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REMOVAL OF NUTRIENTS FROM WASTEWATER EFFLUENT BY THE CULTIVATION OF MICROALGAE IN PHOTOBIOREACTOR (PBR)

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ABSTRACT

Good water quality is vital and essential for human health, social and economical development and additionally also for the ecosystem. As populations develop and natural environments become decrease, ensuring there are enough and secure water elements for everyone is becoming increasingly more challenging. For the safety of water, wastewater needs to be treated before further uses. If these untreated wastewater having nutrients is discharged directly into the natural water bodies, it will cause eutrophication ultimately contaminate the water. For mitigating nutrients, wastewater needs tertiary treatment. In that case, the cultivation of microalgae will be an effective solution as microalgae are capable to assimilate inorganic nitrogen and phosphorus for their growth. Moreover, the production of microalgae biomass is considered as a third-generation renewable resource for bioenergy production. In this study, a photobioreactor (PBR) was designed using a transparent jar for producing microalgal biomass. Transparent jar is more effective and has more capability for receiving sunlight which helps to generate more microalgal growth. The reactor was seeded with Chlorella vulgaris.

After analyzing the data, it is found that the microalgae biomass has the capacity of removing nutrients from the wastewater effluents. The percentage of nitrogen removal is 84% in the form of nitrate and nitrite. The highest rate of phosphate removal was measured at day-5 which is about 97.6%. The efficient growth of microalgae cultivation was found in day-5 in proper sunlight conditions and by using CO2 through photosynthesis, while using wastewater effluent as a nutrient source.

Keywords: *Microalgae, Photobioreactor, Microalgal growth, Cultivation, Nutrients.*

1. INTRODUCTION

In the present world, good water quality is vital and essential for human health, social and economic development and additionally also for the ecosystem. As populations develop and natural environments become decrease, ensuring there are enough and secure water elements for everyone is becoming increasingly more challenging. In the last decades, big mounts of wastewaters have been produced, particularly because of anthropogenic activities, inclusive of agricultural practices, urbanization and industrialization (Aslan & Kapdan, 2006; Rawat, Kumar, Mutanda, & Bux, 2011). The continues disposal of waste waters without necessary treatment can pose extreme pollution. One of the major problems related to the non-stop discharge of effluents into water bodies is eutrophication phenomenon that is the enrichment of water sources in nutrients, specially enrichment with nitrogen and phosphorus.

Although primary and secondary wastewater treatment approaches easily mitigate settle particulate matters, and oxidize organic substances but cannot get rid of nutrients from wastewater. For mitigating nutrients, wastewater desires tertiary treatment. In that case, the cultivation of microalgae could be an effective tertiary biotreatment for wastewater because of microalgae are able to uptaking inorganic nitrogen and phosphorus for their growth. Microalgae have been at the focus of attention in current years as an alternative system for organic wastewater treatment with several applications in wastewater treatment (Kaya & Picard, 1996; Tredici et al., 1992).

Microalgae are photosynthetic organisms that assimilate nitrogen (N) and phosphorus (P) during their growth. They provide a manner for contaminants elimination (nitrogen, phosphorous and carbon) from wastewater while producing biomass that could locate use for the manufacturing of high-value chemicals (algae metabolites) and biogas through anaerobic digestion (Muñoz & Guieysse, 2006). It can also diminish the dangerous outcomes of sewage effluents and reduce eutrophication in good enough environments (Abdel-Raouf, Al-Homaidan, & Ibraheem, 2012). Wang et al. reported a decrease in nitrogen (83% N as NH_4^+) and phosphorous (90% P as PO_4^{3-}) in municipal wastewater by way of *Chlorella sp.*(Wang, Kuo-Dahab, Dolan, & Park, 2014). The main advantages of using microalgae as a source of biomass for biodiesel production are: excessive growth rates and short generation times, minimal land requirements, excessive lipid content, use of wastewater stream as nutrient fed with no need for chemicals. The resulting biomass can be used for the production of bioenergy, food, animal feed and prescription drugs and additionally an oxygenated effluent is discharged into water bodies (Aslan & Kapdan, 2006; Rawat et al., 2011) .

