Austin Environmental Sciences

Open Access

Austin Publishing Group

Research Article

Combined Effect of Nitrate Bioremoval by Aquatic Free Floating Plant an Association with Filamentous Cyanobacteria

Usharani K[1,2]* , Divya K[1] and Sruthilaya K[1]
[1]Department of Environmental Science, Central University of Kerala, India
[2]Department of Environmental Science, Division of Environmental Microbiology, India

***Corresponding author:** Usharani K, Department of Environmental Science, Central University of Kerala, KL-20 Department of Environmental Science, Division of Environmental Microbiology, PSG College of Arts and Science, CBE-14, TN, India

Received: April 29, 2020; **Accepted:** May 18, 2020; **Published:** May 25, 2020

Abstract

Nitrate pollutant shows the way to amplify in the growth of algal bloom those results in eutrophication of fresh water. It has become the most serious environmental problem in our area since it causes algae blooms. Growth of algae decreases the dissolved oxygen concentration of water that final result is the death of fish and other aquatic organisms. This study was focused on waste stabilization and nutrient removal and to examine the removal potential of nitrate by selective aquatic plants of duck weed (*Lemna minor*) and *cyanobacterium* (*Oscillatoria sp*.) from eutrophic lake water sample according to the variance of residence times and concentrations of nitrate. The nitrate level in the synthetic wastewater was estimated at different time intervals by salicylic acid method and absorbance was measured at 410 nm using UV-Vis spectrophotometer. The change in pH was also observed at different concentrations. The maximum nitrate reduction of 68% was shown by *Oscillatoria sp* grown and in case of *Lemna minor* it was found to be 60% at 100ppm nitrate concentration. The results of this study showed that *Oscillatoria sp* and *Lemna minor* are potential for purification of water and effectible on the removal of nitrate and some other nutrients from wastewater. In brief, the amount of *Oscillatoria sp* and *Lemna minor* are increased considerably in wastewater contains high concentration of nitrate. Hence, the combined effect of both aquatic organism of *Lemna minor* and *Oscillatoria sp* are used for biotreatment of nitrate rich wastewater.

Keywords: Synthetic wastewater; NO_3^-; Biotreatment; *Lemna minor*; *Oscillatoria sp*

Introduction

Nitrate and nitrite are in nature occurring ions that are fraction of the nitrogen cycle. The nitrate ion (NO_3^-) is the constant form of combined nitrogen for oxygenated systems. Although chemically un reactive, it can be decreased by microbial action. The nitrite ions (NO_2^-) contain nitrogen in a comparatively unstable oxidation state. Chemical and biological processes can additionally diminish nitrite to a variety of compounds or oxidize it to nitrate [1]. Since years, several different parts of the world have been facing the problem of nitrogen pollution in the environment. As we know, the principal forms of nitrogen are ammonia (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-). Because nitrate is the most stable form of nitrogen species in water, almost all sources of aqueous nitrogen tend to be converted to nitrate. Nitrate (NO_3^-) is an inorganic compound that dissolves easily in water and is used widely in agricultural fields as fertilizer. Nitrate anions are necessary plant nutrients but represent a significant environmental problem when they occur in excess amounts. Nitrate pollutant shows the way to amplify in the growth of algal bloom those results in eutrophication of fresh water. It has become the most serious environmental problem in our area since it causes algae blooms. Growth of algae decreases the dissolved oxygen concentration of water that final result is the death of fish and other aquatic organisms. The highest acceptable concentration for nitrate-nitrogen in Canadian drinking water has been recognized as 10mg N-NO_3^-/L [2]. The major source of nitrogen pollution are the nitrate (NO_3^-) that mainly originated from the emission of industrial wastewater and the use of excess fertilizers in agricultural fields. Plant based biotreatment or remediation (Phytoremediation) is one of the biological wastewater treatment methods or processes to eliminate nitrate contaminant from aquatic system. So as to keep away from the eutrophic formation of fresh water and also to determine the efficiency of nitrate utilization by specific aquatic plants (Duck weed and *Oscillatoria*).

