

Microbial Reduction of Nutrients from Combined Industrial and Municipal Wastewater

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Abstract: *In this paper we have experimentally analysed the utilization of three types of cultured microbial algae for the reduction of nutrients from combined industrial and municipal wastewater. The microbial algae used were Chlorella vulgaris (Walne's medium), Synechocystis salina (Allen medium) and Gloeocapsa gelatinosa (Nelson medium). After the contact period of 5, 10, 15 and 20 days, reduction of nitrates, nitrites were observed significantly*

1. Introduction

Human activities, particularly agriculture and urbanization, have led to increased nitrogen discharge to inland water systems. Wastewater streams rich in the nutrients nitrogen (N) are produced by municipal, industrial, and agricultural processes. These are present in various forms: partially bound to solids and not immediately available to the ambient air and water, and also present in water soluble form.

Preventing fugitive losses through capture and reuse of nutrients can increase overall nutrient use efficiency, partially replace chemical Nitrogen and Phosphorous fertilizers and reduce manufactured Nitrogen and stretch Phosphorous mineral resources, while also reducing adverse environmental impacts. Pollution control and recovery of value of these elements from agricultural or other organic waste can be accomplished for both Nitrogen and Phosphorous.

Eutrophication is due to presence of nutrient level above the limit in marine and surface water due to human activities viz without proper treatment of waste water generated and mixed with surface water. This nutrient enrichment or eutrophication can profoundly alter the structure and function of aquatic ecosystems, potentially endangering human health, biodiversity and ecosystem sustainability. Therefore, nitrogen in wastewater should be properly treated or reused thereby reducing their contaminant effects in aquatic ecosystems.

Microalgae play the significant role in meeting the demand of energy and also serve as the primary feedstock for sustainable products. Microalgae have received a major attention as a source of alternate energy, to make it economical the process should undergo optimization in large scale which will meet the demand. Wastewater which is produced from various industries can also be a source of feed for the algae to grow and hence, play a dual role i.e., nutrient removal and biofuel production

Besides carbon, nitrogen is the second most important nutrient to microalgae since it may comprise more than 10 % of the biomass. Nitrogen exists in many forms, and the most common nitrogen compounds assimilated by microalgae are ammonium (NH₄⁺) and nitrate. The preferred compound

is ammonium, and when this is available, no alternative nitrogen sources will be assimilated. However, ammonium concentrations higher than 20 mg NH₄⁺ per litre are not recommended due to ammonia toxicity. In addition to these nitrogen compounds, urea (CO(NH₂)₂) and nitrite (NO₂⁻) can be used as nitrogen sources. However, the toxicity of nitrite at higher concentrations makes it less convenient. Cyanobacteria are also able to assimilate the amino acids arginine, glutamine and asparagine and some species can fix nitrogen gas (N₂). Of all nitrogen sources, this nitrogen fixation is the most energy demanding and only occurs in some cyanobacteria when no other nitrogen compounds are available in sufficient amounts. Several microalgae can take up nitrogen in excess of the immediate metabolic needs, so called luxury consumption. This can be used later in the case of nitrogen starvation.

2. Methodology

Algae selection

Chlorella vulgaris (Chlorophyceae), *Synechocystis salina* (Cyanophyceae) and *Gloeocapsa gelatinosa* (Cyanophyceae) are used to remove nitrogen content from waste water samples collected from aeration tank outlet and are identified at Laboratory, Manali.

Culturing of Algae

The algal samples were cultured in proper culture media. *Chlorella vulgaris* was cultured in Walne's medium and *Synechocystis salina* and *Gloeocapsa gelatinosa* were cultured in Allen and Nelson medium. The algal culture was incubated at 23±1°C temperature and 2000 lux light. A 16:8 hour light/ dark cycle was provided in order to obtain synchronous culture

Selection of waste water

Municipal wastewater including influent and effluent was taken from a wastewater treatment plant in Manali, Chennai. This plant treated both domestic and industrial wastewaters by the traditional activated sludge method, but the majority wastewater is mainly from domestic sources. The influent and effluent were filtered through a 0.22-µm pore size membrane. After autoclaving, the wastewaters were moved to 500 ml Erlenmeyer flasks for algae cultivation.

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3. Selection of Parameters

Optimization of pH & DO

After optimizing number of days, the pH of synthetic wastewater were to be varied. The synthetic wastewater with different pH to be fed to the reactor with 60 g of each algae. The samples were to be collected after the days optimization analysis for the various parameters. Increase in Dissolved Oxygen of the water sample treated with *Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa* can be determined.

