International Scientific Journal
Environmental Science
http://environment.scientific-journal.com

Parallel Nutrient Removal and Biomitigation of CO_2 by Pure (Chlorella vulgaris) and Mixed Algal Cultures

Burak Cakirlar[1], Gece C. Ogut[1], Tuba H. Erguder[1]
Baris Kaymak[1], Goksel N. Demirer[1]
[1] Environmental Engineering Department, Middle East Technical University, TURKEY

Sibel Uludag-Demirer[2]
[2] Environmental Engineering Department, Karabuk University, TURKEY

Abstract**—The removal of nutrients (TAN and PO_4-P) in the wastewater produced from coking unit of iron and steel production industry was investigated using two different microalgal cultures, namely *Chlorella vulgaris* and mixed microalgae (Arac Creek, Karabuk, Turkey). Lab-scale semi continuous photobioreactors (PBRs) were operated by industrial wastewater with N/P ratio of 6 (g/g) and purged continuously by air containing 4% CO_2 as inorganic carbon source. The hydraulic retention time (HRT) of the wastewater was adjusted to 5, 8, and 12 days by varying the volume of wastewater added per day. The results revealed that as the HRT increases, the PBRs had higher TAN and PO_4-P removal efficiencies (above 95%). The concentration of biomass (TS and TVS) increased as HRT became longer because of lower biomass wastage per day. As a result, CO_2 sequestration was higher in PBRs operated at higher HRT. The optimum HRT was 8 days based on the results of nutrient removal and CO_2 retainment in the system and there was no significant difference in the performance of PBRs inoculated by *Chlorella vulgaris* and mixed microalgal culture.**

Keywords— Algae, Chlorella vulgaris, nutrient, bio-mitigation, CO_2, iron and steel industry wastewater

I. INTRODUCTION

Microalgae are one of the most important bio-resources that are currently receiving a lot of attention due to a number of reasons. For example, they have diverse applications in the field of waste management such as removal of nutrients, organic contaminants, and heavy metals. Microalgal cultures can also be used for the treatment of olive oil, pulp and paper, and iron and steel industry wastewaters [1]. Photosynthetic oxygen production by microalgae reduces the need for external aeration, which means lower energy requirement in the field of aerobic treatment technology. This process is advantageous, in particular, for the treatment of hazardous pollutants to be biodegraded aerobically and might volatilize during mechanical aeration. Moreover, the harvested microalgal biomass itself, constitutes a raw material for the production of different high-value chemicals and bio-fuels such as biodiesel and bio-ethanol [2].

Nutrient discharges to natural waters have contributed to an increase in many problems such as eutrophication. Water quality legislation has increased the standards regarding nutrient removal in order to overcome eutrophication problems in receiving waters. With more stringent standards imposed regarding nutrient removal, processes have been developed to remove compounds containing nitrogen and phosphorus. However, nutrient removal from wastewaters is still a significant concern in many countries due to its high cost. Therefore, effective and low cost technologies for nutrient removal from wastewaters are in great demand [3].

Due to the fast-growing concern about global warming, which can be attributed primarily to the elevated CO_2 level in the atmosphere, the United Nations promoted the Kyoto Protocol (1997) and more than 170 countries have ratified the protocol. Various CO_2 mitigation strategies have been investigated, which can be generally classified into two categories: (1) chemical reaction-based approaches, and (2) biological CO_2 mitigation. Chemical reaction-based methods for capturing CO_2 are relatively costly and energy-consuming, therefore, the mitigation benefits become marginal. Biological CO_2 mitigation has attracted much attention as an alternative strategy because it leads to the production of biomass energy in the process of CO_2 fixation through photosynthesis. It can be carried out by microalgal cultures in addition to the other plants. The microalgae-for-CO_2-mitigation strategy offers numerous advantages especially when it is combined with other processes such as wastewater treatment [4].