Duckweeds are small, fragile, free floating aquatic, smallest flowering plant that grows ubiquitously on fresh or polluted water throughout the world. Their vegetative reproduction can be quick when nutrient densities are most favourable. They grow slowly where nutrient insufficiencies occur or major inequities in nutrients are obvious. They are opportunistic by flushes of nutrients and can put on growth spurts throughout such periods. Ferdoushi et al., [3] experienced the effectiveness of *Lemna* as biofilters of nitrogen and phosphate in fish ponds in Bangladesh and found that they removed the surplus amount of nutrients from the water body and maintained sustainable environmental conditions. Duckweeds have received greatly attention because of their possible to take away contaminants from wastewater [4]. The fundamental theory of a duckweed wastewater treatment system is to farm local duckweed on the wastewater requiring treatment. Duckweed has an elevated mineral absorption

Citation: Usharani K , Divya K and Sruthilaya K. Combined Effect of Nitrate Bioremoval by Aquatic Free Floating Plant an Association with Filamentous Cyanobacteria. Austin Environ Sci. 2020; 5(1): 1043.

capacity and can stand far above the ground of organic loading as well as elevated concentrations of micronutrients. Duckweed wastewater treatment systems eliminate, by bioaccumulation, as much as 99 percent of the nutrients and dissolved solids contained in wastewater [5]. *Oscillatoria* is a unicellular, filamentous cyanobacterium that reproduces by fragmentation, which is named for the oscillation in its movement. *Oscillatoria annae* are a morphologically dissimilar collection of oxygenic photosynthetic prokaryotes, which are phylogenetically closed linked to each other and to chloroplasts. Some filamentous cyanophytes form distinguished cells called heterocyst, that are specific for hydrogen fixation and resting or spore cells called aconites. Most of the bacteria establish in the fresh water, while others are marine arise in damp soil, or even for the time being moistened rocks in deserts. The key purposes of using this system have focused on waste stabilization and nutrient removal. This study was aimed to examine the removal potential of nitrate by selective aquatic plants of duck weed (*Lemna minor*) and algae (*Oscillatoria sp.*) from eutrophic lake water sample according to the variance of residence times and concentrations of nitrate.

Materials and Methods

Preparation of aquatic plant and algae

The aquatic plant and algae used for the removal of nitrate from synthetic medium were Duckweed (*Lemna minor*) and *Oscillatoria*. The plant and algae was collected from a eutrophic lake at Theerthamkara, Kerala, India. The plants were washed thoroughly with distilled water to remove the particles adhering to the plants, and transferred to a container.

Preparation of nitrate solution

Stock solution was prepared using potassium nitrate.1g of potassium nitrate was weighed and transferred in to a standard flask and make up the volume to 1000ml in a standard flask using deionised water . From that stock solution of nitrate solutions working solution were prepared with 8 different concentrations (10, 50, 100, 200, 400, 600, 800 and 1000ppm). Calibration Graphs consists of a plot of absorbance versus concentration for a series of standard solutions whose concentrations are accurately known.

Plant material and Biotreatment (Duck weed (*Lemna minor*) and *Oscillatoria sp*)

After harvesting, plants and algae were washed with deionized water. These were then placed on a blotting paper and allowed to drain for 5 min before weighing. Young *Lemina minor* plants with average weight of 0.58g were taken and used for the nitrate removal experiments. Fresh biomass of *Lemna minor* was then transferred to a100ml beaker at eight different concentrations for one month. One of them was used as a control. About 1g of algal biomass was taken and used for the nitrate removal experiments. Fresh biomass of *Oscillatoria sp* was then transferred to 100ml Erlenmeyer flasks, at eight different concentrations for one month. One of them was used as a control. Control experiments were also performed with the same nitrate solution concentrations, but without aquatic plant. All experiments were performed in triplicates (Figure 1a, 1b).

