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Comparative studies on phycoremediation of sewage water by using blue green algae

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Abstract

Phycoremediation is the process of employing macro or microalgae which has many advantageous over other conventional methods, as it is low cost and environmental friendly. Therefore present investigation was attempted to reduce the toxic pollutants from the municipal wastewater generated in Pune city using *Oscillatoria limosa* and *Nostoc commune*. The experiment was conducted using randomized complete block design with three replications at Department of Environmental of Sciences, University of Pune, Pune-7, (MS) India. The results revealed that both the species of blue green algae were highly efficient and having very good potential to reduce BOD, COD, TH, TA and TDS and the range of reduction of above mentioned parameters was 95 to 98 %. However the range of increase in DO was 62-63 % indicated improvement in quality of wastewater. It was concluded that amongst both the algal species used for phycoremediation, *Oscillatoria limosa* was superior over *Nostoc commune* as it has high bioconversion ability and tolerance to pollution. The phycoremediation was very effective as both the algal species were well adapted to local conditions.

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Introduction

Pollution of water by organic and inorganic pollutants, nutrients and heavy metals is a global problem. The metropolitan cities like Pune generating 567 MLD wastewater and creating serious problem for health of human beings and environment (Shastri and Raval, 2012). The urban and industrial wastewater must be treated before being discharged into the environment to prevent pollution and eutrophication of rivers and other water bodies (Ruiz-Martinez *et al.*, 2012). Phycoremediation has many advantageous over the conventional methods, which are very costly, consuming energy and producing high quantity of sludge and hence it is accepted throughout the world (Ghosh and Singh, 2005; Abdel-Raouf *et al.*, 2012).

This method involves the use of macro or microalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater (Ahmad *et al.*, 2013). Over the last few decades efforts have been made to apply microalgae such as *Chlorella*, *Chlamydomonas*, *Spirulina*, *Scenedesmus*, *Nostoc* and *Oscillatoria* for wastewater treatment (Dubey *et al.*, 2011; Sharma and Khan, 2013). The underlying assumption is that the microalgae are versatile to transform the contaminants into non hazardous materials, enabling the treated water to recycle/reused or safely discharged (Rao *et al.*, 2011). Blue green algae such as *Oscillatoria* and *Nostoc* are widely used in wastewater treatment, as they often grow very fast, possess high nutrient removal capabilities and can grow photo-autotrophically (Vijayakumar *et al.*, 2012; Kotteswari *et al.*, 2012). This technology is low cost and with effective approach to remove excess nutrients, other contaminants in wastewater and producing potentially valuable biomass (Sengar *et al.*, 2011). Cyanobacteria assimilate phytotoxic substances and make use of them for biosynthesis of different metabolites (Martinez *et al.*, 2000). In present investigation an attempt was made for wastewater treatment using locally available species of

Oscillatoria and *Nostoc*, which are highly pollution tolerant and easily adaptable.

Materials and methods

Selection of blue green algae

The algal species used in this study were isolated from polluted water of river Mula, Pune. Standard microbiological methods were followed for isolation of cyanobacteria from the random samples collected, which were microscopically examined and plated on solid agar medium-BG11 (Rippka *et al.*, 1979). The inoculated plates were incubated in culture room (temperature maintained at 25± 2 C, fitted with cool white fluorescent tube emitting 2500 lux for 18 hrs a day) and were regularly examined for the growth of *Nostoc* and *Oscillatoria*. Colonies appearing on solid medium were picked up and transferred to liquid medium by repeated streaking, cultures were made uni-algal and maintained on BG11. Identification of algal forms was made with the help of keys given by Desikachary (1959).

Experimental design

Randomized complete block design with three replicates was used for conducting the experiments and the duration was 25 days, under uniform laboratory conditions at Department of Environmental Sciences, University of Pune, Pune-7, in the year 2012-13. Erlenmeyer conical flasks (250 mL) were used for the experiment and the treatments details were as follows: A1/A2 represents *Nostoc commune* and *Oscillatoria limosa* while wastewater without medium and algae represents control. C: was the concentration of wastewater which was mixed with 25 to 125 mL BG11 medium and inoculated with 0.5 µg of each of *Nostoc* and *Oscillatoria* separately in the corresponding flasks.

