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Microalgae Cultivation in Pilot Scale for Biomass Production Using Exhaust Gas from Thermal Power Plants

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Abstract: Exhaust gases from thermal power plants have the highest amount of carbon dioxide (CO_2), presenting an environmental problem related to a severe impact on ecosystems. Extensively, the reduction of CO_2 from thermal power plants has been considered with the aid of microalgae as a cost-effective, sustainable solution, and efficient biological means for recycling of CO_2. Microalgae can efficiently uptake CO_2 and nutrients resulting in high generation of biomass and which can be processed into different valuable products. In this study, we have taken *Nephroselmis* sp. KGE8, *Acutodesmus obliquus* KGE 17 and *Acutodesmus obliquus* KGE32 microalgae, which are isolated from acid mine drainage and cultivated in a photobiological incubator on a batch scale, and also confirmed that continuous culture was possible on pilot scale for biofuel production. We also evaluated the continuous culture productivity of each cultivate-harvest cycle in the pilot scale. The biomass of the cultivated microalgae was also evaluated for its availability.

Keywords: biomass; microalgae; photobioreactor; power plant exhaust gas; lipid; FAME

1. Introduction

Nowadays, an increase of CO_2 levels in the atmosphere is extensively recognized as a major contributor for global warming. Recent reports are highlighted that atmosphere contains CO_2 level of 450 ppm [1]. The atmospheric CO_2 can be trapped by green plants via photosynthesis. However, terrestrial plants are estimated to reduce only 3–6% of global CO_2 emissions, which is significant given the slow growth rates of plants. On the other hand, microalgae can grow much faster than terrestrial plants, and their CO_2 reduction efficacy was 10–50 times higher than plants [2,3]. The variety of microalgae cultivated in comfortable environmental condition to produce comparably 15–300 times higher energy sources than plants, which also reduce the land area for cultivation and continuously increase the yield per unit area [2,4,5]. Microalgae can biologically store CO_2 through photosynthesis in the form organic compounds and then use microalgal biomass as a feedstock for renewable energy after CO_2 fixation [6]. Moreover, microalgae have been documented as source of valuable biomaterials such as fertilizers, live feed, medicines, and other value-added products.

The large-scale microalgae culture system was divided into two systems, namely the open and closed systems. In the case of the open system, it is difficult to control the amount of light intensity and it may vary depend upon the local time, and also difficult to maintain the temperature. The closed

system, which is a device to overcome these limitations, is able to control the light intensity, external influence, and temperature, though the operation cost and the manufacturing cost are high when compared to the open system. Especially, the closed system microalgal growth rate is 1.5–4 times higher than the open system [7]. The high growth rate of microalgae has a large impact on CO_2 capture and may lead to an increase in biomass production. According to the various research condition, closed system may be designed as airlift column, horizontal tube, stirred tank, and flat panel photobioreactor (PBR) [7,8].

Obviously, industrial exhaust gases contain 10–20% of CO_2 with trace amounts of SO_x and NO_x. The selection of microalgae plays a vital role in CO_2 reduction efficacy and represents a significantly cost-effective route for biomass production. The desirable qualities of microalgae comprise high growth and CO_2 consumption rates, also patience towards trace constituents of exhaust flue gases such as SO_x and NO_x and production of valuable products. Maeda et al. (1996) used *Chlorella* sp. T-1 as a potential microalga for the biological removal of exhausted CO_2 from coal-fired thermal power plants. Aslam et al. (2017) have identified that mixed microalgae societies like *Desmodesmus* spp. can slowly grow in 100% unfiltered exhausted gas from coal combustion with phosphate buffering condition [9]. Kassim and Meng (2017) studied biofixation of CO_2 by two microalgae species such as *Chlorella* sp. and *Tetraselmis suecica* with various CO_2 concentration [10]. Even though the above said studies have been carried out in exhausted gas which adversely affects the microalgal growth. To the best of our knowledge, no study has yet reported on the actual injection of exhaust gas, and there is a lack of research on biomass tendency when continuously injecting the gas into large-scale bioreactor.

Hence, the objective of this study is to evaluate the feasibility of microalgae species like *Nephroselmis* sp. KGE8, *Acutodesmus obliquus* KGE 17 and *Acutodesmus obliquus* KGE32, which were cultivated in a laboratory with the supplementation of power plant exhaust gas. Then, evaluate the growth potential of the microalgae in the semi-continuous photobioreactor (PBR) operating with the exhaust gas injection, and evaluate the microalgae productivity at each cultivate-harvest cycle. Finally, we also assessed the feasibility of biodiesel, lipid and C16-18-FAME contents in recovered microalgae.