Spectrophotometric analysis of Nitrate bioremoval

The nitrate level in the synthetic solution was estimated at different time intervals by Salicylic acid method [6] using spectrophotometer (UV-2600 series SHIMADZU). For the analysis of nitrate solution, about 0.25ml sample solution is pipette out into a 50ml beaker. To that add 0.8ml of 5% (w/v) salicylic acid in conc. H_2SO_4. After 20 minutes add 19ml NaOH, this is to raise the pH above 12.Then the sample is allowed to cool and measured the absorbance in terms of optical density (OD) at 410nm using a UV-Visible spectrophotometer. The amount of nitrate in the samples was detected by UV Spectrophotometer. The complex formation by nitration of salicylic acid under highly acidic conditions absorbs maximally at 410nm in basic (pH>12) solutions. The absorbance of the chromophore is directly proportional to the quantity of nitrate-N present. Ammonium, nitrite and chloride ions do not interfere.

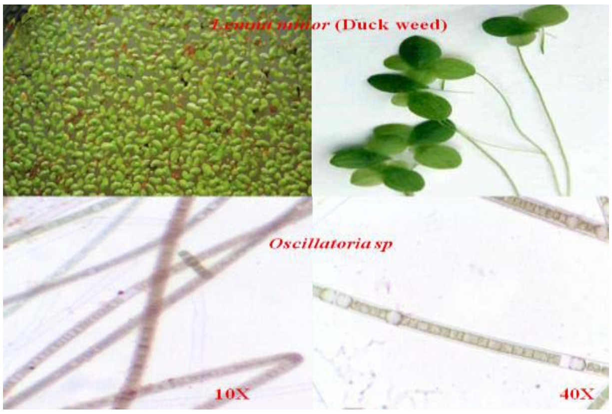

Figure 1a: *Lemna minor* and *Oscillatora sp* used for the study.

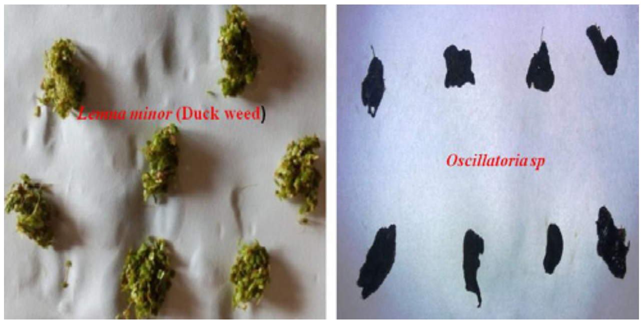

Figure 1b: Wet biomass of *Lemna minor* and *Oscillatora sp* used for the study.

PH analysis

The changes in pH of the treated samples of different concentration at different time intervals were estimated using digital pH meter (Scientific tech, advanced pH meter mode ST-2002).

Results and Discussion

Percentage of Nitrate Bioremoval analysis by Spectrophotometer

Collected plant samples of *Lemna minor* and *Oscillatoria sp*. were allowed to grow in nitrate solution for one month and used for our study. The capacity of the plant to remove nitrate at concentrations from 100 to1000ppm were shown in the below figures. Both the plants showed the nitrate removal property. In this study both showed similar activity that means they are similar in rate of nitrate removal.

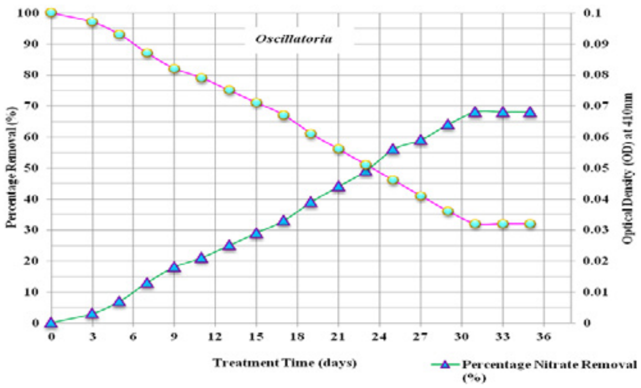

Figure 2: Spectrophotometric analysis of Nitrate removal by *Oscillatoria sp* at 100ppm.

