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ORIGINAL ARTICLE

Outdoor cultivation of microalgae in a coal-fired power plant for conversion of flue gas CO_2 into microalgal direct combustion fuels

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Abstract

Microalgae have piqued renewed interest as a sustainable biofuel feedstock owing to their high CO_2 conversion efficiency. However, the major limitation of microalga-based biofuel production is low productivity. In this study, CO_2 in flue gas emitted from the coal-fired power plants was fixed through mass microalgal cultivation using only sunlight as an energy source. To minimize the cost and energy required to supply the flue gas and efficiently utilize the microalgal biomass, a polycarbonate (PC) greenhouse and polymeric photobioreactors were installed near the power plant stack. Four different microalgal strains (*Chlamydomonas reinhardtii, Chlorella sorokiniana, Neochloris oleoabundans,* and *Neochloris oleoabundans* #13) were subjected to semi-continuous culturing for 1 month. The maximum biomass productivity was achieved by the *N. oleoabundans* #13 strain (0.703 g L^{-1} day^{-1}). Additionally, polymerase chain reaction analysis revealed that the individual microalgal culture was not cross-contaminated with other microalgal cultures in this cultivation system, owing to the structural properties of photobioreactor comprising individual modules. The lipid content and calorific productivity of *N. oleoabundans* #13 biomass were 45.70% and 3.553 kJ L^{-1} day^{-1}, respectively, which indicate satisfactory performance of biomass as a direct combustion fuel. The CO_2 fixation rate, which was calculated based on the carbon content in the biomass, was 0.309 g CO_2 L^{-1} day^{-1}. Therefore, large amounts of CO_2 can be reduced using the large-scale microalgal cultivation system, which enables efficient biological CO_2 conversion and maximizes microalgal biomass utilization.

Keywords Microalgae · Coal-fired power plant · CO_2 conversion process · Direct combustion fuel

Introduction

Climate change caused by greenhouse effect is one of the biggest challenges faced by mankind [1]. The enhanced atmospheric CO_2 concentration is the major factor contributing to global warming. The concentration of CO_2 in the atmosphere has increased by over 40% after industrialization. The global CO_2 emission is approximately 30 Gt $year^{-1}$ with the atmospheric CO_2 concentration increasing by 2 ppm annually [2]. Decreasing the production of energy by fossil fuel combustion is a potential strategy to mitigate the increased carbon concentration in atmosphere. However, fossil fuel combustion is reported to be the major source of energy until 2040 [3]. To address this challenge, there is increased interest in capturing carbon from large-point sources, such as coal-fired and liquefied natural gas (LNG) power plants. Compared to carbon capture and storage (CCS) technology, carbon capture and utilization (CCU) technology has several advantages, such as low infrastructure construction cost and ease of process site selection [4].

Carbon fixation by biological conversion, which is a CCU technology, involves the direct conversion of CO_2 into biomass or biofuel through photoautotrophic cultivation [5, 6]. However, utilizing food crop as a feedstock for energy generation may result in increased food cost and subsequently aggravate food scarcity in countries with poverty

Young Joon Sung and Jeong Seop Lee contributed equally to this work.

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[7]. Additionally, food crop must be subjected to several pretreatment processes for application as a feedstock [8, 9]. Alternatively, microalgal biomass, which is not associated with ethical issues, can be used as a feedstock for energy generation. The conversion of CO_2 into biomass by microalgae is faster than that by food crops [10–12]. Under nitrogen-depleted conditions, microalgae can accumulate a considerable amount of lipids in the intracellular region [13–16]. As reported by Choi et al., high-lipid content microalgae biomass has a high calorific value and can be used in the combustion process as a solid fuel [17]. Microalgae can be easily cultured in the presence of light and simple nutrients. Additionally, SO_x and particular matter emission from microalgal biodiesel combustion are lower than those from conventional diesel combustion [18]. Although microalgae have various advantages as a biofuel feedstock, there are some limitations for its application, including economic feasibility [19].

The major limiting economic factor for microalgal culture is the cost of CO_2 supply [20]. CO_2 can be supplied to the microalgal culture by supplementing air, flue gas, purified CO_2, or bicarbonate. Although the supplementation of purified CO_2 and bicarbonate has high CO_2 transfer efficiency, the cost of per ton CO_2 supply is high. Compared to the supply of atmospheric CO_2, the direct supply of flue gas with high CO_2 concentration has several advantages, such as high efficiency of CO_2 delivery to microalgal cells and reduced CO_2 supply cost [21]. In addition to the type of CO_2 source, the cost of CO_2 supply is affected by the distance between CO_2 source and microalgal cultivation site. In the flue gas transfer method, CO_2 is supplied through pipeline transportation. The proximity of cultivation site and CO_2 emission source decreases the cost of supplying CO_2 [22].

CO_2 emissions from coal-fired power plants account for about 4.5% of global CO_2 emissions [23, 24]. Operating a microalgal mass cultivation facility near a coal-fired power plant, a large-point source of CO_2 emission, enables the supply of CO_2 at high concentrations (10–15%) and low cost. The type of photobioreactor (PBR) is also a major factor that determines the efficiency of CO_2 supply from the coal-fired power plant to the microalgal cultivation system. A closed PBR has better advantages, such as low probability of contamination during cultivation process, increased cell growth, and 30 times high areal productivity when compared with the open pond system [25, 26].