4. Determination of Parameters

Determination of pH using pH Paper

- Take a pH paper strip and place it on a white tile.
- Pour a drop of the sample on the pH paper using a clean dropper.
- Observe the colour of the pH paper.
- Now compare the colour obtained on the pH paper with the different colour shades of the standard colour pH chart and note down the pH value.
- Similarly, find the pH of the remaining samples using a fresh strip of pH paper and a separate dropper for each sample.

Determination of pH using Universal Indicator Solution

- Take a small quantity of the given sample in a test tube using a dropper.
- Using a dropper pour a few drops of the universal indicator solution into the test tube containing the sample.
- Shake the test tube well and note the colour developed in the test tube.
- Now compare the colour produced in the test tube with the different colour shades of the standard colour pH chart and note down the pH value.

Determination of dissolved oxygen (DO)

- Carefully fill a 300ml glass Biological Oxygen Demand (BOD) stopper bottle brim-full with sample water.
- Immediately add 2ml of manganese sulphate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
- Add 2ml of alkali-iodide-azide reagent in the same manner.
- Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or flock will appear. When this flock has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.
- Add 2ml of concentrated sulphuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the flock. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with

aluminium foil and a rubber band during the storage period.

- In a glass flask, titrate 201ml of the sample with sodium thiosulfate to a pale straw colour. Titrate by slowly dropping titrate solution from a calibrated pipette into the flask and continually stirring or swirling the sample water.
- Add 2ml of starch solution so a blue colour forms.
- Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue colour. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue colour.
- The concentration of dissolved oxygen in the sample is equivalent to the number of millilitres of titrant used. Each ml of sodium thiosulfate added in steps 6 and 8 equals 1mg/L dissolved oxygen

Optimization of nitrate and nitrite contents

Nitrite in the water sample can be completely absorbed by the micro algae *Chlorella vulgaris* and *Gloeocapsa gelatinosa*. Percentage reduction in the nitrate content can be observed in water sample treated with *Synechocystis salina*. The concentration of nitrite reduced can be calculated. The methodology adopted for the present undertaken for the study, study consist of various materials used, selection of micro algae, culturing of micro algae, selection of parameters for the removal of nitrogen contents from aqueous samples were also presented.

5. Result

Preliminary analysis of the water sample revealed the pH of the water sample as 6, presence of 2.5 μ mol/l nitrate and 3.2 μ mol/l nitrite. Dissolved Oxygen of the water sample was 2.3mg/l. Change in the water quality after treatment with algae was checked in an interval of 5days for a period of 25days. pH of the water sample treated with *Chlorella vulgaris* showed a drift towards alkalinity. pH has been changed from 6.0 to 8.0 (Table 1). pH was drifted from 6 to 7.9 in the water sample treated with *Synechocystis salina* (Table 2). pH has been changed from 6.0 to 7.6 in the water sample treated with *Gloeocapsa gelatinosa* 2.5 μ mol/l of nitrate had been reduced to 0.5 μ mol/l in the water sample treated with *Chlorella vulgaris* (Table 2). 80% reduction in the nitrate content has been noted. (Table.2). 80% decrease in the nitrate content has been observed in the water sample treated with *Synechocystis salina* The concentration of nitrate has been reduced from 2.5 μ mol/l to 0.5 μ mol/l (Table .2). The concentration of nitrate in the water sample treated with *Gloeocapsa gelatinosa* has been reduced from 2.5 μ mol/l to 0.5 μ mol/l (Table.3).

Nitrite in the water sample had been completely absorbed by the micro algae *Chlorella vulgaris* and *Gloeocapsa gelatinosa* whereas in the control there was only a slight reduction. 59% reduction in the nitrate content has been observed in water sample treated with *Synechocystis salina*. The concentration of nitrite has been reduced from 5.3 μ mol/l to 0.2 μ mol/l (Table.2). The Dissolved Oxygen of the water sample treated with *Chlorella vulgaris* has been increased from 2.3mg/l to 7.6mg/l (Table 1). *Synechocystis*

salina showed an increase in the Dissolved Oxygen from 2.3mg/l to 6.0mg/l Gloeocapsa gelatinosa showed an increase in the Dissolved Oxygen from 2.3mg/l to 5.8mg/l

Table 1: Phycoremediation with *Synechocystis salina* in waste water

Parameters	Concentration				
	0 th day	5 th day	10 th day	15 th day	20 th day
	Samples				
pH	6.0	6.4	7.2	7.6	8.0
Nitrates(μmol/l)	2.5	1.1	1.0	0.9	0.5
Nitrites (μmol/l)	3.2	2.4	2.0	1.2	0.4
DO(mg/l)	2.3	3.9	5.4	7.0	7.6

