Bioremediation of paper mill effluent using some selected cyanobacterial strains

7.1 Introduction

Cyanobacteria have long been recognized as having enormous potential for use in biotechnology, especially in heavy metal removal and wastewater treatment. They possess extraordinary array of activities as biological agents in remediation and amelioration of soil and water environment (Prasanna *et al.*, 2008). Due to their ubiquitous nature, they are often used as 'Marker species' or 'Indicator species' in phytotoxicity test for

environmental monitoring (Vijayakumar, 2012). Species with a natural tendency to aggregate and very high multiplication rate offer an attractive option for intensive mass cultures intended for detoxification of effluents (Darnall *et al.*, 1986) and have an edge over conventional waste water treatment facilities (Modak, J.M.; Natarajan, K. A., 1995).

 Table 7.1: Cyanobacterial strains selected for bioremediation activity from wastewater of

 Cachar Papermill (CPM)

SI No.	Name of the strain	Code used	Study site
1	Lyngbya polysiphoniae	D2	wastewater
2	Oscillatoria formosa	D1	wastewater
3	Nostoc carneum	D3	wastewater

Cyanobacteria possess high potential for removing heavy metals from wastewater (Inthorn *et al.*, 2002). They can sequester heavy metal ions in a short period of time through adsorption and absorption mechanisms (Bajguz, 2000). Moreover, cyanobacteria being photosynthetic in nature provide a favorable condition for removal of heavy metals from the environment because their interior pH is almost two units higher than surrounding liquid (Kuenen *et al.*, 1986), and hence it provides resistance to mass transfer of products out of the biofilm (Liehr *et al.*, 1994). Among the heavy metals, copper is an important functional material found in numerous applications by today's industrial and domestic uses. All aspects of copper production have adverse impact on the surrounding ecosystem.

at high concentration (Kiran et al., 2011). Like copper, chromate compounds used in various industries is found to be toxic, carcinogenic and allergenic (mason's allergy) to man (Costa, 1997) and in microorganisms, no beneficial influence of chromium was found. The complex action of metal absorption by cyanobacteria is attributed not only to the specific surface properties of the organism like, binding of metals to the various functional groups of the different cell wall components like proteins, lipids, polysaccharides etc. (Ari et al., 1999) but also to the cell physiology and as well as external abiotic factors (Arunakumara and Zhang, 2009). Researches show that cyanobacteria regulate the intracellular metal content by the efflux-mediated resistance mechanism (Nies, 2003). The use cyanobacteria in waste remediation are beneficial in different ways since they can bring about oxygenation and mineralization as well as serving as food source for aquatic species. (Thajuddin and Subramanian, 2005). Although cyanobacteria have been successfully used as biomass for wastewater treatment systems because of photoautotrophic growth properties and of great deal of potential as bioremediation and pollution control agents very few have been investigated to determine their papermill wastewater removal abilities. For example, cyanobacterial species such as Oscillatoria salina, Plectonema terebrans, Aphanocapsa sp. and Synechococcus sp. have been successfully used in bioremediation of oil spills in different parts of the world (Raghukumar et al., 2001; Radwan and Al-Hasan, 2001; Cohen, 2002). Industrial wastewater may exert stimulatory or inhibitory influences upon the metabolic activities of cyanobacteria and quantitatively, protein and carbohydrate accumulation within the phytoplankton cells appeared to be related to the pollution stress caused by heavy metals (Angadi and Mathad, 1994, Xylaender and Braune, 1994). Reports on freshwater

cyanobacteria as bioremediation agent are scanty. The present study deals with the screening of three cyanobacterial strains of paper mill waste water origin, for their bioremediation activity.

7.2 Methodology

The detail methodology for evaluation and sensitivity of cyanobacteria for heavy metals and papermill wastewater were already mentioned in **Chapter 3.** The bioremediation analysis involved 3 dominant cyanobacterial strains and the obtained results are depicted in the tables, graphs and figures below.

7.3 Results and discussion

Three species of cyanobacteria belonging to genera *Oscillatoria*, *Lyngbya* and *Nostoc* isolated from wastewater was selected based on their relatively better growth rate and wider occurrence. The purification of the paper mill wastewater subjected to biological treatment using three selected strains was evaluated and compared through measurements of the Removal Efficiencies (RE) of different target contaminants after biotreatment. The untreated wastewater produced by the paper mill was characterized (**Table 7.2**). Significantly higher levels for all the tested parameters (organic, solids and nutrients) were recorded as average levels for pH, BOD, COD, TDS, TSS, chloride, nitrate, phosphate and ammonia respectively. Residue concentrations (RC) of the selected quality parameters were determined (**Table 7.6**) and the Removal Efficiency (RE %) (**Fig. 7.1-7.9**) as well as the percent net removal (**Fig. 7.10-7.13**) as a result of microalgal treatment as a function of experimental duration were examined. **Table 7.3-7.5** shows the changes in physicochemical parameters of water using three test species. In the present investigation,

all the selected species of cyanobacteria drastically reduced the color of the effluent sample with the incubation period of 21 days. The colour was changed from dark brown to light brown with the application of *Nostoc carneum* and it was turned into pale brown in case of Lyngbya polysiphoniae and Oscillatoria formosa. Except all other parameters, pH however showed a slight raise in the value (from the initial 8.7 to maximum 9.5 by Lyngbya *polysiphoniae*). The results obtained for pH value are supported by previous reports (Nagasathya and Thajuddin, 2008; Shah et al., 2001; Nandhini et al., 1214; Kotteswari, 2007; Vijayakumar et al., 2005). In the present investigation, a drastic reduction in the colour of the cyanobacteria screened effluent after the treatment was observed. The production of hydrogen peroxide, hydroxyl anions, and molecular oxygen, released by the cyanobacterium during photosynthesis results into oxidative discoloration of the effluent (Kaushik, 2015). The efficiency of cyanobacteria to remove the color from wastewater was demonstrated by several workers (Nerud et al., 2001; Thajuddin and Subramanyan, 2005; Nandhini et al., 1214; Kaushik, 2015). The highest RE of the BOD and COD recorded was 83.64% and 76.79% by Lyngbya polysiphoniae and Oscillatoria formosa Bory respectively. Similar results for the paper mill wastewater were obtained by Nagasathya and Thajuddin, (2008) and Manoharan and Subrahmanian (1992). The species Lyngbya polysiphoniae showed the maximum biosorption capacity as 63.23% for TDS, 73.65% for TSS and 37.63% for chloride. Similar observation of solids removal using cyanobacteria had already been documented by many workers (Chidambaram, 2004; El-Bestawy, 2008; Nandhini, 2014; David and Rajan, 2014). Concerning the contaminant nutrients, 55.05% and 45.96% was recorded as the highest RE for nitrate and ammonia respectively achieved by Oscillatoria formosa, while for phosphate Nostoc carneum exhibited the maximum RE