There are in particular two most important commercial cultivation systems for microalgae. One is open raceway ponds and some other is closed photobioreactors (Chinnasamy, Bhatnagar, Hunt, & Das, 2010; Chisti, 2007; Muñoz & Guieysse, 2006) . Light is one of the most vital parameters for the cultivation of microalgae and in this regard photobioreactor (PBR) has a top notch influence. Because, PBR use sunlight as a light source that may be effortlessly utilized in microalgae cultivation.

The important purpose of this study is to identify the best conditions for higher microalgae cultivation growth, suitable environments and removal performance of nutrients from wastewater effluent. The efficient growth of microalgae cultivation is mostly preferable at daylight conditions and by using the usage of CO_2 through photosynthesis.

2. METHODOLOGY

2.1 PBR Configuration

The PBR was constructed using locally available transparent water bottle having a capacity of 8000 cc. The transparent jar used for the cultivation of microalgae because of good capability of receiving sunlight. The schematic of the PBR system is shown in figure-1. The PBR system was setup in open space where sunlight can be easily absorbed by microbial culture. An air pump was installed to supply CO_2 in PBR during cultivation period as sunlight, water, nutrients and CO_2 are the primary requirements for microalgal growth. The system was operated by a timer, which was running for 10 min/hr during the 8 days of cultivation periods. The PBRs system were only run at day time during sunlight.

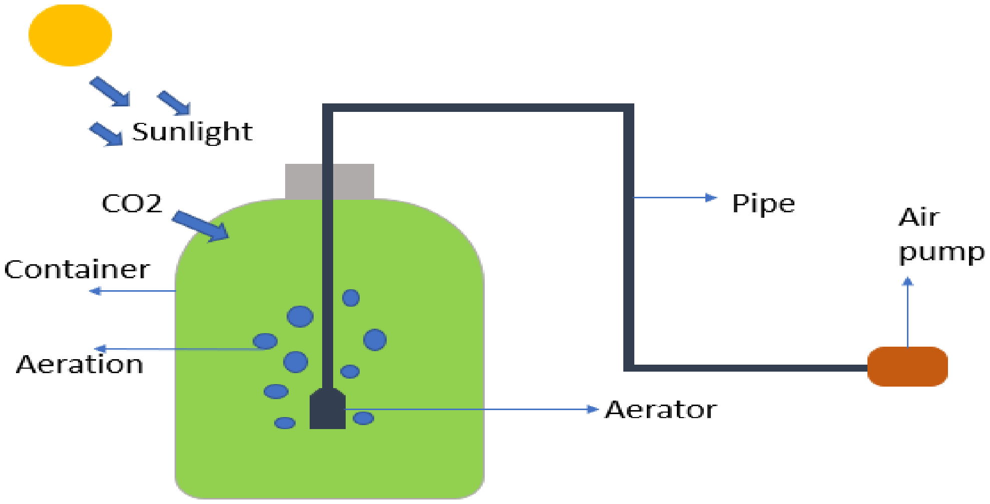


Figure 1: Experimental setup of the PBR system

2.2 Microalgae Cultivation

For the cultivation of microalgae, wastewater effluent was used as nutrients source. The seeds of *Chlorella vulgaris* were poured into the PBR with amount of 20 ml. *Chlorella vulgaris* was seeded due to good tolerance in saline water as the research (Khulna) is located near the coastal zone with high salinity. Moreover, many researchers have been reported that *chlorella vulgaris* have high lipid content.

Figure 2 : Cultivation of microalgae in PBR during experiment.

3. ANALYTICAL PROCEDURES

3.1. Sampling and Nutrients Analysis

A volume of 10 mL microalgae suspension was collected every day from each PBR for nutrient removal analysis starting from inoculation. Then, the samples were appropriately diluted and analyzed for COD, P^H, DO, ORP, Turbidity, NO_2^-, NO_3^-, NH_3^-, PO_4^- by the Spectrophotometer.
(Hach, 2008) The percentage of removal was obtained using the following expression:

$$Percentage\ removal\ W\% = 100\% * (C_o - C_i)/C_o \qquad (1)$$

Where, C_o and C_i are defined as the mean values of nutrient concentration at initial time t_0 and time t_i, respectively.