2. Materials and Methods

2.1. Conditions of Microalgae Cultivation in Batch Scale

Microalgae species were derived from acid mine drainage which include *Nephroselmis* sp. KGE8, *Acutodesmus obliquus* KGE 17, and *Acutodesmus obliquus* KGE32. The batch type cultivation was performed in 140 mL serum bottle with 100 mL of Bold's Basal Medium (BBM) [11] which contained strains with optical density of 0.010 in UV spectroscopy 680 nm region.

The exhaust gas from the Y coal-fired thermal power plant (Gangwon-do, South Korea) was used as a carbon source for microalgae growth. The collected gas was filtered through a 0.2 µm filter and then supplied at a flow rate of 0.5 L min^{-1} for 1 h to complete saturation of the medium. Composition of the exhaust gas details appeared in Table 1. The microalgae cultivation was conducted at 25 °C with 120 µmol photon $m^{-2}s^{-1}$ of light intensity, and the content was agitated in incubator shaker (Witeg, Wisecube WIS-ML, Germany) at 120 rpm to prevent agglomeration for 7 days.

Table 1. Y Power plant gas contents and concentrations located in Gangwon-do.

Gas Contents	Initial Gas Concentration
CO_2 (*v*/*v* %)	14.9 (±0.2)
NO_x (ppmv)	220.2 (±10.5)
SO_x (ppmv)	32 (±5)
CO (ppmv)	1549 (±242)
O_2 (%)	5.47 (±0.03)

2.2. Cultivation of Microalgae in Photobioreactor (PBR)

The culture system used in this study was a multistage photobioreactor (PBR), each PBR has capacity of 2000 L and it is shown in Figure 1. Initially, exhausted gas was injected through PBR 1 and then subsequently passed through the PBR 2, -3 and -4 respectively. Finally, unutilized gas was ejected from the PBR 5, the concentration of CO_2 in each stage was measured and attached in Table 2. The initial and final exhaust gas concentration was analyzed by Testo 350 K emission analyzer (Testo, Germany). According to Blair et al. (2014) red light emitting diode (LED) was installed at each stage for maximize light absorption [12]. The first stage of the pilot scale, the growth rate of each stage of *Nephroselmis* sp. KGE 8 was determined and grown for 20 days. The second cultivation was performed for 22 days, from the 18 days when the specific growth rate (μ_{max}) started to increase, the possibility of continuous cultivation was evaluated by harvest and regrowth. On day 18, the microalgae were recovered and diluted and re-cultured for 16 days and supplemented with BBM to prevent nutrition loss. The interval between collection and incubation was 2 days, and cultures were collected and cultivated three times.

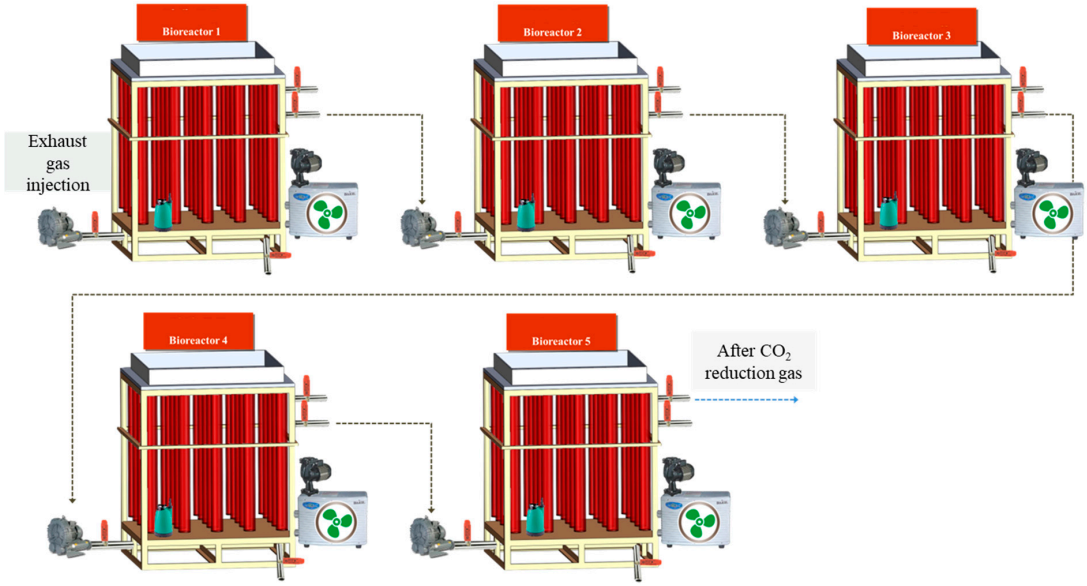

Figure 1. Pilot scale multi-step reactor schematic.

