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# Potential use of cyanobacteria species in phycoremediation of municipal wastewater

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**Abstract**

The treatment of municipal wastewater becomes effective by using microalgae which are superior as the wide range of toxic and other wastes can be reduced with them. The present work is aimed to examine the efficiency of locally available blue green algae such as *Oscillatoria limosa* and *Nostoc commune* in removal of various nutrients to prevent further deterioration of water quality. The phycoremediation experiments were conducted at Department of Environmental Sciences, University of Pune, Pune-7, India using randomized complete block design with three replications of each treatment. The results of present investigation clearly indicated that both the algal species viz *Oscillatoria limosa* and *Nostoc commune* are highly efficient for removal of $NO_3^{-2}$, $PO_4^{-2}$, $SO_4^{-2}$, $Cl^-$ and for reducing EC values. The average reduction was between 84 to 98 %. The pollutant removal efficiency was increased with decreasing concentration of wastewater. Amongst the selected algae *Oscillatoria limosa* was the best candidate as compare to *Nostoc commune*. It was concluded that the cyanophycea members would be the best options for phycoremediation.

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## Introduction

In last two decades, the rapid development in metropolitan cities took place along with very high increase in population size which has generated huge quantity of wastewater which is discharged into the rivers in and around these cities and discharging the wastewater creates burning problems of environmental pollution throughout the globe (Ruiz-Martinez *et al.,* 2012). Many researchers like Dominic *et al.,* (2009), Abdel-Raouf *et al.,* (2012) and Witters *et al.,* (2012) have suggested the use of algae for phycoremediation of wastewater. According to Dominic *et al.,* (2009) phycoremediation is the process of employing algae for improving water quality which can fix carbon dioxide by photosynthesis and remove excess nutrients effectively at minimal cost. The use of algae in purification of wastewater and to eliminate the nutrients was studied widely (Mallick, 2002; Christenson and Sims, 2011). Various types of microalgae cultures have been used successfully to treat wastewater and to reduce different types of nutrients (Yang *et al.,* 2011; Lim *et al.,* 2010). Microalgae culture systems show a versatility that allows them to participate in different processes, such as wastewater treatment, production in animal food, production of fertilizers, and production of common and fine chemicals (De la Noue and De Pauw, 1988). The wastewater treatment by microalgae cultures has a major advantageous such as no additional pollution when the biomass is harvested and it allows efficient recycling of nutrients (Travieso *et al.,* 1992). The blue green algae (cyanobacteria) have extraordinary vitality in urban wastewater, registering faster growth rate, tolerating a wide range of temperature, pH and high load of pollutants making them versatile for sewage purification (Kessler, 1991). Similar to green algae the members of blue green algae are widely used for phycoremediation of municipal wastewater. Dubey *et al.,* (2011) have investigated the potential use of *Oscillatoria, Nostoc, Synechococcus, Nodularia* and *Cyanothece* for bioremediation of industrial effluents. Sri-Kumaran *et al.,* (2011) Vijayakumar *et al.,* (2012) and Nanda *et al.,* (2010) reported the bioremediation of polluted water using different species of *Oscillatoria* and *Nostoc.* The review of literature indicated that the phycoremediation studies using blue green algae (cyanobacteria) was less attempted and the research on findings, the potential of different species of theses algae by diluting the wastewater was not investigated in details. Therefore present investigation was conducted with following objectives e.g. to determine the efficiency of *Nostoc* and *Oscillatoria* in removal of nutrients like $PO_4^{-2}$, $NO_3^{-2}$, $SO_4^{-2}$ and $Cl^-$ from wastewater of Pune Municipal Corporation.

## Materials and methods

*Selection of blue green algae*

The blue green algal species viz *Oscillatoria* and *Nostoc* used for phycremediation experiment were isolated from polluted water of river Mula-Mutha, Pune and standard methods were followed for their collection, isolation and identification (Desikachary, 1959). Pure culture of both the species was maintained on solid agar medium-BG11 (Rippka *et al.,* 1979). The cultures 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 algae. Colonies appearing on solid medium were picked up and transferred to liquid medium.

*Experimental set up*

The experiment was conducted in the year 2012-13 at Department of Environmental Sciences, University of Pune, Pune-7 (MS), India using randomized complete block design with three replications, under uniform laboratory conditions. 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. The wastewater was mixed with 25 to 125 mL BG11 medium and inoculated with 0.5 µg of each algae (*Nostoc* and *Oscillatoria*) separately in the corresponding flasks.