In this study, a buffer solution system that can maintain the pH of the culture solution at a high concentration of CO_2 (15%) was used for effective outdoor mass cultivation of microalgae using the flue gas of coal-fired power plant as a source of carbon [27]. Additionally, the closed PBR comprising transparent polymer film was utilized to increase cell growth and CO_2 transfer rate and prevent contamination. Furthermore, various microalgal species (*Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, and *Neochloris oleoabundans*), including a mutant strain of *Neochloris oleoabundans,* exhibiting high lipid content in a previous study, were subjected to outdoor cultivation [28]. This study demonstrated efficient fixation of CO_2 present in high concentrations in the flue gas of coal-fired power plants through mass outdoor microalgal cultivation. Moreover, a strategy for using biomass as a direct combustion fuel has been proposed as the strategy that can utilize the microalgae biomass produced near the power plant most efficiently. The microalgae biomass produced using flue gas near the power plant can replace the existing solid fuel such as coal and woody biomass and can be directly applied to the power plant boiler. This approach can minimize the generation of additional greenhouse gas (GHG). Besides, the chances of additional GHG being released into the atmosphere are low because the combustion flue gas containing GHG is reused for microalgal culture.

Materials and methods

Microalgal strains and culture conditions

Chlamydomonas reinhardtii strain CC–125 (AGG1; mt+) was obtained from the *Chlamydomonas* Resource Center (University of Minnesota). *Chlorella sorokiniana* strain UTEX 2714 and *Neochloris oleoabundans* strain UTEX 1185 were obtained from the Algal Culture Collection at the University of Texas. The microalgal seed culture was prepared in a 250-mL Erlenmeyer flask under photoautotrophic culture conditions using Tris-phosphate (TP, TAP medium without acetate) medium at 5% CO_2 and 23 °C. The microalgal cells were pre-adapted to the CO_2 bubbling system, which comprises a glass cylinder and a stone sparger, before mass cultivation. The cells were allowed to grow till early stationary phase. Next, the cells were inoculated into the 10-L polymeric PBR for mass cultivation [28].

Outdoor microalgal cultivation system using coal-fired flue gas

PC greenhouse (5 m (width) × 10 m (length) × 3.7 m (height)) was installed at the Taean Coal Power Plant (Chungcheongnam-do, Republic of Korea) to protect the polymeric PBR from external hazards. Coal-fired flue gas was supplied to the PBR at a maximum flow rate of 40 m^3 h^{-1} and a maximum pressure of 3000 mmAq through the ring blower connected to the power plant stack. The flue gas was supplied to the bottom of PBR at a flow rate of 0.1 vessel volumes per min (vvm) through a stone sparger. The residual gas that passed through the culture medium was returned to

the power plant stack through an outline connected to the upper part of the reactor.

Quantification of dry weight of microalgal biomass

The dry cell weight of the microalgal biomass was determined using the filter-assisted gravimetric method. The Whatman GF/C glass microfiber filter (pore size of 1.2 μm and diameter of 47 mm) was washed with deionized (DI) water and dried in the oven. The dry filter weight was determined as the initial filter weight. Next, 3 mL of the cell suspension was loaded on the dried filter. The cell-loaded filter was rinsed with DI water to remove the residual salt. The filter was dried in an oven for 2 days to remove water. The difference between the initial and final filter weights was determined as the biomass concentration of the cells.

Lipid extraction and analysis of algal cells

The lipid-accumulated cell suspension was sampled from the PBR to determine the lipid content in the microalgal cells. The cell suspension (3.0 mL) was transferred to a 15-mL screw-capped glass tube and centrifuged at 3000 rpm for 10 min. Lipid was extracted from the cells following the Bligh-Dyer lipid extraction method [29]. Briefly, the cell pellet was resuspended in 3.9 mL of chloroform/methanol (1:2, v/v) mixture. Next, 1.3 mL of chloroform and 2.0 mL of DI water were added sequentially. The samples were subjected to centrifugation at 3000 rpm for 10 min to separate the aqueous and organic phases. The organic phase (2 mL) was transferred to a pre-weighed empty glass tube. The sample was dried overnight at 50 °C to evaporate the organic solvent. The glass tube was weighed to determine the amount of lipid extracted from the microalgal cells. The amount of fatty acid methyl esters (FAME) was determined using acid-catalyzed transesterification of total lipids in the presence of 10 mg of pentadecanoic acid (C15:0, internal standard) and sulfuric acid. FAME was detected using the gas chromatography-flame ionization detection (GC-FID) method and a DB–23 column (Agilent, USA). The detailed experimental conditions for detection of FAME has been reported elsewhere [30]. The amount of FAME was quantified via the correlation between peak area and concentration of internal standard.

