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Volatile fatty acid recovery by anaerobic fermentation from blue-green algae: Effect of pretreatment

Hyun Uk Cho [a,b,1], Hye Gyeong Kim [a,1], Young Mo Kim [e], Jong Moon Park [a,b,c,d,*]

[a] School of Environmental Science and Engineering, Pohang University of Science and Technology, 77 Cheongam-ro, Nam-gu, Pohang, Gyeongbuk 37673, Republic of Korea
[b] Bioenergy Research Center, Pohang University of Science and Technology, 77 Cheongam-ro, Nam-gu, Pohang, Gyeongbuk 37673, Republic of Korea
[c] Department of Chemical Engineering, Pohang University of Science and Technology, 77 Cheongam-ro, Nam-gu, Pohang, Gyeongbuk 37673, Republic of Korea
[d] Division of Advanced Nuclear Engineering, Pohang University of Science and Technology, 77 Cheongam-ro, Nam-gu, Pohang, Gyeongbuk 37673, Republic of Korea
[e] School of Earth Science and Environmental Engineering, Gwang-ju Institute of Science and Technology, 123 Cheomdangwagi-ro, Buk-gu, Gwang-ju 61005, Republic of Korea

HIGHLIGHTS

- Cyanobacterial biomass solubilization was augmented by all pretreatments.
- The highest biomass solubilization did not result in the highest VFA production.
- The highest VFA yield was obtained using alkaline pretreatment.
- VFAs derived from cyanobacterial biomass were converted into lipids by *C. curvatus*.
- The combination of alkaline pretreatment and *C. curvatus* cultivation is efficient.

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ABSTRACT

The aims of this study were to quantify how pretreatment affects production of volatile fatty acids (VFAs) from cyanobacterial biomass and production of subsequent microbial lipid by an oleaginous microorganism that uses the VFAs as carbon sources. The highest biomass solubilization was obtained using thermal-alkaline (th-alkaline) pretreatment (33.1%), followed by alkaline pretreatment (29.1%), and thermal pretreatment (7.2%), but the highest VFA yield was obtained using alkaline pretreatment (0.54 ± 0.02 g/g VS), followed by the untreated condition (0.47 ± 0.03 g/g VS), and th-alkaline pretreatment (0.44 ± 0.02 g/g VS). Although VFA yield was higher using alkaline pretreatment condition than in the untreated condition, the difference was not great. However, lipid productivity by *Cryptococcus curvatus* after the alkaline pretreatment condition was 2.0-fold higher than that under the untreated condition. This study confirmed the feasibility of using biologically produced VFAs from cyanobacterial biomass for microbial lipid production by the oleaginous microorganism.

1. Introduction

Cyanobacterial blooms are a worldwide problem in various water bodies. Recently in Korea, serious cyanobacterial blooms have occurred every summer in the Han, Geum, Nakdong, and Yeongsan rivers (Srivastava et al., 2015), which threatens the quality of drinking water due to the release of toxic compounds by cyanobacteria. 18 areas have been designated as the priority control area of cyanobacterial blooms along the four rivers by Ministry of Environment (South Korea) since 2015. Among them, the Nakdong River, the longest river (525 km) in South Korea, includes the highest number of priority control areas (10) and it has been the most seriously affected by cyanobacterial blooms. To mitigate cyanobacterial blooms and reduce eutrophication in the rivers, alga-harvesting barges have been launched on the rivers for the past few years during summer (Byeon et al., 2016). This operation is considered to be one of the most direct and efficient strategies to reduce the intensity of cyanobacterial blooms. However, secondary environmental pollution can occur on land unless they are subsequently treated.

Although microalgae are promising feedstocks for the production of biodiesel, its generation only extracts the lipids stored in the biomass (Gonzalez-Fernandez et al., 2013). In general,

* Corresponding author at: Department of Chemical Engineering, Pohang University of Science and Technology, 77 Cheongam-ro, Nam-gu, Pohang, Gyeongbuk 37673, Republic of Korea.
E-mail address: jmpark@postech.ac.kr (J.M. Park).
[1] These authors contributed equally to this work.