Physicochemical and statistical analysis

The initial physicochemical analysis of wastewater was made before inoculation of algae and at final stage, the total content in each flask was filtered to remove algae and then used for the analysis of various parameters (AHPA, AWWA and WEF, 2005).

The results was analyzed statistically by using MSTATC computer software and a comparison of noted data was done on the basis of Duncan's multiple range tests at Alfa level 5 %.

Results and discussion

BOD

The statistical analysis of data (Table 1) revealed that combination treatment of blue green algae and different concentrations had significant effect on BOD of wastewater at final stage. Maximum reduction (98

%) was recorded in presence of *Oscillatoria* and it was followed by *Nostoc* (96 %) at 50 and 40 % concentrations of wastewater respectively as compare to control (Table 2). According to Ganapathy-Selvam *et al.*, (2011) the value of BOD indicates level of toxicity of wastewater and they further reported the reduction in BOD of distillery effluent by 53 % using *Nostoc* species. According to Abdel-Raouf *et al.*, (2012) BOD indicates the respiratory demand of bacteria and algae metabolizing the organic matter present in wastewater and excess BOD usually depletes the dissolved oxygen.

Table 1. Mean squares of variance analysis for BOD, COD and DO of municipal wastewater.

Sources	df	BOD		COD		DO	
		Initial	Final	Initial	Final	Initial	Final
Replication	2	1.12	58.8	124.6	130.6	0.004	0.003
Treatment	10	0.000 ^{ns}	42833 ^{**}	0.000 ^{ns}	177242 ^{**}	0.002 ^{ns}	6.4 ^{**}
Error	20	212.8	28.5	656.61	74.2	0.008	0.04
C. V (%)		4.08	5.06	3.25	3.84	4.79	3.83

*, **: significant at 5 and 1 %, respectively, ns: not significant.

Many researchers like Kshirsagar (2013) and Sengar *et al.*, (2011) have reported very high reduction in BOD using different algal species such as *Chlorella* and *Gloeocapsa* who confirmed that microalgae are the best candidates for purification of wastewater and improvement in its physicochemical parameters.

According to Kalaivani *et al.*, (2009) the microalgae was very efficient for reduction of BOD when sewage water was diluted in different concentrations. Results of present investigation using *Oscillatoria* and *Nostoc* on reduction in BOD of municipal wastewater are inconformity with the above findings.

Table 2. Effect of different algal species and wastewater concentrations on BOD, COD and DO of municipal wastewater.

Treatment	BOD (mg/L)		COD (mg/L)		DO (mg/L)	
	Initial	Final	Initial	Final	Initial	Final
Control	357.7 a	430.3 a	787.3 a	845.3 a	1.9 a	2.2 a
A1 C1	357.7 a	146.3 b	787.3 a	376.0 b	1.9 a	5.4 cd
A1 C2	357.7 a	124.0 c	787.3 a	286.3 d	1.9 a	5.4 cd
A1 C3	357.7a	54.7 f	787.3 a	111.0 g	1.9 a	5.4 cd
A1 C4	357.7a	17.0 h	787.3 a	67.7 h	1.9 a	4.1 f
A1 C5	357.7a	30.7 g	787.3 a	38.7 i	1.9 a	5.8 ab
A2 C1	357.7a	145.7 b	787.3 a	353.7 c	1.9 a	5.0 e
A2 C2	357.7a	112.0 d	787.3 a	209.0 e	1.9 a	5.1 de
A2 C3	357.7a	66.0 e	787.3 a	133.3 f	1.9 a	5.9 ab
A2 C4	357.7a	29.0 g	787.3 a	35.0 i	1.9 a	6.0 a
A2 C5	357.7a	5.7 i	787.3 a	12.3 j	1.9a	5.6 bc

Means with different letters are significantly different at P=0.05, using Duncan's Multiple Range Test.