DNA analysis of photobioreactor culture

DNA analysis was performed to evaluate if the microalgal culture in the closed PBR was contaminated. The cell cultures collected from PBR were centrifuged to obtain cell pellets. The genomic DNA (gDNA) was extracted from the cell pellet using the PureHelix™ Genomic DNA Prep Kit (Nanohelix Co., Ltd., Yuseong-Gu, Daejeon, Republic of Korea). The 18S rRNA gene was amplified using UTEX1185 18S rRNA primers [31], while the 16S rRNA gene was amplified using the following primer pairs: bacteria, 27Fmod and 519Rmod,archaea, archea349F and 806R; eukaryotes, Euk7F and 570R [32]. The 16S/18S rRNA gene was amplified using PrimeSTAR® GXL DNA polymerase (Takara). The polymerase chain reaction (PCR) products were purified using the eCube PCR Purification Kit (PhileKorea, Yuseong-Gu, Daejeon, Republic of Korea). The size of PCR product was confirmed using agarose gel electrophoresis (1.5 wt%, 4 V cm^{-1}, 40 min). The PCR product was sequenced at Macrogen, Inc., Geumcheon-gu, Seoul, Republic of Korea) using the ABI 3730xl System. The microbial species in the PBR was identified by subjecting the sequencing data to basic local alignment search tool (BLAST) analysis using the National Center for Biotechnology Information (NCBI) database.

Determination of the elemental compositions of dry algal biomass

The elemental composition was analyzed in the dry cell biomass. The microalgal biomass was harvested by subjecting it to continuous flow centrifugation using an industrial centrifuge (J-045, Hanil Science Medical, Yuseong-gu, Daejeon, Republic of Korea) at 100 L h^{-1} and 19,000 × g. The biomass was lyophilized at − 55 °C using a freeze-dryer (FDB-5503; Operon Co., Ltd.). Carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) were analyzed using an elemental analyzer (EA2000; Thermofinnigan) in the presence of catalysts (tungstic anhydride and copper wire) at 1000 °C. Oxygen (O) was analyzed using an EA1112 analyzer in the presence of catalysts (nickel-plated carbon and quartz turnings) at 1060 °C. BBOT (2,5-bis(5-tert-butyl-benzoxazole-2-yl) thiophene), L-cystine, and sulfanilamide were used as standards to determine the amount of each element. The trace elements, such as, Cd, Cr, Cu, and Pb were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) in 720 ICP-OES (Agilent, USA) equipped with a VistaChip II CCD detector. Mercury (Hg) was detected using an automatic mercury analyzer (MA-3000; Nippon Instrument Corporation) by determining the atomic absorption at a wavelength of 253.7 nm via an oxygen carrier gas.

Results and discussion

Utilization of coal-fired flue gas for outdoor cultivation of microalgae

As shown in Fig. 1, the microalgal cultivation facility using coal-fired flue gas as a carbon source comprises an air-lift type closed PBR, a ring blower, and a continuous centrifuge.

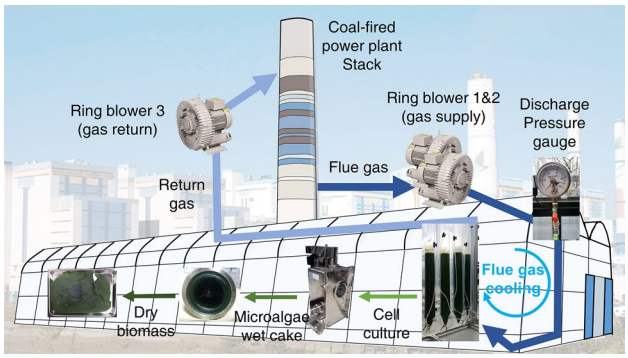

Fig. 1 Outdoor microalgal cultivation using coal-fired power plant flue gas as a carbon source. Flue gas emitted from coal-fired power plant stack was supplied using the ring blowers. Pressure of discharged flue gas from ring blower was checked using a pressure gauge installed on pipeline. The long pipeline path enabled the hot flue gas to cool down. The gas was injected into the photobioreactor. The microalgal cells were cultivated in an air-lift type polymer film photobioreactor. Downstream process was conducted using the tubular type continuous centrifuge and freeze dryer

