



Microalgae culture for the removal of Nitrogen & Phosphorous from Wastewater

Mallikarjuna Ruchir Grandhi¹, Gokulan Ravindiran *²

¹ Year-12, Jumeirah College, Dubai, United Arab Emirates

² Department of Civil Engineering, GMR Institute of Technology, Rajam, Andhra Pradesh, India

ABSTRACT

The wastewater discharged from domestic and industries contains a high level of nitrogen and phosphorus. This is considered one of the major sources of the eutrophication process in water bodies. The present study investigated the removal of nitrogen and phosphorus from the groundwater using five different microalgae strains namely *Nanochloropsis*, *Tetraselmis*, *Charoceros*, *Isochrysis*, and *Thalassiosira* in a batch culture system. The batch culture stream was conducted for 15 days. Initially, the nitrogen and phosphorus content in the wastewater was measured as 62 and 38 mg/L, whereas after 15 days the nitrogen and phosphorus content was reduced to 36 and 17.5 mg/L. The maximum removal of nitrogen (41.9%) and phosphorus (53.9%) was achieved by *Nanochloropsis*.

KEYWORDS: *Nanochloropsis*; *Tetraselmis*; *Charoceros*; *Isochrysis*; *Thalassiosira*.

Received 08 November, 2021; Revised: 22 November, 2021; Accepted 24 November, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION

Ever-increasing population and industries resulted in increasing usage of water. This had resulted in the generation of a huge quantity of wastewater from the domestic and industries that are not properly treated and discharges into nearby water bodies (rivers, lakes, and ponds) [1]. In the wastewater treatment system, the inorganic pollutants namely ammonium, phosphate, and nitrate had been an emerging problem. This inorganic pollutant is essential for aquatic growth and results in the eutrophication process. Eutrophication is the condition of a gradual increase in the concentration of Phosphorus, Nitrogen, and other plant nutrients in a water body resulting in excessive plant and Algal growth. This is one of the most widespread challenges faced by freshwater systems and considered a challenging issue in water management. The most prominent effect of eutrophication of water resources is the formation of blooms of foul-smelling. These will reduce water clarity and might degrade the water quality. The growth of such blooms disturbs light penetration and destroys the growth of aquatic life in water bodies.

So, a cost-effective treatment is need to be adopted for the removal of the inorganic pollutants. In the past several types of treatment methods namely mechanical (sedimentation), chemical (disinfection), and biological processes (trickling filter and activated sludge process) were used for the removal of organic pollutants [2]. But these methods are considered as costly and creates secondary pollutants in the form of sludge. To over the disadvantage of these methods, an alternate biological method called microalgae is considered as a low-cost treatment method for the removal of nutrients from the wastewater [3]. The removal efficiency of the nitrogen and phosphorus content by microalgae depends on media and several other environmental conditions namely nutrient concentration, light/dark period, light intensity, and type of algal species [4].

Many researchers reported that the utilization of microalgae for the removal of the nutrient has a great impact on the removal of nitrogen and phosphorus [5]. Microalgae are naturally overgrown in all regions of the country in soil and water bodies. Further growth of microalgae will not cause any secondary pollutants to the environment [6]. It is also reported that many by-products can be obtained from the biomass of the microalgae namely biodiesel and protein-rich supplement. Major sources for the eutrophication process are fertilizer runoff during the rainy season and wastewater discharged from domestic and industrial activities. The utilization of microorganisms for the removal of nitrogen and phosphorus in a sewage treatment plant before discharging into the environment will act as an effective tool for the eutrophication process. The main objective of the present study is to remove nitrogen and phosphorus from wastewater in a batch culture system. Five different

microalgae strain namely *Nanoochloropsis*, *Tetraselmis*, *Chartoceros*, *Isochrysis*, and *Thalassiosira* were investigated for nutrient removal.