Table 2. Injection CO_2 concentration in each stage.

Stage	CO_2 (*v*/*v* %)	NO_x (ppmv)	CO (ppmv)	O_2 (*v*/*v* %)
PBR1	14.90 (±0.18)	220.2 (±10.5)	1548.5 (±242)	5.47 (±0.03)
PBR2	8.08 (±0.52)	124.4 (±21.8)	941.1 (±41.9)	12.42 (±0.93)
PBR3	6.72 (±0.73)	99.3 (±16.0)	703.3 (±51.4))	13.89 (±0.99)
PBR4	4.99 (±0.52)	72.3 (±12.1)	537.2 (±21.6)	15.81 (±0.86)
PBR5	3.87 (±0.52)	51.9 (±9.3)	421.1 (±35.2)	17.03 (±0.95)
Out	3.11 (±0.60)	51.8 (±8.18)	337.0 (±5.1)	16.86 (±1.41)

2.3. Analysis of Microalgal Growth

The growth rate of microalgae cultivated in pilot plant was obtained by analysis of OD_{680}, from spectrophotometer (Hach DR/2800, Loveland, CO, USA) which values were converted to dry cell weight (DCW) concentration (g L^{-1}). DCW of *Nephroselmis* sp. KGE 8 was calculated by:

$$\text{Dry weight (g L}^{-1}) = 0.3997 \times OD_{680} - 0.0471 \ (R^2 = 0.9871) \quad (1)$$

Further, the specific growth rate (SGR) was calculated by Equation (2):

$$\mu = (\ln X_2 - \ln X_1)/(t_2 - t_1) \quad (2)$$

where X_1 and X_2 are the mass of initial and final weight of microalgae, respectively, which is used to calculate DCW in this study, and t_1 and t_2 are the initial and final incubation times respectively.

2.4. Algal Harvest

In pilot scale, the algal harvest was performed by sludge pump, and collected to storage tank. The algae of each stage were harvested, and the harvested algae were precipitated and recovered by separating the supernatant and algae.

2.5. Analysis of Lipid and C16–C18 Fatty Acid Methyl Ester (FAME)

The modified Bilgh and Dyer method (Ji et al. (2016)) was used to analyze the lipids and fatty acids in the harvested microalgae [13]. Fatty acids were identified by the modified Lepage and Roy method from Yun et al. (2015), which convert fatty acid into fatty acid methyl esters through esterification and is analyzed by Gas chromatography with a flame ionization detector (GC-FID) using HP-INNOWax capillary column (Agilent Technologies, USA) [14].

3. Results and Discussion

3.1. Growable Microalgae in Exhaust Gas Condition

Batch scale experiment results shows that both *Nephroselmis* sp. KGE 8 and *Acutodesmus obliquus* KGE 17 have lag phase up to two days and showed exponential growth phase until fifth day (Figure 2). At this moment, *Nephroselmis* sp. KGE 8 exhibited the maximum growth when compared to the *Acutodesmus obliquus* KGE 17. Another microalga like *Acutodesmus obliquus* KGE 32 exhibits the lag phase until three days, and the exponential growth phase was until five days, and it has the stationary growth phase. The growth rates of microalgae with a supply of exhaust gas are presented in Table 3. Ji et al. (2017) and Yun et al. (2016) evaluated the potential for biofuel production according to changes of CO_2 concentration in exhaust gas. Compare with these previous studies, algae production was faster. Also Tang et al. (2011) was focused on growth potential in high concentration of CO_2 and effective concentration of CO_2, growth rate and lipid contents was lower than this study. *Nephroselmis* sp. KGE 8 have the OD_{680} value of 1.341 and the maximum specific growth rate (μ_{max}) was 1.41 d^{-1} between 3–4 days of culture. Conversely, *Acutodesmus obliquus* KGE 32 and *Acutodesmus obliquus* KGE 17 possess the OD_{680} values of 0.970 and 0.553 and μ_{max} were 1.08 d^{-1} and 1.37 d^{-1} respectively. The microalgae, which applied in this study, showed higher specific grow rates (1.08 to 1.37 d^{-1}) than previous study (Table 3). Continuous and excessive exposure of NO_x and SO_x gases to cells could leads to inhibition of microalgae growth rate [15,16]. Praveenkumar et al. (2014a) reported that algal FAME content and productivity increased from 129 to 168 mg fame/g cells and from 59 to 118 mg fame/L d, respectively, in coal-fired flue-gas inlet condition [17]. They also conclude that stress conditions could lead to improve algal lipid productivity [18].

