BIOREMEDIATION OF INDUSTRIAL EFFLUENT USING CYANOBACTERIAL SPECIES: *PHORMIDIUM MUCICOLA* AND *ANABAENA AEQUALIS*

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ABSTRACT

Industries discharge effluent into different water body subjected to severe levels of pollution that can cope with the high pollution load in the water. Textile and Pharmaceutical industries, Mandideep, Bhopal has discharge industrial effluent into river. The main objective of the present study was to investigate the biodegradation and biosorption capacity of some potential cyanobacterial species; *Phormidium mucicola* and *Anabaena aequalis* dominating the river ecosystem. Industrial effluents are contaminated with heavy metal. The effluents were subjected to biological treatment using axenic cyanobacterial strains as batch system for 7 days. Removal efficiencies of the different contaminants were evaluated and compared. Results confirmed the high efficiencies of the investigated species for the removal of the target contaminants which were species and contaminant-dependent. BOD and COD recorded 91.18 and 82.54% as maximum removal efficiencies achieved by *Anabaena aequalis.* The highest removal efficiencies of the total suspended solids recorded 53.23% achieved by *Phormidium mucicola*, while 41.61% was recorded as the highest TDS. Concerning the contaminant metals, *Phormidium mucicola* showed the highest biosorption capacity where 86.12 and 94.63% removal efficiencies were achieved for Zn and Cu, respectively. In conclusion, results of the study confirmed the advantageous potential of using the tested cyanobacterial species for the bioremediation of industrial effluent and clearly showed the quality improvement of the discharged effluent which in turn will eliminate or at least minimize the expected deterioration of the receiving environment.

Keywords: Bioremediation, cyanobacterial species, heavy metals, industrial effluent

1. INTRODUCTION

Textile and Pharmaceutical industrial effluents discharge directly into water source like, close Water Lake in Bhopal city. Besides nutrients, the river water and sediments show terrible levels of organic matter, and heavy metals in worldwide. This is mainly due to continuous discharge of huge quantities of the effluents lead to deterioration in the water quality of this river (El-Bestawy E., 1993; El-Bestawy E.et al., 2007; Mansy And El-Bestawy, 2002).

Cyanobacteria group is more resistant for that pollutant, dominately cover the water body in summer. These species characterized by great ability to tolerate such high levels of pollution and proved high efficiency for degrading highly organic contaminants and accumulating heavy metals (El-Bestawy E et al.,2007; Podda et al.,2000 and Palmer, C.M. 1980). Therefore, they could be efficiently used in advanced technologies for bioremediation of the industrial effluents.

Cyanobacteria group is one of the most diverse groups of prokaryotes. They are gram-negative oxygenic photosynthetic bacteria. They can be found in a wide range of water bodies especially where the excess nutrient input of anthropogenic activities. The rapid evolution of cyanobacteria in different water importance, relative success and predominance during the land environments is related to their capacity for both growth season in phytoplankton communities. Water temperature above 25°C, low light cytoplasm near the cell periphery intensity in water, low N: P ratio and stability of the water.

Any change in pH of water bodies as a result of influx of effluent; can cause serious change in water chemistry, which can affect resources especially around the river area. These effects on water bodies can be very significant. Traditional methods for the clean up pollutants usually involve the removal of unwanted materials through sedimentation and filtration, and subsequent chemical treatment such as flocculation, neutralization and electro-dialysis before disposal.

Many species of cyanobacteria have nitrogenase enzyme, they convert atmospheric Nitrogen into ammonium in vesicles, which enable regulation of the buoyancy aerobic conditions. Recently, there have been increasing interest about using cyanobacteria as pollution control agents since they possess many advantages over other microorganisms isolated from soil. Their photoautotrophic nature and the ability of some species to fix atmospheric nitrogen enable them to be producers, as opposed to consumers, and make their growth and maintenance inexpensive [Castenholz et al., 1989; Somashekar, R.K. and Ramaswamy, S.N.1983). Metabolic activities are not affected by the decrease in the levels of the biodegradable pollutants that they may break down. Cyanobacteria have been used efficiently as a low-cost method for remediating all industrial effluents as well as transformation and removal of heavy metals (Lefebvre et al., Budd K 2007; Podda et al.,2000). Remediation capabilities of cyanobacteria toward environmental pollutants can be improved and enhanced through genetic engineering technologies (Kuritz and Wolk 1995; Mansy and El-Bestawy E. 2002 and Palmer, C.M. 1980). However, the beneficial application of cyanobacteria in remediation of contaminated waters and industrial effluents is still not optimally manipulated (Jeganathan, 2006 and Kannan, 2006).