Therefore, this study investigated nutrient removal coupled with greenhouse gas mitigation using microalgal cultures. This configuration does not only tackle with waste management issues (wastewater treatment and CO_2 mitigation) but also generate bio-fuel (biogas, bio-hydrogen) and bio-product

This study was funded by The Scientific and Technological Research Council of Turkey through Grant Number 111Y205.

(fertilizer) if the produced algal biomass is further processed. Within the scope of this study, a wastewater from iron and steel producing industry was treated by a photobioreactor (PBR) containing both pure and mixed microalgal cultures. The reactors which were operated at three different hydraulic retention time (HRT) were fed with a gas mixture containing 4% CO_2 simulating flue gas originating from industry, such as, iron and steel production. HRT is an important parameter in designing a reactor and it controls microalgal growth, associated removal of nutrients and CO_2 sequestration [5].

II. MATERIALS AND METHODS

A. Microalgae Cultivation Photobioreactors (PBRs)

Two types of microalgal cultures were used in the experiments. The axenic *Chlorella vulgaris* culture was obtained from CCAP (UK) and freshwater microalgal culture was collected from Arac Creek in the vicinity of Karabuk University Campus, Karabuk (mixed culture). The growth medium of the cultures was refreshed on a weekly basis by using sterilized enhanced bold's basal medium (3N-BBM+V) [6]. The cultivation PBRs received continuous illumination (200 μmol/m^2.s) using 18 cool white fluorescent lamps (OSRAM, L 18W/685). They were continuously aerated with 0.5 L/minute using air pumps (RESUN 9602, PRC) (Fig. 1).

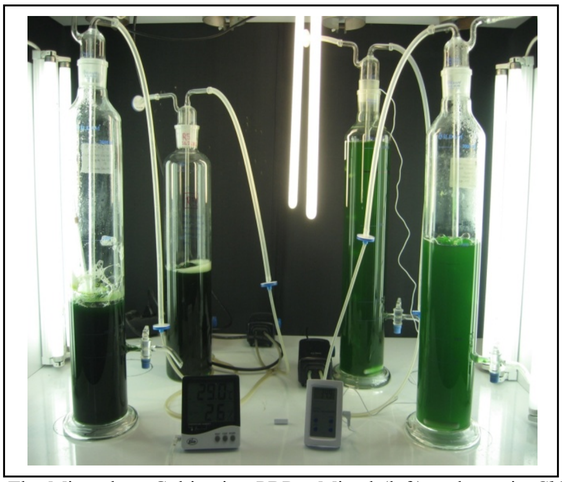

Fig. 1. The Microalgae Cultivation PBRs: Mixed (left) and axenic *Chlorella vulgaris* (right).

B. Wastewater

The industrial wastewater used in this study was collected from the coking unit effluent of the iron and steel making industry located in Karabuk, Turkey. The results from characterization studies showed that the industrial wastewater was in excess of total ammonium nitrogen (TAN) with trace concentration of PO_4^{3-}. Since microalgal growth in the coke wastewater would be limited by P, a source for P was investigated. The use of the effluent from the primary thickener in the local urban wastewater plant was decided to be used as P resource. The composition of coke wastewater and effluent from the thickener unit are provided in Table 1.

The results show that coke wastewater is seriously contaminated by heavy metals and phenol, which allows testing PBRs performance in the existence of chemical agents, which may affect biological activities adversely. The mixture of coke wastewater and effluent from the thickener was made by 1:50 to be able to operate the PBRs with N/P input ratio of 6 (g/g). This ratio was determined as the optimum inlet N/P supporting microalgal growth and nutrient removal previously in this study.