II. METHODS AND MATERIALS

2.1 Microalgae Collection

Five different microalgae isolated strains namely *Nanoochloropsis*, *Tetraselmis*, *Chartoceros*, *Isochrysis* and *Thalassiosira* were collected from the Centre for Marine and Fisheries Research Institute (CMFRI), Visakhapatnam. The 250 ml of sample was collected and stored in a closed container at a temperature of 4 °C. Then the collected samples were stored in an incubator in the laboratory at a temperature of 20 °C.

2.2 Wastewater Collection

The wastewater used in the present investigation was collected from Mettavalasa village, Srikakulam, Andhra Pradesh. The poultry farming was very close to the collected sample and the nitrogen (Nitrate) and phosphorus (orthophosphate) content was measured as 62 and 38 mg/L. This high level of nitrogen and phosphorus content was maybe the waste from poultry farming mixed with the nearby surface water and in turn, resulted in the contamination of the groundwater. The required amount of sample was collected in 10 L cans and pre-treatment of the sample was carried in the laboratory and stored in an incubator at a temperature of 20 °C.

2.3 Batch removal Studies

The batch experiments were conducted in a 250 ml conical flask with a working volume of 100 ml. The wastewater was inoculated with 25 ml of the microalgae strains and it is closed with cotton. To avoid contamination and moisture content, the conical flask head was covered with aluminum foil. The conical flask was kept in a temperature-controlled orbital shaker that was rotated at 150 rpm. The light to dark period was maintained as 15:9 (15 hours light and 9 hours dark). A fluorescent lamp at a light intensity of 230 mmol/m²/s was used. A 5 ml sample was taken for the analysis of the nitrogen and phosphorus content. Nitrogen was measured in the form of Nitrate at 210 nm and phosphorus was measured in the form of Phosphate at 420 nm. A potassium dihydrogen phosphate (KH₂PO₄) was used to prepare a standard stock solution of 1000 ppm (4.39 in 1000 ml) and successive solution was prepared by diluting it to 5, 10, 25, 50, 100 ppm for measuring phosphate. Similarly Potassium nitrate was used to prepare a standard stock solution of 1000 ppm (1.63 g in 1000 ml) and successive solution was prepared by diluting it to 5, 10, 25, 50, 100 ppm for measuring nitrate [7].

III. RESULT AND DISCUSSION

3.1 Nanoochloropsis

Tables 1 and 2 summarized the uptake of nitrogen and phosphorus for 15 days. Figure 1 illustrated the removal efficiency of nitrogen and phosphorus by *Nanoochloropsis*. From the batch culture system, it was concluded that maximum removal efficiency of 41.94 and 53.95 % was achieved after 15 days for nitrogen and phosphorus. From the results, it is also clear that the maximum removal efficiency was high for the first three days. Almost 50 % of the total removal was achieved on the 3rd day. For instance, the 3rd-day removal efficiency of 43.95 and 22.58 % was achieved for phosphorus and nitrogen. From the result, it is also clear that the removal efficiency was maximum for the first 7 days and a further increase in days resulted in very little removal of the nitrogen and phosphorus. For instance, on the 9th day, the removal efficiency of phosphorus and nitrogen was 50.26 and 38.06 %, whereas, on 15th day, the removal efficiency was increased to 53.95 and 41.94% respectively. This shows only a small difference of 3.68 and 3.87% of phosphorus and nitrogen removal. This may be the biomass growth reached the saturation phase and the growth of the microalgae is decreased.