recorded 59.38%. Intensive studies have been made previously regarding the cyanobacterial protocol to detoxify the nutrient rich wastewater (Chidambaram, 2004; Nandhini, 2014; Nagasathya and Thajuddin, 2008; Vijaykumar and Manoharan, 2012; Jeganathan, 2006; Rai *et al.*, 2000; Shashirekha et al., 2008).

Parameters	ers Average values MINAS ^a		CPCB (Permissible) ^b	WHO guidelines ^c		
pH ^a	8.7	5.5-9.0	-	-		
BOD ^a	550	30	-	-		
COD ^a	2240	350	-	-		
TDS ^b	2037	-	2000	-		
TSS ^a	148	-	-	-		
Chloride ^b	380	-	1000	-		
Nitrate ^c	3.70	-	-	50		
Phosphate ^c	0.96	-	-	0.1		
Ammonia	1.90	-	-	-		
Copper ^c	0.04	-	-	2		
Chromium ^c	0.05	-	-	0.05		
Nickel ^c	0.01	-	-	0.07		
Cadmium ^c	0.03	-	-	0.03		
Manganese	0.02	-	-			

Table 7.2: Comparison of treated effluents with standards value

Values are arithmetic mean of four replicates

^aMINAS (1985), ^bCPCB Report (1995), ^cWHO, Guidelines for drinking water quality-Geneva, 1999 (2) ED; All the values are represented in (mg/litre) except pH

Days		Colour	pН	BOD	COD	TDS	TSS	Chloride	Nitrate	Phosphate	Ammonia
0 th day	Control	Dark brown	8.7 ± 0.30	550 ± 26.46	2240 ± 121.66	2037 ± 37.00	148 ± 4.00	380 ± 17.32	3.7 ± 0.26	0.96 ± 0.08	1.9 ± 0.44
	10%	Light brown	7.3 ± 1.15	300 ± 10	960 ± 26.45	222 ± 40.21	17.50 ± 4.13	160 ± 8.67	1.75 ± 0.25	0.32 ± 0.08	1.11 ± 0.86
	30%	Light brown	7.3 ± 0.46	370 ± 17.32	1280 ± 91.65	625 ± 23.33	46 ± 11	190 ± 6.00	1.94 ± 0.06	0.45 ± 0.13	1.3 ± 0.2
	50%	Light brown	7.6 ± 0.87	480 ± 10	1600 ± 173.20	1028 ± 37.00	75.50 ± 4.00	210 ± 5.20	2.28 ± 0.28	0.54 ± 0.15	1.5 ± 0.3
	70%	Dark brown	8.0 ± 0.78	510 ± 10	1600 ± 180.28	1431 ± 37.07	104 ± 4.00	230 ± 17.32	2.72 ± 0.37	0.6 ± 0.13	1.62 ± 0.12
	90%	Dark brown	8.2 ± 1.06	520 ± 10	1920 ± 190.78	1835 ± 40.00	133 ± 4.00	360 ± 8.66	2.99 ± 0.02	0.8 ± 0.14	1.73 ± 0.42
7 th day	Control	Dark brown	8.9 ± 0.3	216 ± 4.58	1410 ± 85.44	1480 ± 20.02	90 ± 6.00	304 ± 9.54	2.92 ± 0.08	0.63 ± 0.06	1.59 ± 0.08
	10%	Light brown	7.5 ± 0.30	120 ± 6.00	459 ± 21.52	163 ± 12.00	11 ± 3.25	104 ± 4.00	0.88 ± 0.10	0.15 ± 0.03	0.28 ± 0.03
	30%	Light brown	7.7 ± 0.35	140 ± 6.08	590 ± 26.46	428 ± 23.09	29 ± 4.11	130 ± 4.04	1.24 ± 0.21	0.25 ± 0.006	0.50 ± 0.08
	50%	Light brown	8 ± 0.46	198 ± 5.29	740 ± 10.00	750 ± 26.54	46 ± 9.06	144 ± 2.65	1.45 ± 0.43	0.3 ± 0.02	0.82 ± 0.04
	70%	Dark brown	8.5 ± 0.5	179 ± 2.65	810 ± 26.46	1042 ± 25.33	63 ± 5.12	165 ± 2.00	1.97 ± 0.10	0.32 ± 0.02	1.06 ± 0.05
	90%	Dark brown	8.6 ± 0.61	180 ± 5.00	1150 ± 86.60	1334 ± 25.20	81 ± 11.00	276 ± 6.93	2.26 ± 0.12	0.5 ± 0.26	1.29 ± 0.56
14 th day	Control	Dark brown	9.2 ± 0.72	109 ± 2.00	920 ± 26.46	1000 ± 20.00	50 ± 7.00	257 ± 7.00	2.21 ± 0.71	0.47 ± 0.07	1.4 ± 0.1
	10%	Pale brown	7.8 ± 0.2	40 ± 1.73	224 ± 6.93	103 ± 8.56	7 ± 2.10	73 ± 3.46	0.48 ± 0.10	-	-
	30%	Pale brown	8 ± 0.2	46 ± 2.65	283 ± 15.72	320 ±13.22	18 ± 6.25	90 ± 5.00	0.73 ± 0.086	-	0.14 ± 0.04
	50%	Light brown	8.3 ± 0.10	61 ± 2.65	399 ± 26.51	501 ±15.11	26 ± 5.40	112 ± 3.61	0.85 ± 0.06	0.18 ± 0.02	0.48 ± 0.03
	70%	Light brown	8.7 ± 0.52	87 ± 5.20	460 ± 26.46	680 ± 31.19	35 ± 7.81	131 ± 2.65	1.26 ± 0.15	0.20 ± 0.05	0.77 ± 0.03
	90%	Dark brown	9 ± 0.4	90 ± 2.65	720 ± 20	885 ± 20.27	45 ± 3.20	229 ± 1.73	1.63 ± 0.11	0.36 ± 0.03	1.17 ± 0.17
21th day	Control	Light brown	9.5 ± 0.5	90 ± 3.00	650 ± 30	749 ± 4.00	39 ± 3.00	237 ± 3.00	1.95 ± 0.04	0.42 ± 0.05	1.3 ± 0.26
	10%	Colorless	8.1 ± 0.17	21 ± 3.60	125 ± 22.91	83 ± 6.03	6.2 ± 2.21	56 ± 2.00	0.29 ± 0.10	-	-
	30%	Colorless	8.5 ± 0.87	30 ± 2.65	164 ± 19.70	227 ± 9.08	13 ± 2.82	72 ± 2.65	0.47 ± 0.06	-	-
	50%	Pale brown	8.8 ± 0.35	40 ± 1.73	234 ± 23.52	381±11.52	21 ± 4.00	89 ± 1.73	0.56 ± 0.06	-	0.3 ± 0.05
	70%	Pale brown	9 ± 0.7	57 ± 2.65	281 ± 10.15	530 ± 9.20	28 ± 4.07	105 ± 2.00	0.94 ± 0.03	0.16 ± 0.03	0.5 ± 0.17
	90%	Light brown	9.3 ± 0.58	70 ± 2.65	480 ± 17.32	606 ± 15.83	35 ± 3.50	200 ± 1.73	1.39 ± 0.10	0.32 ± 0.03	0.96 ± 0.03