TABLE I. THE COMPOSITION OF THE WASTEWATERS USED IN THE MICROALGAL GROWTH IN PBRS

Parameter	Wastewater Composition (mg/L)	
	Effluent from Coking Unit	***Effluent from sludge thickener***
TS	8471 ± 311	880 ± 42
TVS	136 ± 4	488 ± 20
TS(%TVS)	2	55
tCOD	11827 ± 150	587 ± 3.6
dCOD	10225 ± 61	328 ± 6.2
TN	3600 ± 90	47.2 ± 1.5
TAN	3352 ± 78	41.7 ± 2.1
NO3-N	4 ± 0.2	< 0.1
NO2-N	<0.01	< 0.01
Organic-N	244	5.48
Ortho PO_4-P	1 ± 0.1	19.9 ± 0.2
Sulfate[a]	1509	-
Cyanide[a]	0.0125	-
Arsenic[a]	0.767	-
Mercury[a]	0.003	-
Iron[a]	0.009	-
Cadmium[a]	0.017	-
Total chromium[a]	<0.001	-
Phenol[a]	950	-

a. *Analyses were made in a privately owned commercial accredited laboratory*

C. Photobioreactors (PBRs)

Six 1-L gas washer columns made of glass (Fig. 2) were used as the PBRs, where three of them were inoculated by axenic *Chlorella vulgaris* and the other three were inoculated with mixed microalgal cultures and fed by wastewater from the coking unit of the iron and steel industry (Karabuk, Turkey) and 4% CO_2-enriched air with 0.5 L/min flow rate (vvm) (air pump RESUN 9601, China). The wastewater feeding was carried out manually every day and air flow was continuous and controlled by using flow meters attached to air pump and pure CO_2 tank. The illumination was continuous (24 hour day) and supplied by cool white 18 W fluorescent lamps (OSRAM, L 18W/685, Korea) providing 120 μmol/m^2.s. pH adjustment in the PBRs was achieved by purging the system with 4% CO_2-enriched air and temperature was maintained at 28±2°C using the ventilation system. The PBRs were operated at HRTs of 5, 8, and 12 days corresponding to the addition of 200, 125 and 83.3 mL of wastewater per day. The withdrawn volume of the wastewater (effluent of the PBRs) was used for the analyses.

D. Analyses

The samples taken from the effluent of the PBRs were analyzed for optical density at 685 nm (OD) (HACH Dr-2800 Spectrophotometer), total solids (TS), total volatile solids (TVS) (Standard methods 2540 B and 3540 E; APHA 1998)

[7], pH (Eutech, Cyberscan pH510), total and dissolved COD, TN, TAN, ortho-PO_4-P (Lovibond GmbH, Aqualytic), following a monitoring program to observe the steady state conditions to establish in the reactors. The concentrations of NO_3-N and NO_2-N were measured for characterization purposes using the kits (Aqualytic). The photosynthetic active radiation (PAR) was measured by the light meter (Li-Cor LI-250A). The concentrations of chlorophyll-a and pheophitin a were measured by colorimetric methods (Standard method 10200H; APHA 1998) [7]. The measurements of CO_2 in inlet and outlet of gaseous phase were performed using GC (Agilent 6890N) equipped with thermal conductivity detector and capillary column (CP-Sil 8, Varian).

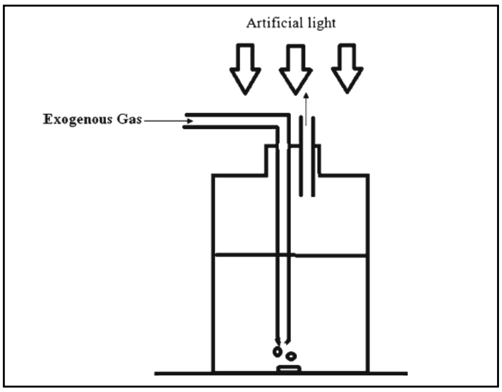

Fig. 2. The gas washer column used as PBR in the study (semi-continuous in wastewater addition, continuous in aeration and illumination)

III. RESULTS AND DISCUSSION

The pH in PBRs was in the range of 6-7 throughout their operation and it was clear that dissolved CO_2 provided a buffering capacity for the reaction medium. The microalgal growth (biomass production) in the PBRs operating at 5, 8 and 12 days of HRT was monitored regularly since it correlates with nutrient uptake and CO_2 usage directly. The parameters indicating the microalgal growth were optical density measured at 685 nm and total solids (TS) concentrations in this study. The total volatile solids (TVS) were measured to be able to report approximate quantity of the microalgal biomass. The majority of the biomass in the reactors was assumed to be composed of microalgae in the study, but the presence of other bacteria carried into the PBRs via the effluent from primary thickener is also recognized.