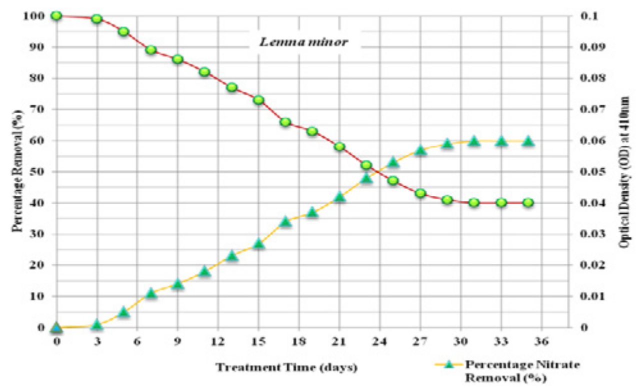

Figure 3: Spectrophotometric analysis of Nitrate removal by *Lemna minor* at 100ppm.

Figure 2&3, showed the removal of nitrate concentration at 100ppm concentration of nitrate solution at different treatment time. This reveals that the OD value decreases from the initial day to the final day. The decrease in OD value means the decrease in nitrate concentration in the solution. Decrease in nitrate concentration was due to the uptake of nitrate by the aquatic plant and algae. Dominic et al., [7] accounted that the 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. In general, cyanobacteria have a variety of characteristics that make them well-suited to wastewater treatment. Fay [8] stated that they have a high nutrient removal capacity as they can accumulate inorganic phosphorus and nitrogen and store them as polyphosphates and cyanophycin. Cyanophycin granules consist largely of proteinaceous reserve material [9]. Their elevated productivity and resourceful nitrogen removal make them suitable for wastewater treatment [10] and as biofilters in fish ponds [3].

The figures showed that the removal of nitrate concentration at 100ppm of *Lemna minor* and *Oscillatoria sp*. In comparison with *Lemna minor*, *Oscillatoria sp*. shows higher nitrate reduction from the medium at 100ppm. The nitrate reduction was observed to be higher in *Oscillatoria sp* when compared to *Lemna minor* from the treated wastewater containing 100ppm nitrate concentration. The maximum nitrate removal was observed in the 31st day of the experiment.

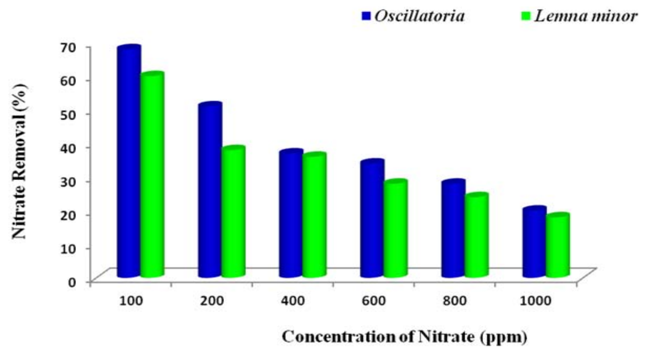

Figure 4: UV-Vis spectrophotometric of Nitrate bioremoval by Duck weed (*Lemna minor*) and algae (*Oscillatoria sp.*) after 30 days.

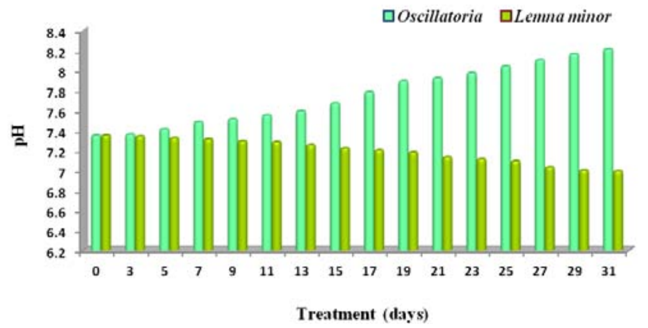

Figure 5: Change in pH of nitrate solution by *Oscillatoria sp* and *Lemna minor.*

