

# Application of the Thin Flat-Plate Photo-Bioreactor to the Removal of Nitrate and Phosphate Using Marine *Nannochloropsis oculata*

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**Abstract**—Many environmental issues of marine pollution and its causes and consequences were often learned from News. Algae in the ocean play the major role in the conversion of carbon dioxide into oxygen. This study used  $\text{NaHCO}_3$  as a main carbon source to replace the two-percent-carbon-dioxide gas mixture that are often used in a large-scale algal field. The results show that the addition of 7.6 g/L  $\text{NaHCO}_3$  obtains the best algal productivity and yield of about 4 g/L and 0.52 g/L/day, respectively. The shake-flask test shows that the cheap and easy-handling  $\text{NaHCO}_3$  can be used as an alternative carbon source for replacement of  $\text{CO}_2$  gas mixture in cultivation of microalgae and maintain the culture of effectiveness. In the thin flat-plate photo-bioreactor (FPPBR), designed to reduce the distance of the light pass, can solve the traditional drawback in the limit of the effective transmittance of light when cultured concentration increased, thereby inhibiting the growth of algae. The fed-batch FPPBR operated during 432 hr could reach a maximum algal concentration of 7.1 g/L in association with the periodical removal of substrate and nutrients (nitrate and phosphate) every three days. Its capacity for nitrate and phosphate were 0.22 g/m<sup>2</sup>/day and 0.15 g/m<sup>2</sup>/day, respectively referred to about 0.5-1.0 acre required for one-day spill for using FPPBR in the sea or in the adjacent coast area for treatment of an accidentally emitted domestic wastewater.

**Index Terms**—Estuary pollution, *Nannochloropsis oculata*, flat-plate photobioreactor.

## I. INTRODUCTION

The marine pollution is often ignored because of the misconception that the ocean is large enough to provide an infinite assimilative capacity for pollutants or because the marine pollutants would go away to other countries. Many environmental issues of marine pollution and its causes and consequences were learned from the Magazines or News. In Taiwan, not only the Environmental Protection Agency but

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also the local Environmental Protection Bureau (EPB) of the City or County has taken an action on the cleaning of the sea shore and coastal area. Many projects working on the auditing of the marine pollutants were executed for many years. The results can be found in the website of every local Environmental Protection Bureau, for example, the website of the EPB in Hsinchu County [1].

In the ocean algae play the major role in the conversion of carbon dioxide into oxygen. In rivers and reservoir, the eutrophication such as algae bloom is caused by the high load of nitrogen and phosphorus into the surface water. Consequently, algae have the capability to treat nitrogen and phosphorus with the oxygen produced through the algal growth, if they can be adequately utilized with continuously removal of the accumulated biomass. Therefore, in this study, *Nannochloropsis oculata* was chosen and associated with a high efficient photobioreactor.

*Nannochloropsis oculata* grows on temperate waters at 10-35 °C (the maximum growth rate at 25-31 °C). It can tolerate the high salinity between 5-77 ppt (the maximum growth rate at 20-35 ppt) and the strong light intensity from 12000 lux to 30000 lux (the most efficient at 12000 lux).

The algal photobioreactors have been studied for a long period of time. The commercial large-scale cultivation prefers the autotrophic mode using bicarbonate for the algal growth, requires a large surface area for capture of the light, and prevents the contamination of microorganisms [2]. Therefore, the carbon source using  $\text{NaHCO}_3$  was considered in this study. Reference [3] reviewed many large-scale photobioreactor and classified them according to their efficiencies in mass transfer or light transmission. For example, (1) Open Pond is widely used because of its simple structure, but its major backs are on the poor mass transfer efficiency and the often-occurred interference by the weather [4]-[6]. (2) Flat-plate photobioreactor (FPPBR) was studied by Milner earlier in 1953. The major benefit is the high light utilization rate and mass transfer rate [7], [8]. (3) Tubular photobioreactor can be made with many modifications, such as horizontal [9], [10], spiral [11], vertical [12], Cone-shaped pipe [13], and inclined pipeline type [14], [15]. It has a very good mass transfer efficiency but poor light transmission, once the diameter of the pipe becomes large. (4) Vertical-column photobioreactor is designed to capture more light into the water phase, but light transmission becomes less when the scale in association with the diameter of column goes up [16]-[19]. (5) Internally-illuminated photobioreactor is designed to solve the problem of poor light transmission. However, it is only suitable for small scale of photobioreactor [20]. It becomes too complicated for set-up of light sources once the scale goes