The change of OD (685 nm) in the PBRs inoculated with *Chlorella vulgaris* and mixed microalgal culture is shown in Fig. 3 and 4 respectively for each HRT tested. The start-up period of the PBRs, which was approximately the first 20 days of operation (Fig. 3 and 4), was followed by steady state conditions. The prevalence of steady state conditions in terms of biomass concentration was robust in HRT 8 and 12 days PBRs as opposed to HRT 5 day reaction, which had higher flow rate (0.2 L/day) and growth rate because of higher concentrations of nutrients in C5 and D5 than the other two PBRs [8].

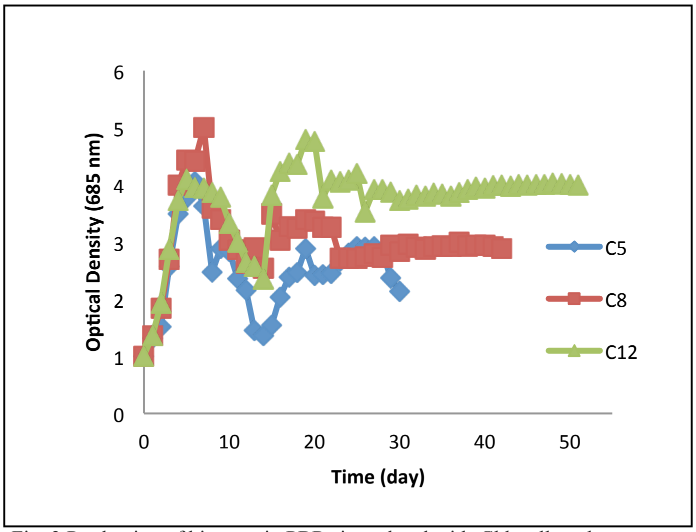

Fig. 3 Production of biomass in PBRs inoculated with *Chlorella vulgaris*

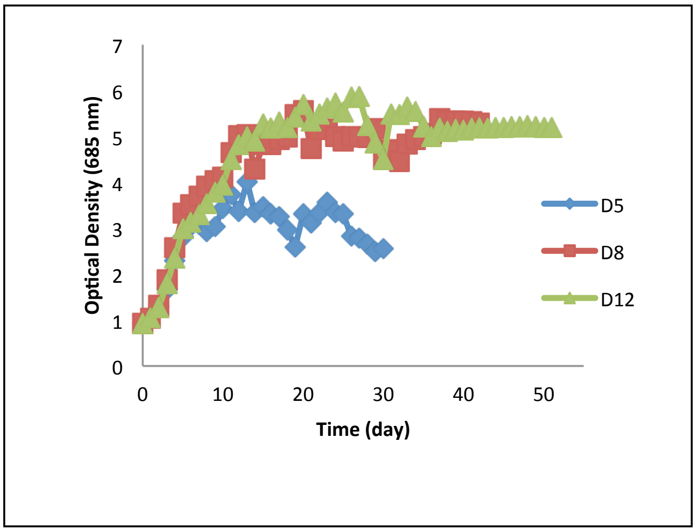

Fig. 4. Production of biomass in PBRs inoculated with mixed microalgal culture collected from Arac Creek (Karabuk, Turkey)

The observed increase in the concentrations of TS and TVS from the start-up period to steady state conditions is provided in Table 2.