Table 7.3: Changes in physicochemical parameters of water using *Lyngbya polysiphoniae*:

Days		Colour	pH	TDS	TSS	BOD	COD	Chloride	Nitrate	Phosphate	Ammonia
0 th day	Control	Dark brown	8.7 ± 0.30	2037 ± 37.00	148 ± 4.00	550 ± 26.46	2240 ± 121.66	380 ± 8.89	3.7 ± 0.26	0.96 ± 0.08	1.9 ± 0.44
	10%	Light brown	7.3 ± 1.15	222 ± 20.21	17.50 ±4.13	300 ± 10	960 ± 26.45	160 ± 8.72	1.75 ± 0.25	0.32 ± 0.08	1.11 ± 0.86
	30%	Light brown	7.3 ± 0.46	625 ± 23.33	46 ± 11	370 ± 17.32	1280 ± 91.65	190 ± 10.00	1.94 ± 0.06	0.45 ± 0.13	1.3 ± 0.2
	50%	Light brown	7.6 ± 0.87	1028 ± 37.02	75.50 ± 4.00	480 ± 10	1600 ± 173.20	210 ± 10.00	2.28 ± 0.28	0.54 ± 0.15	1.5 ± 0.3
	70%	Dark brown	8.0 ± 0.78	1431 ± 26.05	104 ± 4.00	510 ± 10	1600 ± 180.28	230 ± 7.00	2.72 ± 0.37	0.6 ± 0.13	1.62 ± 0.12
	90%	Dark brown	8.2 ± 1.06	1835 ± 40.00	133 ± 4.00	520 ± 10	1920 ± 190.78	360 ± 8.67	2.99 ± 0.02	0.8 ± 0.14	1.73 ± 0.42
7 th day	Control	Dark brown	8.8 ± 0.26	1507 ± 20.03	85 ± 4.00	240 ± 26.46	1200 ± 100.00	330 ± 8.89	2.89 ± 0.18	0.73 ± 0.23	1.45 ± 0.03
	10%	Light brown	7.5 ± 0.87	173 ± 17.30	11.5 ± 3.55	140 ± 20.00	415 ± 13.23	120 ± 5.00	0.76 ± 0.06	0.18 ± 0.01	0.53 ± 0.12
	30%	Light brown	7.5 ± 0.5	466 ± 10.11	27 ± 6.07	170 ± 20	560 ± 10.00	150 ± 2.00	0.85 ± 0.15	0.26 ± 0.02	0.6 ± 0.3
	50%	Light brown	8.1 ± 0.17	763 ± 19.85	44 ± 5.01	200 ± 14	790 ± 17.32	170 ± 3.00	1.05 ± 0.06	0.32 ± 0.02	0.98 ± 0.02
	70%	Dark brown	8.3 ± 0.52	1060 ± 30.31	60 ± 8. 11	220 ± 36.05	836 ± 118.46	185 ± 8.67	1.39 ± 0.01	0.36 ± 0.04	1.15 ± 0.05
	90%	Dark brown	8.5 ± 0.46	1358 ± 52.02	76 ± 5.90	210±17.32	1120 ± 150.99	290 ± 8.72	2.21 ± 0.16	0.51 ± 0.05	1.3 ± 0.07
14 th day	Control	Dark brown	9.00 ± 0.92	1036 ± 10.50	65 ± 6.00	130 ± 26.46	790 ± 17.32	290 ± 7.00	2.09 ± 0.07	0.58 ± 0.03	1.20 ± 0.06
	10%	Pale brown	7.6 ± 0.53	107 ± 15.22	9.2 ± 2.36	70 ± 8.72	195 ± 13.23	75 ± 5.00	0.31 ± 0.10	-	0.18 ± 0.02
	30%	Light brown	7.9 ± 0.26	322 ± 20.00	21 ± 5.89	90 ± 10	270 ± 10.00	100 ± 4	0.37 ± 0.04	0.15 ± 0.03	0.2 ± 0.10
	50%	Light brown	8.2 ± 0.35	528 ± 14.36	34 ± 4.40	100 ± 8.72	420 ± 20.00	120 ± 8.72	0.46 ± 0.08	0.20 ± 0.03	0.57 ± 0.04
	70%	Light brown	8.6 ± 0.36	731 ± 9.34	46 ± 5.67	110 ± 17.32	470 ± 7.00	155 ± 6.25	0.85 ± 0.14	0.25 ± 0.04	0.75 ± 0.07
	90%	Dark brown	8.8 ± 0.75	934 ± 38.01	59 ± 8,01	115 ± 13.23	536 ± 100.17	250 ± 8.73	1.60 ± 0.10	0.40 ± 0.04	1.00 ± 0.20
21th day	Control	Light brown	9.1 ± 0.36	912 ± 20.00	56 ± 6.00	110 ± 26.46	520 ± 8.67	260 ± 2.00	1.66 ± 0.05	0.47 ± 0.07	1.03 ± 0.03
	10%	Colorless	7.9 ± 0.17	109 ± 23.03	8.3 ± 9.52	42 ± 11.14	116 ± 10.26	59 ± 3.46	0.14 ± 0.03	-	-
	30%	Colorless	8.2 ± 0.35	277 ± 27.16	18 ± 3.12	60 ± 13.23	160 ± 20	85 ± 2.65	0.22 ± 0.07	-	-
	50%	Pale brown	8.5 ± 0.50	452 ± 20.32	29 ± 4.00	68 ± 15.62	250 ± 43.59	105 ± 4.36	0.27 ± 0.03	0.12 ± 0.03	0.4 ± 0.10
	70%	Light brown	8.6 ± 0.53	630 ± 67.55	40 ± 8.10	80 ± 10	300 ± 8.00	135 ± 4.58	0.61 ± 0.09	0.18 ± 0.08	0.58 ± 0.97
	90%	Light brown	8.8 ± 0.35	819 ± 50.31	50 ± 7.72	96 ± 3.47	376 ± 22.65	220 ± 4.00	1.14 ± 0.03	0.3 ± 0.06	0.82 ± 0.12

