

1 **BIOREMEDIATION OF INDUSTRIAL EFFLUENT USING CYANOBACTERIAL**

2 **SPECIES: *Phormidium mucicola* AND *Anabaena aequalis*.**

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9 **ABSTRACT**

10

Different Industries discharge effluent in different water bodies, which is the only reason of pollution. 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* in Textile and Pharmaceutical industries, Mandideep, Bhopal Madhya Pradesh, India. 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.

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

12 **1. INTRODUCTION**

13 Water covers 71% of the earth and makes up 65% of the our bodies. All organisms
14 contain it, live in it, drink it.. Plants and animals require water that is moderately pure and
15 they can not survive if their water is loaded with toxic chemical and harmful microorganisms
16 (Encarta Encyclopedia, 2009). Effluents discharge from different sources is directly

17 responsible for water pollution. This is mainly due to continuous discharge of huge quantities
18 of the effluents lead to deterioration in the water quality of the rivers (El-Bestawy E., 1993;
19 El-Bestawy E. et al., 2007; Mansy And El-Bestawy, 2002). Any change in pH of water bodies
20 as a result of influx of effluent; can cause serious change in water chemistry, which can
21 affect resources especially around the river area. These effects on water bodies can be very
22 significant. Besides nutrients, the river water and sediments show terrible levels of organic
23 matter, and heavy metals in worldwide. To overcome water pollution problem some physical
24 and chemical methods are used, but they are expensive and generative concentrated waste
25 that require subsequent treatment and disposal (Komori et al., 1990). Traditional methods for
26 the cleanup pollutants usually involve the removal of unwanted materials through
27 sedimentation and filtration, and subsequent chemical treatment such as flocculation,
28 neutralization and electro-dialysis before disposal. Biological treatment may provide a
29 suitable means for treatment from waste water (Lovely and Coates 1997 and Rittmann et al.,
30 2004). Cyanobacterial species characterized by great ability to tolerate such high levels of
31 pollution and proved high efficiency for degrading highly organic contaminants and
32 accumulating heavy metals (El-Bestawy et al., 2007; Podda et al., 2000 and Palmer, 1980).
33 Therefore, they could be efficiently used in advanced technologies for bioremediation of the
34 industrial effluents.

35 Cyanobacteria group is one of the most diverse groups of prokaryotes. They are
36 gram-negative oxygenic photosynthetic bacteria. They can be found in a wide range of water
37 bodies especially where the excess nutrient input of anthropogenic activities. Many species
38 of cyanobacteria have nitrogenase enzyme, they convert atmospheric nitrogen into
39 ammonium. Their photoautotrophic nature and the ability of some species to fix atmospheric
40 nitrogen enable them to be producers, as opposed to consumers, and make their growth and
41 maintenance inexpensive [Castenholz et al., 1989; Somashekar, and Ramaswamy, 1983].
42 Metabolic activities are not affected by the decrease in the levels of the biodegradable
43 pollutants that they may break down. Cyanobacteria have been used efficiently as a low-cost
44 method for remediating all industrial effluents as well as transformation and removal of
45 heavy metals (Lefebvre et al., Budd 2007; Podda et al., 2000).

46 Recently, there have been increasing interest about using cyanobacteria as pollution control
47 agents since they possess many advantages over other microorganisms isolated from soil.
48 Remediation capabilities of cyanobacteria toward environmental pollutants can be improved
49 and enhanced through genetic engineering technologies (Kuritz and Wolk 1995; Mansy and
50 El-Bestawy, 2002 and Palmer, 1980). However, the beneficial application of cyanobacteria in
51 remediation of contaminated waters and industrial effluents is still not optimally manipulated
52 (Jeganathan, 2006 and Kannan, 2006).

53

54 **2. AIMS AND OBJECTIVES**

55 The main objective of the present study was to investigate the remediation capacity of some
56 potential cyanobacterial species isolated from Textile and Pharmaceutical industrial effluent;

57 **3. MATERIAL AND METHODS**

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

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

73 Standard microbiological methods were followed for the isolation of cyanobacteria. Algal
74 samples were microscopically examined and the selected cyanobacterial species were
75 grown in Chu No.10 (1942) media was used as culture media. and the pH was adjusted to
76 7.2 with HCl. Media has sterilized by autoclaving separately at 121⁰C for 20 min.
77 Micronutrient solution was sterilized by filtration through 0.22 mm polycarbonate membrane
78 to avoid interaction and precipitation of heavy metals. Chu No.10 media was freshly
79 prepared from A and B where 1.0 ml of each component of solution A and 1.0 ml of Solution
80 B were combined and diluted to 1.0 l, sterilized as mentioned and used for selective culturing
81 of the selected species. After inoculation, all the selected species were incubated at room
82 temperature (28⁰C) and day light with manual shaking every 24 h to avoid adhesion of the
83 algae on the walls of the glass vessels until heavy growth appeared within 3 weeks.

