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Short Communication

# Improved biomass and lipid production in a mixotrophic culture of *Chlorella* sp. KR-1 with addition of coal-fired flue-gas

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## HIGHLIGHTS

- $CO_2$-rich coal-fired flue gas could be effectively used for mixotrophic algal growth.
- Fed-batch system with air supply in dark resulted in the highest lipid productivity.
- Bacterial community of KR-1 depended markedly on organic feeding(s) and aeration.
- Fatty acid composition of KR-1 meets the key biodiesel standards.

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## ABSTRACT

Industrial $CO_2$-rich flue-gases, owing to their eco-toxicity, have yet to be practically exploited for microalgal biomass and lipid production. In this study, various autotrophic and mixotrophic culture modes for an oleaginous microalga, *Chlorella* sp. KR-1 were compared for the use in actual coal-fired flue-gas. Among the mixotrophic conditions tested, the fed-batch feedings of glucose and the supply of air in dark cycles showed the highest biomass (561 mg/L d) and fatty-acid methyl-ester (168 mg/L d) productivities. This growth condition also resulted in the maximal population of microalgae and the minimal population and types of KR-1-associated-bacterial species as confirmed by particle-volume-distribution and denaturing-gradient-gel-electrophoresis (DGGE) analyses. Furthermore, microalgal lipid produced was assessed, based on its fatty acid profile, to meet key biodiesel standards such as saponification, iodine, and cetane numbers.



## 1. Introduction

Exhaust flue-gases with high concentrations of $CO_2$ (~15%, v/v) from various industries such as thermal power, cement, steel, and incineration have gained much interests as an inexpensive and $CO_2$ source for photosynthetic microalgal biomass production (Hende et al., 2012; Kao et al., 2014; Praveenkumar et al., 2014). However, the excess $CO_2$ and the presence of inhibitory compounds such as $NO_x$, $SO_x$ and CO seriously repress the metabolic activities of microalgae (Chiu et al., 2011; Hende et al., 2012).

Some microalgal species can use both inorganic ($CO_2$) and organic carbon substrates in the presence of light, growing via a form of mixotrophic metabolism (Ceron-Garcia et al., 2013; Praveenkumar et al., 2014; Wan et al., 2011). These show improved growth rates when compared with their autotrophic or heterotrophic counterparts. Organic carbon is utilized to prevent night biomass loss due to respiration and to achieve continuous cell growth under light–dark cycles. Synergistic effects of light and organic carbon for higher biomass and lipid production have been reported (Cheirsilp and Torpee, 2012; Wan et al., 2011). Therefore, a mixotrophic cultivation system combining high concentrations of $CO_2$, especially from industrial-exhaust-gas sources, and an organic substrate can be practical means of mass production of microalgae for biofuel feedstocks (Cheirsilp and Torpee, 2012; Lee et al., 2013a,b; Praveenkumar et al., 2014). However, most of the research reported previously has focused on the feasibilities of mixotrophic cultures and isolations of proper microalgae using synthetic $CO_2$-enriched air and therefore efforts to apply actual industrial flue-gases are very limited. Recently, Praveenkumar et al. (2014) reported mixotrophic algal lipid production of

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118 mg fatty-acid-methyl-ester (FAME)/L d with 0.2 vvm actual coal-burned flue-gas and 5 g/L glucose under the 24 h lighting condition. However, when the flue-gas was increased to 0.6 vvm, the lipid productivity was significantly reduced to 59 mg FAME/L d, and with a very low glucose consumption efficiency of 6%, due to the toxic effect of flue-gas on microalgal growth (Praveenkumar et al., 2014).

Some microalgae such as *Chlorella* sp. lives in symbiosis with bacteria, and the associations are species-specific (Eigemann et al., 2013; Lee et al., 2014). Co-existing bacteria can enhance or inhibit microalgal growth, depending on environmental and nutritional conditions (Lakaniemi et al., 2012). This can significantly affect microalgal culture process performance measures such as biomass and lipid productivities. However, little information on mixotrophic cultures, especially with industrial $CO_2$-rich flue-gas, is available in the literature.

The purpose of this study was to examine the possibility of improving the biomass and lipid (FAME) productivities of mixotrophic *Chlorella* sp. KR-1 using real coal-fired flue-gas and operational strategies such as fed-batch feedings of glucose and air supply during dark cycles. Changes in the bacterial consortium and population were determined through the denaturing-gradient-gel-electrophoresis (DGGE) technique and particle-volume-distribution measurement. The quality of the microalgal lipid was further characterized based on key biodiesel parameters such as saponification, iodine and cetane numbers from fatty acid composition.

