Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant

Rawiwan Boonchai, Gyu Tae Seo, Da Rang Park, and Chung Yeol Seong

***Abstract*—To determine the performance of microalgae photobireactor for advanced wastewater treatment and microalgal biomass production, *Chlorella vulgaris* was cultured with final effluent from sewage treatment plant in batch condition. The average specific growth rate was 0.103 d^{-1} because low P concentration inhibited algal growth. 60% of N and P concentrations were removed from the system in 2 days. When the system operated under a semi-continuous condition with hydraulic retention time (HRT) 2 days, the microalgae showed growth rate of 0.452 d^{-1} and 0.277 d^{-1} in primary effluent and final effluent, respectively. 30% of N and 53% of P were removed from primary effluent and 44% of N and 84.2% of P were removed from final effluent. These results suggest that semi-continuous mode offers higher biomass production and appropriate HRT were needed for high N and P removals.**

***Index Terms*—Nitrogen and phosphorus removal, microalgae, photobioreactor, wastewater.**

I. Introduction

Conventional technology has been widely applied in wastewater treatment. The process removes most of the organic contaminants, nitrogen (N) and phosphorus (P) in the wastewater. However, the secondary effluent still contains high level of N and P, which may cause eutrophication and water pollution. To avoid eutrophication, South Korean government has listed the effluent quality standard as Total Nitrogen (TN): 20mg/L, Total Phosphorus (TP): 2mg/L, and TP trends to be lower than 0.2 mg/L in the near future. To meet this requirement, microalgae can utilize nitrogen and phosphorus from wastewater for their growth. Moreover, microalgae can also fix carbon dioxide from atmosphere as in photosynthesis, thus reducing green house gas emission. Also, microalgal biomass can be used for biofuel which is considered as renewable energy.

So far, more research was done on microalgal culture for lipid production for biodiesel. Currently, there is little research focus on nitrogen and phosphorus removal using real wastewater in their experiments. The main reason for this may be due to the difference and variety in compositions. Therefore, investigations on the characteristics of nutrients removal by microalgae, and real effluent wastewater are needed.

The N/P ratio and initial nutrient concentration are considered to be the significant factors that affect algal growth and nutrient removal efficiency [1]-[5]. The optimal N/P ratio for *Chlorella vulgaris* was 8 [6]. Moreover, the elemental composition of Chlorella also changes at different nutrient concentration [7], [8]. In order to enhance N, P removal efficiency, cultivation of microalgae had been carried out in photobioreactors which offer high illumination surfaces, high mass transfer rates, reducing contaminants and requiring small space. However, to apply microalgae photobioreactor more practically as a tertiary treatment, a process needs to be considered certain hydraulic retention time (HRT) and to run the system in a continuous process started up in a batch or semi-continuous mode [9].

The propose of this study was to determine the performance of microalgae photobioreactor operating in batch and semi-continuous conditions using real wastewater from sewage treatment plant by analysis cell growth, N, and P removal efficiency.

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II. Material and Methods

A. Microalgae and Culture Medium

A species of microalgae *Chlorella vulgaris* KMMC 9 from Korean Marine Microalgae Culture Center was used in this study. The algae were kept in BG11 medium [10] as follows: $NaNO_3$1.5 g/L, K_2HPO_4 0.04g/L, $MgSO_4.7H_2O$ 0.075g/L, $CaCl_2.2H_2O$ 0.036 g/L, NA_2CO_3 0.02g/L, Citric acid $C_6H_8O_7$ 0.006 g/L, $Fe(NH_4)3(C_6H_5O_7)_3$ 0.06 g/L, EDTA 0.001 g/L , trace elements A_5 1 ml/L containing H_3BO_3 2.86g/L, $MnCl_2.4H_2O$ 1.81g/L, $ZnSO_4.7H_2O$ 0.22 g/L, $Na_2MoO_4.2H_2O$ 0.39 g/L, $CuSO_4.5H_2O$ 0.08g/L and $Co(NO_3)2.6H_2O$ 0.05g/L. The medium was adjusted to have a pH of 7.1 before autoclaving. Two weeks after inoculation, microalgae were centrifuged at 2000 x g for 5 minutes. Then, the cells were washed twice with distilled water.

B. Characteristic of Wastewater Used in This Study

Primary effluent and final effluent (after UV radiation treatment) from sewage treatment plant in Jinhae, South Korea, were used. For all experiments, we attempted to remove microorganisms and particles from real effluent before using it as a culture medium by filtration (Whatman GF/C).

TABLE I: Initial Average Values of TN and TP in Primary and Final Effluent.