The closed air-lift type PBR used in this microalgal cultivation system was prepared by thermally bonding a thin and transparent polymer film to the reactor to maximize light transmittance. A sintered stone sparger was installed inside the PBR to inject flue gas in the form of micro-scale air bubbles to increase the CO_2 transfer efficiency. Additionally, a syringe filter was installed in the tube connected to each PBR to prevent bacterial contamination and microalgal cross-contamination. The long-term stable supply of gas to the closed PBR was achieved using a double-stage three-phase ring blower (IHB-302, IN HA electric CO., LTD., Republic of Korea). To prevent overheating of the ring blower, alternating work/stop operation (12 h working/12 h stop) was performed. The pressure generated by the culture medium volume in the reactor must be overcome to ensure smooth supply of flue gas to the air-lift type PBR. In this cultivation system, approximately 1300 mmAq water pressure must be overcome by the ring blower. An aeration of 0.1 vvm was required to prevent the biomass from settling in the PBR and to mix the culture medium in the reactor. Therefore, a double-stage ring blower (discharge pressure: 1400 mmAq, flow rate: 1100 L min^{-1}) was used to ensure a minimum discharge pressure of 1300 mmAq and a minimum flow rate of 500 L min^{-1} (entire culture volume: 5 ton). Additionally, the flue gas pipe was installed along the inner wall of the greenhouse to lengthen the flue gas path, which enables the cooling of flue gas discharged from the ring blower at 50 ℃. The exhaust gas exiting the reactor was returned to the power plant stack through a downstream ring blower to prevent flue gas accumulation inside the greenhouse. A continuous operation centrifuge is more suitable to rapidly recover the culture medium of at least tens of liters than a batch type centrifuge. In this microalgal cultivation process, a tubular type continuous centrifuge, which is easy to operate and maintain, was used to enable dewatering process with minimal human resource. The samples were subjected to centrifugation at 19,000×*g* and 1.6 L min^{-1} to obtain the dense microalgal wet cake. Microalgal wet cake was dried using a bench top type freeze dryer (FDB-5503; Operon Co., Ltd.) at − 50 ℃ for 24 h. The completely dried biomass was homogenized using a household blender to obtain the microalgal biomass powder.

Culture conditions for outdoor cultivation of microalgae at the Taean coal power plant

The greenhouse for outdoor microalgal cultivation was established at the Taean Coal Power Plant (Fig. 2a). It is important to minimize the shading effect caused by the frame structure and maximize the penetration of sunlight required for microalgal cell growth. Thus, PC was used for the construction of greenhouse to minimize the frame thickness. The light transmittance of PC is similar to that of glass, while the weight of PC is lesser than that of glass. During the cultivation period in September 2019, the temperature inside the greenhouse was maintained between 21.5 and 36.5 °C at noon (Fig. 2b). Although high temperatures were often recorded due to the greenhouse effect caused by sunlight, optimal temperatures that did not markedly inhibit cell growth were maintained. Sunlight was used as the sole light source for cost-effective microalgal cultivation. The fluctuations in light intensity are reported to differentially affect cell growth [33] depending on the weather conditions. However, the microalgal cultivation process could be operated without additional energy input (Fig. 2b). The concentration of SO_x is high in coal-fired flue gas, which can lead to acidification of cell growth medium. Therefore, the SO_x concentration in flue gas must be decreased before the flue gas is directly supplied to the microalgal cultivation system. According to the Clean Air Conservation Act (Republic of Korea), all combustion gases must be exhausted through the stack after the desulfurization process. The composition of flue gas resulting from the desulfurization process is suitable for microalgal cell growth (Fig. 2c). The effect of simulated flue gas mixture (SO_x 25 ppm, NO_x 35 ppm, and CO_2 15%) on the pH of bicarbonate/phosphate (BP) buffer system was investigated to examine the effect of flue gas on the pH of culture medium (Fig. 2d). The pH changed from 11.59 to 6.53 upon supplementation of the simulated flue gas. Additionally, the buffer system enabled the maintenance of near-neutral pH at various concentrations of CO_2. However, the final pH slightly decreased at a CO_2 concentration of 15% (pH 6.67), which was due to the effect of SO_x [27]. In water, NO_x concentrations lower than 300 ppm can promote cell growth by acting as a nitrogen source [34]. Thus, the