3.2. Determination of Microalgae Growth

A correlation between the optical density(od) of *Chlorella sp.* at 680 nm and the dried biomass was pre-determined. od_{680} was measured every day using spectrophotometer (Jingke 722N, Shanghai, China). The correlation is shown below:

$$Dry\ weight\ (mg/L) = 3.387 * od_{680}; \qquad R^2=0.9913 \qquad (2)$$

The specific growth rate y in the exponential phase of algal growth was measured by using following equation (Issarapayup, Powtongsook, & Pavasant, 2009):

$$y\ (day^{-1}) = \ln(N_2/N_1)/(t_2 - t_1) \qquad (3)$$

Where, N_1 and N_2 are defined as dry biomass (mg/L) at time t_1 and t_2, respectively. The biomass productivity (P) was calculated according to the following formula:

$$P = (DW_i - DW_0)/(t_i - t_0) \qquad (4)$$

Where, DW_i and DW_0 are dry biomass (mg/L) at time t_i and t_0 (initial time), respectively.

4. RESULT AND DISCUSSION

4.1. Wastewater Characteristics

Some parameters wastewater effluent like P^H, DO, ORP, Turbidity, NO_2^-, NO_3^-, PO_4^-, COD were measured before microalgae cultivation which is summarized in table-1.

Table 1: Characteristics of wastewater effluent

Parameters	Units	Value
P^H	-	8.08
DO	mg/l	5.0
ORP	mV	88
Turbidity	NTU	19
NO_2^-	mg/l	0.012
NO_3^-	mg/l	0.2
NH_3^-	mg/l	25.2
PO_4^-	mg/l	7.2
COD	mg/l	20

After pouring the seeds of *chlorella vulgaris* into the PBR system the system was running for consecutive 8 days. For the analysis of nutrients removal performance and calculation of microalgal growth the others parameters (such as P^H, DO, ORP, Turbidity, NO_2^-, NO_3^-, PO_4^-, COD and algae concentration) were observed every day during the cultivation periods, which is summarized in table-2.

Table 2: Monitoring parameters during the cultivation periods

Parameters	**Units**	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Day-6**	**Day-7**	**Day-8**
P^H	-	8.08	8.47	8.59	9.57	10.0	10.22	10.3	9.96
DO	mg/l	5.0	7.2	8.1	8.3	6.8	8.0	8.6	7.4
ORP	Mv	88	214	219	111	119	98	91	93
Turbidity	NTU	19	16.2	10.3	27.8	57.8	18.6	10.8	10.8
NO_2^-	mg/l	0.012	0.014	0.037	0.048	0.014	0.023	0.031	0.022
NO_3^-	mg/l	0.2	0.4	0.2	0.022	0.2	.04	0.4	0.1
NH_3^-	mg/l	25.2	24.6	20.1	15.4	14.84	11.48	8.6	4.91
PO_4^-	mg/l	7.2	0.3	0.3	0.51	0.17	0.17	0.17	0.16
COD	mg/l	20	131.7	124.4	117.12	224.48	114.68	102.5	12.2
Algae Conc.	mg/l	0.135	0.112	0.152	0.586	0.758	0.285	0.132	0.179

4.2. Microalgal Biomass and Growth Rate

The concentration of micralgal biomass is converted into weight from optical density by equation-2. From the experimental data which is showen in table-2, the maximum growth of microalgae is found out at day-5 and the amount is about 0.758 mg/l . In Fig-3, at first microalgal biomass is increased with time. But after day-5, the microalgal biomass growth started to reduce.

