

# Bioremedial efficacy of *Nostoc carneum* Agardh in industrial effluents treatment

## Abstract

The present work was done as laboratory experiment to evaluate the efficacy of *Nostoc carneum*— a heterocystous, nonpoisonous cyanobacterium as bioremedial agent of waste water particularly from removal of some commonly available heavy metals in industrial effluents of Jharkhand. The effluents were collected in sterile glass bottles and transported in cold condition to the laboratory for physicochemical analysis viz. pH, BOD, COD, Chloride, Sulphate and Phosphate. 3 sets of Erlenmeyer flasks were used for the experimental setup. 1st set having waste water without medium and algae represents control. To the 2nd set 100% waste water was added along with 50 mL of BG-11 medium (BNM) and 2mL of *Nostoc carneum*. One more set having 100% waste water, along with *Nostoc* but without BNM represents 3rd set. All the Erlenmeyer flasks were maintained at the temperature  $25\pm2^\circ\text{C}$  under continuous white light (2200lux) under aseptic condition. The experimental set ups were illuminated properly to facilitate the cyanobacterial growth. Initial pH value of effluent was 7.80 which become 8.3, 8.43 and 7.81 on 30th day respectively in 1st, 2nd and 3rd setup. Chloride shows 11.58%, 23.27% and 5.06% reduction from initial value, similarly deviations in values of other parameters of all the 3 setups are described in detail in research paper.

**Keywords:** *nostoc carneum*, effluent, aseptic, bg-11 medium

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## Introduction

Bioremediation is a technology of using microorganisms to reduce, eliminate or transformation of contaminants to benign products present in soils, sediments, water and air. It is not a new technology; it has been practiced by humankind since the beginning of recorded history.<sup>1</sup> Evidence of kitchen middens and compost piles data back to 6000BC and the more modern use of bioremediation began over 100 years ago with the opening of the first biological sewage treatment plant in Sussex UK in 1891. However the word “Bioremediation” is fairly new. Its first appearance in peer revised scientific literature was in 1987.<sup>2</sup> Bioremediation is an alternative to traditional remediation technologies such as land filling or incineration. It works by either transforming or degrading contaminants to non hazardous or less hazardous chemicals, respectively known as Biotransformation and Biodegradation.<sup>3</sup> Although metals and radio nuclides cannot be biodegraded but microorganisms can interact with these contaminants and transform them into another state or increasing their mobility so that they can more easily be flushed from the environment. In some cases metal and radio nuclides are precipitated out leading to their immobilization.

In last 25 years, application of bioremediation has been increased because of rapid urbanization and industrialization.<sup>4</sup> Industries generate huge quantity of waste water and discharge them into river without predisposal treatment which in turn generate sequences of environmental and ultimately health problems. The industrial effluents contain several types of chemicals such as dispersants, leveling agents, acids, alkalies, carriers and various dyes, phenol, carbonates, alcohols, cyanide, heavy metals etc.<sup>5</sup> A release of these effluents into aquatic ecosystems alters the pH, increases the BOD and COD and gives the water intense colourations.<sup>6</sup>

In general treatment of effluents includes physicochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation.<sup>7</sup> But due to high cost and intense experimental setup.<sup>8,9</sup> biological treatment methods are used which include various

bacteria, fungi and cyanobacteria. Bioremediation is a natural, efficient, low cost and rapid degradation process and is therefore perceived by public as an acceptable waste treatment process. The role of algae in the removal of various kinds of inorganic and related substances has been studied by several workers during the last several years.<sup>10-14</sup> Algae serve as indicators of water pollution since they respond typically to many ions and toxicants.<sup>15</sup> Blue green algae are ideally suitable to play a dual role of treating waste water in the process of effective utilization of different constituents essential for growth leading to enhanced biomass production. The algal biomass can be utilized for various productive purposes. Therefore the present investigation was undertaken to study bioremedial efficacy of *Nostoc carneum* in reducing the pollution load from waste water.