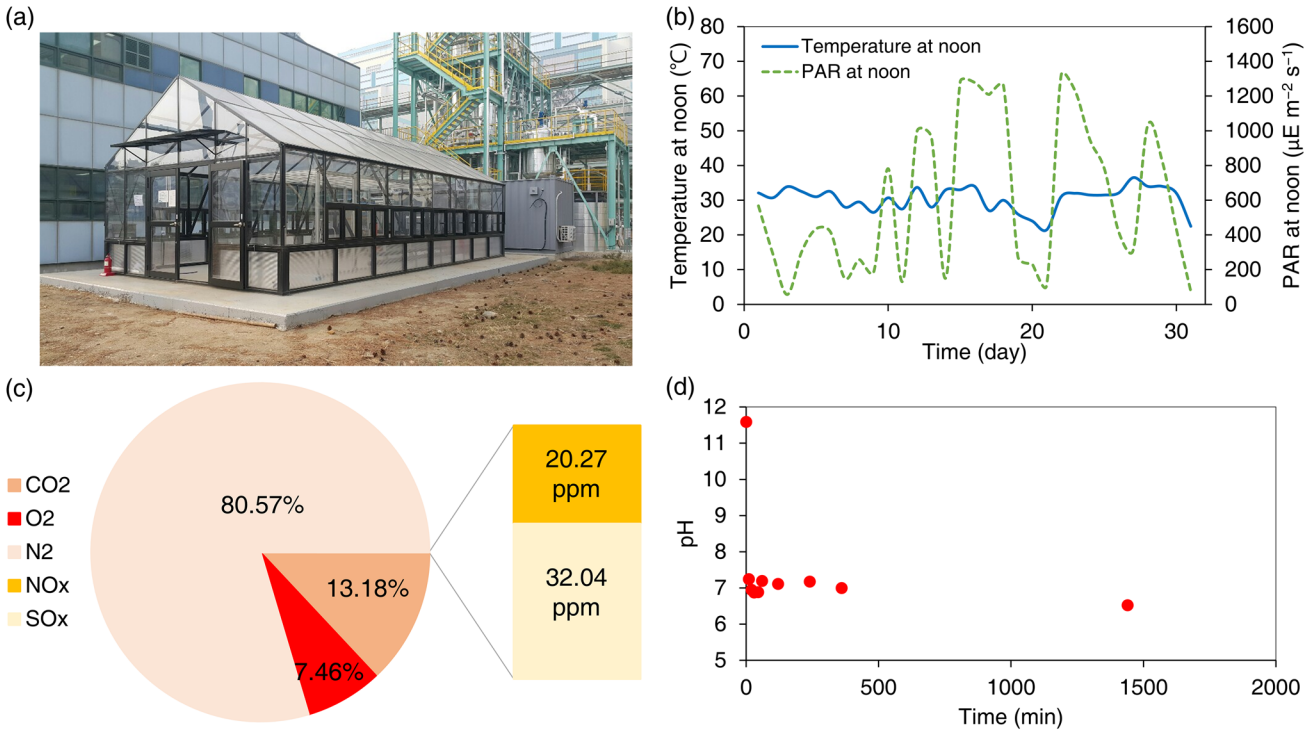

Fig. 2 Outdoor microalgal cultivation facility and culture conditions at the Taean coal power plant. **a** The greenhouse established near the power plant stack. The gas valves for supplying the flue gas, photobioreactor (PBR) for cell culture, and PBR holder are installed inside the greenhouse. **b** The changes in temperature and photosynthetically active radiation (PAR) inside the greenhouse at noon during microalgal cultivation. **c** Composition of flue gas supplied to the PBR after desulfurization. **d** The effect of simulated coal-fired flue gas (SO_x 25 ppm, NO_x 35 ppm, and CO_2 15%) supplementation on the pH of culture medium comprising the bicarbonate/phosphate (BP) buffer system over time. The data are represented as mean ± standard deviation (SD) from three replicates

microalgae could be stably cultivated using coal-fired flue gas directly without additional cost and energy input.

Biomass production of different microalgal strains during autumn season

During autumn (3rd September–30th September), four different microalgal strains (*Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Neochloris oleoabundans*, and *Neochloris oleoabundans* mutant #13 [28] were cultivated in a 10-L scale polymeric PBR in BP buffer system. During the first 13 days of cultivation, the biomass concentration of all strains increased steadily (Fig. 3a). The growth rate of *C. reinhardtii* is lower than that of other strains. As reported previously [17], the biomass concentration of *C. reinhardtii* was 0.713 g L^{-1} after 13 days, which was only 39.2% of the biomass concentration of *N. oleoabundans* #13 strain (1.819 g L^{-1}). A semi-continuous culture system was used to achieve maximum algal biomass productivity based on cell adaptation to the flue gas [35]. When the biomass concentration of *N. oleoabundans* #13 strain exceeded 1.8 g L^{-1}, 55% of total culture volume was harvested and replaced with the same volume of fresh medium. Thus, the culture period required to reach a biomass concentration of 1.8 g L^{-1} reduced from 13 days to a maximum of 3 days. The biomass productivity of each culture cycle may vary depending on the climate conditions. This is consistent with the results of Yoon et al., who reported that the productivity of biomass increases in a semi-continuous culture [35]. Unlike lab-scale indoor cultivation, the light intensity and temperature vary frequently depending on climate changes during outdoor cultivation. As the PBR is located inside the greenhouse, the change in temperature was low but the fluctuation in light intensity was high (Fig. 2b). The cell growth rate may be largely dependent on the changes in light intensity when the cells are grown using only sunlight and CO_2 without additional carbon sources. As shown in Fig. 3b, the daily biomass productivity pattern was directly proportional to the light intensity pattern. All microalgal strains exhibited maximum cell growth rates during the culture period of 22–23 days. This period is the third culture cycle in which the cells are fully adapted to the culture conditions and is associated with high light intensity at noon (1272 μE m^{-2} s^{-1}). The maximum biomass productivities of *Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *N. oleoabundans*, and *N. oleoabundans* mutant #13 strains were