2. AIMS AND OBJECTIVES

The main objective of the present study was to investigate the remediation capacity of some potential cyanobacterial species isolated from Textile and Pharmaceutical industrial effluent (Gohl and Vilensky 1987; James et al., 1979; Stewart et al., 1970; Tien and Kirk 1984).

3. MATERIAL AND METHODS

Survey of different sites of industrial effluent for identification of different algal forms from taxonomic point of view will be undertaken. Samples were collected from two industries such as Textile and pharmaceuticals industries, from Mandideep, Bhopal, M.P. India. Effluents were collected in large sterilized containers and polythene bags respectively. Thus, it is expected that the effluents contain industrial pollutants such as heavy metals which are not likely to be removed by that primary treatment of the industries. Grab samples representing all effluents entering the plant during 24 h were collected from both plants to avoid the fluctuation in the flow and the strength of the effluent.

Physico-chemical characteristics of effluents were carried out by standard methods (APHA, 1995).Such as biochemical oxygen demand (BOD); chemical oxygen demand (COD); total suspended solids (TSS); total dissolved solids (TDS); and two heavy metals (Zn, Cu) were characterized before and after treatment to determine the effectiveness of the remediation process (Boominathan 2005, Cairns, J. Jr. & Dickson,K.L. 1971). All the investigated parameters were determined using the standard techniques described by (Celesseri et al., 1999) in the standard methods for the examination of water and effluent.

Standard microbiological methods were followed for the isolation of cyanobacteria. Algal samples were microscopically examined and the selected cyanobacterial species were grown in Chu No.10 (1942) media was used as culture media. and the pH was adjusted to 7.2 with HCl. Media has sterilized by autoclaving separately at 121^0C for 20 min. Micronutrient solution was sterilized by filtration through 0.22 mm polycarbonate membrane to avoid interaction and precipitation of heavy metals. Chu No.10 media was freshly prepared from A and B where 1.0 ml of each component of solution A and 1.0 ml of Solution B were combined and diluted to 1.0 l, sterilized as mentioned and used for selective culturing of the selected species. After inoculation, all the selected species were incubated at room temperature (28^0C) and day light with manual shaking every 24 h to avoid adhesion of the algae on the walls of the glass vessels until heavy growth appeared within 3 weeks.

Identification was confirmed based upon the keys given by (Geitler, 1932 and Desikachary, 1959) for microscopic parameters. The isolated cyanobacteria were identified with the help of classical manuals. Two different cyanobacterial species; *Anabaena aequalis* and *Phormidium mucicola*; were selected for further study.

2.1 Axenity and bioassay

Unialgal cultures usually remained contaminated with bacteria and therefore to free them from bacteria is a pre requisite for further studies (Ash and Jenkins, 2006; Anagnostidis, El-Nahhal et al, 2013, Komárek, 1985 and Safi et al, 2014). The cultures were made bacteria free by ultraviolet irradiation for varying periods and inoculated in the medium. Axenic cultures were prepared of these isolate species. However, before using these strains in the bioremediation of the contaminated industries effluents, their axenity was checked using agar phototactic response method. Semi solid standard agar medium was prepared and liquated into test tubes and sterilized. Each tube was inoculated with 100 µl of cyanobacterial culture (two replicates per culture). Light was prevented to reach the top 10 cm of the tube using aluminum foil. All the tubes were incubated in optimal conditions (28^0C) in an illuminated incubator. Based on the phototactic response phenomena the cyanobacterial filaments were grown toward light direction through the semi solid agar, but bacteria did not grown. After 7days incubation of the agar column was dragged out the tube on sterilized Petri dish. The agar column was sliced into ten slices 1 cm per each. Each slice was stranded longitudinally and transversally cut under common sterilized conditions to separate the algal filaments surrounded with a small piece of agar. Each agar piece involving cyanobacterial growth was inoculated into standard selective liquid medium. After incubation, each inoculated culture was tested for contamination using general bacterial medium (nutrient agar). In bioremediation bioassay, the tested species were checked for their axenity, and the liquid cultures were tested by plating on bacterial nutrient medium and incubating at 28^0C for 7days. Only axenic cultures were involved in the assays. The selected species were inoculated individually in 100 ml culturing medium (three replicates) and incubated for 2 weeks till heavy growth was obtained. Effluents from both industries (textile and pharmaceutical) was dispensed (900 ml each) in 18 sterilized conical flasks, nine flasks for each effluent. Each culture (100 ml) was separately seeded at a final volume of 1l each (three replicates/strain/effluent) and incubated under the previously mentioned conditions for