 Table 7.4: Changes in physicochemical parameters of water using Oscillatoria formosa:

Days		Colour	pН	BOD	COD	TDS	TSS	Chloride	Nitrate	Phosphate	Ammonia
0 th day	Control	Dark brown	8.7 ± 0.30	550 ± 26.46	2240 ± 121.66	2037 ± 37.00	148 ± 4.00	380 ± 17.32	3.7 ± 0.26	0.96 ± 0.08	1.9 ± 0.44
	10%	Light brown	7.3 ± 1.15	300 ± 10	960 ± 26.45	222 ± 40.21	17.50 ± 4.13	160 ± 26.46	1.75 ± 0.25	0.32 ± 0.08	1.11 ± 0.86
	30%	Light brown	7.3 ± 0.46	370 ± 17.32	1280 ± 91.65	625 ± 23.33	46 ± 11	190 ± 17.32	1.94 ± 0.06	0.45 ± 0.13	1.3 ± 0.2
	50%	Light brown	7.6 ± 0.87	480 ± 12	1600 ± 173.20	1028 ± 37.00	75.50 ± 4.00	210 ± 20	2.28 ± 0.28	0.54 ± 0.15	1.5 ± 0.3
	70%	Dark brown	8.0 ± 0.78	510 ± 10	1600 ± 180.28	1431 ± 37.00	104 ± 4.00	230 ± 17.32	2.72 ± 0.37	0.6 ± 0.13	1.62 ± 0.12
	90%	Dark brown	8.2 ± 1.06	520 ± 19	1920 ± 190.78	1835 ± 40.00	133 ± 4.00	360 ± 26.46	2.99 ± 0.02	0.8 ± 0.14	1.73 ± 0.42
7 th day	Control	Dark brown	8.7 ± 0.3	380 ± 50	1420 ± 10.00	1290 ± 20.00	95 ± 5.00	320 ± 8.54	3.22 ± 0.70	0.60 ± 0.02	1.7 ± 0.20
	10%	Light brown	7.4 ± 0.40	180 ± 13.23	580 ± 10.00	147±13.66	12±3.11	120 ± 5.00	1.02 ± 0.04	0.13 ± 0.01	0.4 ± 0.10
	30%	Light brown	$7.5 \pm .050$	230 ± 26.46	776 ± 6.25	401±10.01	30±5.06	160 ± 8.00	1.19 ± 0.08	0.20 ± 0.02	0.7 ± 0.10
	50%	Light brown	7.7 ± 0.52	290 ± 10	900 ± 10.00	665±23.09	49±6.15	180 ± 6.00	1.70 ± 0.26	0.31 ± 0.03	1 ± 0.17
	70%	Dark brown	8.1 ± 0.17	330 ± 10	1000 ± 17.44	909±20.17	67±12.92	190 ± 10.00	2.21 ± 0.21	0.4 ± 0.04	1.24 ± 0.01
	90%	Dark brown	8.4 ± 0.26	360 ± 13.23	1220 ± 17.32	1163 ± 30.05	85±20.13	310 ± 4.36	2.55 ± 0.45	0.46 ± 0.05	1.3 ± 0.20
14 th day	Control	Dark brown	8.8 ± 0.26	230 ± 12.49	850 ± 13.23	1000 ± 20.00	60 ± 6.00	290 ± 2.65	2.89 ± 0.25	0.45 ± 0.06	1.5 ± 0.36
	10%	Pale brown	7.4 ± 0.3	90 ± 2.00	280 ± 9.17	117±12.55	8.70±2.21	80 ± 2.65	0.68 ± 0.07	0.05 ± 0.01	-
	30%	Light brown	7.6 ± 0.35	120 ± 5.00	680 ± 20.00	314±30.23	20±4.39	116 ± 3.45	0.85 ± 0.10	0.1 ± 0.02	0.45 ± 0.04
	50%	Light brown	7.9 ± 0.10	150 ± 12.77	550 ± 10.39	510±37.03	31±3.16	140 ± 3.60	1.36 ± 0.16	0.17 ± 0.01	0.61 ± 0.02
	70%	Dark brown	8.1 ± 0.17	170 ± 8.72	550 ± 8.72	706±23.40	42±10.20	160 ± 2.65	1.70 ± 0.26	0.22 ±0.03	1 ± 0.20
	90%	Dark brown	8.5 ± 0.50	200 ± 26.46	710 ± 11.79	902±43.90	54±8.07	270 ± 3.61	2.21 ± 0.10	0.30 ± 0.02	1.1 ± 0.20
21th day	Control	Light brown	8.9 ± 0.30	170 ± 26	590 ± 8.89	937 ± 37.00	50 ± 6.00	270 ± 3.46	2.68 ± 0.16	0.39 ± 0.02	1.4 ± 0.10
	10%	Colourless	7.5 ± 0.44	50.67 ± 17.78	150 ± 4.00	220±10.06	7±2.21	50 ± 1.73	0.34 ± 0.08	-	-
	30%	Pale brown	7.9 ± 0.20	76 ± 4.58	240 ± 10.00	281±16.33	17±4.30	88 ± 2.65	0.66 ± 0.11	-	0.3 ± 0.17
	50%	Light brown	8 ± 0.30	100 ± 8	320 ± 5.00	468±17.40	26±5.05	110 ± 5.29	1.04 ± 0.06	0.1 ± 0.02	0.43 ± 0.03
	70%	Light brown	8.3 ± 0.30	120 ± 10	350 ± 6.25	655 ±23.72	35±5.77	136 ± 4.58	1.43 ± 0.06	0.18 ± 0.03	0.7 ± 0.20
	90%	Light brown	8.7 ± 0.26	146 ± 8.71	480 ± 17.44	843±26.05	45±9.22	240 ± 1.73	1.92 ± 0.13	0.25 ± 0.03	0.93 ± 0.25