Figure 4, showed the percentage nitrate removed from the synthetic nitrate solution among different concentration of nitrate solution .From the figure it was understood that maximum nitrate was removed by the algae *Oscillatoria sp* and least percentage of nitrates was removed by *Lemna minor* grown at 100ppm of nitrate solution. The percentage nitrate removal was given in figure 4. It was found that the maximum nitrate removal (68%) was observed at 100ppm and the minimum percentage removal (20%) was observed at 1000ppm by *Oscillatoria sp*. In the case of *Lemna minor* percentage nitrate removal was occurred maximum (60%) at 100ppm and the minimum percentage removal (18%) was occurred at 1000ppm. *L. minor* can regulate NO_3^- uptake via fronds or roots depending on light intensity [11].

The growth of the plant and algae and thus the removal efficiency are dependent not only on the type of nutrients available but also on the interactions between environmental factors such as pH [12,13,14,3] reported that relatively improved performance was detected in the treatment using *Lemna sp*. than treatment by *Azolla sp*.

PH change

Figure 5, shows the change in pH of nitrate solution by *Lemna minor* and *oscillatoria* during the treatment days. In the case of Duckweed (*Lemna minor*) the pH value is decreased from 7.35 to 6.99. This could be due to respiration by Duckweed plants. But in the presence of *Oscillatoria* the pH value increased from7.35 to 8.21. Such neutral or slightly alkaline conditions are favourable for denitrification.

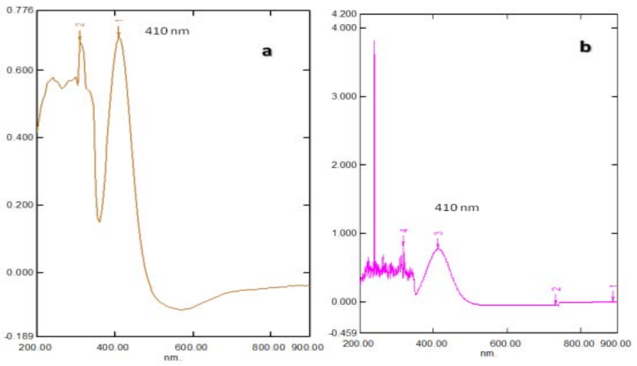

Figure 6: Spectrum of (a) *Oscillatoria* and (b) *Lemna minor* at 100ppm nitrate concentration after 30 days of treatment.

During this study the pH value increased from 7.35 to 8.21 in presence of *Oscillatoria* and in case of duckweed the pH value is decreased from 7.35 to 6.99. The increase in pH in all the treatments might be due to the photosynthetic activity of the algae which utilized CO_2 and produced less amount of carbonic acid [15].

Studies have revealed that significant increase in the pH of the culture medium is due to CO_2 uptake for photosynthesis. This leads to a decrease in CO_2 partial pressure, when CO_2 replacement is slower than the utilization [16,17].

Spectral analysis

Figure 6a, showed the spectral analysis of nitrate reduction by *Oscillatoria sp*. at 100ppm concentration of nitrate solution. The entire graph showed the Optical Density (OD) value at 410nm. OD value of different plant at different concentration compared with the OD value of corresponding standard nitrate solution. While comparing with standard solution the treated solution showed decrease in the amount of nitrate. Figure 6b, showed the spectral analysis of nitrate reduction by *Lemna minor* at 100ppm concentration of nitrate solution. The entire graph showed the Optical Density (OD) value at 410nm. OD value of different plant at different concentration compared with the OD value of corresponding standard nitrate solution. While comparing with standard solution the treated solution showed decrease in the amount of nitrate.