up.

The purpose of this study was to determine the characteristics of the pre-chosen FPPBR under the autotrophic mode feeding  $\text{NaHCO}_3$  as carbon source. The resulting nitrogen and phosphorus removal were expected for cleaning the marine pollution in the sea, coastal area, or estuary.

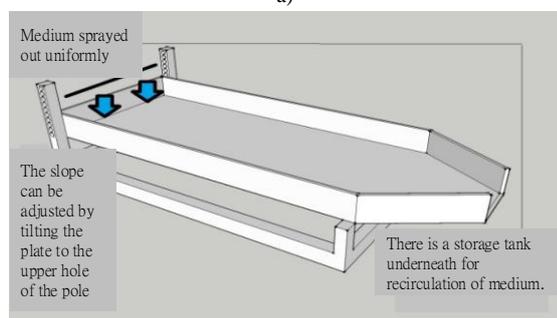
## II. MATERIALS AND METHODS

### A. Microbe

The culture of marine *Nannochloropsis oculata* was obtained from the Dr. Su, Huei-Meei at Tungkuang Marine Laboratory, Taiwan Fisheries Research Institute. The microscopical examination was shown in Fig. 1 a).



a)



b)

Fig. 1. *Nannochloropsis oculata*: a) the microscopical examination with a 200 × magnification, b) the schematic diagram of FPPBR.

### B. Culture Medium

The F/2 medium shown in Table I was chosen for growth of marine *Nannochloropsis oculata* [21]. It contained sterilized seawater which was made with 20 g/L of NaCl diluted into R.O. water and then sterilized at 121 °C and 1.2 kg<sub>f</sub>/cm<sup>2</sup> for 20 min. The stock solution of F/2 medium was normally stored in a 4 °C refrigerator and wrapped with aluminum foil before use. The Vit solution must be placed in an amber bottle. The trace metal solution and Vit solution were also stored in a 4 °C refrigerator.

### C. The Flat-Plate Photobioreactor

The steel-made FPPBR (30 cm × 100 cm) was arranged with a slope of 3 % (Fig. 1b). There was a 20-L plastic tank underneath for storage of the circulation medium. The circulation of fluid was conveyed through a submersed water pump in the tank. The flowrate were set at 4 or 8.5 L/min.

### D. Analysis

The algal concentration was obtained through the conversion of the algal optical density (O.D.) at 685 nm with the inversed calibration equation described by equation (1). The analysis of nitrate and phosphate was based on the methods of NIEA W436.51C and NIEA W427.53B, respectively, based on the Water Quality Standard Analysis Protocols announced by Environmental Analysis Laboratory, Environmental Protection Agency, Taiwan.

$$\text{Biomass Conc. (g/L)} = 0.517 \times OD_{685} + 0.0156 \quad (1)$$

$$R^2 = 0.995$$

## III. RESULTS AND DISCUSSION

### A. Microbial Characteristics

The environmental influencing factors were investigated through a series of shake-flask cultivation. They were optimal at pH of 8, at the initial substrate concentration of 7.6 g/L as  $\text{NaHCO}_3$ , and at the aeration rate of 0.15 L/min to keep algae suspended in a 20-L storage tank. Table II shows the comparisons of different carbon sources such as adding a 2%  $\text{CO}_2$  or 7.6-g/L  $\text{NaHCO}_3$  after 7-day cultivation. Both of them were validated to be the optimal concentrations for their own. According to the Table II, the results of microbial characteristics are comparable. Therefore, adding  $\text{NaHCO}_3$  of 7.6 g/L was selected for the following experiment to make sure the light intensity is a dominant operating factor without the interference of the mass transfer efficiency for  $\text{CO}_2$ .