## Materials and methods

### Sample collection and its characterization

Industrial effluents were collected from a thermal power station situated in Jharkhand. Because of some confidential issues its name is not discussed anywhere in this article. The effluents were collected in sterile glass bottles and transported in cold condition to the laboratory for physicochemical analysis. Industrial effluent quality parameters included pH, BOD, COD, Chloride, Sulphate and Phosphate. All these parameters were characterized before beginning of experiment i.e. on 0 day, then on 15th day and lastly on 30th day of experiment. All the investigated parameters were determined by using standard methods as described by APHA.<sup>16</sup> and the experiments were conducted in triplicates.

### Source of organism and culture medium

The pure culture of *Nostoc carneum* Agardh was obtained from the regional Agricultural Research Institute, Chipilima, Orissa. The cyanobacteria were cultured in BG-11 medium.<sup>17</sup> The culture medium was composed of 0.04g  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ ; 0.075g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.036g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; 0.006g Citric acid; 0.006g Ferric ammonium

Citrate; 0.001g EDTA (Disodium magnesium salt); 0.02g Na<sub>2</sub>CO<sub>3</sub>; 2.86g H<sub>3</sub>BO<sub>3</sub>; 1.81g MnCl<sub>2</sub>.4H<sub>2</sub>O; 0.222g ZnSO<sub>4</sub>.7H<sub>2</sub>O; 0.049g CO(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O; 0.39g Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O; 0.079g CuSO<sub>4</sub>.5H<sub>2</sub>O and trace elements were dissolved in 1000mL of distilled water.<sup>18</sup> The pH was kept at 7.4. Before the inoculation, the culture medium was subjected to autoclaving for sterilization.

## Experimental setup

3 sets of Erlenmeyer flasks of size 250mL, each with 3 replicates were used for the experimental setup. 1st set having waste water without medium and algae represents control. To the 2<sup>nd</sup> set 100% waste water was added along with 50 mL of BG-11 medium (BNM) and 2 mL of *Nostoc carneum*. One more set having 100% waste water, along with *Nostoc* but without BNM represents 3<sup>rd</sup> set. All

the Erlenmeyer flasks were maintained at the temperature 25±2°C under continuous white light (2200 lux) under aseptic condition. The experimental set ups were illuminated properly to facilitate the cyanobacterial growth. The flasks were sterilized before using them for culture.

## Results and discussion

In the experiment, *Nostoc carneum* was grown in 100% waste water with BNM (concentration where it gives the maximum growth) and waste water without BNM (Concentration where it gives least growth). Effluent samples were analyzed for their physicochemical characteristics before i.e. on the inoculation day (0 day), day of maximum growth (15th day), and on the senescence phase (30th day) as shown in Table 1-3.

**Table 1** Reduction in pollution load in waste water through algal treatment in control

Control (Waste Water without BNM and without Algae)				
Parameters	0 Day	15 <sup>th</sup> Day	30 <sup>th</sup> Day	% Reduction (over 0-Day)
Colour	Dark Brown	Light Brown	Tan	
pH	7.8	8.35	8.30	6.41
BOD(mg/L)	245	280	323	31.83
COD(mg/L)	0	0	0	0
Chloride	19.77	17.48	17.48	11.58
Sulphate	97.26	73.45	45.71	52.99
Phosphate	19	4.71	4.38	76.95

**Table 2** Reduction in pollution load in waste water through algal treatment in 100% waste water with BNM

100% WW with BNM (Waste Water with Algae and BNM)				
Parameters	0 Day	15 <sup>th</sup> Day	30 <sup>th</sup> Day	% Reduction (over 0-Day)
Colour	Dark Brown	Light Brown	Tan	
pH	7.8	8.5	8.43	8.07
BOD(mg/L)	245	105.48	60.73	75.21
COD(mg/L)	720	180	156	78.33
Chloride	19.77	15.23	15.17	23.27
Sulphate	97.26	27.96	25.13	74.16
Phosphate	19.77	2.07	1.97	90.03

**Table 3** Reduction in pollution load in waste water through algal treatment in 100% waste water without BNM

100% WW without BNM (Waste Water with Algae)				
Parameters	0 Day	15 <sup>th</sup> Day	30 <sup>th</sup> Day	% Reduction (over 0-Day)
Colour	Dark Brown	Light Brown	Tan	
pH	7.8	7.93	7.81	0.13
BOD(mg/L)	282	196.8	169.2	40
COD(mg/L)	800	602	412	48.5
Chloride	19.77	18.23	18.77	5.06
Sulphate	100	75.13	71.13	24.87
Phosphate	19.77	15.12	14.82	25.04