Table 7.5: Changes in physicochemical parameters of water using *Nostoc carneum*:

Table 7.6: Residue concentrations (RC) of the quality parameters from the contaminated CPM effluents using the selected cyanobacteria

Time (Week)) Control								Lyngbya polysiphoniae									
	pН	BOD	COD	TDS	TSS	Chloride	Nitrate	Phosphate	Ammonia	pН	BOD	COD	TDS	TSS	Chloride	Nitrate	Phosphate	Ammonia
0 th	8.70	550	2240	2037	148	380	3.70	0.96	1.9	8.70	550	2240	2037	148	380	3.70	0.96	1.9
1^{st}	8.79	510	1996	2000	124	357	3.55	0.88	1.68	8.9	216	1410	1480	90	304	2.92	0.63	1.59
2^{nd}	8.80	495	1920	1930	110	325	3.43	0.79	1.62	9.2	109	920	1000	50	257	2.21	0.47	1.4
3 rd	8.90	490	1905	1900	107	321	3.33	0.77	1.7	9.5	90	650	749	39	237	1.95	0.42	1.3
Time (Week)	Oscilla	toria fe	ormosa							Nostoc carneum								
	pН	BOD	COD	TDS	TSS	Chloride	Nitrate	Phosphate	Ammonia	pН	BOD	COD	TDS	TSS	Chloride	Nitrate	Phosphate	Ammonia
0 th	8 70	550	2240	2037	148	380	3.70	0.96	1.9	8.70	550	2240	2037	148	380	3.70	0.96	1.9
	0.70		22.0	2007	1.0													
1^{st}	8.8	1480	85	240	1200	330	2.89	0.73	1.45	8.7	380	1420	1290	95	320	3.22	0.60	1.7
1^{st} 2^{nd}	8.8 9.00	1480 1000	85 65	240 130	1200 790	330 290	2.89 2.09	0.73 0.58	1.45 1.20	8.7 8.8	380 230	1420 850	1290 1000	95 60	320 290	3.22 2.89	0.60 0.45	1.7 1.5



Fig 7.1 Removal Efficiency (RE %) of pH from papermill effluent using different cyanobacteria at different exposure time



Fig 7.2 Removal Efficiency (RE %) of BOD from papermill effluent using the different cyanobacteria at different exposure time



Fig 7.3 Removal Efficiency (RE %) of COD from papermill effluent using the different cyanobacteria at different exposure time



Fig 7.4 Removal Efficiency (RE %) of TDS from papermill effluent using the different cyanobacteria at different exposure time



Fig 7.5 Removal Efficiency (RE %) of TSS from CPM effluent using the different cyanobacteria at different exposure time



Fig 7.6 Removal Efficiency (RE %) of Chloride from CPM effluent using the different cyanobacteria at different exposure time



Fig 7.7 Removal Efficiency (RE %) of Nitrate from papermill effluent using the different cyanobacteria at different exposure time



Fig 7.8 Removal Efficiency (RE %) of Phosphate from CPM effluent using the different cyanobacteria at different exposure time



Fig 7.9 Removal Efficiency (RE %) of ammonia from CPM effluent using the different cyanobacteria at different exposure time



Fig 7.10 Percent net reduction in selected physicochemical parameters of the control medium (100% wastewater) after the experiment (21 days)



Fig 7.11 Percent net reduction in selected physicochemical parameters of the medium treated with *Lyngbya polysiphoniae* after the experiment (21 days)



Fig 7.12 Percent net reduction in selected physicochemical parameters of the medium treated with *Oscillatoria formosa* after the experiment (21 days)



Fig 7.13 Percent net reduction in selected physicochemical parameters of the medium treated with *Nostoc carneum* after the experiment (21 days)

The investigation of wastewater from papermill after biotreatment attributed to significant reduction in heavy metals present. Copper, chromium, cadmium, lead, manganese and nickel were found in raw effluent were significantly reduced or almost completely removed after treatment. *L. polysiphoniae* recorded the highest REs% for Cu (100) at 100% of wastewater. As far as the uptake of copper by *O. formosa* was concerned, here also the highest uptake (60%) took place at 100% concentration followed by *N. carneum* (75.95%) at 10% recording RCs of 0.00, 0.12 and 0.07 mg/l by the three species, respectively, after 21 days (Fig. 7.16). With regard to chromium, the uptake by *L. polysiphoniae* was found to increase with increase in concentration till 70% with RE