## 2. Methods

### 2.1. Microalga and seed culture

*Chlorella* sp. KR-1 used in this study was isolated from a freshwater stream near a thermal power plant in Korea (Na et al., 2011). This strain showed a good tolerance for actual coal-burned flue-gas (Praveenkumar et al., 2014) and had a relatively high lipid content of ~41% (w/w) (Na et al., 2011; See Fig. S1 for transmission electron microscopic image of KR-1). For the experimentation, modified N8 medium was prepared and filter-sterilized through a 0.2 μm membrane, and its pH was maintained at 6.5 (Praveenkumar et al., 2014). The cells were cultivated for 120 h in a Pyrex glass bubble-column photo-bioreactor (b-PBR) (length, 35 cm; inner dia., 3.7 cm; working vol., 500 mL) and used as the inoculum. The b-PBR was continuously supplied with $CO_2$-enriched air (10%, v/v; 0.6 vvm) via a 0.2 μm PTFE filter (Minisart 2000, Satorius Stedium Biotech., Germany). The flow rate and composition of the gas was controlled using mass flow controllers (MK Precision, Korea) and flow meters (Dwyer Instruments Inc., USA). The reactor was maintained under continuous illumination (white fluorescent lamps, *ca.* 170 μmol/$m^2$ s) in a temperature-controlled room (28–31 °C). More details are previously reported by Praveenkumar et al. (2014).

### 2.2. Microalgal cultivation using actual flue-gas

Autotrophic and mixotrophic cultures with addition of actual coal-fired flue-gas were performed for 120 h utilizing the same b-PBRs as employed for the seed culture. The phosphate concentration in the N8 medium was increased to 30 mM by adding 3054 mg $KH_2PO_4$ and 1072 mg $Na_2HPO_4$ in order to avoid significant pH drop by adding $CO_2$-rich flue-gas (Praveenkumar et al., 2014). The initial pH of the medium was adjusted to 6.5. The inoculum was adjusted to an optical density (OD) of 0.2 at 660 nm. The b-PBR was supplied either continuously with flue-gas or intermittently with air during the dark cycles at the flow rate of 0.2 vvm.

For the mixotrophic cultures, filter-sterilized glucose (0.2 μm; Minisart High-Flow, Satorius Stedium Biotech., Germany) was added initially at 3 g/L (batch mode) or 3 times at 1 g/L at 18, 42, and 66 h, respectively during the dark cycles (fed-batch mode). Light was supplied initially for 18 h and then on a 12:12 h dark/light cycle using white fluorescent lamps at both sides of the b-PBR (285 μmol/$m^2$ s). All of the experiments were carried out in a temperature-controlled room (27–31 °C). The flue-gas flow rate (0.2 vvm) and the glucose concentration (3 g/L) were selected based on previous experiments under the 24 h light condition (Praveenkumar et al., 2014).

### 2.3. Coal-fired flue-gas

The flue-gas used in this study was collected from a 2.1 MW demonstration-scale coal-burning power plant located at the Korea Institute of Energy Research (KIER) in Daejeon, Korea. Details on the storage, pre-processing and transfer of flue-gas are available elsewhere (Praveenkumar et al., 2014). The typical composition of the flue-gas was $CO_2$, 13.3%; $O_2$, 7.6%; CO, 39.1 ppm; and $NO_x$, 6.9 ppm (Fig. 3a). A schematic illustration of the flue-gas based cultivation system for *Chlorella* sp. KR-1 is shown in Fig. S2.

### 2.4. Analytical methods

The biomass concentration was measured as ash-free dry-cell weight (AFDW; g/L). Ash content was measured based on GF/C filtration (Whatman, UK) and incineration of algal biomass at 550 °C for 15 min. A linear correlation was obtained from optical density values of the culture at 660 nm against AFDW. The pH and light intensity were determined using a pH meter (DKK-TOA Co., Japan) and a quantum meter (LI-250A, LI-COR Inc., USA), respectively. The nitrate and glucose concentrations were analyzed by nitrate (D5030-11, Humas Co., Korea) and glucose (AceChem, YD-Diagnostics, Korea) assay kits, respectively, according to the protocols provided by the suppliers. The microbial-community composition of the *Ch.* sp. KR-1-associated bacteria was investigated using the DGGE technique and the Dcode™ Universal Mutation Detection system (Bio-Rad Laboratories, USA). The bands on the DGGE gel were eluted, amplified, sequenced, and identified in BLAST analyses at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The bio-volumes of the microalgae and bacteria were analyzed by Multisizer™ 4 Coulter counter (Beckman Coulter, USA), assuming spherical shapes in the ranges of 0.4–1.5 μm and 2–6 μm, respectively, based on microscopic measurements (Microscope Axio Imager.A2, Carl Zeiss, Germany).

Flue-gas was collected in a Tedlar® gas-sampling bag (Sigma–Aldrich, USA), and its composition was analyzed by a potable flue-gas analyzer (Vario Plus, MRU Instruments Inc., Germany). The fatty acid content of microalgal biomass was determined by the direct transesterification method followed by gas chromatography (GC; Agilent 6890, Agilent Technologies, USA) analysis. All of the analytical methods are detailed in Supplementary Information and are also reported previously (Lee et al., 2013a,b; Lee et al., 2014; Praveenkumar et al., 2014).

### 2.5. Biodiesel parameter estimation

Biodiesel properties such as the saponification, iodine and cetane numbers were estimated from the fatty acid composition based on the empirical formulae proposed by Lei et al. (2012).

### 2.6. Statistical analysis

Data were statistically analyzed using SPSS Statistics 11.5 software (IBM Corporation, USA) and the results were expressed as