3.2. Pilot Scale Cultivation

The pilot scale cultivation result discloses that, lag phase period of *Nephroselmis* sp. KGE 8 was increased from 2 days to 10 days when compared with batch scale results due to the stress present in the exhaust gas (Figure 3). Same trend was also observed in previous study by Borowitzka et al. (2018) and mentioned that adaptation by stress due to CO_2 [21].

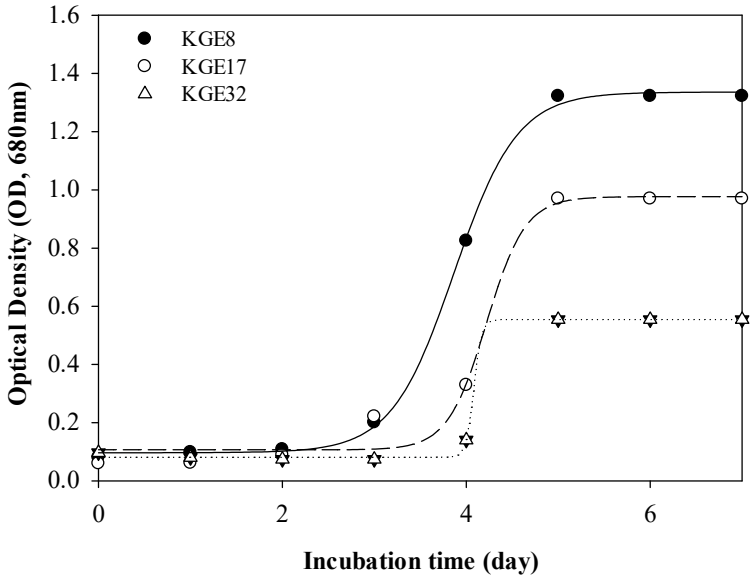

Figure 2. Growth curve of *Nephroselmis* sp. KGE 8, *Acutodesmus obliquus* KGE 17, and *Acutodesmus obliquus* KGE 30 microalgae in purged power plant gas.

Table 3. Specific growth rate and lipid contents of various microalgal strains cultivated at different carbon dioxide concentration.

Species	Carbon Dioxide Concentration (%)	Incubation Condition	Medium	Lipid Contents (%)	Specific Growth Rate (μ_{max}, d^{-1})
Scenedesmus obliquus KGE 9 [a]	14.1	Batch	BBM	22.8	1.00
Chlorella pyrenoidosa SJTU-2 [b]	10.0	Batch	BG11	24.2	0.78
Acutodesmus obliquus KGE 30 [c]	14.1	Batch	BBM	17.5	1.09
Acutodesmus obliquus KGE 32 [d]	14.1	Batch	BBM	-	1.08
Acutodesmus obliquus KGE 17 [d]	14.1	Batch	BBM	-	1.37
Nephroselmis sp. KGE 8 [d]	14.1	Batch	BBM	59.4	1.41
0 Nephroselmis sp. KGE 8 [d]	14.1	Pilot scale	BBM	60.9	0.26

[a] Ji et al. (2017) [19]; [b] D. Tang et al. (2011) [20]; [c] HS Yun et al. (2016) [14]; [d] This study.

Further, the microalgae growth was not similar with the batch scale (Figure 3). Due to the different character of coal and also the generated exhaust gas from the thermal power plant does not contain constant amount of CO_2, it may lead to irregular growth of microalgae in the pilot scale. Cheng et al. (2019) also reported that biomass yields were not constant for every cycle, even gas-adapted microalgae were injected with a constant concentration of mixed gas [22]. These results indicated that *Nephroselmis* KGE 8 is a microalga species that could adaptively grow, even when the exhaust gas was continuously injected.

In continuous culture potential evaluation experiment, *Nephroselmis* sp. KGE 8 reached the exponential growth phase at 17 days after initiated the cultivation (Figure 4). According to Tan et al. (2018), the productivity of microalgae tended to decrease with increasing amount of cultivation [23]. The growth of *Nephroselmis* sp. KGE 8 was different in each stage. PBR 2 show a microalgae concentration of 0.6002 g L^{-1} for the first time and 0.4932 g L^{-1} for the second cultivation. Also the microalgae concentration in PBR 3, PBR 4, and PBR 5 was decreased from 0.5644 g L^{-1} to 0.4955g L^{-1}, 0.5343 g L^{-1} to 0.4722 g L^{-1}, and 0.4421 g L^{-1} to 0.4116 g L^{-1} respectively. In contrast, the microalgae growth in PBR 1 has increased from 0.4996 g L^{-1} to 0.5710 g L^{-1}, unlike other stages. Biomass productivity is affected by growth factors, and according to Sun et al. (2018), the growth

factors like high temperature and large N source will increase biomass growth [24]. When NO_x is dissolved in water, it tends to form nitrite, which can help to grow the biomass [25].