TABLE I: THE COMPOSITION OF F/2 MEDIUM (PER LITER)

Composition	Amount	*Trace metal solution	Amount, g/L
$\text{KNO}_3$	0.075 g	EDTA $\text{Na}_2$	4.16
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	0.00565 g	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.15
*Trace metal solution	1 c.c.	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.01
**Vit solution	1 c.c.	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.022
**Vit solution	Amount, g/L	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.01
Cyanobalamin ( $\text{B}_{12}$ )	0.0005	$\text{Na}_2\text{MoO}_4 \cdot 6\text{H}_2\text{O}$	0.006
Thiamine HCl	0.1	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.18
Biotin	0.0005		

TABLE II: THE COMPARISONS OF THE ALGAL GROWTH BETWEEN ADDING 2%  $\text{CO}_2$  AND ADDING 7.6-G/L  $\text{NAHCO}_3$

Carbon Source	$\mu$ (day <sup>-1</sup> )	The maximum algal concentration (g/L)	The average algal productivity (g/L/day)
$\text{CO}_2$	0.301	4.23	0.55
$\text{NaHCO}_3$	0.348	4.08	0.52

### B. The Effect of the Flow Depth or the Water Circulation Rate on the Growth of *Nannochloropsis oculata*

The experiments for different flow depths (0.2 cm and 0.5 cm) on the surface of the FPPBR under the light intensity of 15000 lux were performed by adjusting the water circulation rate to 4 and 8.5 L/min, respectively. If the nutrient supply is limited due to a high demand in algal growth, the higher water circulation rate will provide more nutrient per time to improve the growth condition. However, the thicker flow depth caused by the higher water circulation rate will have a less light absorbance efficiency if the algal concentration is kept the

same. The results are shown in Fig. 2. Obviously, in this case, the higher algal concentration of 4.42 g/L was achieved at the water circulation rate of 8.5 L/min (Fig. 2 a), due to the nutrient limitation occurred at the lower water circulation rate of 4 L/min (Fig. 2 b) and Fig. 2 c).

