

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

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6  
7 **ABSTRACT**  
8

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.

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

10 **1. INTRODUCTION**

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

16 2002).Cyanobacteria group is more resistant for that pollutant, dominately cover the water  
17 body in summer. These species characterized by great ability to tolerate such high levels of  
18 pollution and proved high efficiency for degrading highly organic contaminants and  
19 accumulating heavy metals (El-Bestawy **E** et al.,2007; Podda et al.,2000 and Palmer, **C.M.**  
20 1980). Therefore, they could be efficiently used in advanced technologies for bioremediation  
21 of the industrial effluents.

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

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

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

51

52 **2. AIMS AND OBJECTIVES**

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

56 **3. MATERIAL AND METHODS**

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

65 Physico-chemical characteristics of effluents were carried out by standard methods (APHA,  
66 1995). Such as biochemical oxygen demand (BOD); chemical oxygen demand (COD); total  
67 suspended solids (TSS); total dissolved solids (TDS); and two heavy metals (Zn, Cu) were  
68 characterized before and after treatment to determine the effectiveness of the remediation  
69 process (Boominathan 2005, Cairns, J. Jr. & and Dickson, K.L. 1971). All the investigated  
70 parameters were determined using the standard techniques described by (Celesseri et al.,  
71 1999) in the standard methods for the examination of water and effluent.

72 Standard microbiological methods were followed for the isolation of cyanobacteria. Algal  
73 samples were microscopically examined and the selected cyanobacterial species were  
74 grown in Chu No.10 (1942) media was used as culture media. and the pH was adjusted to  
75 7.2 with HCl. Media has sterilized by autoclaving separately at 121°C for 20 min.  
76 Micronutrient solution was sterilized by filtration through 0.22 mm polycarbonate membrane  
77 to avoid interaction and precipitation of heavy metals. Chu No.10 media was freshly  
78 prepared from A and B where 1.0 ml of each component of solution A and 1.0 ml of Solution  
79 B were combined and diluted to 1.0 l, sterilized as mentioned and used for selective culturing  
80 of the selected species. After inoculation, all the selected species were incubated at room  
81 temperature (28°C) and day light with manual shaking every 24 h to avoid adhesion of the  
82 algae on the walls of the glass vessels until heavy growth appeared within 3 weeks.

83 Identification was confirmed based upon the keys given by (Geitler, 1932 and Desikachary,  
84 1959) for microscopic parameters. The isolated cyanobacteria were identified with the help  
85 of classical manuals. Two different cyanobacterial species; *Anabaena aequalis* and  
86 *Phormidium mucicola*; were selected for further study.

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## 87 **2.1 Axenity and bioassay**

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

114 7 days. Another six flasks (three flasks for each industry) were supplied by 1ml each of the  
 115 effluent of both industries without seeding with cyanobacteria to serve as control for the  
 116 bioassay. They were incubated under the same conditions. For the determination of heavy  
 117 metals and other parameters residues, samples were collected at 24 h interval. At each  
 118 sampling time, 130 ml from each flask were aseptically drawn, where all the investigated  
 119 parameters were determined and their removal efficiencies using the selected species were  
 120 determined by PERKIN ELMER OPTIMA 5300 DV ICP-OES (Inductively Coupled Plasma  
 121 Optical Emission Spectrophotometer) method and result were obtained from SAIF  
 122 (Sophisticated Analytical Instrument Facility) Indian Institute of Technology Madras,  
 123 Chennai.

### 124 3. RESULTS AND DISCUSSION

125 Following species have been collected from Textile and pharmaceutical industries.

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

128

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

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

129 Present (+) Absent (-)

130

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

138

139

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

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

143 Time(day)	BOD	COD	TSS	TDS	ZN	CU
144 Raw water	140	360	167	1,150	0.11	0.04
145 2	89	289	132	887	0.09	0.01
146 3	80	243	100	766	0.05	0.02
147 4	81	200	101	756	0.12	0.04
148 5	70	187	98	611	0.09	0.05
149 6	120	387	154	900	0.14	0.06
150 7	143	331	143	998	0.12	0.07

151

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

153	Time (days)	BOD	COD	TSS	TDS	ZN	CU
154	Raw water	198	445	387	1430	0.01	0.03
155	2	187	465	411	1457	0.04	0.01
156	3	199	412	376	1432	0.03	0.04
157	4	176	398	311	1200	0.01	0.04
158	5	166	345	298	1100	0.03	0.06
159	6	178	411	321	1289	0.05	0.02
160	7	200	378	365	1008	0.02	0.01