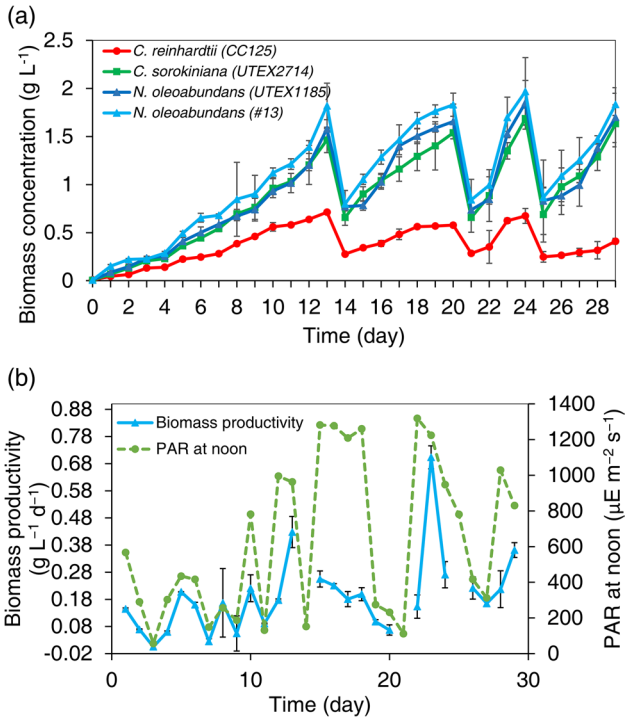

Fig. 3 Microalgal biomass production in a 10-L scale polymeric photobioreactor (PBR) directly supplied with coal-fired flue gas. **a** Biomass concentration of four different microalgal strains (*Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Neochloris oleoabundans*, and *Neochloris oleoabundans* mutant #13 [28] during autumn (3rd September–30th September). **b** Comparison of daily biomass productivity and photosynthetically active radiation (PAR) at noon. The data are represented as mean ± standard deviation (SD) from three replicates

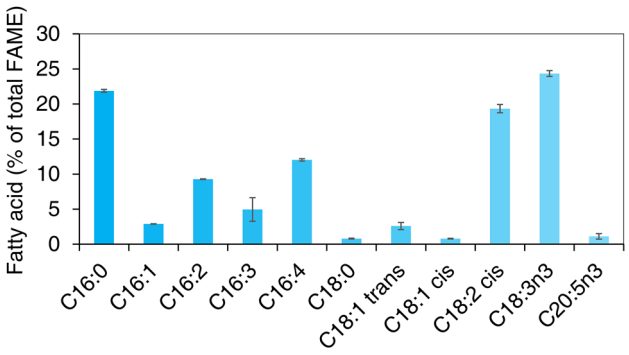

Fig. 4 Lipid content and fatty acid distribution in microalgal biomass. Distribution of fatty acid methyl esters (FAME) in #13 biomass. Each fatty acid is represented by the ratio of fatty acids to total FAME. The data represent mean ± standard deviation (SD) from three replicates

0.272, 0.465, 0.683, and 0.703 g L^{-1} day^{-1}, respectively, in this culture system.

Content of high-salinity stress-induced lipids in microalgal biomass

The content of high-salinity stress-induced lipids in various strains was analyzed. Additionally, the performance of these strains as a direct combustion fuel was investigated. The use of microalgal biomass with high lipid content as a direct combustion fuel is reported to be the most efficient and direct method to reduce CO_2 [17]. The oleaginous algal species, *N. oleoabundans* exhibited a higher total lipid content (39.89%) than other species [*Chlamydomonas reinhardtii* (30.12%) and *Chlorella sorokiniana* (38.48%)]. One study [28] reported that the maximum biomass productivity of *N. oleoabundans* mutant #13 and wild-type *N. oleoabundans* strains was similar ($P < 0.05$). However, the lipid content of *N. oleoabundans* mutant #13 (45.70%) was higher than that of wild-type strain. This high total lipid content can directly contribute to the high biomass energy density. In contrast to using only lipids, the energy yield can be improved when the entire biomass is applied to the combustion process as a biofuel. Microalgal biomass is reported to be a potential feedstock for biodiesel production. Thus, the fatty acid (FA) composition of *N. oleoabundans* mutant #13 strain was analyzed to examine its potential as a biodiesel feedstock (Fig. 4). According to European standards (EN14214), the proportion of linolenic acid in an ideal biodiesel should be less than 12%, while that of polyunsaturated FA (> 4 double bonds) should be less than 1% [36]. However, the proportions of linolenic acid and polyunsaturated FA in the *N. oleoabundans* #13 strain cultured using flue gas were 24.3 and 12.0%, respectively. The proportion of total polyunsaturated FA is 71.06%, which provides many reactive sites during the oxidation process and leads to low oxidation stability of biodiesel [37]. These results indicate that the #13 strain biomass is not suitable as a feedstock for biodiesel production. As this cultivation system directly utilizes the coal-fired flue gas from the power plant, the cultivation system was designed near the power plant. Based on the biomass productivity and total lipid content of strain #13 in this culture system, this strain can be expected to show excellent performance as a direct combustion fuel.