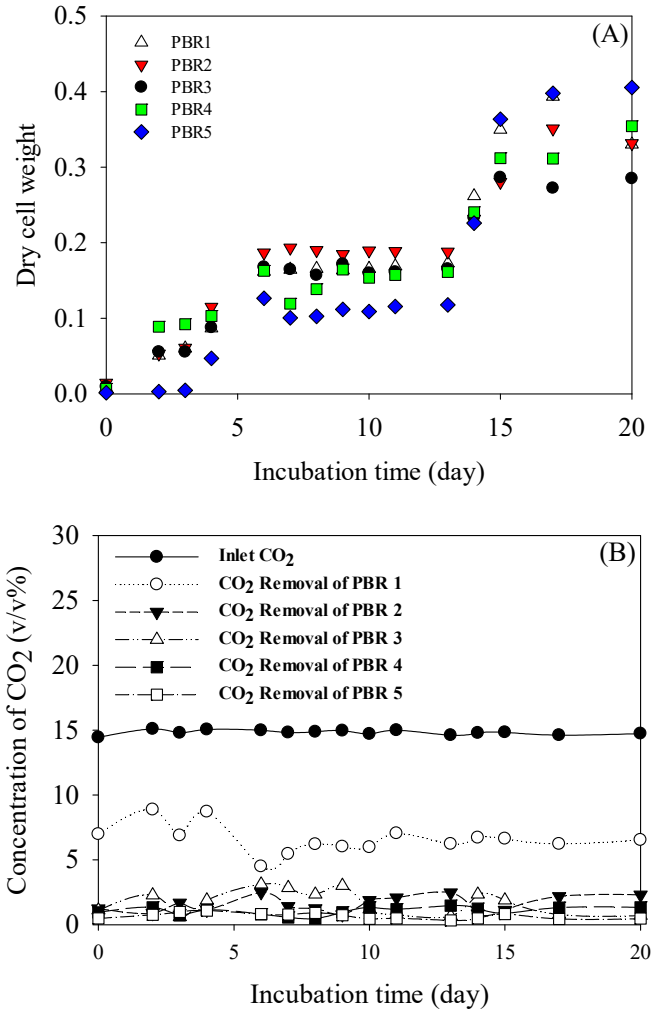

Figure 3. The growth of *Neproselmis* sp. KGE 8 when injecting exhaust gas from thermal power plant using multi-step reactor. Growth rate was shown to (**A**), and removal CO_2 concentration was shown to (**B**).

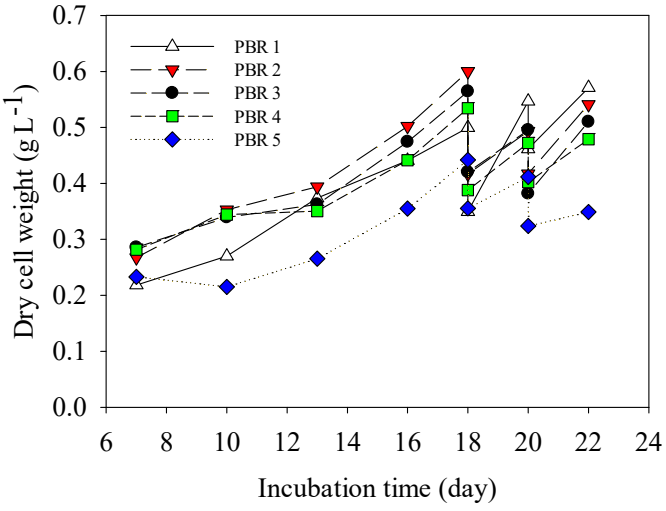

Figure 4. Microalgae growth curve in each incubator re-cultivation process.

3.3. Lipid and Fatty Acid Productivity

We have compared the biomass productivity of *Nephroselmis* sp. KGE 8 in both batch and pilot scale. In batch scale, the biomass productivity was found to be 0.696 g L^{-1} d^{-1}, also contain 59.4% and 95.1% of lipid and C16–C18 FAME respectively. In contrast, the biomass productivity, lipid, and C16-C18 FAME contents were decreased to 0.163 g L^{-1} d^{-1}, 39.4%, and 77.8% respectively in pilot scale experiments (Table 4). Park et al. (2013) reported that the maximum lipid content of the *Nephroselmis* sp. KGE 8 species was 38.8% [26].