(79.72%) followed by a decline. Compared to Lyngbya sp., lower Cr REs were achieved by the rest two test species. Cr removal recorded 56.83 and 71.79% achieved as the highest Cr REs% by O. formosa and N. carneum (0.30 and 0.27 mg/l, respectively at 50% and 30% respectively) at the end of the experiment (Fig. 7.15). As far as the uptake of cadmium by L. polysiphoniae was concerned the highest uptake took place at 50% (77.78) RE %). The uptake of cadmium at concentrations above 50% was found to decrease. 70 and 48.39% RE of Cd were achieved by O. formosa and N. carneum (0.07 and 0.16 mg/l RC), at 30% and control culture respectively, after 21 days (Fig. 7.14). Concerning lead, much lower REs% were recorded for wastewater effluent by all the three selected species compared to those obtained for Cu, Cr and Cd removal. Pb removal recorded 54.70, 38.13 and 35.71% (9.07, 17.33 and 18.01 mg/l, respectively) as the highest Pb RE by L. polysiphoniae, O. formosa and N. carneum at 50%, 30% and 30% (Fig. 7.17), respectively. With regard to manganese, again highest removal of Mn was achieved by the L. polysiphoniae and O. formosa from the raw wastewater reaching a maximum of 100% (0.00 mg/l) as in Cu after 21 days, while 61.98% (0.10 mg/l) was recorded as the highest Mn removal from 10% wastewater by the *N. carneum* (Fig. 7.18). In case of Ni, same trend is followed by the L. polysiphoniae and O. formosa attaining 100% removal of the metal Ni from the wastewater at the end of the experiment. While the strain N. carneum, 100% removal was recorded at the 70% concentration (Fig. 7.19). In conclusion, results confirmed that the most effective species for heavy metal removal from the papermill effluents are in the following order L. polysiphoniae > O. formosa > N. *carneum* which may be attributed to the selective uptake of the investigated metals by the tested cyanobacterial species.



Fig 7.14: Reduction of cadmium concentration of wastewater during phycoremediation by the selected test species (D1- *L. polysiphoniae*, D2- *O. formosa* and D3- *N. carneum*)



Fig 7.15: Reduction of chromium concentration of wastewater during phycoremediation by the selected test species (D1- *L. polysiphoniae*, D2- *O. formosa* and D3- *N. carneum*)



Fig 7.16: Reduction of chromium concentration of wastewater during phycoremediation by the selected test species (D1- *L. polysiphoniae*, D2 - *O. formosa* and D3- *N. carneum*)



Fig 7.17: Reduction of lead concentration of wastewater during phycoremediation by the selected test species (D1- *L. polysiphoniae*, D2 - *O. formosa* and D3- *N. carneum*)



Fig 7.18: Reduction of manganese concentration of wastewater during phycoremediation by the selected test species (D1- *L. polysiphoniae*, D2 - *O. formosa* and D3- *N. carneum*)



Fig 7.19: Reduction of manganese concentration of wastewater during phycoremediation by the selected test species (D1- *L. polysiphoniae*, D2 - *O. formosa* and D3- *N. carneum*)

Cyanobacterial species, tested for heavy metal removal is found that L. polysiphoniae is able to remove copper and chromium better than other microbes. An increase in the % metal ion removal by the algae with increasing copper concentrations in the medium was recorded (Fig: 7.20). For 0.1 ppm concentration, % metal removal was 67% and for 0.3, 0.5 and 0.7 mg/l they were 61%, 78% and 53% respectively. However, the uptake of copper at concentrations above 0.5 mg/l was found to decrease. The alga was able to remove almost 33-51% Cu ions when the concentrations were 0.9 and 1.0 mg/l. The average removal capacity was about 60%. With regard to chromium (Fig: 7.21), the lowest uptake by Lyngbya took place at 10 mg/l while the highest at 7 mg/l. Unlike copper, the uptake of chromium by Lyngbya was found to increase with increase in concentration till 7 mg/l followed by a decline. The average removal of Cu ions for Oscillatoria sp. was 44% while that for Nostoc sp. was only 37% after 21 days. The average removal capacity of Cr ions for Lyngbya was found to be 74%, for Oscillatoria sp. it was 63% while that for Nostoc sp. was only 30% after 21 days. The results also revealed that N. carneum was the most sensitive alga to the two metal ions even at lower concentrations (3 and 5mg/ L for Cu and 5 and 7 mg/L) while L. polysiphoniae and O. formosa were more tolerant to high metal concentrations. The bioremoval of heavy metal ions by L. polysiphoniae from aqueous solution showed that the highest percentage of metal bioremoval was recorded 73% (Cu) and 88% (Cr). From the present study, for both the metals analyzed, the % removal of Cu and Cr with increasing metal concentrations shows interspecific variation. Lyngbya showed significantly greater sorptive capacity for Cu and Cr than all other strains tested.



Fig 7.20: Percent reduction in heavy metal concentration (CuSO₄) during phycoremediation by the selected test species



Fig 7.21: Percent reduction in heavy metal concentration (K_2CrO_4) during phycoremediation by the selected test species