Table 1: Removal of Nitrogen by different microalgae's (concentration- mg/L)

Days	<i>Nanoochloropsis</i>	<i>Tetraselmis</i>	<i>Chartoceros</i>	<i>Isochrysis</i>	<i>Thalassiosira</i>
0	62	62	62	62	62
1	48.3	48.1	57.3	47.5	59.8
3	45	46	55	45	58
5	40.9	45.3	45.8	43.2	56
7	39.5	44.5	43.8	41.1	54
9	38.4	43.2	42.5	40.1	52
11	37.3	41.4	41.3	39.1	51

13	36.5	40.3	40.5	38	47
15	36	39.8	39.5	37.5	46

Table 2: Removal of phosphorus by different microalgae's (concentration- mg/L)

Days	<i>Nanochloropsis</i>	<i>Tetraselmis</i>	<i>Charoceros</i>	<i>Isochrysis</i>	<i>Thalassiosira</i>
0	38	38	38	38	38
1	22.5	34	28.5	26	23
3	21.3	32.1	26.1	23.9	21.5
5	20.1	31.4	24.3	22.9	20.9
7	19.8	31	24	22.3	20.5
9	18.9	29	23	21.5	19.5
11	18	27.3	22.1	19.9	19
13	17.8	26	21.6	19.5	18.7
15	17.5	24.3	21	19.3	18.3

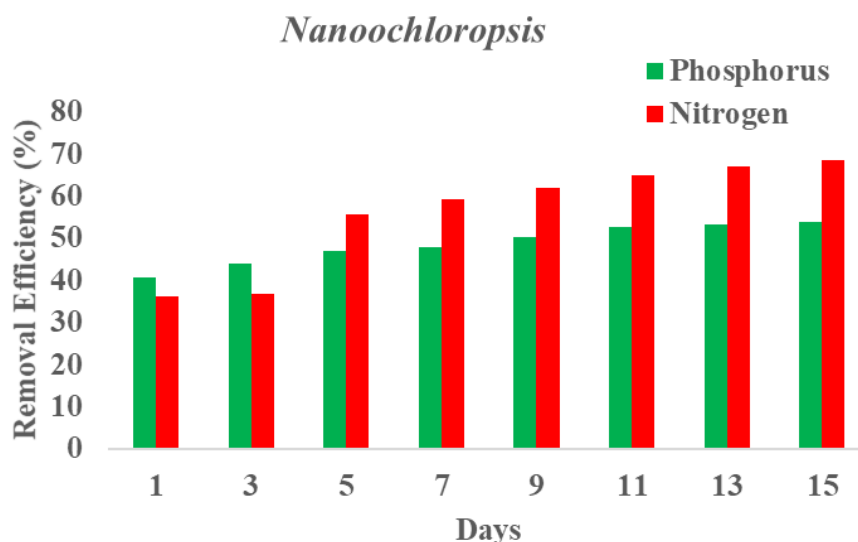


Figure 1: Removal Efficiency of Nitrogen and Phosphorus using *Nanochloropsis*

3.2 Tetraselmis

Tables 1 and 2 summarized the uptake of nitrogen and phosphorus for 15 days. Figure 2 illustrated the removal efficiency of nitrogen and phosphorus by *Tetraselmis*. From the batch culture system, it was concluded that maximum removal efficiency of 35.81 and 36.05% was achieved after 15 days for nitrogen and phosphorus. From the results, it is also clear that the maximum removal efficiency was high for the first three days. Almost 50 % of the total removal was achieved on the 3rd day. For instance, the 3rd-day removal efficiency of 15.53 and 25.81 % was achieved for phosphorus and nitrogen. From the result, it is also clear that the removal efficiency was maximum for the first 7 days and a further increase in days resulted in very little removal of the nitrogen and phosphorus. For instance, on the 9th day, the removal efficiency of phosphorus and nitrogen was 23.68 and 30.32%, whereas, on 15th day, the removal efficiency was increased to 36.05 and 35.81% respectively. This shows only a small difference of 12.37 and 5.48% of phosphorus and nitrogen removal. This may be the biomass growth reached the saturation phase and the growth of the microalgae is decreased.