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cyanobacteria accumulate low levels of lipids, and lipid production of cyanobacteria cannot reach that of eukaryotic algae (Hannon et al., 2010). Therefore, energy recovery from the harvested cyanobacteria by anaerobic fermentation can be more suitable because all three macromolecules (protein, carbohydrate, and lipid) in the biomass can be used as substrates.

Volatile fatty acids (VFAs), which can be produced by anaerobic fermentation of organic wastes, have been widely utilized as external carbon sources (Cho et al., 2015b) for generation of electricity (Chen et al., 2013), biological removal of phosphorus (Pijuan et al., 2004), and production of microbial oil (Cho et al., 2015a). VFAs production through acidogenic fermentation is highly influenced by the type of substrates. Most studies have considered VFA production from organic wastewaters or typical organic wastes such as sewage sludge and food waste; use of microalgal biomass in VFA production has been rarely reported (Fernandes et al., 2016; Wang et al., 2014; Chen et al., 2013; Zhang et al., 2009; Cho et al., 2015b; Suresh et al., 2013), and even less information is available regarding VFA production from cyanobacteria.

Microalgal biomass is more difficult to degrade than sewage sludge because of the high resistance of cell walls (Park et al., 2013), so a pretreatment step is required to accelerate their hydrolysis, which is the major bottleneck during anaerobic fermentation (Passos and Ferrer, 2015; Lee et al., 2014; Mendez et al., 2013; Gonzalez-Fernandez et al., 2012; Passos et al., 2013). For VFA production from biomasses to be economically feasible, maximization of biomass conversion is significant, but the effect of pretreatment on VFA production can differ greatly for individual types of algae due to their different structures of cell wall. The lack of cellulose and other complex polymers renders cyanobacterial biomass as an ideal substrate for biogas production compared to microalgal biomass (Mendez et al., 2015), but little information is available about the effect of pretreatment on VFA production from cyanobacterial biomass.

Therefore, the influence of thermal, alkaline, and thermal-alkaline (th-alkaline) pretreatments on cyanobacterial biomass solubilization and its effect on VFA production was evaluated in this study. In addition, combining the produced VFAs with cultivation of oleaginous microorganisms was tested in this study to determine whether the VFAs from cyanobacterial biomass can be stably converted to microbial lipids for biodiesel production.

2. Materials and methods

2.1. Substrate and pretreatments

The Nakdong River, the longest river (525 km) in South Korea, is located in the southeast of the Korean peninsula. The cyanobacteria were collected from the Nakdong River about 7 km downstream from Dalsung weir during a summer cyanobacterial bloom. *Microcystis* sp. was a dominant strain in the collected biomass; it had 22.1 ± 0.6 g/L of total solids (TS), 20.5 ± 0.5 g/L of volatile solids (VS), 30.0 ± 1.1 g/L of total chemical oxygen demand (TCOD), 0.21 ± 0.03 g/L of soluble total nitrogen (STN), 0.03 ± 0.00 g/L of soluble total phosphorus (STP), and 6.67 ± 0.10 of pH. The main component of *Microcystis* sp. is carbohydrate (56.42% carbohydrate, 6.96% protein, 0.35% lipid, and 25.69% ash) (Kim et al., 2012). Three pretreatments were applied to the cyanobacterial biomass to evaluate their effects on biomass solubilization. Thermal pretreatment was conducted by autoclaving the biomass at 60 °C for 20, 40, or 60 min. Alkaline pretreatment was conducted by adding 5.0 N NaOH to the biomass and mixing it carefully for 60 min until pH reached 8, 10, or 12. Th-alkaline pretreatment was conducted sequentially: the pH of the biomass was adjusted to 12 by adding 5.0 N NaOH, then the alkali-pretreated biomass was autoclaved at 60 °C for 20, 40, or 60 min. Biomass solubilization (%) was calculated as follows: $[(SCOD_a - SCOD_b)/(TCOD - SCOD_b)] \times 100$ where $SCOD_a$ denotes SCOD after pretreatment and $SCOD_b$ denotes SCOD before pretreatment.