	TN	TP	pH
Primary effluent	38.76±2.5	3.17±0.5	6.69
Final effluent	24±9.7	0.68±0.5	6.75

C. Photobioreactor Design

The 10-L photobioreactors were set as a bubble column

type reactor and made of 7-mm thick transparent PVC. The inner diameter for the inside pipe and the outside pipe were10 and 20.6 cm, with their 50-cm height. Two of 20W fluorescent lamps were placed inside the inner pipe at a distance of 5 cm from the culture column providing white light around 50 μmol/m^2·s. The light:dark cycle of 14:10 was controlled by a timer. Air was provided continuously for mixing without additional CO_2 source. On top of the reactor was ports for degassing, pH, temperature probes and refilling port. The pH was buffered with air supply in the range of 7.5-8.5. The photobioreactors were kept in a temperature of 26±3℃.

D. Batch Culture

Microalgae were inoculated with a cell concentration of 140 mg/mL to final effluent 8L. The Air was aerated with flow rate of 10 L/min. The experiment lasted 7 days. The cell growth in batch culture experiment was monitored by dry weight. The biomass dry weight was measured by separating the cells from culture by membrane filtration (Whatman GF/C). The filter membrane was pre-weighted and then dried to a constant weight at 105˚C for 24 hours.

E. Semi-Continuous Culture

Microalgae were inoculated to reach initial cell density 2.5 x 10^6 cells/mL with a working volume of 5L ran under the same conditions as the batch culture except for the 5 L/min of air flow which was employed to the photobioreactors. The first 2 days, microalgae were incubated without wastewater replacement (pre-cycle). While the microalgae were in the exponential phase, half of the culture (2.5L) was separated from the microalgae for water analysis, then, the microalgae were returned to the reactor, and finally, fresh wastewater was added to maintain HRT 2 days. The operation ran for 8 days. Two bioreactors were set, one with primary effluent and another one with final effluent. Cell growth was measured by cell counting.

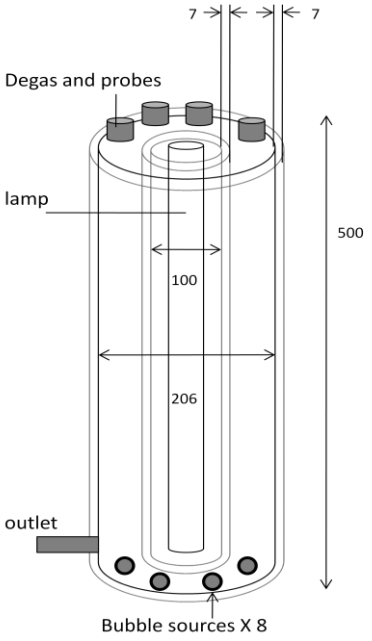


Fig. 1. Schematic view of the bubble column photobioreactor.

F. Analytical Method

The specific growth rate (μ, d^{-1}) was expressed as

$$\mu\,(-d^{-1}) = \frac{\ln(X2 - X1)}{t2 - t1}$$

where $X1$ and $X2$ represent dry weight or cell number at time $t1$ and $t2$, respectively.

Algae culture were filtered through 0.45 μm syringe filters everyday or every two days. The filtered water was taken for TN and TP analysis.

III. Results and Discussion

A. Algal Growth under Batch Culture Condition

As show in Fig. 2, a lag phase could not be observed in the growth curve, indicating that *C. vulgaris* can adapt well in final effluent. The microalgae cell density increased with culture duration. After 8 days of cultivation, maximum cell density was reached at 320 mg/L and then went to the death phase. In the exponential phase, the specific growth rate was 0.103 d^{-1}, considered low compared to *C. vulgaris* in final effluent which was 0.186 d^{-1} [11]. The average specific growth rates of *Chlorella* sp. in wastewater before primary settling, wastewater after primary settling, wastewater after aeration tank and wastewater from sludge centrifuge were 0.412, 0.429, 0.343, and 0.948 d^{-1} respectively [12]. The main reason of slow growth because N/P ratio in final effluent used in this study was considerably much higher (*N*/*P* ratio 192.6) compared to the *N*/*P* ratio in other studies. However, the growth rate was comparable to [13], where a membrane photobioreactor operated using *Scenedesmus* sp. under low N and *P* concentration (*P* < 1.5 mg/L, *N* < 20 mg/L) and the growth rate was 0.09 d^{-1}.

B. Removal of N and P under Batch Condition

During microalgae cultivation, TN and TP concentrations in the liquid culture decreased with differing patterns (Fig. 3). TN concentration initially decreased rapidly from 28.7 mg/L to 11 mg/L which meant that N removal efficiency was 61.7% within 2 days. Then it went to stabilization. After 7 days of operation, TN concentration of 10.44 mg/L remained in the reactor indicating that nitrogen could not be further removed from the effluent when low TP concentration was induced.