A change in colour of the effluents was an initial indication of biodegradation. The initial effluent colour at the time of collection was dark brown and finally after treatment for 4 weeks it turned tan. The total suspended solids, minerals and synthetic dyes make the water bodies coloured and hampers light penetration which is a very critical factor for aquatic life forms.<sup>19</sup> However after a due course of discharge of the effluents in the water bodies there is a marked loss in colouration between 10 to 15%.<sup>20</sup> As the chief ingredients of BG11 medium are salts, hence the supplementation of tannery effluents into the minimal medium acted as the carbon source for the cyanobacteria to metabolize it and reduce its concentration from the medium.<sup>21</sup>

In the present study pH was found to increase in control and 100% waste water with BNM whereas there was almost no change in pH in 100% waste water without BNM. Manoharan et al.<sup>13,14</sup> found a rise in pH value up to 10th day of growth in waste water and ossein effluent inoculated with BGA and after that it decreased. The acceptable limit for discharge of waste waters to both surface waters and sewers varies in range between pH 5.5 to 10.<sup>22</sup> In the present paper, initial pH of the effluent was 7.8 and on 30th day the pH value was 8.3. This increase in alkalinity is due to the rate of aerobic decomposition, this may be because of some carbohydrates or fatty acids present in effluent which on degradation produces CO<sub>2</sub> and pH increases to more than 8.

BOD and COD are generally considered as a major indicator of organic pollution in water. In this study Nostoc carneum reduced 75.21% of BOD and 78.33% of COD from the culture medium containing 100% waste water with BNM and 40% of BOD and 48.50% of COD from 100% waste water without BNM. The high BOD creates septic conditions, generating foul smelling Hydrogen sulphide, which in turn precipitates+ iron and any dissolved salts, turning the water black and highly toxic for aquatic life.<sup>23</sup> According to Ganapathy– Selvam et al.<sup>24</sup> 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.<sup>25</sup> BOD indicates the respiratory demand of bacteria and algae metabolizing the organic matter present in wastewater and excess BOD usually depletes the dissolved oxygen. Many researchers like Kshirsagar & Sengar et al.<sup>26,27</sup> 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.<sup>28</sup> the microalgae was very efficient for reduction of BOD when sewage water was diluted in different concentrations. In the present experiment, BOD and COD of waste water showed deteriorating trend by algal treatment. Similar reducing trend of COD was reported by Govindan.<sup>29</sup> when acclimatized algal cultures were used for treatment of different types of waste water. Sharma et al.<sup>30</sup> recorded substantial removal of COD (90%) using Chlorella and Nostoc species without adverse effect on their growth. Elumalai et al.<sup>31</sup> also observed considerable reduction in CD 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.<sup>25</sup>

Chlorides are generally considered as one of the major pollutants in the effluents which are difficult to be removed by conventional biological treatment methods. The reduction in chloride value was found to be 11.58% in control and 23.27% in 100% waste water with BNM and 5.06% in 100% waste water without BNM (Table 1–3). Uma et al.<sup>32</sup> observed a 30% chloride reduction under laboratory conditions by Halobacterium and only additional 12–17% with cyanobacteria in ossein effluent. Elumalai et al.<sup>31</sup> observed very high reduction in chloride ions of effluent from textile industry using Chlorella, Synedesmus and consortiums. Ahmad et al.<sup>33</sup> reported very high reduction in chloride using Chlorella and mixed algal culture during phycoremediation of sewage water. Similar was the trend noted by Jafari et al.<sup>34</sup> who reported significant fall in Chloride value with Oscillatoria, Anabaena, Nostoc and Spirogyra. The reduction in Chloride was attributed to its bioconversion and absorption by algal species.