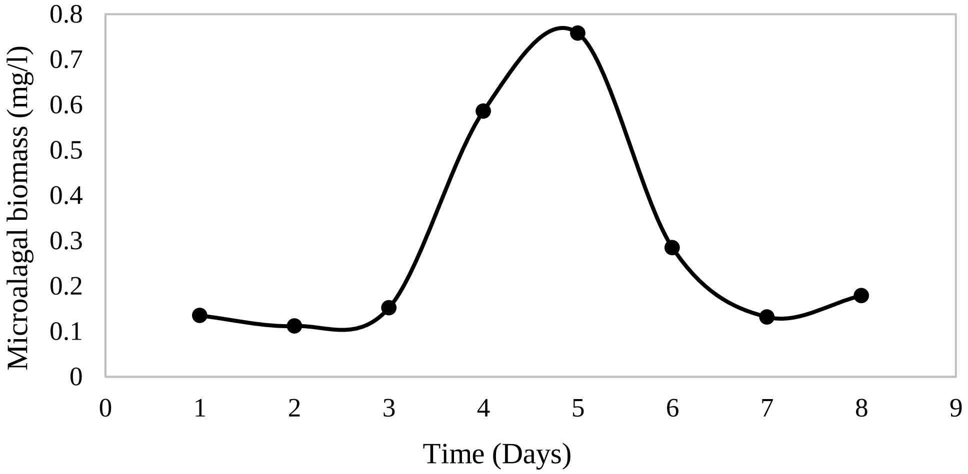


Figure 3: Microalgal biomass (mg/l) vs Time (Days) Graph.

Because , after day-5 the microalgae started to settle into the water bodies. As a result, the concentration of microalge started to reduce. The mean growth rate is found after day-8 is about 0.0353 mg/l using the equation-3. The mean biomass productivity of the PBR system is about 0.0409 mg/l using the equation-4.

4.3. Percentage of Phosphate Removal

Microalgae also removes the nutrients from the wastewater. Microalgal biomass use these nutrients as like the nutrients source for the photosyenthesis process. The mean removal percentage of phosphate is about 96.5%. The highest phosphate removed at day-5 which is around 97.6% because of the highest microalgae biomass. It has been found that , at day-5 microalgae biomasss grew the most. As a result, more phosphate used for microalgal growth which increases the removal percentage of phosphate.

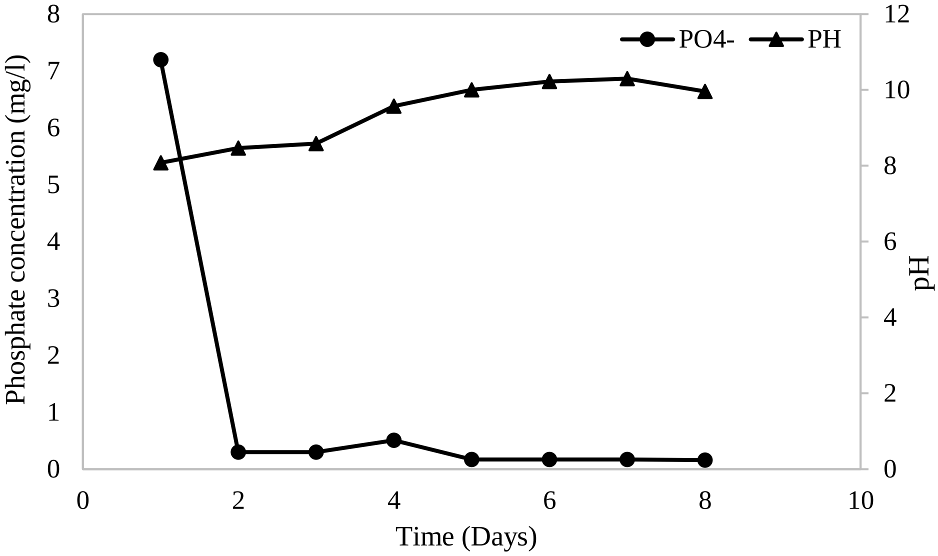


Figure 4: Relation between pH and Phosphate (mg/l).

Fig-4, showing the variation of pH and Phosphate concentration with the variation of time. For pH values above 8.0 and high oxygen concentrations, phosphorus precipitation may occur (Su, Mennerich, & Urban, 2012; Wang et al., 2014). From the Fig-4, it can be seen that, when the pH is larger than 8 the phosphate decreased. When the microalgae is most in the wastewater the phosphate found around 0.17 mg/l only (table-2). Because, most of the phosphate used in photosynthesis process for producing ATP and precipitated in waterbodies

4.4. Percentage of Nitrogen Removal

Microalgae also removed nitrogen from the wastewater. The mean removal percentage of nitrogen is about 84%. Most of the nitrogen moves when the microalgae grew most which is at day-5. The main reason for this is also the highest microalgal biomass growth at day-5.