Purity of microalgal culture in photobioreactor

The microalgal culture is vulnerable to contamination through water and air when cultured outdoors. Bacterial contamination was not observed during the early stage of cultivation. However, bacteria can grow in this cultivation system using the dead microalgae as nutrients with the progression of cultivation. Additionally, the risk of cross-contamination with other microalgal species increases when culturing several types of microalgae at one cultivation site. Therefore, it is important to prevent contamination and routinely check for contamination when the microalgae are mass cultivated

outdoors. The purity of microalgal culture was confirmed by 16S/18S rRNA gene analysis (Fig. 1). The bacterial and archaeal contaminations were confirmed via PCR analysis of 16S rRNA gene sequences. The bacterial contamination in the microalgal culture resulted in the amplification of a sequence with a size of 500 bp, whereas the archaeal contamination in the microalgal culture resulted in the amplification of a sequence with a size of 450 bp. In this study, only a band corresponding to eukaryotic 18S rRNA gene (563 bp) was amplified and no amplification of bacterial or archaeal 16S rRNA gene was observed (Fig. 5a). This indicated that the culture was not contaminated with bacteria and archaea. Additional analysis was performed to confirm whether there was cross-contamination with microalgae. The sequence of *N. oleoabundans* 18S rRNA gene registered in the NCBI is not complete. Therefore, a primer set that can amplify the fragment of *N. oleoabundans* 18S rRNA gene registered in NCBI was used [31]. Similarly, a unique primer set was used to amplify *C. reinhardtii* 18S rRNA gene. This is because the sequence near 3′ untranslated region (UTR) of the 18S rRNA gene of *Chlamydomonas reinhardtii* differs from that of the 18S rRNA gene of *Chlorella sorokiniana*. The 18S rRNA sequencing analysis revealed that the culture media contained only 18S rRNA gene sequence of the inoculated species (Fig. 5b). This indicated that there was no cross-contamination between the PBRs. The prevention of bacterial contamination and microalgal cross-contamination, which minimizes the inflow of contaminants from outside, in a closed PBR can be achieved by sanitization of PBR using hypochlorous acid before inoculation and installing filters in flue gas transportation tubes in each PBR.

Performance of dry algal biomass for use as direct combustion fuel

The performance of biomass obtained from culturing the microalgae using the flue gas as a direct combustion fuel was evaluated (Table 1). The harvested microalgal biomass was subjected to dehydration, drying, and grinding to obtain a dry powder. Heavy metals generated from the combustion of coal can be adsorbed to the microalgal biomass as the coal-fired flue gas is directly supplied to the cultivation system. These heavy metals, which are abundant in fly ash during combustion, cause environmental pollution [38]. The levels of As, Cd, Cr, and Pb in the microalgal biomass were less than 0.1 ppm (Supplementary file 1), which are lower than those reported in a previous study [39]. In the Taean power plant, the flue gas emitted from the power plant is supplied to the microalgal culture medium after a desulfurization process due to various environmental regulations in Republic of

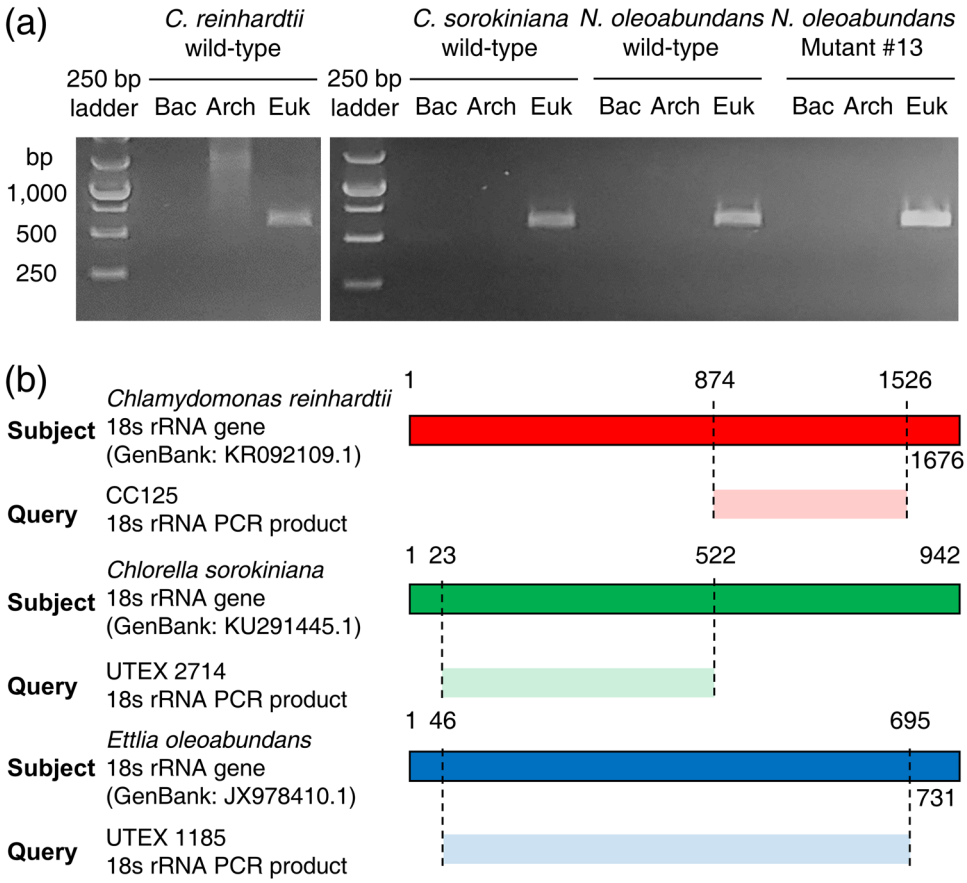

Fig. 5 Analysis of contamination in cultivated microalgae. **a** The PCR analysis of microalgal culture in a closed photobioreactor (PBR). The 16/18S rRNA gene was amplified to examine bacterial or archaeal contamination [Bac: bacterial 16S rRNA amplicon (492 bp), Arch: archaeal 16S rRNA amplicon (457 bp), Euk: Eukaryote 18S rRNA amplicon (563 bp)]. **b** The basic local alignment search tool (BLAST) analysis of 18S rRNA gene of each strain. Subject means sequence database registered on National Center for Biotechnology Information (NCBI). Query is sequence of fragment of 18S rRNA DNA amplified from microalgal culture