The pigment content (chlorophyll a) of Lyngbya, Oscillatoria and Nostoc treated with different concentrations of wastewater is illustrated in Figure 4.22-4.36. Though the low concentrations (10, 20 and 30%) resulted in slight stimulations (0.2, 3.8 and 6.5%) respectively) at 1st week, wastewater inhibited the growth in a dose-dependent manner as incubation progressed as long-term stress. Compared to the control, inhibitions at the end of 24th day incubation were 8.4, 12.4, 13.9, 29.1, 42.9 and 47.6% respectively for 10, 20, 30, 50, 70 and 100% of wastewater in case of Lyngbya polysiphoniae. The corresponding figures for Oscillatoria formosa were 12.2, 33.6, 43.7, 46.9, 62.5 and 67.9% and for Nostoc carneum it was 19.3, 30.7, 49.5, 56.2, 67.9 and 73.7 respectively for 10, 20, 30, 50, 70 and 100% of wastewater. Fig.7.37, 7.38 and 7.39 depicts the Chl a accumulation treated with different concentrations of CuSO4 and K₂CrO₄ in the BG11 medium over 24 days of incubation along with the control. The present study of growth pattern of Oscillatoria formosa, Lyngbya polysiphoniae and Nostoc carneum suggest that the tolerance capacity of Lyngbya is more compared to Oscillatoria and Nostoc for both the metals. However, all the algae tolerated higher doses of chromium compared to copper. Intensive studies have been done previously regarding the inhibitory impact of higher concentrations of various heavy metals on algae (Gupta and Arora, 1978; El-Sheekh et al., 2003; Osman et al., 2004; Muwafq and Bernd, 2006; Romera et al., 2007; Kiran and Thanasekaran, 2011; Priyadarshini and Rath, 2012; Sikarwar and Singh, 2012; Begam et al., 2014). Chlorophyll concentrations were found to be maximum in control for all the test species. Absorption Spectra for chlorophyll indicate that all the algae showed rapid growth up to 15th day in case of control and treated (0.5 mg/L for Lyngbya sp., 0.1 mg/L for Oscillatoria and 0.3 mg/L for Nostoc sp. for copper and 5 mg/L for

Lyngbya sp., 1 mg/L for Oscillatoria and control for Nostoc sp. for chromium respectively). Metal treatment favored the growth of all the three cyanobacteria with increasing chlorophyll concentration up to some days, however, exposure of the cyanobacteria beyond these concentrations led to progressive decrease in the growth. Growth inhibition in cyanobacteria is well known due to metal toxicity and found to be related to the absorption of bio-available contaminants, to the amount of intracellular metal (Ma et al., 2003) and to the chemical nature of the metal. In this respect, Kiran and Thanasekaran (2011) found that high concentrations of copper were associated with reductions in the chlorophyll contents in the cyanobacteria. All the species were affected by the metal treatment, but as compared to Oscillatoria and Nostoc, Lyngbya polysiphoniae showed more favorable response to metal treatment and the species Nostoc *carneum* was found to be more sensitive. The reduction in the chl *a* contents could be related to the alterations of the thylakoid membrane and disruption in the photosynthetic structures as reported by Carfagna et al. (2013). According to Qiu et al. (2006), the decrease of Chl a in *Chlorococcum* sp. AZHB is related to the increasing concentrations of Cu or Cd treatment. LC₅₀ values for Lyngbya, Oscillatoria and Nostoc sp. was found to be 0.89 mg/L, 0.69 mg/L and 0.63 mg/L respectively for copper while it was 7 mg/L, 5.8 mg/L and 3.96 mg/L respectively for Lyngbya, Oscillatoria and Nostoc sp. respectively for chromium. For wastewater, LC_{50} values were 79.98 % for Lyngbya polysiphoniae, 69.18% for Oscillatoria formosa and 48.98% for Nostoc carneum. The value of protein and carbohydrate contents in all the test species under copper treatment was found to be highest at the 0.1mg/L concentration of CuSO₄ and in control culture respectively (Fig 7.43 and 7.46). Carbohydrate and protein contents tended to increase

significantly in response to chromium and were observed to be maximum at control and 1mg/L metal concentration respectively (Fig 7.44 and 7.47). However, a steady decline of all the constituents was observed with elevated concentrations of both the metals as well as in wastewater (Fig 7.45 and 7.48) in all the three test organisms. There was no appreciable growth after 15th days of incubation. As compared to the Oscillatoria and *Nostoc sp.*, batter tolerance was achieved by the *Lyngbya polysiphoniae*. Concerning the cultures of three species, the results revealed that the treatment with low concentration of $CuSO_4$, K_2CrO_4 and wastewater stimulated the chlorophyll *a* and carbohydrate contents, but inhibited the accumulation of protein contents. The toxic effects of lethal concentration of these metals on growth and biochemical constituents were more pronounced in Oscillatoria and Nostoc sp., than in Lyngbya polysiphoniae. Catalase (CAT), an enzyme of H_2O_2 scavanger generated mostly in stress condition in plant was studied following the treatment of cyanobacterium L. polysiphoniae, O. formosa and N. carneum with the various doses of wastewater, Cu, and Cr (Fig 7.49, 7.50 and 7.51 respectively). After 24th days of treatment of wastewater by the test species stimulated catalase activity which is dose dependent increase. Control, 10%, 30%, 50% and 90% for Lyngbya, control, 10%, 30% and 70% for Oscillatoria and control, 10%, 30% and 90% for Nostoc respectively. At highest concentration ie. 100% wastewater, all the selected species showed a reduction in the catalase activity. For copper stress, Lyngbya and Oscillatoria showed higher concentration of CAT activities up to 90% concentration but for *Nostoc*, the enzymatic activity reduced at the higher concentrations. Unlike copper, the CAT activity under chromium stress showed less reduction in value at the higher concentrations, showing more toxicity of copper than chromium. Different concentrations

of wastewater, Cu and Cr enhanced glutathione reductase activities (**Fig 7.52**, **7.53** and **7.54** respectively) at control and lower concentrations for all the three species, but at higher concentrations the value was remarkably decreased compared to catalase. The SEM study clearly indicates the surface morphology and texture at different magnifications. It was observed that the cyanobacterial filaments were intact in case of control set up. The micrographs structures were observed with large surface area as similar to *Chlorella pyrenoidosa* (Rezei *et al.*, 2011). In case of chromium treated cells, the number of filaments damaged was less and thick sheath was observed but in case of the cells exposed to copper and wastewater, the filaments were rough, scattered, ruptured and shrinkage of gelatinous sheath and heterocyst was observed. A clear morphological alteration of cell organelles were obviously recorded in Cu treated cells (**Plate. 7.5, 7.6** and **7.7**) more than in Cr treated ones and waste water. The least damage was observed in Cr treated ones.