Sulphate is widely distributed in nature. It directly effects the growth of algae because it has an important role in the formation of chlorophyll. This is also an important constituent of proteins and organic acids. In the present study the reduction of sulphate was found to be 52.99% in control, 74.16% in 100% waste water with BNM and 24.87% in 100% waste water without BNM (Table 1–3). Mittal et al.<sup>35</sup> studied the capacity of two species of cyanobacteria for the uptake of sulphate when grown in different concentration of stock solutions. They found that Oscillatoria perornata uptakes about 34–55% of sulphate and Scenedsmus quadricauda var. longispina about 12–16% when grown in those media. The results of present experiment are in agreement with studies of Chandra et al.<sup>36</sup> who reported more than 99%

reduction in sulphate of tannery effluent with Nostoc. Same trend was recorded by Ahmad et al.<sup>33</sup> who also reported considerable reduction in sulphate using Chlorella and mixed algal culture. Elumalai et al.<sup>31</sup> reported removal of very high amount of sulphate using consortium of algae as compare to single culture of Chlorella and Scyndesmus. Kumar et al.<sup>37</sup> reported very high reduction in sulphate in municipal wastewater by using microbiological technology.

Reduction of phosphate level was found to be 76.95% in control, 90.03% in 100% waste water with BNM and 25.04% in wastewater without BNM (Table 1–3). These results are in agreement with studies of Chandra et al.<sup>36</sup> who reported more than 99% reduction in sulphate of tannery effluent with Nostoc. Manoharan et al.<sup>12–14</sup> found a total or near total removal of all types of phosphates by Oscillatoria either alone or in combination with natural population of microbes. The capacity of cyanobacteria to remove large amount of phosphorus from the waste water has also been demonstrated by several workers.<sup>38</sup> Further the cyanobacteria are known to absorb and store large amounts of phosphorus and polyphosphate granules.<sup>39</sup>

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## Conflicts of interest

None.

## References

1. Heng LL, Ofori G, Choo MLL, et al. Sustainability matters. 2010;2:492.
1. Hazen TC. Bioremediation. In: Haldeman D (Eds.) Microbiology of the Terrestrial Subsurface. CRC Press, USA. 1997:247–266.
2. Ulrich W. Contaminant soil areas, different countries and contaminant monitoring of contaminants. In: HJ Rehm & G Reed (Eds.), Environmental Process II. Soil Decontamination Biotechnology. 2000;11:5–42.
3. Gadd GM. Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Curr Opin Biotech*. 2000;11(3):271–279.
4. Cooper P. Colour in dyehouse effluent. Society of dyers and colourists, The Alden Press, Oxford, UK;1995.
5. Asad S, Amoozegar MA, Pourbabaee AA, et al. Decolorization of textile dyes by newly isolated halophilic and halotolerant bacteria. *Bioresource Technol*. 2007;98(11):2082–2088.
6. Olukanni OD, Osuntoki AA, Gbenle GO. Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. *African Journal of Biotechnology*. 2006;5(20):1980–1984.
7. Do T, Shen J, Cawood G, et al. Biotreatment of textile effluent using *Pseudomonas* spp. Immobilized on polymer supports. In: Advances in biotreatment for textile processing. University of Georgia Press, USA; 2002.
8. Maier J, Kandelbauer A, Erlacher A, et al. A new alkali- thermostable azoreductase from *bacillus* sp. Strain SF. *Applied Environmental Microbiology*. 2004;70(2):837–844.
9. Benemann JR. A system analysis of bioconversion with microalgae. Symposium on clean fuels from biomass and wastes. Orlando, florida, USA. 1977:101–126.
10. Sengar RMS, Sharma KD. Tolerance of *Phormidium corium* and *Chlamydomonas* sp. Against chemical elements present in polluted water of Yamuna river; 1987.
11. Manoharan C, Subramanian G. International action between paper mill effluent and the cyanobacterium *Oscillatoria Pseudogeminata* var. Uni-granulate. *Poll Res*. 1992a;11(2):73–84.