Table 2

Parameters	Concentration				
	0 th day	5 th day	10 th day	15 th day	20 th day
	Sample				
pH	6.0	6.2	7.2	7.5	7.9
Nitrates(μmol/l)	2.5	1.0	0.8	0.7	0.5
Nitrites (μmol/l)	3.2	4.1	3.5	2.0	1.3
DO(mg/l)	2.3	3.4	4.3	5.5	6.0

In Table 2, it was found that the nitrates and nitrites concentration got reduced indicating that the nitrates and nitrites in the samples are assimilated by the microalgae

Table 3: Phycoremediation with *Gloeocapsa gelatinosa* in waste water

Parameters	Concentration				
	0 th day	5 th day	10 th day	15 th day	20 th day
	Sample				
pH	6.0	6.3	6.8	7.2	7.6
Nitrates(μmol/l)	2.5	1.5	0.8	0.7	0.5
Nitrites (μmol/l)	3.2	3.5	2.8	2.4	1.4
DO(mg/l)	2.3	4	4.7	5.5	5.8

Table 4: Efficiency comparison of each algae

Parameter studied	<i>Chlorella vulgaris</i> ,	<i>Synechocystis salina</i>	<i>Gloeocapsa gelatinosa</i>
pH(%)	33.3	31.6	26.6
Nitrate(%)	80	80	80
Nitrite(%)	87.5	59	56.2
DO(%)	230.4	160.8	152.17

Analysis of various physiochemical parameters before and after treatment revealed that the micro algae *Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa* could effectively improve water quality by removal of pollutants. The studies on the nutrient uptake by these micro algae clearly reveal the efficiency of algae to remove nutrients from polluted water bodies. All the three algae showed high efficiency in the removal of nutrients.

pH of the water sample treated with *Chlorella vulgaris* showed a drastic change of 35.79%. 32.61% pH change has been observed in the water sample treated with *Synechocystis salina*. 31.19% change in the water sample treated with *Gloeocapsa gelatinosa* was observed

6. Conclusion

From the results is can be concluded that the pH of the water

sample treated with *Chlorella vulgaris* is 35.79%. 80% reduction nitrate content of the water sample treated with *Chlorella vulgaris*. Dissolved Oxygen in the water sample treated with *Chlorella vulgaris* had been found increasing by 230.4%. hence it can concluded that the *Chlorella vulgaris* is more efficient and reliable than *Synechocystis salina* and *Gloeocapsa gelatinosa*

References

- [1] Abeliovich, A., & Azov, Y. (1976). Toxicity of ammonia to algae in sewage oxidation ponds. *Applied and Environmental Microbiology*, 31, 801–806
- [2] Abomohra, A. E. F., El-Sheekh, M., & Hanelt, D. (2014). Pilot cultivation of the chlorophyte microalga *Scenedesmus obliquus* as a promising feedstock for biofuel. *Biomass and Bioenergy*, 64, 237–244.
- [3] Algae–bacteria interactions: Evolution, ecology and emerging applications. *Biotechnology Advances*, 34, 14–29. [http](http://)
- [4] An, J.Y., S.J. Sim, J.S. Lee and B.W. Kim: Hydrocarbon production from secondarily treated piggery wastewater by green alga *Botryococcus braunii*. *J. Appl. Phycol.* , 15 , 185-191(2003).
- [5] Aslan, S. and I.K. Kapdan: Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng.* , 28 , 64-70 (2006)
- [6] Bashan, L.E., M. Moreno, J. Hernandez and Y. Bashan: Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense* . *Water Res.* , 36 , 29412948 (2002).
- [7] Chinnasamy, S., A. Bhatnagar, R.W. Hunt and K.C. Das: Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Biores. Technol.* , 101 , 3097-3105 (2010).
- [8] Clarens, A.F., E.P. Resurreccion, M.A. White and L.M. Colosi: Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environ. Sci. Technol.* , 44 , 1813-1819 (2010). 36
- [9] Ecological roles of nitrification. In M. G. Klotz, B. B. Ward, D. J. Arp, and American Society for Microbiology (Eds.), *Nitrification* (pp. 3–8). Washington, DC
- [10] Effectiveness of flashing light for increasing photosynthetic efficiency of microalgal cultures over a critical cell density. *Biotechnology and Bioprocess Engineering*, 6, 189–193. <http://dx.doi.org/10.1007/BF02932549> PerezGarcia, O., De-Bashan, L., Hernandez, J., & Bashan, Y. (2010).
- [11] Efficiency of growth and nutrient uptake from wastewater by heterotrophic, autotrophic, and mixotrophic cultivation of *Chlorella vulgaris* immobilized with *Azospirillum brasilense*. *Journal of Phycology*, 46, 800–812.
- [12] González, L.E., R.O. Cañizares and S. Baena: Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus* . *Biores. Technol.*, 60 , 259-262 (1997).