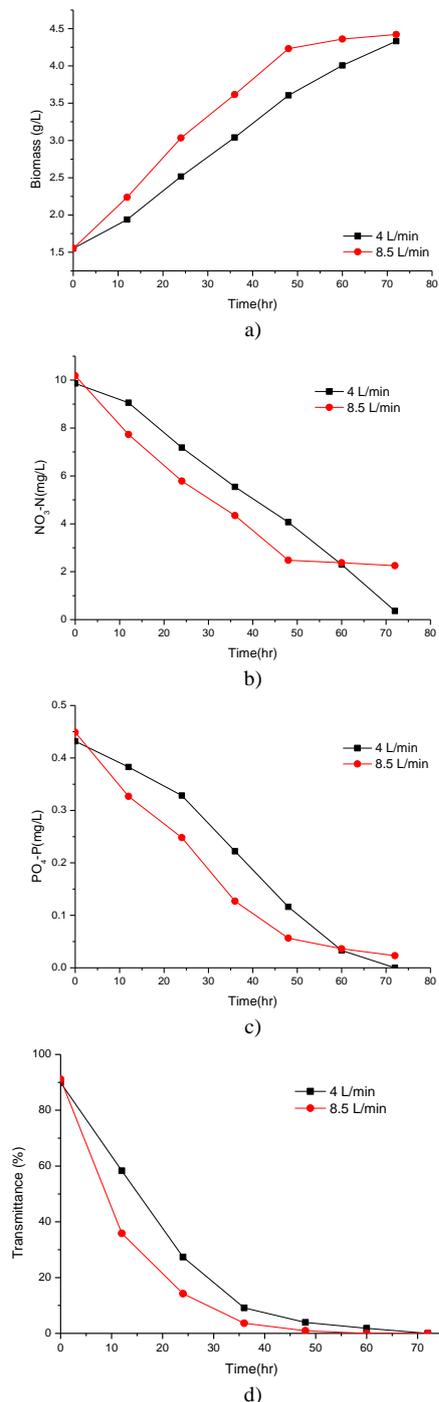


Fig. 2. The time courses of the performance of FPPBR under different water circulation rates between 4 and 8.5 L/min: a) the growth of *Nannochloropsis oculata*, b) the nitrate concentration, and c) the phosphate concentration, d) the transmittance of light passing through the water.

For both of them, before nutrients were completely consumed, the algal concentration increased faster during the period between the first 12 to 48 hr with the specific growth rate of 0.414 and 0.424 day<sup>-1</sup> for the water circulation rate of 4 and 8.5 L/min, respectively (Table III). In the end of the experiment, algal concentration stopped increasing earlier at the water circulation rate of 8.5 L/min, indicating the higher

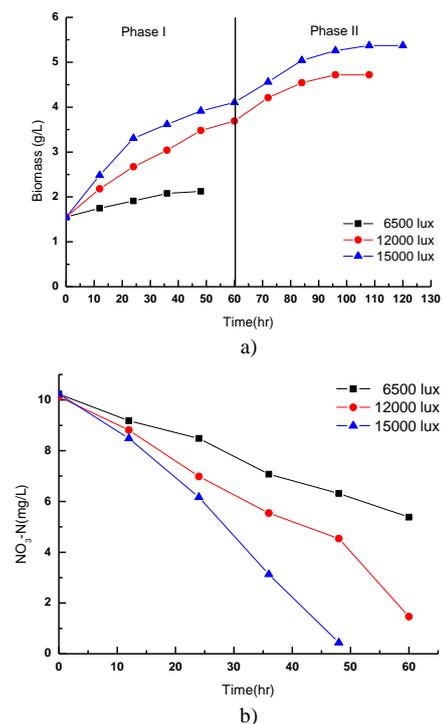
algal biomass blocked the light traveling path through the water. There is an evidence from Fig. 2 d). The transmittance decreased immediately after the experiment started for the water circulation rate of 8.5 L/min.

TABLE III: THE PERFORMANCE IN THE GROWTH OF *NANNOCHLOROPSIS OCULATA* UNDER VARIOUS WATER CIRCULATION RATES

The medium recirculation rate (L/min)	$\mu$ (day <sup>-1</sup> )	The maximum algal concentration (g/L)	The average algal biomass productivity (g/L/day)
4	0.414	4.33	0.93
8.5	0.424	4.42	0.96

### C. Effect of the Light Intensity on the Growth of *Nannochloropsis oculata*

Experiments were also done in the FPPBR under the water circulation rate of 4 L/min. The light intensity was regulated by adjusting the distance of the light source, for example, 5 cm for 15000 lux, 15 cm for 12000 lux, and 45 cm for 6500 lux on the surface of the FPPBR. The initial algal biomass was 1.5±0.03 g/L. The growth of *Nannochloropsis oculata*, and the removal of nitrate and phosphate nutrients are shown in the Fig. 3. The higher the light intensity, the higher the algal concentration was obtained (Fig. 3 a). However, the more algal biomass, the higher the demand on the nutrients of nitrate and phosphate. According to the Fig. 3b and 3c, the nitrate and phosphate were used up in 48 hr for the highest light intensity of 15000 lux. This is the reason why the biomass was accumulated slowly in the end of experiment. It is also proved by the following addition of the nutrients to the system in 60 hr. The algal concentration for the light intensity of 15000 lux increased again from the first plateau of 4.1 g/L to 5.4 g/L. On the opposite side, the maximum algal concentration for light intensity of 6500 lux was only 2 g/L. Much more nutrients were remained in 60 hr. It indicates that the light intensity is a key factor on the removal of the nutrient of nitrate and phosphate.