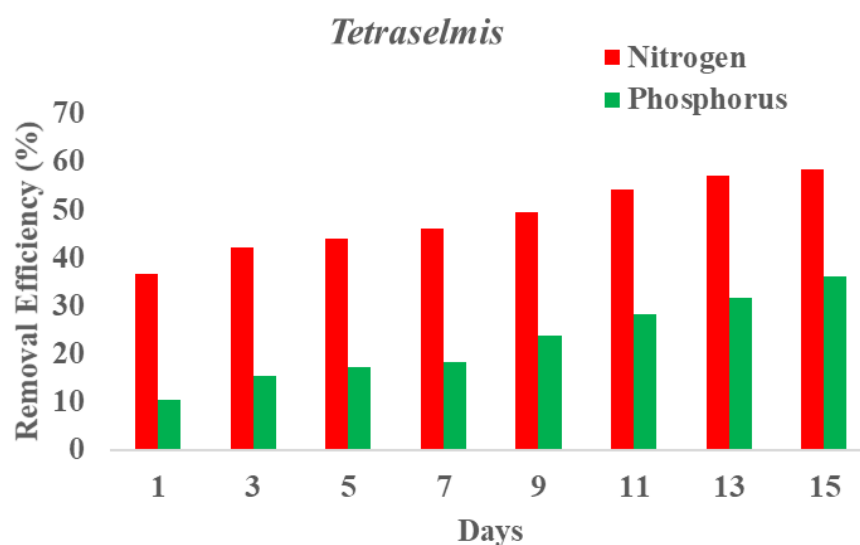


Figure 2: Removal Efficiency of Nitrogen and Phosphorus using *Tetraselmis*

3.3 Charcoerous

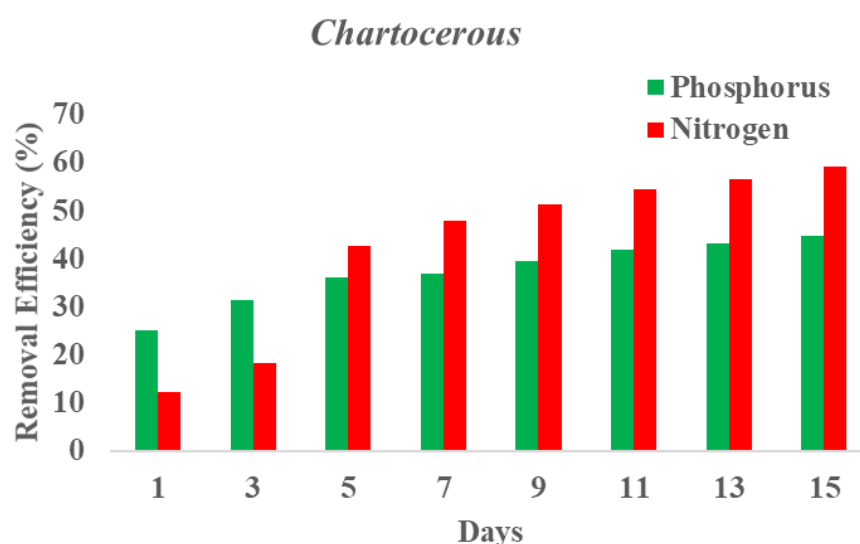


Figure 3: Removal Efficiency of Nitrogen and Phosphorus using *Chartoceros*

Tables 1 and 2 summarized the uptake of nitrogen and phosphorus for 15 days. Figure 3 illustrated the removal efficiency of nitrogen and phosphorus by *Chartoceros*. From the batch culture system, it was concluded that maximum removal efficiency of 36.29 and 44.74 % was achieved after 15 days for nitrogen and phosphorus. From the results, it is also clear that the maximum removal efficiency was high for the first three days. Almost 50 % of the total removal was achieved on the 3rd day. For instance, the 3rd-day removal efficiency of 31.32 and 26.13 % was achieved for phosphorus and nitrogen. From the result, it is also clear that the removal efficiency was maximum for the first 7 days and a further increase in days resulted in very little removal of the nitrogen and phosphorus. For instance, on the 9th day, the removal efficiency of phosphorus and nitrogen was 39.47 and 31.45%, whereas, on 15th day, the removal efficiency was increased to 44.74 and 36.29% respectively. This shows only a small difference of 5.26 and 4.84% of phosphorus and nitrogen removal. This may be the biomass growth reached the saturation phase and the growth of the microalgae is decreased.