7 days. Another six flasks (three flasks for each industry) were supplied by 1ml each of the effluent of both industries without seeding with cyanobacteria to serve as control for the bioassay. They were incubated under the same conditions. For the determination of heavy metals and other parameters residues, samples were collected at 24 h interval. At each sampling time, 130 ml from each flask were aseptically drawn, where all the investigated parameters were determined and their removal efficiencies using the selected species were determined by PERKIN ELMER OPTIMA 5300 DV ICP-OES (Inductively Coupled Plasma Optical Emission Spectrophotometer) method and result were obtained from SAIF (Sophisticated Analytical Instrument Facility) Indian Institute of Technology Madras, Chennai.

3. RESULTS AND DISCUSSION

Following species have been collected from Textile and pharmaceutical industries.

Table 1: Occurrence of cyanobacteria in effluents of different sites of Pharmaceutical, Textile industries

Cyanobacterial species	Industries					
	Pharmaceutical			Textile		
	Site I	Site II	Site III	Site I	Site II	Site III
Chroococcus dispersus	+	+	+	+	+	+
Rhabdoderma irregulare	-	-	-	-	-	+
Aphanocapsa elachista	-	-	-	+	-	-
Holopedium irregular	-	-	-	+	-	-
Gloeothece linearis	-	-	-	-	-	-
Gomphosphaeria aponina var.cordiformis	-	-	-	+	-	-
Microcystis aeruginosa	+	+	+	+	+	+
Spirulina major	+	+	+	-	-	-
Oscillatoria amoena	-	-	-	+	+	+
Phormidium inundatum	+	+	+	+	+	+
Phormidium mucicola	+	+	+	+	+	+
Schizothrix lacustris	+	+	+	-	-	-
Schizothrix tinctoria	-	-	-	-	-	-
Anabaena aequalis	+	+	+	+	+	+

Cyanobacterial species	Industries					
	Pharmaceutical			Textile		
	Site I	Site II	Site III	Site I	Site II	Site III
Anabaena affinis	+		+			
Nostoc verrucosum	+	+	+	+	+	+
Nodularia spumigena	-	-	-	-	-	-
Aphanizomenon flos-aquae	+	+	+	+	+	+
Cylindrospermum catenatum	-	-	+	-	-	-
Scytonema crispum	-	+	-	-	-	-
Plectonema wollei	-	-	-	+	+	+
Stigonema mesentericum	-	-	+	+	-	-
Stigonema ocellatum	+	+	+	+	+	+

Present (+) Absent (-)

Phormidium mucicola and *Anabaena aequalis* investigated as free-living cells for their ability for organic matter biodegradation and heavy metal removal from the effluent. They were selected based on their dominance and survival in the highly polluted water of Pharmaceutical industries and textile industries where they acquired high resistance and acclimatized to deal with high loads of different contaminants. They were also proven high ability for degradation of the heavy metals. Therefore, the selected species were considered promising candidates for biological treatment of the industrial effluents.

Table 2: Residue concentrations (RC) of the quality parameters from the contaminated industrial effluents

Textile industry effluent (Before treatment value in mg/l)

Time(day)	BOD	COD	TSS	TDS	ZN	CU
Raw water	140	360	167	1,150	0.11	0.04
2	89	289	132	887	0.09	0.01
3	80	243	100	766	0.05	0.02
4	81	200	101	756	0.12	0.04
5	70	187	98	611	0.09	0.05
6	120	387	154	900	0.14	0.06
7	143	331	143	998	0.12	0.07

Pharmaceutical industry effluent (Before treatment value in mg/l)

Time (days)	BOD	COD	TSS	TDS	ZN	CU
Raw water	198	445	387	1430	0.01	0.03
2	187	465	411	1457	0.04	0.01
3	199	412	376	1432	0.03	0.04
4	176	398	311	1200	0.01	0.04
5	166	345	298	1100	0.03	0.06
6	178	411	321	1289	0.05	0.02
7	200	378	365	1008	0.02	0.01

Table 3: Residue concentrations (RC) of the quality parameters from the contaminated industrial effluents using the selected cyanobacteria at different exposure time icpoes.