Table 4. Biomass productivity, C16-C18 ratio, and lipid content of *Nephroselmis* sp. KGE8 at laboratory scale and pilot scale.

Strain	Volumetric Productivity of Biomass at μ_{max} (g L^{-1} day^{-1})	C16–C18 Ratio (wt %)	Lipid Content (wt %)
KGE8 cultivated in Laboratory	0.696	95.1	59.4
KGE8 Cultivated in Pilot scale	0.163	77.8	60.9

The average lipid content of harvested microalgae in PBR 1 was 41.24%, which was higher than that of other stages. Arief et al. (2009) reported that the content of lipid in microalgae increased with increasing CO_2 concentration. The fatty acid content of the recovered *Nephroselmis* sp. KGE 8 illustrated in Figure 5. The average of C16 to C18 FAME contents in recovered microalgae at each harvest cycle illustrated Figure 5A. The fatty acid content was 74.38% (*w*/*w*), and the highest fatty acid content showed the highest fatty acid content as 87.29% in PBR 5. However, as the number of continuous cultures increased, the fatty acid content of PBR 1 also increased, while that of PBR 2 to PBR 5 tended to decrease (Figure 5B). In particular, PBR 5, which showed the highest fatty acid content at the initial stage, showed a sharp decrease in fatty acid content as the number of times increased. Sharmaet al. (2012), and Nayaket al. (2018) reported an increase in Oleic acid (C18: 1) in the fatty acids of cultured algae in coal combustion gases [27,28].

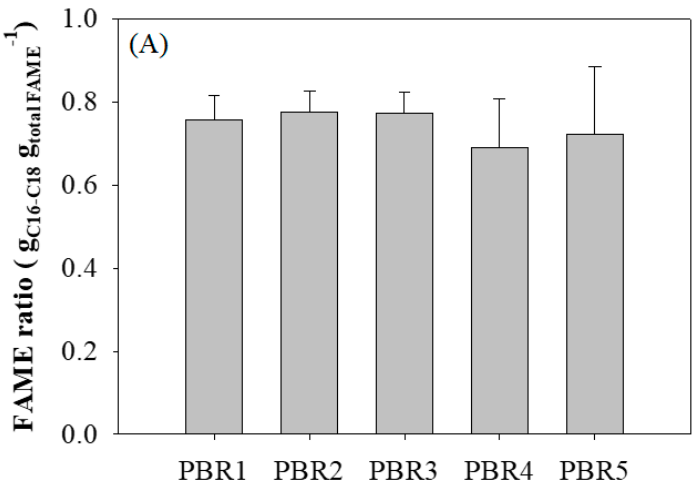

Figure 5. *Cont.*

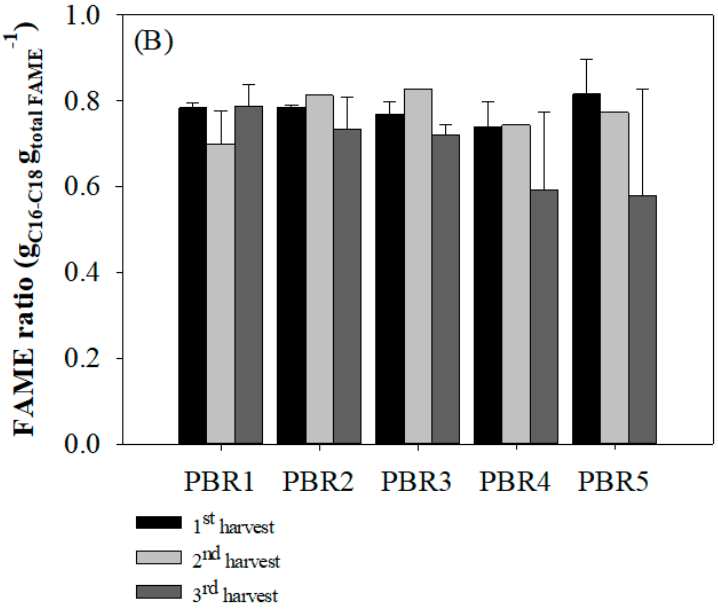

Figure 5. C16–C18 FAME yield from each photo bioreactor.