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

88 **2.1 Axenity and bioassay**

89 Unialgal cultures usually remained contaminated with bacteria and therefore to free
90 them from bacteria is a pre requisite for further studies (Ash and Jenkins, 2006;
91 Anagnostidis, El-Nahhal et al, 2013, Komárek, 1985 and Safi et al, 2014). The cultures were
92 made bacteria free by ultraviolet irradiation for varying periods and inoculated in the medium.
93 Axenic cultures were prepared of these isolate species. However, before using these strains
94 in the bioremediation of the contaminated industries effluents, their axenity was checked
95 using agar phototactic response method. Semi solid standard agar medium was prepared
96 and liquated into test tubes and sterilized. Each tube was inoculated with 100 µl of
97 cyanobacterial culture (two replicates per culture). Light was prevented to reach the top 10
98 cm of the tube using aluminum foil. All the tubes were incubated in optimal conditions (28⁰C)
99 in an illuminated incubator. Based on the phototactic response phenomena the
100 cyanobacterial filaments were grown toward light direction through the semi solid agar, but
101 bacteria did not grown. After 7days incubation of the agar column was dragged out the tube
102 on sterilized Petri dish. The agar column was sliced into ten slices 1 cm per each. Each slice
103 was stranded longitudinally and transversally cut under common sterilized conditions to
104 separate the algal filaments surrounded with a small piece of agar. Each agar piece
105 involving cyanobacterial growth was inoculated into standard selective liquid medium. After
106 incubation, each inoculated culture was tested for contamination using general bacterial
107 medium (nutrient agar). In bioremediation bioassay, the tested species were checked for
108 their axenity, and the liquid cultures were tested by plating on bacterial nutrient medium and
109 incubating at 28⁰C for 7days. Only axenic cultures were involved in the assays. The selected
110 species were inoculated individually in 100 ml culturing medium (three replicates) and
111 incubated for 2 weeks till heavy growth was obtained. Effluents from both industries (textile
112 and pharmaceutical) was dispensed (900 ml each) in 18 sterilized conical flasks, nine flasks
113 for each effluent. Each culture (100 ml) was separately seeded at a final volume of 1l each
114 (three replicates/strain/effluent) and incubated under the previously mentioned conditions for

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

125 3. RESULTS AND DISCUSSION

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

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

129

Cyanobacterial species	Industries					
	Pharmaceutical			Textile		
	Site I	Site II	Site III	Site I	Site II	Site III
<i>Chroococcus disperses</i>	+	+	+	+	+	+
<i>Rhabdoderma irregular</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>	+	+	+	+	+	+

130 Present (+) Absent (-)

131

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

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

141 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

142

143

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

Time(day)	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

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

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

Days	<i>Anabaena aequalis</i>						<i>Phormidium mucicola</i>					
	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	0.1
7	62	98	112	983	0.02	0.01	78	249	156	1012	0.1	0.1

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

Days	<i>Anabaena aequalis</i>						<i>Phormidium mucicola</i>					
	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	1287	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

149 **3.1 Industrial effluent characteristics**

150 Effluent produced by the two industries were characterized (Table2, control).BOD, COD,
151 TSS, TDS, Zn and Cu recorded averages of 140, 360, 167, 1150, 0.11and 0.04 mg/l,
152 respectively, in the effluent of the textile industry. Significantly higher levels for almost all the
153 tested parameters were detected in the pharmaceutical effluent where 198, 445, 387, 1430,
154 0.01, and 0.03 mg/l were recorded as average. However, Zn recorded much lower average
155 in the pharmaceutical effluent (0.01mg/l) compared to that of the textile effluent (0.11 mg/l)
156 while no significant differences were recorded in the Cu levels among the two industry (0.04
157 and 0.03 mg/l in the textile and pharmaceutical effluents). Nitrogen and phosphorus content
158 in both effluents (El-Bestawy et al., 2005 and Ellis 1977) along with the toxic industrial
159 contaminants suppressed the growth of cyanobacteria or any other algae.

160 **3.2 Treatability and removal efficiency of effluent**

161 **3.2.1 Contaminants**

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

167 **3.2.2 Organic matter removal**

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

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

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

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

184

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

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

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

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

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

203 **3.2.3 Solids removal**

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

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

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

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

215

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

218 1. The maximum TDS REs% obtained for the effluent by the tested species ranged between
219 a maximum of 16.66% (1,078 mg/l) achieved by *Phormidium mucicola* and a minimum of
220 12.00% (1,119 mg/l) obtained by *Anabaena aequalis* after 7 exposure days. *Phormidium*

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

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

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

229 **3.2.4 Heavy metals removal**

230 Results revealed the following points:

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

234 2. Although low Zn levels were detected in the pharmaceutical effluent, lower Zn REs were
235 achieved compared to those obtained for the textile effluent. Zn removal recorded 78.2, and
236 65.00% achieved as the highest Zn REs% by *Phormidium mucicola*, and *Anabaena*
237 *aequalis*, respectively (0.0123, and 0.0182 mg/l, respectively) after 7 days.

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

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

246 **4. CONCLUSION**

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

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