*Physicochemical analysis*

Pre and post experimental analysis of wastewater was carried out by using (AHPA, AWWA and WEF, 2005).

*Statistical analysis*

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**

*Nitrate*

The results shown in Table 1 clearly indicated significant effect of both the algae on $NO_3^{-2}$ content of wastewater at final stage. The reduction in nitrate was very high due to *Oscillotaria* (97 %) as well as *Nostoc* (96 %) at final stage in the treatment of 50 and 30 % concentrations of wastewater and BGA over control respectively (Table 2). These results corroborate with findings of Kshirsagar, (2013) and Rao *et al.,* (2011) who reported 78 and 70 % reduction in $NO_3^{-2}$ using *Scenedesmus* and *Chlorella*.

**Table 1.** Mean squares of variance analysis for $NO_3^{-2}$, $PO_4^{-2}$ and $SO_4^{-2}$ of municipal wastewater.

| Sources | df | $NO_3^{-2}$ Initial | $NO_3^{-2}$ Final | $PO_4^{-2}$ Initial | $PO_4^{-2}$ Final | $SO_4^{-2}$ Initial | $SO_4^{-2}$ Final |
|---|---|---|---|---|---|---|---|
| Replication | 2 | 81.85 | 0.295 | 0.008 | 0.000 | 5.2 | 0.295 |
| Treatment | 10 | $0.000^{ns}$ | $6034.2^{**}$ | $0.000^{ns}$ | $40.2^{**}$ | $0.000^{ns}$ | $1051.8^{**}$ |
| Error | 20 | 58.7 | 3.12 | 0.491 | 0.100 | 9.3 | 0.495 |
| C. V (%) | | 5.00 | 4.24 | 4.29 | 6.78 | 4.55 | 3.58 |

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

Tartte, (2010) observed effective removal of nitrogenous contaminants from wastewater using *Anabeana* and *Nostoc*. Similar trend was recorded by findings of Sengar *et al.,* (2011) are supporting the above trend who also noted 91 % reduction in $NO_3^{-2}$ using mixed algal population. The uptake and utilization of $NO_3^{-2}$ by algal species for their growth may be the reason for reduction in nitrate.

**Table 2.** Effect of algal species and wastewater concentrations on $NO_3^{-2}$, $PO_4^{-2}$ and $SO_4^{-2}$ of municipal wastewater.

| Treatment | $NO_3^{-2}$ (mg/L) Initial | $NO_3^{-2}$ (mg/L) Final | $PO_4^{-2}$ (mg/L) Initial | $PO_4^{-2}$ (mg/L) Final | $SO_4^{-2}$ (mg/L) Initial | $SO_4^{-2}$ (mg/L) Final |
|---|---|---|---|---|---|---|
| Control | 153.3 a | 153.7 a | 16.3 a | 14.7 a | 67.0 a | 65.7 a |
| A1 C1 | 153.3 a | 75.0 b | 16.3 a | 5.0 c | 67.0 a | 29.3 c |
| A1 C2 | 153.3 a | 51.7 d | 16.3 a | 4.7 cd | 67.0 a | 22.7 e |
| A1 C3 | 153.3 a | 6.0 h | 16.3 a | 3.0 f | 67.0 a | 10.0 g |
| A1 C4 | 153.3 a | 11.7 g | 16.3 a | 4.0 e | 67.0 a | 7.3 i |
| A1 C5 | 115.3 a | 11.3 g | 16.3 a | 2.3 g | 67.0 a | 5.3 h |
| A2 C1 | 153.3 a | 63.3 c | 16.3 a | 6.0 b | 67.0 a | 30.7 b |
| A2 C2 | 153.3 a | 47.7 e | 16.3 a | 4.3 de | 67.0 a | 25.0 d |
| A2 C3 | 153.3 a | 22.7 f | 16.3 a | 4.3 de | 67.0 a | 12.7 f |
| A2 C4 | 153.3 a | 13.7 g | 16.3 a | 2.7 fg | 67.0 a | 3.7 j |
| A2 C5 | 153.3 a | 4.3 h | 16.3 a | 1.0 h | 67.0 a | 2.7 j |

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

*Phosphate*

The data presented in Table 1 revealed that at final stage of treatment both the blue green algal species had considerable effect on $PO_4^{-2}$ content. For removal of $PO_4^{-2}$ content from wastewater *Oscillotaria* was highly efficient (93 %) than *Nostoc* (84 %) at final stage of phycoremediation (in the 50 % concentration for both algal species). Many other researchers like Dubey *et al.,* (2011) and Sengar *et al.,* (2011) also noted similar trend during phycotreatment of industrial effluents and sewage water with the help of different species of BGA and Chlorophyceae. However they recorded highest reduction in $PO_4^{-2}$ using *Nostoc*, *Oscillotaria*, *Scnedesmus* and *Glococapsa* as theses algal species had good potential and tolerance to polluted water.