4. Conclusions

Batch scale studies reveal that *Nephroselmis* sp. KGE 8 showed the best growth under exhaust gas conditions. *Nephroselmis* sp. KGE 8 showed growth potential (0.696 g L^{-1}) in the semi-continuous PBR operation with the exhaust gas injection. The lipid content and C16-C18 FAME content were 39.4% and 77.8% in PBR1, respectively. The microalgae productivity of five reactors showed range from 0.4116 g L^{-1} to 0.5468 g L^{-1} at each cultivate-harvest cycles. PBR 1 showed highest microalgae productivity during PBR operation.

When exhaust gas is directly injected, changes in NO_x and temperature condition accelerate the microbial energy conversion. Singh et al. (2014) reported that some algal species obtained maximum biomass in 15% CO_2 [29]. Based on the result, it was concluded that direct injection of exhaust gas is the most suitable condition for utilization of energy source of microalgae. This microalgal cultivation system could be a suitable process for the massive cultivation of microalgae with exhaust gas from power plants.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, S.P. and H.-S.Y.; Methodology, H.-S.Y.; Software, S.P.; Validation, H.-S.Y.; Investigation, M.-K.J.; Data Curation, S.P.; Writing-Original Draft Preparation, S.P.; Writing-Review & Editing, Y.A. and K.P.; Visualization, H.-S.Y.; Supervision, J.-Y.C.; Project Administration, J.-Y.C.; Funding Acquisition, J.-Y.C.".

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shen, Y. Carbon dioxide bio-fixation and wastewater treatment via algae photochemical synthesis for biofuels production. *RSC Adv.* **2014**, *4*, 49672–49722. [CrossRef]
2. Yuan, C.; Wang, S.; Cao, B.; Hu, Y.; Abomohra, A.E.-F.; Wang, Q.; Qian, L.; Liu, L.; Liu, X.; He, Z.; et al. Optimization of hydrothermal co-liquefaction of seaweeds with lignocellulosic biomass: Merging 2nd and 3rd generation feedstocks for enhanced bio-oil production. *Energy* **2019**, *173*, 413–422. [CrossRef]