12. Manoharan C, Subramanian G. Sewage-cyanobacterial interaction- A case study. *Indian J Env Prot.* 1992b;12(4):251–258.
13. Manoharan C, Subramanian G. Feasibility studies on using cyanobacteria in Ossein effluent treatment. *Indian J. Env. Health.* 1993;35(2):88–96.
14. Elnabarawy MT, Welter AN. Utilization of algal cultures and assays by industry. Algae as ecological indicators. LE Shubert (Eds.), Academic Press, USA. 1984:317–328.
15. APHA Standard methods for estimation of water and waste water. (17<sup>th</sup> edn), American public health association, Washington, USA. 1989:1–541.
16. Stanier RY, Kunisawa MM, Cohen-Bazire G. Purification and properties of unicellular blue green algae (order Chroococcales). *Bact Res.* 1971;35(2):171–201.
17. Rippka R, Deruelles J, Waterbury J, et al. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol.* 1979;111:1–61.
18. Goncalves IMC, Gomes A, Bras R, et al. Biological treatment of effluent containing textile dyes. *Coloration Technology.* 2000;116(12):393–397.
19. Vaidya AA, Datye KV. Environmental pollution during chemical processing of synthetic fibers. *Colourage.* 1982;14:3–10.
20. Nanda S, Sarangi PK, Abraham J. Cyanobacterial remediation of industrial effluents I. Tannery effluents. *New York Science J.* 2010;3(12):32–36.
21. Bosnic M, Buljan J, Daniels RP. Pollutants in tannery effluents. United Nations Industrial Development Organization, Austria. 2000:1–26.
22. Akbar NM, Khwaja MA. Study on Effluents from Selected Sugar Mills in Pakistan: Potential Environmental, Health, and Economic Consequences of an Excessive Pollution Load. Sustainable Development Policy Institute (SDPI) Islamabad, Pakistan, 2006;1–41.
23. Ganapathy SG, Baskaran R, Mohan PM. Microbial diversity and bioremediation of distilleries effluent. *Journal of Research in Biology.* 3; 2011;153–162.
24. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences.* 2012;19(3):257–275.
25. Kshirsagar AD. Bioremediation of wastewater by using microalgae: An experimental study. *International Journal of Life Sciences Biotechnology and Pharma Research.* 2013;2(3):338–346.
26. Sengar RMS, Singh KK, Singh S. Application of phytoremediation technology in the treatment of sewage water to reduce pollution load. *Indian J Sci Res.* 2011;2(4):33–39.
27. Kalaivani S, Mahalakshmi A, Priya S, et al. Phytoremediation of sewage using microalgae Chlorella sp. *Nature Environment and Pollution Technology.* 2009;8(1):187–192.
28. Govindan VS. Treatment of tannery wastewater by stabilization pond method. *Ind J Env Health.* 1985;27:58–66.
29. Sharma GK, Khan SA. Bioremediation of sewage wastewater using selective algae for manure production. *International Journal of Environmental Engineering and Management.* 2013;4(6):573–580.
30. Elumalai S, Saravanan GK, Ramganesh S, et al. Phytoremediation of textile dye industrial effluent from tirupur district, Tamil Nadu, India. *IJSID.* 2013;3(1):31–37.
31. Uma L, Subramanian G. Effective use of cyanobacteria in effluent treatment. Proc Nati symp. Cyanobacteria in Nitrogen fixation, IARI, New Delhi, India. 1990:437–443.
32. Ahmad F, Khan AU, Yasar A. Comparative phytoremediation of sewage water by various species of algae. *Proceeding of Pakistan Academy of Sciences.* 2013;50(2):131–139.
33. Jafari N, Alavi SS. Phytoplankton community in relation to physico-chemical characteristics of the Talar River, Iran. *Journal of Applied Sciences and Environmental Management.* 2010;14(2):51–56.
34. Mittal S, Sengar RMS. Toxic effect of sulphate and its uptake in algae. *National Academy of Science Letters.* 1989;12(7):17–19.
35. Chandra R, Pandey PK, Srivastava A. Comparative toxicological evaluation of untreated and treated tannery effluent with *Nostoc muscorum* L. (algal assay) and microtox bioassay. *Environmental Monitoring and Assessment.* 2004;95(1–3):287–294.
36. Kumar V, Chopra AK. Monitoring of physicochemical and microbiological characteristics of municipal waste water at treatment plant Haridwar City, Uttarakhand. *Journal of Environmental Science and Technology.* 2012;5(2):109–118.
37. Tam NFY, Wong YS. Waste nutrient removal by *Chlorella pyrenoidosa* and *Scenedesmus* sp. *Environmental Pollution.* 1989;58(1):19–34.
38. Fogg GE, Stewart WDP, Fay P, et al. The blue-green algae. Academic Press, London, UK; 1973.