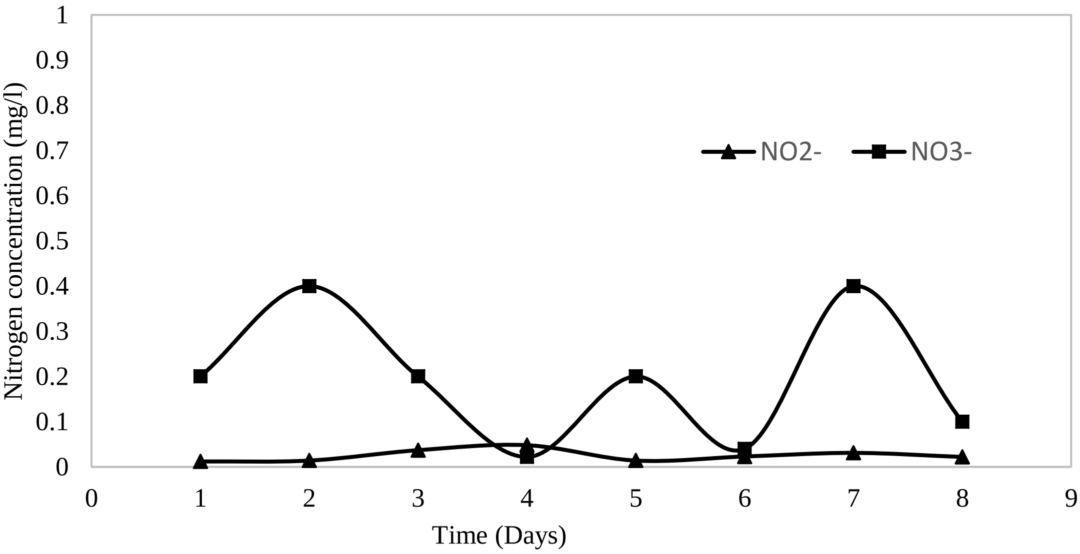


Figure 5: Nitrogen concentration (mg/l) Vs Time (Days) Graph.

Fig-5 shows that, the concentration of the NO_2^- and NO_3^- in wastewater. Microalgae biomass growth has less effect on nitrate (NO_2^-) whereas it has a great influence on nitrite (NO_3^-). Most of the nitrite removes at day-4 and day-6 when the wastewater has a large number of microalgae. After that, the nitrite increase because of the settlement on microalgae. Fig-6 shows that, the ammonia removes with the increase of microalgal biomass and time. The average ammonia removal is about 70% and removes about 18% of nitrogen from wastewater.

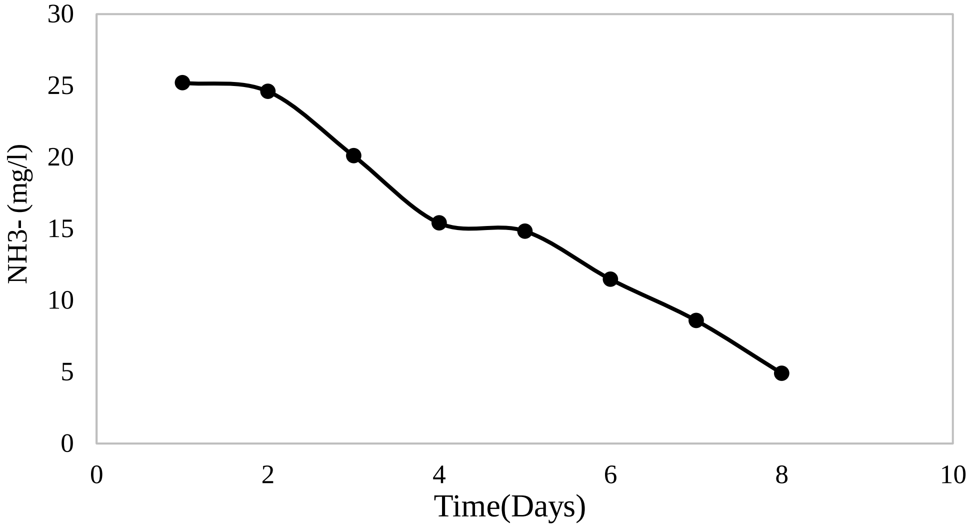
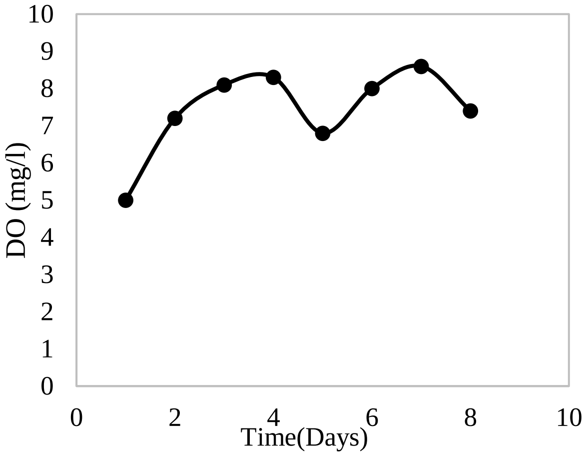