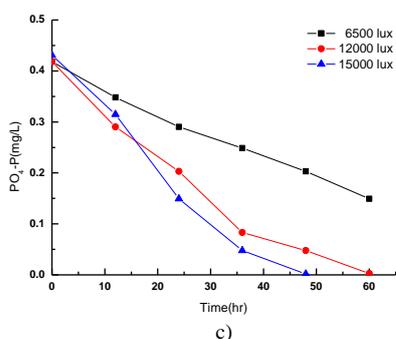


Fig. 3. The time courses of the performance of FPPBR under various light intensity values from 6500 lux to 15000 lux: a) the growth of *Nannochloropsis oculata*, b) the nitrate concentration, and c) the phosphate concentration.

The algal concentration and biomass productivity was calculated in Table IV. It indicates that the light intensity of 12000 lux was more efficient and comparable to the light

intensity of 15000 lux. In addition, the transmittance of light in the FPPBR was zero (Table IV) for the light intensity of 15000 lux, indicating the algal growth in the flow depth of 0.2 cm was limited by the amount of light absorbance when the incident light intensity was above 12000 lux.

TABLE IV: THE PERFORMANCE IN THE GROWTH OF *NANNOCHLOROPSIS OCVLATA* UNDER VARIOUS INCIDENT LIGHT INTENSITY VALUES

The incident light intensity (lux)	$\mu$ ( $\text{day}^{-1}$ )	The maximum algal concentration (g/L)	The average algal biomass productivity (g/L/day)	The transmittance of light in the FPPBR during 120 hr (%)
15000	0.376	5.37	0.76	0
12000	0.332	4.72	0.7	6.1
6500	0.173	2.12	0.29	28.9

TABLE V: THE ESTIMATED REQUIRED AREA FOR FPPBR TO TREAT AN EFFLUENT OF DOMESTIC WASTE WATER TO MEET THE STANDARD OF EFFLUENT WATER QUALITY IN TAIWAN<sup>#1</sup>

Target Pollutants	Concentration to be removed (mg/L)	Flowrate (CMD)	Mass flow rate (g/day)	Capacity <sup>#2</sup> of FPPBR (g/m <sup>2</sup> /day)	Estimated area for one day spill (m <sup>2</sup> )
NO <sub>3</sub> -N	30	250	7500	0.22	11364
PO <sub>4</sub>	6	250	1500	0.15	5000

<sup>#1</sup>The standard of effluent water quality in Taiwan is 50 mg/L for NO<sub>3</sub>-N and 4 mg/L for ortho-phosphate.

<sup>#2</sup>Capacity for nitrate = 10 mg/L\*20 L/3 days/(0.3 m×1.0 m) and Capacity for ortho-phosphate = 1.5 mg/L\*20 L/2 days/0.3 m<sup>2</sup>\*(31+64)/31

#### D. The Fed-Batch Cultivation of *Nannochloropsis oculata* Using FPPBR

The FPPBR was designed for improvement of the conventional photobioreactor that has the major drawback on penetration of light into the deeper place of the reactor when the algal biomass was significantly accumulated. Although, the operating conditions of the incident light intensity and the water circulation rate were tuned already for the FPPBR, the nutrients were always insufficient. Nevertheless, there is a place that can provide the adequate substrate and nutrients periodically. That is the estuary of a river. The better way to simulate the cleaning process in the estuary of the river is doing the fed-batch reaction in FPPBR. The substrate of NaHCO<sub>3</sub> was provided again whenever the biomass concentration increased slowly and maintained at least for 12 hr, and the F/2 medium was replaced constantly every 60 hr. Since *Nannochloropsis oculata* was kept suspended in the medium, it was centrifuged twice at 1800 rpm for 5 min in 50-mL Eppendorf tubes using a High Speed Refrigerated Centrifuge, and then the supernatant was replaced with the new F/2 medium.