Table 2. Biomass concentrations in the PBRs at the start-up and steady state conditions

Reactor	TS (mg/L)	TVS (mg/L)
Start-up conditions		
C (*C. vulgaris*)	1293±10	887±6
D (mixed microalgae)	1110±6	645±4
Steady-state conditions		
C5	1651 ± 146	703 ± 55
C8	1920 ± 112	944 ± 34
C12	2347 ± 121	1029 ± 121
D5	1612 ± 119	669 ± 35
D8	2427 ± 140	890 ± 71
D12	2525 ± 162	1031 ± 72

As can be seen from the results listed in Table 2, the steady state concentration of biomass in the PBRs inoculated with *Chlorella vulgaris* and mixed microalgae increased as HRT increased [5, 8]. The biomass production in C8 and C12 was lower than that in D8 and D12, which may be due to the coexistence of microalgal and bacterial biomass in the inoculum used to start up the PBRs. The microalgal biomass production in PBRs is significant from different perspectives; for instance, production of biofuels from microalgal biomass requires higher growth rate (short HRT) while removal of nutrients and sequestration of CO_2 increases as the concentration of biomass increases (long HRT). The concentration of nutrients, TAN and PO_4-P, in the PBRs was measured from the start-up until the steady state conditions were established and the results of the analyses are shown graphically in Fig. 5-8.

The removal of nutrients was determined based on the TAN and PO_4-P concentrations in the effluent of PBRs. The complete removal of nutrients was achieved in the reactors operating with 8 and 12 day of HRT regardless of the microalgae used for inoculation. The removal of TAN was 90 and 98% in C8 and C12, respectively and the efficiencies of D8 and D12 was at the same level as 99% removal. The PO_4-P removal was above 95% in all of the PBRs with HRTs longer than 5 days. The effluent concentrations of the nutrients were lower than 3 mg/L for TAN and 1 mg/L for PO_4-P, which makes the effluent in compliance with the Turkish Environmental Regulation on Water Pollution and Control (31/12/2004-No.25687).

However, the PBR with 5 day HRT inoculated with *Chlorella vulgaris* (C5) resulted in 70% and 50% removals for TAN and PO_4-P, respectively, which was lower than the nutrient removal efficiency of the reactor D5 (80% TAN and 93% PO_4-P). Prior to the steady state conditions observed in C5 and D5, there was a sudden decrease in the concentrations of the nutrients followed by a rise along with the decrease in biomass concentration (Fig. 3 and 4). The findings clearly indicated that 8 day HRT can be accepted as the optimum retention time regarding the nutrient removal efficiencies for the PBRs inoculated by *Chlorella vulgaris* and mixed microalgal culture. However, to be able to increase the amount of microalgal biomass harvested from the effluent of PBRs, the HRT should be kept short (5 day in this study) forcing the system have a higher growth rate in nutrient replete conditions. The CO_2 sequestration in the PBRs was calculated based on the CO_2 concentrations in the air flow (0.5 L/min). The inflow air composition had 4% CO_2 by volume and the amount of CO_2 retained within the reactors correlated well with the biomass concentration. PBRs with 5 day HRT resulted in lowest CO_2 sequestration since they have the lowest concentration of biomass concentration at the steady state conditions. The level of CO_2 removal from the air was in the range of 4-9% in C5 and D5. In the PBRs with 8 and 12 day HRT, CO_2 retained in the levels of 17-19% as a result of high biomass concentration at steady state conditions. The research study carried out by Chiu et al. [9] reported 0.32 g/hour CO_2 removal in the PBRs inoculated by *Chlorella vulgaris* and fed by 5% CO_2 containing air. The results in this study revealed a similar level of CO_2 sequestration as 0.37 g/hour in C12. Another study by Yoo et al. [10] confirm the results in this study by observing 24% CO_2 removal by *Scenedesmus obliquus* culture purged by 5.5% CO_2 containing air.