3.4 Isochrysis

Tables 1 and 2 summarized the uptake of nitrogen and phosphorus for 15 days. Figure 4 illustrated the removal efficiency of nitrogen and phosphorus by *Isochrysis*. From the batch culture system, it was concluded that maximum removal efficiency of 39.52 and 49.21 % was achieved after 15 days for nitrogen and

phosphorus. From the results, it is also clear that the maximum removal efficiency was high for the first three days. Almost 50 % of the total removal was achieved on the 3rd day. For instance, the 3rd-day removal efficiency of 37.11 and 27.42 % was achieved for phosphorus and nitrogen. From the result, it is also clear that the removal efficiency was maximum for the first 7 days and a further increase in days resulted in very little removal of the nitrogen and phosphorus. For instance, on the 9th day, the removal efficiency of phosphorus and nitrogen was 43.42 and 35.32%, whereas, on 15th day, the removal efficiency was increased to 49.21 and 39.52% respectively. This shows only a small difference of 5.79 and 4.19% of phosphorus and nitrogen removal. This may be the biomass growth reached the saturation phase and the growth of the microalgae is decreased.

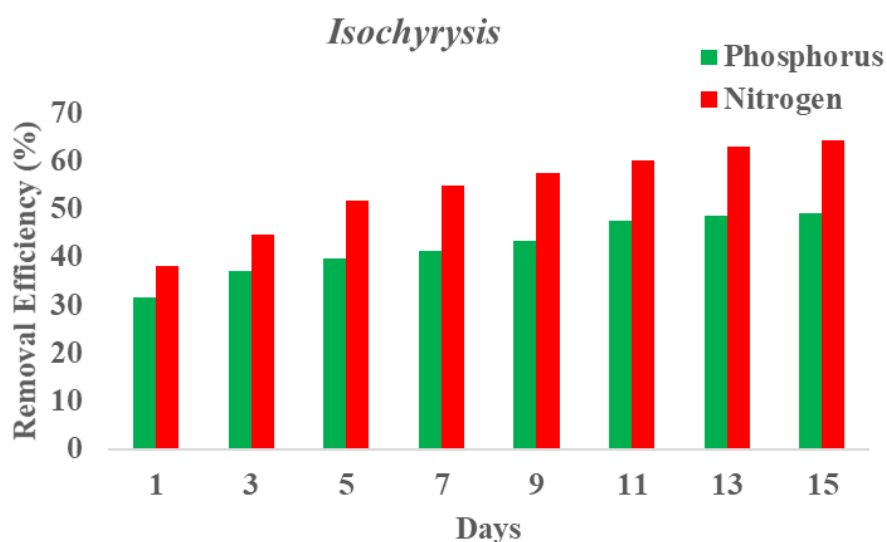


Figure 4: Removal Efficiency of Nitrogen and Phosphorus using *Isochrysis*

3.5 *Thalassiosira*

Tables 1 and 2 summarized the uptake of nitrogen and phosphorus for 15 days. Figure 5 illustrated the removal efficiency of nitrogen and phosphorus by *Thalassiosira*. From the batch culture system, it was concluded that maximum removal efficiency of 25.82 and 51.84% was achieved after 15 days for nitrogen and phosphorus. From the results, it is also clear that the maximum removal efficiency was high for the first three days. Almost 50 % of the total removal was achieved on the 3rd day. For instance, the 3rd-day removal efficiency of 37.11 and 6.45% was achieved for phosphorus and nitrogen. From the result, it is also clear that the removal efficiency was maximum for the first 7 days and a further increase in days resulted in very little removal of the nitrogen and phosphorus. For instance, on the 9th day, the removal efficiency of phosphorus and nitrogen was 43.42 and 16.13 %, whereas, on 15th day, the removal efficiency was increased to 51.84 and 25.81% respectively. This shows only a small difference of 3.16 and 9.68% of phosphorus and nitrogen removal. This may be the biomass growth reached the saturation phase and the growth of the microalgae is decreased.