Textile industries effluent (After treatment value in mg/l)

	Anabaena aequalis						*Phormidium mucicola*					
Days	BOD	COD	TSS	TDS	ZN	CU	BOD	COD	TSS	TDS	ZN	CU
Raw water	149	312	200	1012	0.11	0.05	148	324	234	1134	0.12	0.07
2	141	301	199	1011	0.09	0.05	143	321	223	1124	0.10	0.07
3	132	298	189	1001	0.06	0.03	132	309	212	1120	0.5	0.05
4	121	288	170	998	0.05	0.02	122	289	206	1103	0.5	0.03
5	100	198	150	988	0.03	0.02	101	281	189	1087	0.3	0.02
6	76	122	132	986	0.03	0.02	98	256	167	1079	0.1	0.01
7	62	98	112	983	0.02	0.01	78	249	156	1012	0.1	0.01

Pharmaceutical industries effluent (After treatment value in mg/l)

	Anabaena aequalis						Phormidium mucicola					
Days	BOD	COD	TSS	TDS	ZN	CU	BOD	COD	TSS	TDS	ZN	CU
Raw water	254	432	321	1349	0.08	0.07	231	421	401	1401	0.06	0.07
2	233	428	311	1323	0.06	0.05	211	421	387	1400	0.04	0.06
3	212	412	306	1321	0.06	0.04	201	401	381	1387	0.04	0.04
4	198	398	285	1309	0.04	0.04	192	356	372	1345	0.02	0.03
5	167	378	265	1287	0.03	0.02	187	241	324	1302	0.01	0.03
6	121	321	209	1270	0.02	0.01	123	209	283	1265	0.01	0.01
7	98	243	187	1201	0.02	0.00	98	198	223	1230	0.00	0.01

3.1 Industrial effluent characteristics

Effluent produced by the two industries were characterized (Table2, control).BOD, COD, TSS, TDS, Zn and Cu recorded averages of 140, 360, 167, 1150, 0.11and 0.04 mg/l, respectively, in the effluent of the textile industry. Significantly higher levels for almost all the tested parameters were detected in the pharmaceutical effluent where 198, 445, 387, 1430, 0.01, and 0.03 mg/l were recorded as average. However, Zn recorded much lower average in the pharmaceutical effluent (0.01mg/l) compared to that of the textile effluent (0.11 mg/l) while no significant differences were recorded in the Cu levels among the two industry (0.04 and 0.03 mg/l in the textile and pharmaceutical effluents). Nitrogen and phosphorus content in both effluents (El-Bestawy E et al., 2005 and Ellis 1977) along with the toxic industrial contaminants suppressed the growth of cyanobacteria or any other algae.

3.2 Treatability and removal efficiency of effluent

3.2.1 Contaminants

Residue levels of the selected quality parameters were determined (Table 2) and the removal efficiencies (RE %) as results of the biological treatment using the selected species were calculated. As a general trend, the two tested species exhibited positive correlation between their RE% of all the tested parameters and the exposure time up to the last exposure day for both types of effluents.

3.2.2 Organic matter removal

Biochemical oxygen demand Removal of BOD from industrial effluents of both industries using the selected algae revealed the following points:

1. High REs% were obtained for BOD removal from industrial effluent by the selected species with *Anabaena aequalis* (90.65%) and finally *Phormidium mucicola* (81.9%).
2. Despite the RE variations of BOD achieved by the tested species, RC(s) of the BOD in the industrial effluent reached acceptable limits (19, 20 and 32 mg/l by *Anabaena aequalis* and *Phormidium mucicola*, respectively) after 7 days exposure which is much lower than the maximum permissible limit (APHA 1998) of 60 mg/l stated by the Environmental Laws for safe discharge into surface water courses. When these figures compared with those obtained by the control it was showed that the natural microbial population of the effluent achieved a maximum removal of 50% after 5 days equivalent to 60.7 mg/l (MPL of the BOD) after which there was a sharp decline in the efficiency associated by increasing the RC reaching 140 mg/l and 3.20% RE after 7 exposure of days.
3. Comparing BOD removal by the selected cyanobacteria revealed very high efficiency for all of them in the degradation of biodegradable organic matter which is stimulated by increasing the levels of the pollutant in the wastewater.

Chemical oxygen demand Removal of COD from the industrial effluents using the selected species revealed the following points:

1. *Anabaena aequalis* considered the most effective for removing COD from the industrial effluent achieving the maximum RE of 83.68% compared to the RE achieved by *Phormidium mucicola* (45.00%) after 7 exposure days. However, *Phormidium mucicola* exhibited higher COD RE% within the first 24 h compared to Anabaena aequalis.