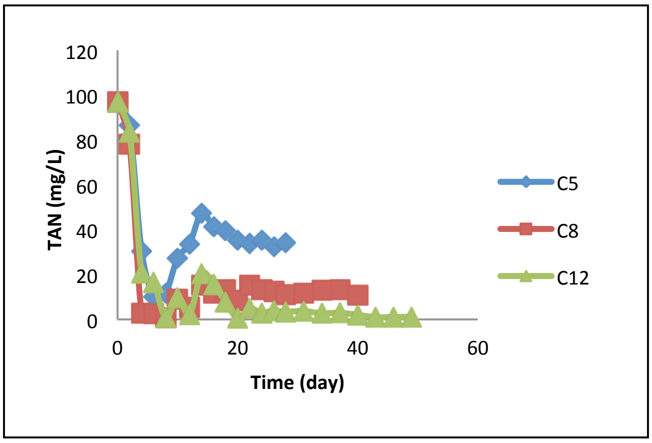

Fig. 5. The concentration of TAN (mg/L) in the effluent of PBRs inoculated with *C. vulgaris*

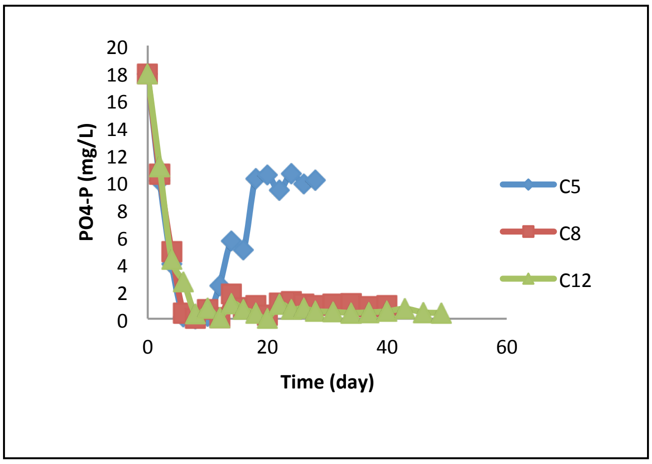

Fig. 6. The concentration of PO_4-P (mg/L) in the effluent of PBRs inoculated with *C. vulgaris*

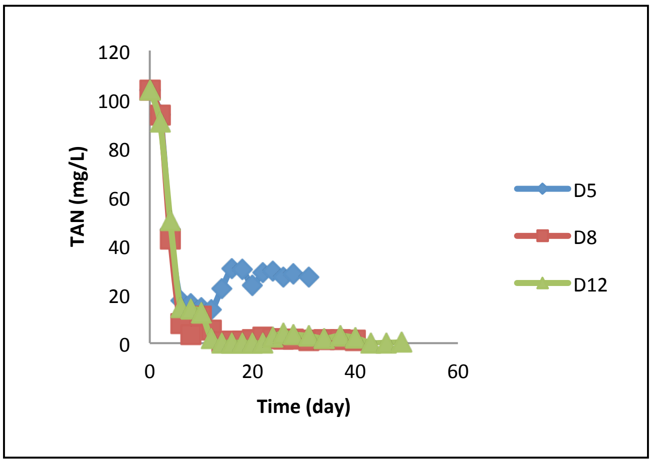

Fig. 7. The concentration of TAN (mg/L) in the effluent of PBRs inoculated with mixed microalgal culture from Arac Creek (Karabuk, Turkey)

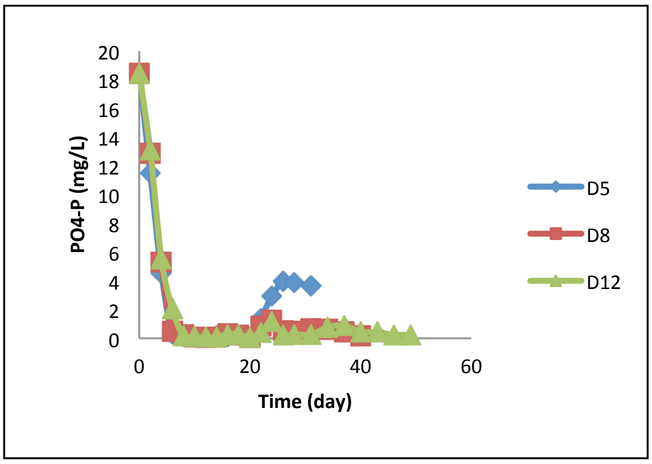

Fig. 8. The concentration of PO_4-P (mg/L) in the effluent of PBRs inoculated with mixed microalgal culture from Arac Creek (Karabuk, Turkey)