**Table 3.** Mean squares of variance analysis for EC and $Cl^-$ of municipal wastewater.

| Sources | df | $Cl^-$ | | EC | |
|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final |
| Replication | 2 | 161.49 | 2.2 | 4482.3 | 79.5 |
| Treatment | 10 | $0.121^{ns}$ | $240917^{**}$ | $0.000^{ns}$ | $3282802^{**}$ |
| Error | 20 | 1837.1 | 98.6 | 35961.7 | 1996.5 |
| C. V (%) | | 4.36 | 3.65 | 5.31 | 4.83 |

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

The removal of $PO_4^{-2}$ by 81 % and 83 % using *Chlorella* and *Scnedesmus* was noted by Kshirsagr, (2013) and Kim *et al.,* (2007). They clearly indicated that both the algal species had very high efficiency to eliminate $PO_4^{-2}$ from wastewater. They further claimed that the optimum removal in $PO_4^{-2}$ was due to utilization of phosphorous for the growth of algae employed in the wastewater treatment. The results of present investigation are in agreement with above findings and explanations cited for reduction of $PO_4^{-2}$.

**Table 4.** Effect of different algae species and wastewater concentrations on $Cl^-$ and EC of municipal wastewater.

| Treatment | $Cl^-$ (mg/L) | | EC (µmohs/cm) | |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| Control | 983.0 a | 972.0 a | 3570 a | 3556.0 a |
| A1 C1 | 983.0 a | 464.7 b | 3570 a | 1652.0 b |
| A1 C2 | 983.0 a | 348.3 d | 3570 a | 1247.0 d |
| A1 C3 | 983.0 a | 130.3 g | 3570 a | 456.7 f |
| A1 C4 | 983.0 a | 71.3 h | 3570 a | 188.7 g |
| A1 C5 | 983.0 a | 46.7 i | 3570 a | 62.7 h |
| A2 C1 | 983.0 a | 438.3 c | 3570 a | 1558.0 c |
| A2 C2 | 983.0 a | 307.0 e | 3570 a | 657.0 e |
| A2 C3 | 983.0 a | 151.3 f | 3570 a | 493.0 f |
| A2 C4 | 983.0 a | 48.7 i | 3570 a | 230.7 g |
| A2 C5 | 983.0 a | 14.0 j | 3570 a | 64.7 h |

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

*Sulphate*

Significant effect was observed on $SO_4^{-2}$ content by both the selected algal species at final stage (Table 1). Similarly the results recorded in Table 2 had clearly shown significant reduction of $SO_4^{-2}$ in wastewater at final stage, which was by 95.8 and 92 % in presence of *Oscillotaria* and *Nostoc* respectively at 50 % concentration of wastewater. These results are in agreement with studies of Chandra *et al.,* (2004) who reported more than 99 % reduction in $SO_4^{-2}$ of tannery effluent with *Nostoc*.

Same trend was recorded by Ahmad *et al.,* (2013) who also reported considerable reduction in $SO_4^{-2}$ using *Chlorella* and mixed algal culture. Elumalai *et al.,* (2013) reported removal of very high amount of $SO_4^{-2}$ using consortium of algae as compare to single culture of *Chlorella* and *Scynedesmus*. Kumar and Chopra, (2012) recorded very high reduction in $SO_4^{-2}$ in municipal wastewater by using microbiological technology. In present study the $SO_4^{-2}$ removal capacity of both the algae was at par indicating equal efficiency for eliminating of $SO_4^{-2}$ from wastewater which may be contributed to its high uptake from polluted water for growth of algal species.

*Chlorides*

The results presented in Table 3 clearly showed that both the species of cyanobacteria had significant influence on $Cl^-$ content at final stage. The data recorded in Table 4 revealed that $Cl^-$ were reduced to the maximum level by 98.6 % in presence of *Oscillotaria* and by 95.2 % with *Nostoc* over control at final stage of treatment which was 50 % concentration of wastewater. The optimum performance by both the algal species was reported in lowest concentration of wastewater.