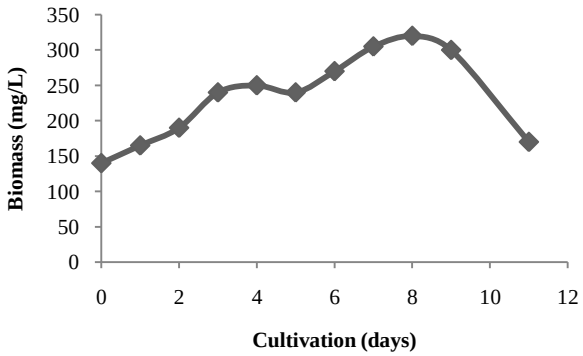
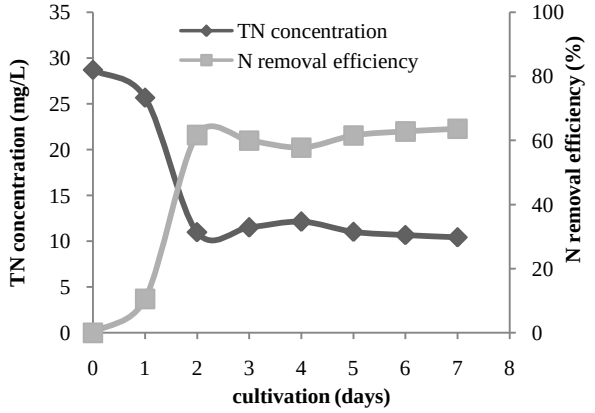


Fig. 2. Growth curve of microalgae in final effluent.

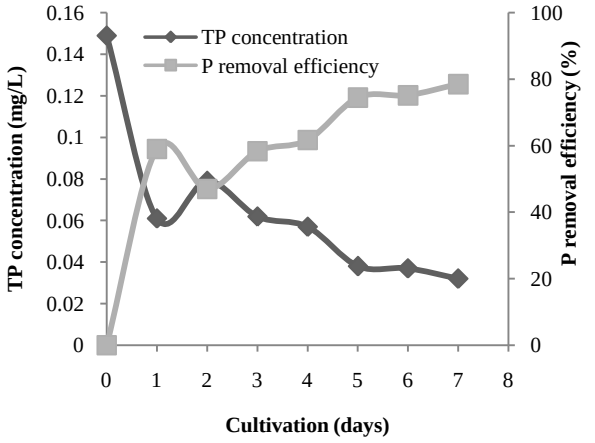
The N removal efficiency on day 7 was 63.62%. TP concentration decreased quickly from 0.149 mg/L to 0.061 mg/L as *P* removal efficiency of 59% within only 1 day. Then, it slightly decreased to 0.032 mg/L until day 7 which the *P* removal efficiency was 78.52%. The *P* removal rate was 0.017 mg/L/d. indicating that there was not enough *P* to support algal growth. Thus, low *P* concentration in the

effluent was the main factor that inhibited microalgae cell growth and *N* removal efficiency. The result compared to other work such as [14], reported that the removal efficiency of TN and TP from secondary effluent (low TN, low TP) by *C. ellipsoidea* YJ1 achieved 99% and 95%, respectively. Moreover, *C. zofingiensis* in *N* deficiency and P deficiency conditions and found that biomass productivity of *C. zofingiensis* in *P* deficiency condition was much lower than those under *N* deficiency [15].

In this study, *N* and *P* removal efficiencies reached 60% within short HRT compared to [16], which took 4 days to achieve removal efficiency of approximately 62% *N* and 55% *P* from septage wastewater by *C.vulgaris*. Also, *C. vulgaris* removed half of nitrogen from wastewater effluent in a batch mode in 2 days [17].



(A)



(B)

Fig. 3. TN concentration and N removal efficiency (A), TP concentration and P removal efficiency (B).

C. Algal Growth under Semi-Continuous Condition

Since *C. vulgaris* fed by final effluent in batch condition seems to be inhibited by an unbalanced N/P ratio and limited phosphorus. The result suggests that *C.vulgaris* could show better growth in a different source of wastewater which offers a higher nutrient concentration or that operates the culture in a semi-continuous condition when fresh medium is added. In this study, the experiments were carried out for 8 days where the first 2 days is pre-cycle for microalgae to reach the exponential phase. From the result in batch condition, this period could allow *C. vulgaris* to remove 60% of both N and P.