Conclusion

The results of this study showed that *Oscillatoria sp* and *Lemna minor* are potential for purification of water and effectible on the removal of nitrate and some other nutrients from wastewater. In brief, the amount of *Oscillatoria sp* and *Lemna minor* are increased considerably in wastewater contains high concentration of nitrate. In the other hand, the amount of nitrate decline significantly as same time as the algae and plant biomass increased. Among the two free floating aquatic organism, *Oscillatoria sp* achieved the maximum removal efficiency of nitrate from water. Maximum nitrate reduction was shown by *Oscillatoria sp* grown in 100ppm (68%). In case of *Lemna minor* maximum reduction was shown in100ppm (60%). This study reveals that *Oscillatoria sp* can perform well in wastewater .Through this experiment, it could be concluded that the nutrient removal load in wastewater can be reduced through *Lemna minor* and *Oscillatoria sp* and thus eutrophication could be avoided. Therefore the combined effect both aquatic organism of *Lemna minor* and *Oscillatoria sp* are used for biotreatment or Phytoremediation of nitrate rich wastewater. There by it was also possible to improve the quality of different wastewaters and the treated wastewater can be used for agriculture and aquaculture activities.

Acknowledgement

The author Usharani K wishes to thank the Department of Environmental Science, Central University of Kerala, South India. She also wishes to extend her sincere gratitude to editor and reviewers.

References

1. ICAIR Life Systems Inc. Drinking water criteria document on nitrate/nitrite. Washington, DC, US Environmental Protection Agency, Office of Drinking Water. 1987.
2. WHO. Nitrate and nitrite in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. World Health Organization, Geneva (WHO/SDE/WSH/07.01/16). 2007.
3. Zannatul F, Haque F, Khan S, Haque M. The Effects of two Aquatic Floating Macrophytes (*Lemna* and *Azolla*) as Biofilters of Nitrogen and Phosphate in Fish Ponds. Turkish Journal of Fisheries and Aquatic Sciences. 2018; 8: 253-258.
4. Leng RA, Stambolie JH, Bell R. Duckweed- a potential high-protein feed resource for domestic animals and fish. AAAP Conf. Proc., Bali. 1995; 103-114.
5. Paul S, William S, William J. Duckweed Aquaculture: A New Aquatic Farming System for Developing Countries. A world bank Publication. US. 1993.
6. APHA. American Public Health Association. American Water Works Association. Water Environment Federation. Standard Methods for the Examination of Water and Wastewater. 1998.
7. Dominic VJ, Murali S, Nisha MC. Phycoremediation efficiency of three *micro algae Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa*: Academic Review. 2009; 138-146.
8. Fay P. The Blue-Greens. Edward Arnold Publishers Ltd, London. 1983; 87.
9. Oliver RL, Ganf GG. Freshwater blooms. Whitton & Potts, editors. In: The Ecology of Cyanobacteria, their Diversity on Time and Space. Kluwer Academic Publishers. The Netherlands. 2000.
10. Rabah ZO, van der Steen P, Gijzen H. Effect of Organic Surface Load on Process Performance of Pilot-Scale Algae and Duckweed-Based Waste Stabilization Ponds. Journal of Environmental Engineering. 2005; 131: 587.
11. Cedergreen N, Madsen TV. Light regulation of root and leaf NO_3^- uptake and reduction in the floating macrophyte Lemna minor. New Phytol. 2004; 161: 449-457.
12. Muñoz R, Guieysse B. Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Research. 2006; 40: 2799-2815.
13. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. Review: Microalgae and wastewater treatment. Saudi Journal of Biological Sciences. 2012; 19; 257-275.
14. Zheng Y, Chen Z, Lu H, Zhang W. Optimization of carbon dioxide fixation and starch accumulation by Tetraselmis subcordiformis in a rectangular airlift photobioreactor. African Journal of Biotechnology. 2013; 10: 1888-1901.
15. Ozengin N, Elmaci A. Performance of duckweed (*Lemna minor*) on different types of wastewater treatment; Journal of Environmental Biology. 2007; 28: 307-314.
16. Dubinsky Z, Rotem J. Relations between algal populations and the pH of their media. Oecologia. 1974; 16: 53-60.
17. Chen Celia Y, Edward G Durbin. Effects of pH on the growth and carbon uptake of marine phytoplankton. Marine ecology progress series. Mar Ecol Prog Ser. 1994; 109: 83-94.