Elumalai *et al.,* (2013) observed very high reduction in $Cl^-$ of effluent from textile industry using *Chlorella*, *Synedesmus* and consortiums. Ahmad *et al.,* (2013) reported very high reduction in $Cl^-$ using *Chlorella* and mixed algal culture during phycoremediation of sewage water. Similar was the trend noted by Jafari and Alavi, (2010) who reported significant fall in $Cl^-$ value with *Oscillotaria*, *Anabeana*, *Nostoc* and *Spirogyra*. The reduction in $Cl^-$ was attributed to its bioconversion and absorption by algal species. Results of present study are inconformity with above findings and same may be the reason for reduction of $Cl^-$ with the biological system used for phycoremediation.

*Electrical conductivity*

Both the selected algal species had shown significant effect on EC at final stage of treatment (Table 3). *Oscillotaria* and *Nostoc* had remarkable effect on of EC value of wastewater which was lowered down to its maximum level (98.1 %) at final stage when compare to control. The algal species had shown approximately equal performance in decreasing the EC value during phycoremediation at lowest concentration of wastewater (Table 4). The EC value of water is more or less liner function of the concentration of dissolved ions in it, therefore its measurements can be used as a quick way to locate potential water quality problems (Kumar and Chopra, 2012).

The results of present study are in agreement with many other researchers such as Velan and Saravanane. (2013) and Kotesswari *et al.,* (2012). The reduction in EC was correlated with concurrent removal of various salts such as $SO_4^{-2}$, $PO_4^{-2}$, $NO_3^{-2}$, $Cl^-$ etc which lead to considerable reduction in EC of municipal wastewater.

**Summary and conclusion**

The study revealed that both the algal species are effective for the reduction of various nutrients and chlorides as well as EC from wastewater. The highest potential for phycoremoval of all above parameters was noted in the lowest concentration of wastewater. Amongst the selected algae *Oscillatoria* was having better potential and hence it may function as better candidate for large scale treatment. Consequently the blue green algae best treatment can be used as a sustainable technique for wastewater treatment. Not only these but both the species also have very good potential for nitrogen fixation and the biomass produced can be a novel bioresource for paddy cultivation.

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## References


**Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM.** 2012. Microalgae and wastewater treatment. Saudi Journal of Biological Sciences 19, 257-275. http://dx.doi.org/10.1016/j.sjbs.2012.04.005

**Ahmad F, Khan AU, Yasar A.** 2013. Comparative phycoremediation of sewage water by various species of algae. Proceeding of Pakistan Academy of Sciences **50,** 131-139.

**APHA, AWWA, WEF,** 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association Publication, Washington, D.C. 21th edition.

**Chandra R, Pandey PK, Srivastava A.** 2004. Comparative toxicological evaluation of untreated and treated tannery effluent with *Nostoc muscorum* L. (algal assay) and microtox bioassay. Environmental Monitoring and Assessment **95,** 287-294. http://dx.doi.org/10.1023/B:EMAS.0000029909.87977.a5.

**Christenson L, Sims R.** 2011. Production and harvesting of microalgae for wastewater treatment, biofuels and bioproducts. Biotechnology Advances **29,** 686-702. http://dx.doi.org/10.1016/j.biotechadv.2011.05.015.

**De La Noue J, De Pauw N.** 1988. The potential of microalgal biotechnology. A review of production and uses of microalgae. Biotechnology Advances **6,** 725-770. http://dx.doi.org/10.1016/0734-9750(88)91921-0.

**Desikachary TV.** 1959. *Cyanophyta*, Indian Council of Agriculture Research. New Delhi. 686 p.

**Dominic VJ, Murali S, Nisha MC.** 2009. Phycoremediation efficiency of three micro algae *Chlorella vulgaris, Synechocystis salina* and *Geloeocapsa gelatiosa*. Academic Review XVI, 138-146.

**Dubey SK, Dubey J, Mehra S, Tiwari P, Bishwas AJ.** 2011. Potential use of cyanobacterial species in bioremediation of industrial effluents. African Journal of Biotechnology **10,** 1125-1132.

**Elumalai S, Saravanan GK, Ramganesh S, Sakhtival R, Prakasam V.** 2013. Phycoremediation of textile dye industrial effluent from tirupur district, Tamil Nadu, India. International Journal of Science Innovations and Discoveries **3,** 31-37.