The CO_2 sequestration by microalgal culture in PBRs can be as high as 70% [11] depending on the concentration of CO_2 in the air supply and its flow rate.

IV. CONCLUSIONS

The results obtained in this study pointed out the indifference of using axenic *Chlorella vulgaris* and mixed microalgal culture in the removal of nutrients from the industrial wastewater and CO_2 sequestration. This is found to be promising since mixed microalgal culture was collected from a creek nearby the iron and steel industry producing the wastewater tested in the research. To remove nutrients from the iron and steel producing industry and capture CO_2 at the acceptable levels, HRT of a PBR is to be minimum 8 days according to the findings from the study. However, for a dual purpose of harvesting microalgal biomass for biofuel production and nutrient and CO_2 removal, further investigation is necessary to report the optimum HRT for PBRs.

ACKNOWLEDGMENT

This is a study undertaken under the Project 111Y205, which is supported by the Scientific and Technological Research Council of Turkey (TUBITAK). The authors are thankful for the support through the study.

REFERENCES

[1] S. Chinnasamy, A. Bhatnagar, R.W. Hunt, K.C. Das, “Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications,” Bioresource Technology, vol. 101, pp. 3097–3105, 2010.

[2] Y. Chisti, “Biodiesel from microalgae,” Biotechnology Advances, vol. 25, pp. 249–306, 2007.

[3] R. Boonchai, G.T. Seo, D.R. Park, Y.C. Seong, “Microalgae photobioreactor for nitrogen and phosphorus removal from wastewater of sewage treatment plant,” International Journal of Bioscience, Biochemistry and Bioinformatics, vol. 6, pp. 407-410, 2012.

[4] B. Wang, L.Yanqun, N. Wu, C.Q. Lan, “CO_2 bio-mitigation using microalgae,” Appl. Microbiol. Biotechnol., vol. 79, pp. 707-718, 2008.

[5] L. Wang, Y. Wang, P. Chen, R. Ruan, “Semi-continuous cultivation of Chlorella vulgaris for treating undigested and digested dairy manures,” Appl. Biochem. Biotechnol., vol. 162, pp. 2324-2332, 2010.

[6] R. Andersen, Algal Culturing Techniques, Elsevier Academic Press, 2005, pp. 437-438.

[7] APHA (American Public Health Association), 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

[8] H. Tang, M. Chen, K.Y. Simon Ng, S.O. Salley, “Continuous Microalgae Cultivation in a Photobioreactors,” Biotechnology and Bioengineering, vol. 109, pp. 2468-2474, 2012.

[9] S.Y. Chiu, C.Y. Kao, C.H. Chen, T.C. Kuan, S.C. Ong, C.S. Lin, “Reduction of CO_2 by a high-density culture of *Chlorella sp.* in a semicontinuous photobioreactor,” Bioresour. Technol., vol. 99, pp. 3389–3396, 2008.

[10] C. Yoo, S.Y. Jun, J.Y. Lee, C.Y. Ahn, H.M Oh, “Selection of microalgae for lipid production under high level of carbon dioxide,” Bioresour. Technol., vol. 101, pp.71–74, 2010.

[11] J.C.M. Pires, M.C.M. Alvim-Ferraz, F.G. Martins, M. Simoes, “Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept,” Renewable and Sustainable Energy Reviews, vol.16, pp. 3043-3053, 2012.