COD

Results shown in (Table 1) indicated significant impact of both the algal species and concentration on

COD of wastewater. The reduction in COD was by 98.5 and 95 % in presence of *Oscillatoria* and *Nostoc* respectively in lowest concentration of wastewater at final stage. Our results are inconformity with Chandra *et al.*, (2004) who also recorded 94.6 % reduction in COD of tannery effluent using *Nostoc* species. This clearly indicates significant role of microalgae in removal of toxic material and improving the quality of wastewater. Similar observation was noted by Ahmad *et al.*, (2013) during comparative study of phycoremediation of sewage water using various species of algae like *Chlorella*, *Spirogyra* etc, who reported about 98 % reduction in COD.

Sharma and Khan (2013) recorded substantial removal of COD (90 %) using *Chlorella* and *Nostoc* species without adverse effect on their growth. Elumalai *et al.*, (2013) also observed considerable reduction in COD by using *Chlorella* and *Scenedesmus* and further indicated that consortium of algae was very efficient for reducing of COD. The chemical oxidations of carbon present in organic pollutants releasing carbon dioxide is responsible for reduction of COD value, similarly faster biodegradation and bioconversion of organic matter due to algae might be the additional reason (Abdel-Raouf *et al.*, 2012).

Table 3. Mean squares of variance analysis for TH, TA and TDS of municipal wastewater.

Sources	df	TH		TA		TDS	
		Initial	Final	Initial	Final	Initial	Final
Replication	2	25.7	52.4	175.73	1.18	625.49	37.91
Treatment	10	0.000 ^{ns}	151182 ^{**}	0.000 ^{ns}	156359 ^{**}	0.000 ^{ns}	1416024 ^{**}
Error	20	674.000	18.83	438.33	109.12	5158.02	644.842
C. V (%)		3.32	2.04	2.69	4.69	3.06	3.92

*, **: significant at 5 and 1 %, respectively, ns: not significant.

DO

The results presented on DO in Table 1 clearly shown that application of both the algal species had significant influence at final stage of wastewater treatment. There was increase in DO during phycoremediation of wastewater and *Oscillatoria* was slightly better (63 %) than *Nostoc* (62 %) at final stage

over control in low concentration of wastewater (Table 2). Results of present study are in agreement with Kalaivani *et al.*, (2009) who reported increase in DO of sewage wastewater after diluting it in different concentrations by using *Chlorella* and the wastewater after treatment was suitable for irrigation, fisheries etc.

Table 4. Effect of different algal species and wastewater concentrations on TH, TA and TDS of municipal wastewater

Treatment	TH (mg/L)		TA (mg/L)		TDS (mg/L)	
	Initial	Final	Initial	Final	Initial	Final
Control	781.7 a	769.0 a	779.0 a	779.7 a	2351.0 a	2322.0 a
A1 C1	781.7 a	365.7 b	779.0 a	384.0 b	2351.0 a	1130.0 b
A1 C2	781.7 a	284.7 d	779.0 a	288.0 d	2351.0 a	827.7 d
A1 C3	781.7 a	99.7 g	779.0 a	108.0 f	2351.0 a	326.3 f
A1 C4	781.7 a	44.7 h	779.0 a	40.3 h	2351.0 a	117.7 g
A1 C5	781.7 a	33.7 i	779.0 a	38.7 h	2351.0 a	42.7 h
A2 C1	781.7 a	352.7 c	779.0 a	362.0 c	2351.0 a	1055.0 c
A2 C2	781.7 a	189.0 e	779.0 a	253.7 e	2351.0 a	778.0 e
A2 C3	781.7 a	133.7 f	779.0 a	115.7 f	2351.0 a	334.0 f
A2 C4	781.7 a	47.3 h	779.0 a	66.7 g	2351.0 a	147.7 g
A2 C5	781.7 a	15.0 j	779.0 a	12.3 i	2351.0 a	38.3 h

Means with different letters are significantly different at P=0.05, using Duncan's Multiple Range Test.