2.2. Anaerobic fermentation of cyanobacterial biomass

Both the alkali-pretreated biomass at pH 12 and the th-alkali-pretreated biomass at pH 12 and 60 °C for 20 min were selected for VFA production due to relatively higher biomass solubilization. VFA production from raw biomass was also conducted as a control. The experiments were performed in batch mode under different pretreatment conditions (raw, alkaline, and th-alkaline) using anaerobic fermenters with a working volume of 1L. All fermenters containing each substrate and inoculum were mechanically stirred at 150 rpm and operated at 35 °C. The inoculum was taken from a full-scale mesophilic anaerobic digester treating sewage sludge in Daegue, Korea and it was heated at 100 °C for 2 h to suppress methanogens; it had 25.4 ± 0.1 g/L of TS and 14.6 ± 0.1 g/L of VS. pH was adjusted at 6.5 ± 0.1 by a pH controller and aluminum foil was used to cover the fermenters to suppress photosynthesis.

2.3. Combining VFA production with microbial lipid production

VFAs produced from the cyanobacterial biomass under three different pretreatment conditions (raw, alkaline, and th-alkaline) were used as carbon sources for microbial lipid production by *Cryptococcus curvatus* (*C. curvatus*). *C. curvatus* was obtained from Korean Collection for Type cultures (Daejeon, South Korea) and pre-cultured in 250 mL Erlenmeyer flasks containing 100 mL of a sterilized YPD medium (10 g/L of yeast extract, 20 g/L of peptone, and 20 g/L of glucose). Cultivation was performed in a shaking incubator (200 rpm) at 30 °C.

Each anaerobically fermented broth was centrifuged at 8000 rpm for 15 min, then the supernatant was passed through a 1.2-μm glass microfiber filter and autoclaved at 121 °C for 20 min. *C. curvatus* was cultivated in batch mode in raw, alkaline, and th-alkaline media in Erlenmeyer flasks with a working volume of 400 mL. When inoculum cultivated in the pre-culture medium was in the late growth phase, 10% (v/v) inoculum was added to the media. Initial pH was controlled at 7.0 ± 0.1 with 2.0 N NaOH and 2.0 N HCl. All cultivations were performed in duplicate using a shaking incubator (200 rpm) at 30 °C.

2.4. Analytical methods

TS, VS, SCOD, TCOD, TN, and TP were quantified, following the standard methods (Clesceri et al., 1998). Volatile fatty acids (VFAs) were measured utilizing a high performance liquid chromatography (HPLC-1100, Agilent Technology, USA) equipped with a column (Aminex HPX-87H, Biorad Inc., USA), refractive index detector, and diode array detector (Cho et al., 2013). Ammonium concentration was analyzed utilizing an ion chromatography (ICS 1100, Dionex, USA) equipped with a column (Ionpac CS12A, Dionex, USA). Soluble fractions were measured after samples were filtered through 0.45-μm membrane filters (Millipore, USA). Microbial biomass concentration was determined by taking samples from the flasks, passing the samples through pre-weighed GF/C filters (Whatman, UK), and drying at 105 °C for 24 h. Lipid extraction was conducted using modified methods of Bligh and Dyer (1959) (Bourque and Titorenko, 2009). Lipid was extracted from lyophilized biomass by using a mixture of chloroform and methanol (2:1 of v/v); the mixture was centrifuged to dissolve the lipids in the solvent, then a nitrogen evaporator (Organomation Associates Inc., USA) was used to evaporate the solvent with nitrogen gas (Xu et al., 2015). The lipids were converted to fatty acid

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