Figure-6: Ammonia removal (mg/l) vs Time (Days) Graph.

4.5. DO and ORP Variation

DO (Dissolved Oxygen) and ORP (oxidation reduction potential) also varies with the amount of microalgae present in wastewater. In Fig-7, the DO is increased with time of microalgae cultivation. The DO increase because of the microalgal biomass. Microalgae produce oxygen by photosynthesis process using carbon dioxide from air. But DO reduced at day-5 because of starting the settlement of microalgae. ORP also varies with microalgal biomass which produces oxygen in water by photosynthesis process (Fig-8).



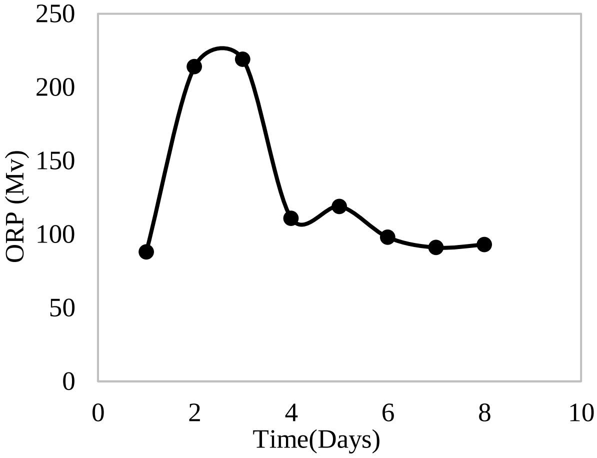


Figure-7: DO (mg/l) vs Time (Days) Graph.

Figure-8: ORP (Mv) vs Time (Days) Graph.

4.6. Turbidity

The turbidity of wastewater effluents varies with the increase of microalgae biomass. In Fig-9, the turbidity of wastewater increases most when the wastewater has the most microalgal biomass in the water which is at day-5. The turbidity is increased about 46% at day-5 comparing with Day-1.

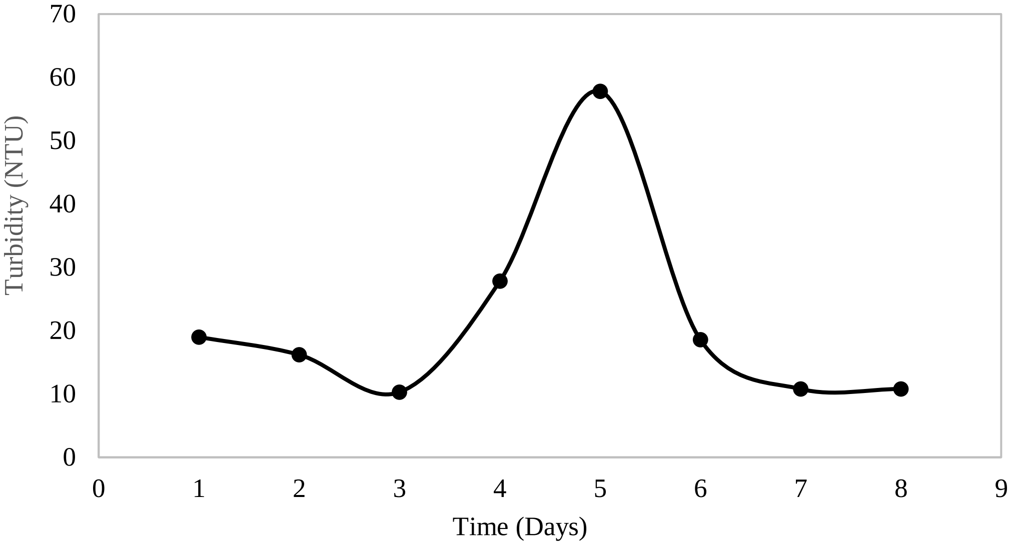


Figure-9: Turbidity (NTU) vs Time (Days) Graph.