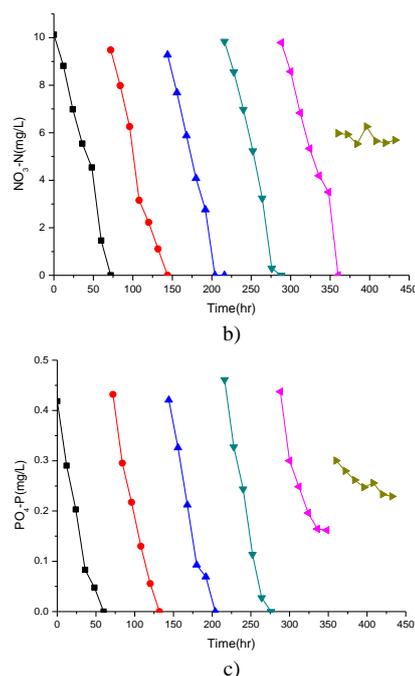
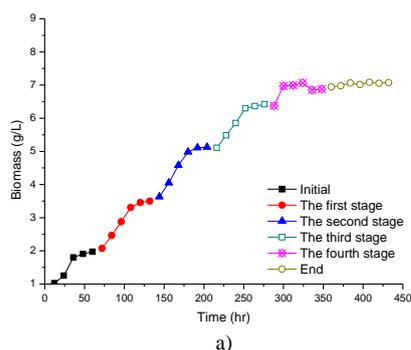


Fig. 4. The time course of the performance of the fed-batch FPPBR under the light intensity of 12000 lux and the water circulation rate of 4 L/min: (a) the concentration of *Nannochloropsis oculata*, (b) the removal of nitrate, and (c) the removal of phosphate.

The performance of FPPBR in a fed-batch mode were resulted in the accumulation of algal biomass reaching a maximum algal concentration of 7.1 g/L in association with the periodical removal of substrate and nutrients (nitrate and phosphate) every three days (Fig. 4). It demonstrated that the algal system can maintain functioning in the FPPBR during 432 hr, almost for a half month. The amount of nitrate and phosphate in F/2 medium was consumed efficiently at the first two days in a three-day cycle, indicating the cycle time might

be increased more if the concentration of pollutants was higher. The limitation of operating time was believed to be caused by no light penetration into the concentrated algal cells. Nevertheless, the maximum algal concentration of 7.1 g/L was more than twice as many as the tubular column reactor had in our laboratory.

The capacity of the FPPBR for nitrate and phosphate are 0.22 g/m<sup>2</sup>/day and 0.15 g/m<sup>2</sup>/day, respectively (Table V), indicating the estimated required area of FPPBR is about 0.1-0.5 acre in the sea or in the adjacent coastal area for treatment of an accidentally emitted domestic wastewater.

#### IV. CONCLUSIONS

The shake-flask test shows that the cheap and easy-handling NaHCO<sub>3</sub> can be used as an alternative carbon source for replacement of CO<sub>2</sub> gas mixture in cultivation of microalgae and maintain the culture of effectiveness. The optimal operating factors of FPPBR were at 4 L/min and 12000 lux. The fed-batch FPPBR operated during 432 hr could reach a maximum algal concentration of 7.1 g/L in association with the periodical removal of substrate and nutrients (nitrate and phosphate) every three days. Its capacity for nitrate and phosphate were 0.22 g/m<sup>2</sup>/day and 0.15 g/m<sup>2</sup>/day, respectively referred to about 0.5-1.0 acre required for using FPPBR in the sea or in the adjacent coast area for treatment of an accidentally emitted domestic wastewater.

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