161

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

164

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

166	<i>Anabaena aequalis</i>							<i>Phormidium mucicola</i>					
167	Days	BOD	COD	TSS	TDS	ZN	CU	BOD	COD	TSS	TDS	ZN	CU
168	Raw water	149	312	200	1012	0.11	0.05	148	324	234	1134	0.12	0.07
169	2	141	301	199	1011	0.09	0.05	143	321	223	1124	0.10	0.07
170	3	132	298	189	1001	0.06	0.03	132	309	212	1120	0.5	0.05
171	4	121	288	170	998	0.05	0.02	122	289	206	1103	0.5	0.03
172	5	100	198	150	988	0.03	0.02	101	281	189	1087	0.3	0.02
173	6	76	122	132	986	0.03	0.02	98	256	167	1079	0.1	0.01
174	7	62	98	112	983	0.02	0.01	78	249	156	1012	0.1	0.01

175

176

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

178

179	<i>Anabaena aequalis</i>							<i>Phormidium mucicola</i>					
180	Days	BOD	COD	TSS	TDS	ZN	CU	BOD	COD	TSS	TDS	ZN	CU
181	Raw water	254	432	321	1349	0.08	0.07	231	421	401	1401	0.06	0.07
182	2	233	428	311	1323	0.06	0.05	211	421	387	1400	0.04	0.06
183	3	212	412	306	1321	0.06	0.04	201	401	381	1387	0.04	0.04
184	4	198	398	285	1309	0.04	0.04	192	356	372	1345	0.02	0.03
185	5	167	378	265	1287	0.03	0.02	187	241	324	1302	0.01	0.03
186	6	121	321	209	1270	0.02	0.01	123	209	283	1265	0.01	0.01
187	7	98	243	187	1201	0.02	0.00	98	198	223	1230	0.00	0.01

### 188 **3.1 Industrial effluent characteristics**

189 Effluent produced by the two industries were characterized (Table2, control).BOD, COD,  
190 TSS, TDS, Zn and Cu recorded averages of 140, 360, 167, 1150, 0.11and 0.04 mg/l,  
191 respectively, in the effluent of the textile industry. Significantly higher levels for almost all the  
192 tested parameters were detected in the pharmaceutical effluent where 198, 445, 387, 1430,  
193 0.01, and 0.03 mg/l were recorded as average. However, Zn recorded much lower average  
194 in the pharmaceutical effluent (0.01mg/l) compared to that of the textile effluent (0.11 mg/l)  
195 while no significant differences were recorded in the Cu levels among the two industry (0.04  
196 and 0.03 mg/l in the textile and pharmaceutical effluents). Nitrogen and phosphorus content  
197 in both effluents (El-Bestawy [E](#) et al., 2005 and Ellis 1977) along with the toxic industrial  
198 contaminants suppressed the growth of cyanobacteria or any other algae.

### 199 **3.2 Treatability and removal efficiency of effluent**

#### 200 **3.2.1 Contaminants**

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

#### 206 **3.2.2 Organic matter removal**

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

209 1. High REs% were obtained for BOD removal from industrial effluent by the selected  
210 species with *Anabaena aequalis* (90.65%) and finally *Phormidium mucicola* (81.9%).

211 2. Despite the RE variations of BOD achieved by the tested species, RC(s) of the BOD in  
212 the industrial effluent reached acceptable limits (19, 20 and 32 mg/l by *Anabaena aequalis*  
213 and *Phormidium mucicola*, respectively) after 7 days exposure which is much lower than the  
214 maximum permissible limit (APHA 1998) of 60 mg/l stated by the Environmental Laws for  
215 safe discharge into surface water courses. When these figures compared with those  
216 obtained by the control it was showed that the natural microbial population of the effluent  
217 achieved a maximum removal of 50% after 5 days equivalent to 60.7 mg/l (MPL of the BOD)  
218 after which there was a sharp decline in the efficiency associated by increasing the RC  
219 reaching 140 mg/l and 3.20% RE after 7 exposure of days.

220 3. Comparing BOD removal by the selected cyanobacteria revealed very high efficiency for  
221 all of them in the degradation of biodegradable organic matter which is stimulated by  
222 increasing the levels of the pollutant in the wastewater.

223



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

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

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

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

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

### 242 **3.2.3 Solids removal**

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

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

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

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

254

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

257 1. The maximum TDS REs% obtained for the effluent by the tested species ranged between  
258 a maximum of 16.66% (1,078 mg/l) achieved by *Phormidium mucicola* and a minimum of  
259 12.00% (1,119 mg/l) obtained by *Anabaena aequalis* after 7 exposure days. *Phormidium*

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

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

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

#### 268 **3.2.4 Heavy metals removal**

269 Results revealed the following points:

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

273 2. Although low Zn levels were detected in the pharmaceutical effluent, lower Zn REs were  
274 achieved compared to those obtained for the textile effluent. Zn removal recorded 78.2, and  
275 65.00% achieved as the highest Zn REs% by *Phormidium mucicola*, and *Anabaena*  
276 *aequalis*, respectively (0.0123, and 0.0182 mg/l, respectively) after 7 days.

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

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

#### 285 **4. CONCLUSION**

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

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