Table 1 Elemental composition of microalgal biomass and its performance as a direct combustion fuel

UTEX 1185 #13 mutant	
Biomass productivity	0.190 ± 0.001 g L^{-1} day^{-1}
Lower heating value (LHV)	18.715 ± 0.261 kJ g^{-1}
Elemental composition	
C	$44.410 \pm 0.531\%$
H	$6.693 \pm 0.031\%$
O	$32.417 \pm 1.818\%$
N	$5.153 \pm 0.816\%$
S	$0.053 \pm 0.092\%$
Calorific productivity (CP)	3.553 kJ L^{-1} day^{-1}
CO_2 fixation potential (CFP)	1.628 ± 0.019 g-CO_2 g-biomass^{-1}
CO_2 fixation rate (R_{CO_2})	0.309 g-CO_2 L^{-1} day^{-1}

Biomass productivity was determined as the average biomass productivity over the entire culture period. The elemental composition represents the weight ratio of each element in the total biomass weight. Calorific productivity (CP) was calculated based on lower heating value (LHV) obtained from bomb calorimeter. In addition, CO_2 fixation potential (CFP) and CO_2 fixation rate (R_{CO_2}) were calculated from the carbon content and biomass productivity

Korea. Thus, the flue gas may have a small amount of heavy metals (data not shown). Additionally, the microalgae were subjected to four cycles of semi-continuous culture with a cultivation period of 26 days. Therefore, the harvest time of microalgal biomass, which was harvested every 6.5 days on average. The analysis of lower heating value (LHV) determines whether the microalgae biomass can be used as a combustion fuel. The LHV, which was determined using a bomb calorimeter, of #13 biomass was 18.715 kJ g^{-1}. The calorific productivity (*CP*; kJ L^{-1} day^{-1}) of #13 biomass was 3.553 kJ L^{-1} day^{-1} based on the average biomass productivity in this culture period (0.190 g L^{-1} day^{-1}) and the LHV [40]. This value is relatively low when compared with the previously reported value (6.36 kJ L^{-1} day^{-1}) because the average biomass productivity decreases due to fluctuations in outdoor light conditions. Moreover, biochemical analysis revealed that the carbon content of biomass, which is strongly correlated to LHV, was 44.41%. As the carbon source of biomass is derived from CO_2 in the coal-fired flue gas, the ability of #13 biomass- to convert CO_2 to combustion fuel can be evaluated by calculating the CO_2 fixation potential (*CFP*; g CO_2 g biomass^{-1}) and CO_2 fixation rate (R_{CO_2}; g CO_2 L^{-1} day^{-1}) as follows:

$$\mathrm{CFP} = \frac{C}{100} \times \frac{M_{CO_2}}{M_C} \quad (1)$$

$$R_{CO_2} = \mathrm{CFP} \times \text{Biomass productivity} \quad (2)$$

where M_{CO_2} and M_C represent the molecular weight of CO_2 and carbon, respectively. CFP and R_{CO_2} were 1.628 g CO_2 g biomass^{-1} and 0.309 g CO_2 L^{-1} day^{-1}, respectively. The culture scale is directly correlated to the CO_2 reduction effect. The mass cultivation of microalgae using flue gas from the coal-fired power plant decreases the CO_2 emissions and produces biomass, which has excellent performance as a direct combustion fuel.

Conclusions

The *N. oleoabundans* mutant strain #13 achieved maximum biomass productivity of 0.703 g L^{-1} day^{-1} with a total lipid content of 45.70% after cultivation for a month using flue gas from the Taean power plant as a carbon source. PCR analysis revealed that different microalgal strains could be cultured without cross-contamination due to the structural characteristics of PBR composed of individual modules. Additionally, the LHV and carbon content of the microalgal biomass revealed that the calorific productivity and CO_2 fixation rate were 3.553 kJ L^{-1} day^{-1} and 0.309 g CO_2 L^{-1} day^{-1}, respectively. Therefore, CO_2 in the coal-fired flue gas can be efficiently reduced by cultivating microalgae and the microalgal biomass can be used as a direct combustion fuel.

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Author contributions YJS and JSL prepared and revised the manuscript. YJS and JSL analyzed the data. YJS, JSL, HKY, and HK designed and performed the experiments. SJS supervised the study.

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Availability of data and materials All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval Not applicable.

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Consent for publication All authors agree to the publication of this manuscript.

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