4.7. COD Variation

The chemical oxygen demand (COD) also varies with time and the presence of microalgal biomass in wastewater. In Fig-10, COD increased with time when the microalgal biomass increasing. But after day-5, when the microalgae started to settle down the values of COD also started to decrease.

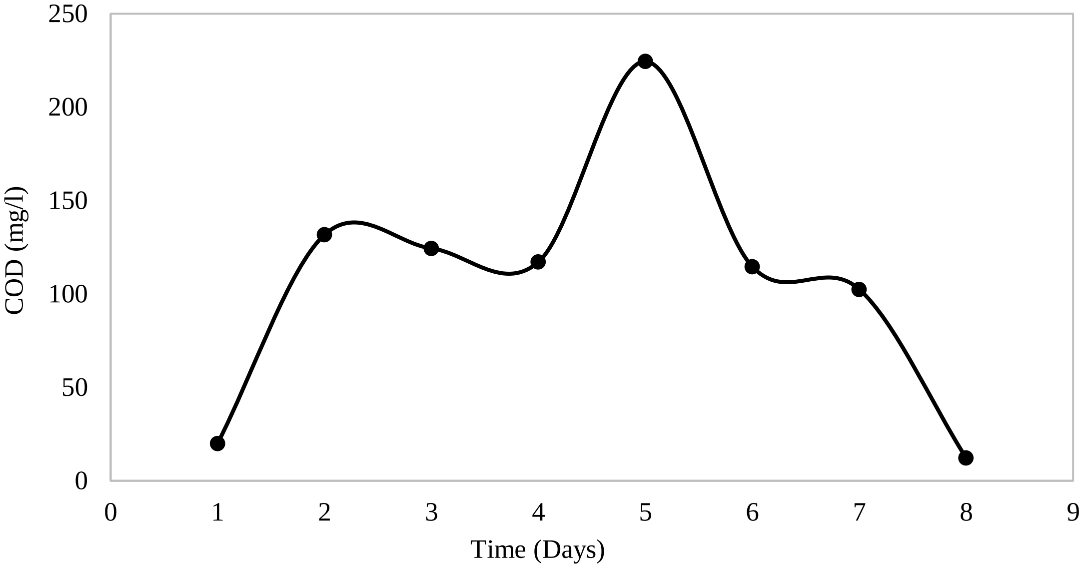


Figure-10: Chemical Oxygen Demand (COD) (mg/l) vs Time (Days) Graph.

5. CONCLUSIONS

In this paper, the proportion of dissolved nitrogen and phosphate removal, microalgal growth rate and productiveness had been studied. A clear information of anaerobic system mechanisms is required with the intention to improve PBR performances in terms of biomass productiveness and wastewater treatment. The percentage of nitrogen removal is 84% in the form of nitrate and nitrite. Ammonia stripping seems one of the mechanisms liable for nitrogen elimination, contributing to as a minimum 18% of N-removal. Nitrification changed into also a pathway for ammonium transformation in our experimental system. Around 96% of phosphate ions have been removed from the wastewater after 8 days. The highest rate of phosphate removal was measured at day-5 which is about 97.6%. The values of DO, ORP, Turbidity also vary with time, microalgal presence and concentration in wastewater effluents. As a conclusion, microalgal treatment of wastewater, via tertiary wastewater treatment mechanisms, may want to constitute an attractive addition to current biological treatments used to purify wastewaters. The advantages of the use of microalgae as a source of biomass for biodiesel production, excessive growth rates and quick generation times, minimum land requirements, excessive lipid content, use of wastewater stream as nutrient fed with no need for chemicals. The resulting biomass may be used for the production of bioenergy, food, animal feed and pharmaceuticals and also an oxygenated effluent which can discharge into water bodies.

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