3. Cuellar-Bermudez, S.P.; Garcia-Perez, J.S.; Rittmann, B.E.; Parra-Saldívar, R. Photosynthetic bioenergy utilizing CO_2: An approach on flue gases utilization for third generation biofuels. *J. Clean. Prod.* **2015**, *98*, 53–65. [CrossRef]
4. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306. [CrossRef]
5. Yun, H. Carbon Dioxide Sequestration of Exhaust Fumes by Freshwater Microalgae with Massive Biomass Production. Bachelor's Thesis, Yonsei University, Seoul, Korea, 2015.
6. Zhao, B.; Su, Y.; Zhang, Y.; Cui, G. Carbon dioxide fixation and biomass production from combustion flue gas using energy microalgae. *Energy* **2015**, *89*, 347–357. [CrossRef]
7. Chew, K.W.; Chia, S.R.; Show, P.L.; Yap, Y.J.; Ling, T.C.; Chang, J.-S. Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: A review. *J. Taiwan Inst. Chem. Eng.* **2018**, *91*, 332–344. [CrossRef]
8. Zhao, B.; Su, Y. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. *Renew. Sustain. Energy Rev.* **2014**, *31*, 121–132. [CrossRef]
9. Aslam, A.; Thomas-Hall, S.R.; Mughal, T.A.; Schenk, P.M. Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas. *Bioresour. Technol.* **2017**, *233*, 271–283. [CrossRef]
10. Kassim, M.A.; Meng, T.K. Carbon dioxide (CO_2) biofixation by microalgae and its potential for biorefinery and biofuel production. *Sci. Total Environ.* **2017**, *584*, 1121–1129. [CrossRef]
11. Bischoff, H.W. Phycological studies. IV. Some Algae from Enchanted Rock and Related Algal Species. *Univ. Texas Publ.* **1963**, *6318*, 95.
12. Blair, M.F.; Kokabian, B.; Gude, V.G. Light and growth medium effect on Chlorella vulgaris biomass production. *J. Environ. Chem. Eng.* **2014**, *2*, 665–674. [CrossRef]
13. Ji, M.-K.; Yun, H.-S.; Hwang, B.S.; Kabra, A.N.; Jeon, B.-H.; Choi, J. Mixotrophic cultivation of Nephroselmis sp. using industrial wastewater for enhanced microalgal biomass production. *Ecol. Eng.* **2016**, *95*, 527–533. [CrossRef]
14. Yun, H.S.; Ji, M.K.; Park, Y.T.; Salama el, S.; Choi, J. Microalga, Acutodesmus obliquus KGE 30 as a potential candidate for CO_2 mitigation and biodiesel production. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 17831–17839. [CrossRef] [PubMed]
15. Li, Y.; Horsman, M.; Wang, B.; Wu, N.; Lan, C.Q. Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 629–636. [CrossRef] [PubMed]
16. Chiu, S.-Y.; Kao, C.-Y.; Huang, T.-T.; Lin, C.-J.; Ong, S.-C.; Chen, C.-D.; Chang, J.-S.; Lin, C.-S. Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. *Bioresour. Technol.* **2011**, *102*, 9135–9142. [CrossRef] [PubMed]
17. Praveenkumar, R.; Kim, B.; Choi, E.; Lee, K.; Cho, S.; Hyun, J.-S.; Park, J.-Y.; Lee, Y.-C.; Lee, H.U.; Lee, J.-S.; et al. Mixotrophic cultivation of oleaginous Chlorella sp. KR-1 mediated by actual coal-fired flue gas for biodiesel production. *Bioprocess Biosyst. Eng.* **2014**, *37*, 2083–2094. [CrossRef] [PubMed]
18. Praveenkumar, R.; Kim, B.; Choi, E.; Lee, K.; Park, J.-Y.; Lee, J.-S.; Lee, Y.-C.; Oh, Y.-K. Improved biomass and lipid production in a mixotrophic culture of Chlorella sp. KR-1 with addition of coal-fired flue-gas. *Bioresour. Technol.* **2014**, *171*, 500–505. [CrossRef] [PubMed]
19. Ji, M.K.; Yun, H.S.; Hwang, J.H.; Salama, E.S.; Jeon, B.H.; Choi, J. Effect of flue gas CO_2 on the growth, carbohydrate and fatty acid composition of a green microalga Scenedesmus obliquus for biofuel production. *Environ. Technol.* **2017**, *38*, 2085–2092. [CrossRef]
20. Tang, D.; Han, W.; Li, P.; Miao, X.; Zhong, J.-J. CO_2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO_2 levels. *Bioresour. Technol.* **2011**, *102*, 3071–3076. [CrossRef]
21. Borowitzka, M.A. The 'stress' concept in microalgal biology—Homeostasis, acclimation and adaptation. *Environ. Boil. Fishes* **2018**, *30*, 2815–2825. [CrossRef]
22. Cheng, D.; Li, X.; Yuan, Y.; Yang, C.; Tang, T.; Zhao, Q.; Sun, Y. Adaptive evolution and carbon dioxide fixation of Chlorella sp. in simulated flue gas. *Sci. Total Environ.* **2019**, *650*, 2931–2938. [CrossRef] [PubMed]
23. Tan, X.B.; Lam, M.K.; Uemura, Y.; Lim, J.W.; Wong, C.Y.; Ramli, A.; Kiew, P.L.; Lee, K.T. Semi-continuous cultivation of Chlorella vulgaris using chicken compost as nutrients source: Growth optimization study and fatty acid composition analysis. *Energy Convers. Manag.* **2018**, *164*, 363–373. [CrossRef]

24. Sun, X.-M.; Ren, L.-J.; Zhao, Q.-Y.; Ji, X.-J.; Huang, H. Microalgae for the production of lipid and carotenoids: A review with focus on stress regulation and adaptation. *Biotechnol. Biofuels* **2018**, *11*, 272. [CrossRef] [PubMed]
25. Brown, L.M. Uptake of carbon dioxide from flue gas by microalgae. *Energy Convers. Manag.* **1996**, *37*, 1363–1367. [CrossRef]
26. Park, Y.-T.; Lee, H.; Yun, H.-S.; Song, K.-G.; Yeom, S.-H.; Choi, J. Removal of metal from acid mine drainage using a hybrid system including a pipes inserted microalgae reactor. *Bioresour. Technol.* **2013**, *150*, 242–248. [CrossRef] [PubMed]
27. Sharma, K.K.; Schuhmann, H.; Schenk, P.M. High Lipid Induction in Microalgae for Biodiesel Production. *Energies* **2012**, *5*, 1532–1553. [CrossRef]
28. Nayak, M.; Dhanarajan, G.; Dineshkumar, R.; Sen, R. Artificial intelligence driven process optimization for cleaner production of biomass with co-valorization of wastewater and flue gas in an algal biorefinery. *J. Clean. Prod.* **2018**, *201*, 1092–1100. [CrossRef]
29. Singh, S.; Singh, P. Effect of CO_2 concentration on algal growth: A review. *Renew. Sustain. Energy Rev.* **2014**, *38*, 172–179. [CrossRef]