2. The lowest residue concentration of 100 mg/l was achieved by Anabaena aequalis which is the maximum acceptable limit stated by the law (MPL for COD = 100 mg/l) while *Phormidium mucicola* could not bring the COD levels of the effluent to better quality. They recorded 180 and 246 mg/l, respectively, and required longer exposures. The highest RE% achieved by the control culture recorded 50.11% (170.59 mg/l) after 5 exposure days.

3. Similar to BOD removal, the natural microorganisms in the control culture were inhibited by the high Strength of the industrial effluent leading to reduction in the COD removal.

4. Although high REs% of the COD was achieved by the selected species, none of them could bring the COD levels in the effluent below the MPL during the investigated exposure time (1 week). This may be attributed to the need for longer time for achieving the proper quality. It could also result from the inhibition in cyanobacterial growth due to the higher COD levels in the pharmaceutical effluent compared to that of the textile effluent.

3.2.3 Solids removal

Total suspended solids (TSS) Removal of TSS from the industrial effluents using the selected species revealed the following points:

1. The highest recorded TSS REs% in the effluent recorded 42.0 and 29.12% achieved by *Anabaena aequalis,* and *Phormidium mucicola* (125 and 133 mg/l RC), respectively, after 7 days.

2. According to the law, 60 mg/l is stated as the MPL of the TSS; therefore none of the tested species reached the required efficiency to bring the TSS in the effluents below the MPL during the tested exposure time. This indicates that they required longer time, heavier biomass or different application using the same species to achieve that quality.

3. In contrast to cyanobateria control culture achieved higher TSS removal form the textile effluent compared to that of the pharmaceutical effluent.

Total dissolved solids (TDS) Removal of TDS from the industrial effluent using the selected species revealed the following points:

1. The maximum TDS REs% obtained for the effluent by the tested species ranged between a maximum of 16.66% (1,078 mg/l) achieved by *Phormidium mucicola* and a minimum of 12.00% (1,119 mg/l) obtained by Anabaena aequalis after 7 exposure days. *Phormidium*

mucicola exhibited higher TDS RE% at the shorter exposures (up to 2nd day) compared to Anabaena aequalis.

2. Similar behavior for TSS removal was shown by bacteria of the control culture where higher TDS removal was achieved form the textile effluent compared to that of the pharmaceutical effluent.

3. Since the TDS content in the effluents were lower than the MPL of the TDS (2,000 mg/l), the residual concentrations of the TDS produced in the final effluents by all the tested species as well as the two controls improved and still within the safe range for discharging.

3.2.4 Heavy metals removal

Results revealed the following points:

1. *Phormidium mucicola* recorded the highest REs% for Zn from EWTP (86.12) and *Anabaena aequalis (*70.88%) recording RCs of 0.0247, and 0.0370 mg/l by three species, respectively, after 7 days.

2. Although low Zn levels were detected in the pharmaceutical effluent, lower Zn REs were achieved compared to those obtained for the textile effluent. Zn removal recorded 78.2, and 65.00% achieved as the highest Zn REs% by *Phormidium mucicola*, and *Anabaena aequalis*, respectively (0.0123, and 0.0182 mg/l, respectively) after 7 days.

3. Although all the average levels of Zn for both effluents were below the MPL of 5 mg/l before the treatment, Zn levels were reduced producing much better effluent quality. Zinc removal was stimulated by increasing its level in the wastewater.

4. Concerning Cu , much higher REs% were recorded for effluent compared to those obtained for Zn removal regardless it's high toxicity. This may be attributed to the high resistance of the selected members which was stimulated by increasing Cu levels in the wastewater. 94.63, 90.99 and 90.64% RE of Cu were achieved by *Phormidium mucicola*, and Anabaena aequalis (0.0031, and 0.0054 mg/l RC), respectively, after 7 days.

4. CONCLUSION

In conclusion, results confirmed that *Phormidium mucicola* and *Anabaena aequalis* are the effective species for BOD, COD, TSS, TDS , Zn and Cu removal from the effluents of the textile and pharmaceutical industries. Zn and Cu both are toxic heavy metals. Effluents discharge in water bodies can affect the living organism. These cyanobacterial species are able to remove these metals from water body. Absorption of metals increase in the higher concentration. Presence of these cyanobacteria can low BOD, COD, TSS, TDS.

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