**Jafari N, Alavi SS.** 2010. Phytoplankton community relation to physic-chemical characteristics of the Talar river, Iran. Journal of Applied Science and Environmental Management **14,** 51-56.

**Kessler E.** 1991. *Scenedesmus*: problems of a highly variable genus of green algae. Botanica Acta **104,** 169-171.

**Kim MK, Park JW, Park CS, Kim SJ, Jeune KH, Chang MU, Acreman J.** 2007. Enhanced production of *Scenedesmus* spp. (green microalgae) using a new medium containing fermented swine wastewater. Bioresource Technology **98,** 2220-2228.

**Koteswari M, Murugesan S, Ranjith-Kumar R.** 2012. Phycoremediation of dairy effluent by using the microalgae *Nostoc* sp. International Journal of Environmental Research and Development **2,** 35-43.

**Kshirsagar AD.** 2013. Bioremediation of wastewater by using microalgae: An experimental study. International Journal of Life Sciences Biotechnology and Pharma Research **2,** 338-346.

**Kumar V, Chopra AK.** 2012. Monitoring of physicochemical and microbiological characteristics of municipal wastewater at treatment plant, Haridwar city (Uttarakhand) India. Journal of Environmental Sciences and Technology **5,** 109-118.

**Lim S, Chu W, Phang S,** 2010. Use of *Chlorella vulgaris* for bioremediation of textile wastewater. Bioresource Technology **101,** 7314-7322. http://dx.doi.org/10.1016/j.biortech.2010.04.092.

**Malik A, Jaiswal R**. 2000. Metal resistance in Pseudomonas strains isolated from soil treated with industrial wastewater. World Journal of Microbiology and Biotechnology **16,** 177-182. http://dx.doi.org/10.1023/A:1008905902282.

**Nanda S, Sarangi PK, Abraham J.** 2010. Cyanobacterial remediation of industrial effluents II. paper mill effluents. New York Science Journal **3,** 37-41.

**Rao PM, Kumar RR, Raghavan BG, Subramanian VV, Sivasubramanian V.** 2011. Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility. Water SA **37,** 7-14.

**Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY,** 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology **111,** 1 - 61. http://dx.doi.org/101099/00221287-111-1-1.

**Ruiz-Martinez A, Martin-Garcia N, Romero I, Seco A, Ferrer J.** 2012. Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent. Bioresource Technology **126,** 247-253. http://dx.doi.org/10.1016/j.biortech.2012.09.022.

**Sengar RMS, Singh KK, Singh S.** 2011. Application of phycoremediation technology in the treatment of sewage water to reduce pollution load. Indian Journal of Scientific Research **2,** 33-39.

**Sri-Kumaran N, Sundaramanicam A, Bragadeeswaran S.** 2011. Adsorption studies on heavy metals by isolated cyanobacterial strain (Nostoc sp) from uppanar water, southeast coast of India. Journal of Applied Sciences Research **7,** 1609-1615.

**Tartte V, Kalla CM, Murthy-Sistla DS, Fareeda G.** 2010. Comparative studies on growth and remediation of wastewater by two cyanobacterial biofertilizers. Agriculture Conspectus Scientifics **75,** 99-103.

**Travieso L, Benitez F, Dupeiron R,** 1992. Sewage treatment using immobilized microalgae. Bioresource Technology **40,** 183-187. http://dx.doi.org/10.1016/0960-8524(92)90207-E.

**Velan M, Saravanane R.** 2013. CO2 sequestration and treatment of municipal sewage by microalgae. International Journal of Innovative Technology and Exploring Engineering **2,** 2278-3075.

**Vijayakumar S, Jeyachandren S, Manoharan C.** 2012. Studies on cyanobacterial population in industrial effluents. Journal of Algal Biomass Utilization **3,** 39-45.

**Witters N, Mendelsohn RO, Van-Slycken S, Weyens N, Schreurs E, Meers F, Carleer R, Vangronsveld J.** 2012. Phycoremediatoion a sustainable remediation technology? Conclusion from a case study. I: Energy production and carbon dioxide abatement. Biomass and Bioenergy **39,** 454-469. http://dx.doi.org/10.1016/j.biombioe.2011.08.016.

**Yang J, Li X, Hu H, Zhang X, Yu Y, Chen Y.** 2011. Growth and lipid accumulation properties of a freshwater microalga, *Chlorella ellipsoidea* YJ1, in domestic secondary effluents. Applied Energy **88,** 3295-3299. http://dx.doi.org/10.1016/j.apenergy.2010.11.029.