Primary effluent from sewage treatment plant contains various kinds of organic compounds such as fatty acids, amino acids and carbohydrates which can be utilized for mixotropic and heterotropic growths by algae [18]. As seen on Fig. 4, *C. vulgaris* treated with primary effluent reached maximum cell density of 9.7×10^6 cells/mL within 3 days, faster than those obtained in final effluent (5 days). This might be because primary effluent contains nitrogen in ammonium form preferred by algae. The specific growth rates in the exponential phase were 0.452 d^{-1} and 0.277 d^{-1} in primary effluent and final effluent, respectively. However, after 4 days of treatment, algae cell density in primary effluent was decreased rapidly similar to algae density in final effluent which decreased after day 7. The results suggested that the death of algae could have been caused by increasing the number of heterotopic bacteria and toxicity in wastewater, which inhibited or competed with microalgal growth.

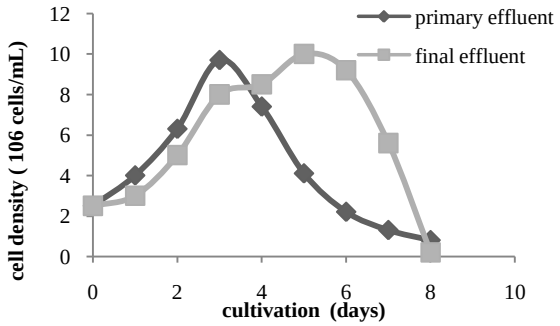
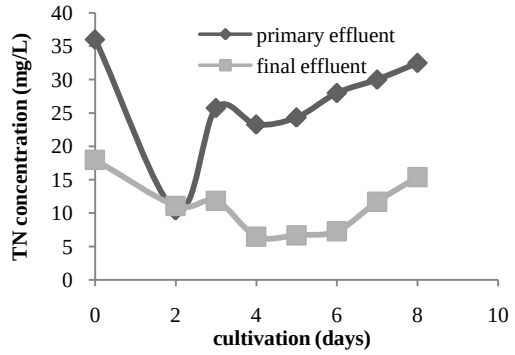
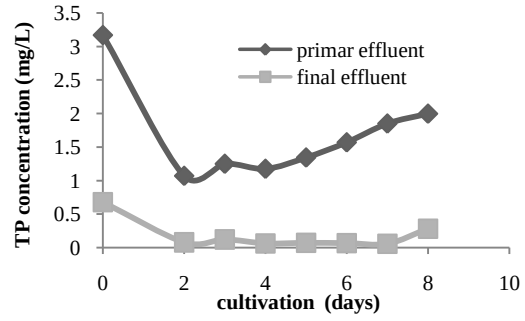


Fig. 4. Growth curves of microalgae in semi-continuous mode.



(A)



(B)

Fig. 5. TN concentration and N removal efficiency compare between primary and final effluent (A). TP concentration and P removal efficiency compare between primary and final effluent (B).

D. Removal of N and P under Semi-Continuous Condition

From Fig. 5, different trends were observed for nitrogen

removal by *C. vulgaris* where in primary effluent. TN concentration decreased from 36 to 10.44 mg/L (71% N removal) and in final effluent TN concentration decreased from 17.97 to 11.10 mg/L (38% N removal) during pre-cycle. However, the average N removals were 30 % and 44% in primary and final effluent, respectively. The system showed lower N uptake in primary effluent even though microalgae were in the exponential phase, that could be occurred because HRT 2 days was too short for low initial density of microalgae ($2.5x10^6$cells/mL) to remove nitrogen. An initial inoculums algae culture of $1x10^7$cells/mL required shorter HRT for nutrient removal [19]. Culture in the final effluent, the N removal efficiency was quite stable until the end of the experiment.

In primary effluent, nitrogen could have been removed by ammonium stripping, considered significant because microalgal photosynthetic activity raised the system temperature to 27-29 ℃ and pH to 8.2- 8.5. However, the N removal trends matched the growth curves suggesting that most of the N removal mechanism was actually used for microalgal growth.

A similar trend was shown for P removal where the system had P removal from 3.17 to 1.07 mg/L (66% P removal) in primary effluent and 0.68 to 0.08 mg/L (88% P removal) in final effluent during pre-cycle. P uptake decrease in primary effluent (after 4 days) and final effluent (after 7 days) due to the decreasing of cell density in the reactors. The average P removals were 53% and 84.2% in primary and final effluent, respectively.

In addition, small particles in wastewater associated with microalgal cells, increased the specific gravity of the microalgal cells leading to their easier settlement and harvesting.

IV. Conclusion

In this study, microalgae photobioreactor operating in batch condition using final effluent, even though biomass production was low, photobioreactor reduced N and P up to 60% in 2 days. When operated in semi-continuous using primary and final effluent, biomass productions were greater than those in batch condition. About 30% of N and 53% of P were removed from primary effluent and 44% of N and 84.2% of P were removed from final effluent. To improve the removal of N and P, an appropriate HRT is needed.

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