Sharma and Khan (2013) reported that phycoremediation of sewage water showed increases in DO using *Chlorella* and *Scenedesmus*. Similar trend was also observed by Sengar *et al.*, (2011) using *Gloeocapsa*, *Euglena*, and *Synedra* for treatment of sewage water and recorded many fold increase in DO using mixed algal population. The photosynthetic efficiency of both the algae may be the reason for increase in DO of municipal wastewater (Promya *et al.*, 2008).

TH

The data recorded in Table 3 clearly showed that both the algal species used for phycotreatment had significant effect on TH at final stage. Similarly the results illustrated in Table 4 revealed that both *Oscillatoria* and *Nostoc* at lowest concentration of wastewater had shown better efficiency in reduction of TH and the percent reduction was by 98 and 95.6 % over control respectively. These results are inconformity with the findings of Kumar and Chopra (2012) who reported reduction in hardness to a great extend during municipal wastewater treatment by activities of microalgae.

As stated by Varghese *et al.*, (2012) hardness of water mainly depends on the concentrations of calcium and magnesium and their reduction by algae is due to heavy uptake and utilization for their growth, same may be the reason for considerable decrease in TH of wastewater by using *Oscillatoria* and *Nostoc*. Sengar *et al.*, (2011) in their studies on phycoremediation of sewage water recorded 97 % reduction in TH supporting the observation of present investigation and claimed that loss of CO₂ and precipitation of CaCO₃ also contribute to depletion of Ca⁺² leading to lowering of hardness of wastewater. Rao *et al.*, (2011) had shown similar trend in reduction of TH of wastewater from leather processing industry using *Chlorella* and noted almost 50 % reduction.

TA

The statistical analysis of data showed that algal specie viz *Oscillatoria* and *Nostoc* had significant impact on reduction of TA of wastewater at final stage

(Table 3). The results indicated that the maximum efficiency of algal species was observed at lowest concentration of wastewater. The TA of wastewater was reduced by 98.4 and 95 % in the presence of *Oscillatoria* and *Nostoc* respectively at 50 % wastewater concentration (Table 4). These results corroborate with Kumar and Sahu (2012) and Kumar and Chopra (2012).

About 80 % reduction in alkalinity of dairy effluents using *Nostoc* was reported by Kotteswari *et al.*, (2012) and they ascribed it to release of CO₂, generated by microalgae in the process of degradation of organic materials and photosynthesis. The reduction in alkalinity by using variety of algae was also noted by Murugesan *et al.*, (2010) and they assigned it to removal of SO₄⁻², PO₄⁻², biocarbonates, chlorides and alkaline metals like Na⁺, K⁺ etc. In present investigation the reduction in TA may be due to removal of Cl⁻, SO₄⁻², Na⁺ and Mg⁺² by *Oscillatoria* and *Nostoc*. Our results are supported and confirmed by above work.

TDS

Results presented in Table 3 had clearly indicated that both the algae had caused significant impact on TDS at final stage. The reduction in TDS by *Oscillatoria* and *Nostoc* was maximum (98 %) in the combination treatment of lowest concentration of wastewater and algae (Table 4). Similar results were reported by many researchers such as Kotteswari *et al.*, (2012), Ahmad *et al.*, (2013) and Elumalai *et al.*, (2013) using different species of cyanophyceae and chlorophyceae for wastewater treatment who reported the average reduction in TDS up to 60 %.

The unique mechanism of bioabsorption/adsorption of different types of dissolved solids in wastewater is responsible to reduce TDS to lowest level (Nanda *et al.*, 2010). Results of present investigation are in close agreement with above researchers and same may be the reason for reduction in TDS by both the algal species used in present study.

Conclusion

From the results it is concluded that recycling and reuse of municipal wastewater is possible by phytotreatment using different algal species like *Oscillatoria*, *Nostoc*, *Chlorella*, *Anabaena* and *Scenedesmus* etc. in present investigation both the algal species had very good potential to reduce the toxic level of almost all physicochemical parameters and the reduction varied from 95 to 99 %. The phycoremediation was very efficient, cost effective and eco-friendly indicating that microalgae has vital role in the removal of different pollutants from wastewater. The pollutants removal efficiency of *Oscillatoria* was higher as compare to *Nostoc* which can be recommended for phycoremediation purpose.

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