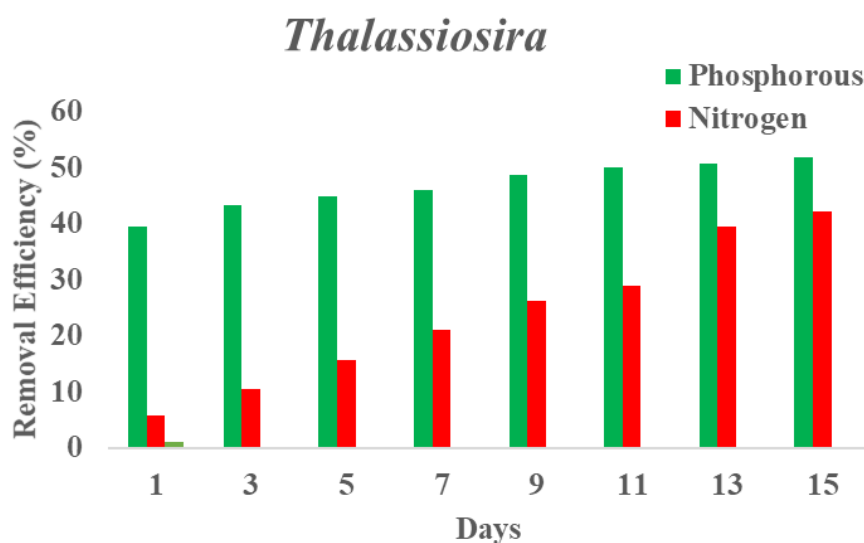


Figure 5: Removal Efficiency of Nitrogen and Phosphorus using *Thalassiosira*

IV. CONCLUSION

The current investigation concluded that microalgae can be effectively utilized for the removal of nitrogen and phosphorus from water and wastewater. The removal efficiency of the nitrogen was in the order of *Nanochloropsis* (53.95%) > *Thalassiosira* (51.84%) > *Isochrysis* (49.12%) > *Charoceros* (44.74%) > *Tetraselmis* (36.05%). Whereas the removal efficiency of the phosphorus was in the order of *Nanochloropsis* (41.94%) > *Isochrysis* (39.52%) > *Charoceros* (36.29%) > *Tetraselmis* (35.81%) > *Thalassiosira* (25.81%). So, it is concluded that *Nanochloropsis* was found to be the best microalgae in the removal of nitrogen and phosphorus.

REFERENCES

- [1]. Martinez, M. E., et al., Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology*, 2000. **73**: P. 263-272.
- [2]. Ruiz-Marin, A., et al., Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology*, 2010. **101**: p. 58-64.
- [3]. Larsdotter, K., Wastewater treatment with microalgae a literature review. *VATTEN*, 2006. **62**: p. 31-8.
- [4]. Aslan, S., and Kapdan, I.K., Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*, 2006. **28**: p. 64-70.
- [5]. Zamani, N., et al., Effect of alginate structure and microalgae immobilization method on orthophosphate removal from wastewater. *Journal of Applied Pycology*, 2011. **24**: p. 649-56.
- [6]. Rasoul-Amini, S., et al., *Chlorella* sp.: A new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor. *Applied Energy*, 2011. **88** (10): p. 3354-6.
- [7]. Gokulan, R., and Ragunath, S., Comparative study on treatment of municipal wastewater with carbondioxide sequestration by microalgae. *International Journal of ChemTech Research*, 2014. **6** (1): p. 609-618