Fig. 7.22 Absorption Spectra of *Lyngbya polysiphoniae* under different wastewater concentrations at 0th day and 3rd day (1-2)





Fig. 7.23 Absorption Spectra of *L. polysiphoniae* under different wastewater concentrations at 6th day and 9rd day (3-4)





Fig. 7.24 Absorption Spectra of *L. polysiphoniae* under different wastewater concentrations at 12th day and 15th day (5-6)





Fig. 7.25 Absorption Spectra of *L. polysiphoniae* under different wastewater concentrations at 18th day and 21th day (7-8)



Fig 7.26 Absorption Spectra of *L. polysiphoniae* under different wastewater concentrations at 24th day (9)



Fig 7.27 Absorption Spectra of *O. formosa* under different wastewater concentrations at 0th day (10)





Fig 7.28 Absorption Spectra of *O. formosa* under different wastewater concentrations at 3rd day and 6th day (11-12)





Fig. 7.29 Absorption Spectra of *O. formosa* under different wastewater concentrations at 9th day and 12th day (13-14)





Fig. 7.30 Absorption Spectra of *O. formosa* under different wastewater concentrations at 15th day and 18th day (15-16)





Fig 7.31 Absorption Spectra of *O. formosa* under different wastewater concentrations at 21th day and 24th day (17-18)





Fig 7.32: Absorption Spectra of *N. carneum* under different wastewater concentrations at 0^{th} day and 3^{rd} day (19-20)





Fig 7.33: Absorption Spectra of *N. carneum* under different wastewater concentration s at 6th day and 9th day (21-22)





Fig 7.34: Absorption Spectra of *N. carneum* under different wastewater concentrations at 12^{th} day and 15^{th} day (23-24)





Fig 7.35: Absorption Spectra of *N. carneum* under different wastewater concentrations at 18th day and 21th day (25-26)



Fig 7.36: Absorption Spectra of *N. carneum* under different wastewater concentrations at 24th day (27)





Fig 7.37 (a-c) Absorption Spectra of *Lyngbya polysiphoniae* under different doses of CuSO₄ stress













Fig 7.40: (a-c) Absorption Spectra of *Lyngbya polysiphoniae* under different doses of K₂CrO₄ stress





Fig 7.41 (a-c) Absorption Spectra of *Oscillatoria formosa* under different doses of K₂CrO₄ stress





Fig 7.42 (a-c) Absorption Spectra of *Nostoc carneum* under different doses of K₂CrO₄ stress



Figure 7.43: Concentration (µgml-1) of protein in *Lyngbya polysiphoniae*, *Oscillatoria formosa* and *Nostoc carneum* after 0 and 21 days at different doses of Copper sulphate.



Figure 7.44: Concentration (µgml-1) of protein in *Lyngbya polysiphoniae*, *Oscillatoria formosa* and *Nostoc carneum* after 0 and 21 days at different doses of Potassium chromate.



Figure 7.45: Concentration (µgml-1) of protein in *Lyngbya polysiphoniae*, *Oscillatoria formosa* and *Nostoc carneum* after 0 and 21 days at different doses of wastewater.



Figure 7.46: Concentration (µgml-1) of carbohydrates in *Lyngbya polysiphoniae*, *Oscillatoria formosa* and *Nostoc carneum* after 0 and 21 days at different doses of Copper sulphate.



Figure 7.47: Concentration (µgml-1) of carbohydrates in Lyngbya polysiphoniae, Oscillatoria formosa and Nostoc carneum after 0 and 21 days at different doses of Potassium chromate.



Figure 7.48: Concentration (µgml-1) of carbohydrates in *Lyngbya polysiphoniae*, *Oscillatoria formosa* and *Nostoc carneum* after 0 and 21 days at different doses of wastewater.



Figure 7.49: Catalase (CAT) activity shown by three test species under wastewater stress



Figure 7.50: Catalase (CAT) activity shown by three test species under copper sulphate stress



Figure 7.51: Catalase (CAT) activity shown by three test species under Potassium chromate stress



Figure 7.52: Glutathione reductase (GR) activity shown by three test species under wastewater stress



Figure 7.53: Glutathione reductase (GR) activity shown by three test species under Copper

sulphate stress









Plate 7.2 A-B SEM of Lyngbya polysiphoniae in Control; C-D SEM of Lyngbya polysiphoniae in waste water



Plate 7.3 A-B SEM of Oscillatoria formosa in Control; C-D SEM of Oscillatoria formosa in waste water



Plate 7.4 A-B SEM of Nostoc carneum in Control; C-D SEM of Nostoc carneum in waste water







Plate 7.6 A-B SEM of Nostoc carneum in Control; C-D SEM of Nostoc carneum in Copper treated solution







Plate 7.8 A-B SEM of Lyngbya polysiphoniae in Control; C-D SEM of Lyngbya polysiphoniae in Chromium treated solution



Plate 7.9 A-B SEM of Nostoc carneum in Control; C-D SEM of Nostoc carneum in Chromium treated solution



Plate 7.10 A-B SEM of Oscillatoria formosa in Control; C-D SEM of Oscillatoria in Chromium treated solution

7.4: Conclusion

A biological treatment process consisted of native cyanobacteria was applied to pulp mill effluents in order to increase the quality of wastewater. During the present study, the selected indigenous cyanobacterial species performed high efficiencies as suspended growth application toward the removal of both organic (BOD and COD), physical contaminants (solids; suspended and dissolved), chemical contaminants (nitrate, phosphate and ammonia) as well as heavy metals (Cu, Cd, Cr, Pb, Mn and Ni) from the paper mill wastewater. Thus, the results obtained in this experiment proved the biotechnological applicability and feasibility of using the tested microalgae for wastewater treatment where promising removal of the investigated contaminants were achieved. Therefore, this study highlighted a novel approach for the application of biological process for a feasible solution for wastewater through quality improvement which in turn will help in meeting the requirements for the wastewater discharge to the water bodies and thus minimizes the expected deterioration of the receiving environment..