated value and therefore there is no significant difference between before and after treatment when considering these parameters.

DISCUSSION

The cyanobacteria is capable of producing oxygen during photosynthesis, high level tolerance of various pollutants, large surface area offered by the minute cells make them highly suitable for effluent treatment (Oswald *et al.*, 1978). There are several review papers on wastewater treatment with microalgae (Abeliovich, 1986; de la Noüe *et al.*, 1986, 1990; Mara and Pearson, 1986; Pantastico, 1987; de la Noüe and DePauw, 1988; Oswald, 1988 a, b, c; Huntley *et al.*, 1989; Redalje *et al.*, 1989).

The *P. tenue* KMD 33 isolated from the salt pans of Kattumavadi of Palk Strait region was used for the treatment of the papermill effluent, since it shows high tolerance and versatility nature. Pouliot *et al.* (1989) stated that the filamentous type of cyanobacteria having many advantages, viz., the simplicity of harvesting by sedimentation, the epiphytic nature of *Phormidium*, resistant to contamination by other kinds of algae and the culture composition therefore remains stable, utilization of CO₂ more efficiently than chlorophyceae.

When treating with *P. tenue* KMD 33 in the papermill effluent, the acidic pH level was changed and maintained to neutral (pH 7-7.4). Gomyo *et al.* (1972) reported that increased pH leads to oxidative discoloration of the effluents. It was observed that *P. tenue* treated effluent leads in the reduction of chloride, salinity, total alkalinity, calcium, magnesium, sulphate, nitrite, nitrate and inorganic phosphate (Table 1a-c). Many studies have demonstrated the success of using algal cultures to remove nutrients from wastewaters rich in nitrogenous and phosphorus compounds (Neos and Varma, 1966; Kalisz, 1974; Saxena *et al.*, 1974; Matusiak *et al.*, 1976; Oswald *et al.*, 1978; Chan *et al.*, 1979; de la Noüe and Proulx, 1988) and hence have been used in stabilization pond (Gloyns, 1971), in lagoons (Neel *et al.*, 1961) and in tertiary treatment of sewage (Hemens and Mason, 1968; Knapp, 1971) for the removal of pollutants from the wastewater. There was a drastic reduction in the BOD and COD content of the treated effluent (Table 1a-c). As stated by Nandan *et al.* (1990) microbial methods increasingly used for the reduction of BOD and COD. More than 95% reduction

of BOD have already been reported by Manoharan and Subramanian (1993) in Ossein effluent and papermill effluent by using *Oscillatoria pseudogeminata var granulata* and Kumar *et al.* (1997) observed 57% reduction of COD of anaerobically digested distillery spent wash. A significant increase in dissolved oxygen was observed in the effluent (Table 1a-c). The use of algae in wastewater treatment could prove beneficial in different ways since they bring about oxygenation and mineralization in addition to serving as food source for aquatic species (Elnabarawy and Welter, 1984). Srinivasan (1959) reported that *Chlorella*, *Hydrodictyon*, *Scenedesmus* and *Mastigocladus* showed oxygen-donating capacities in distillery wastes.

The main aim of the present study was to remove the colour of the papermill effluent on treating with *P. tenue* (KMD 33). A significant colour reduction was observed on 20th day of the treatment. (Table 1a-c). As reported by Dohanyos *et al.* (1978), the removal of dyes from wastewaters by biological process is physical and/or physico-chemical in nature. Adsorption onto the surface of the microorganisms was cited as the primary mechanism of dye removal by the activated sludge (Davis *et al.*, 1977; Dohanyos *et al.*, 1978). Though the result of the preliminary study was promising further systematic field level, large-scale experiments are needed before a technology can be perfected.

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