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Evaluation of thermal, ultrasonic and alkali pretreatments on mixed-microalgal biomass to enhance anaerobic methane production

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HIGHLIGHTS

- A mix-microalgae biomass was used as a feedstock for anaerobic digestion.
- Ultrasonic, alkali and thermal pretreatments were done to promote the digestion.
- Energy recovery on anaerobic digestion of the pretreated biomass was assessed.
- Biochemical methane potential was used for energy recovery assessment.
- The involving microbial community was investigated by PCR-DGGE technique.

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ABSTRACT

Anaerobic digestion was regarded as one of the ways to recover energy from mixed-microalgae biomass in this study. After applying thermal-, ultrasonic-, and alkali-pretreatments to raw microalgae biomass to promote the digestion efficiency, a biochemical methane potential was investigated to evaluate the effectiveness of the pre-treatments for the purpose. As the pretreatment intensity increased, the solubilization of the mixed microalgae increased. However, the increased solubilization was not followed proportionally by the increased methane production. The highest methane productivity was achieved by the thermal-pretreatment at 120 °C (405 mL CH_4/g-VS), which was 1.2 times higher than that of the non-pretreatment condition (336 mL CH_4/g-VS). The net energy analysis revealed that only the pretreatment adjusted to pH 9 yielded a slightly higher energy gains (12.8 kJ/g-VS) than that of non-pretreatment condition (11.9 kJ/g-VS). These findings recommend direct supply of microalgae biomass for anaerobic digestion.

1. Introduction

The rapid growth of microalgae in eutrophication makes the drinking water treatment difficult and disturbs the ecosystem of rivers and lakes. Several microalgae excrete harmful toxins which can damage human health through drinking or skin contact. Therefore, many studies have focused on the proper way of reducing microalgal bloom such as flocculent settling using clay (Sengco and Anderson, 2004) or barley stew (Barrett et al., 1996), ultrasonic destruction (Nakano et al., 2001), and a combination of ultrasonic irradiation and coagulation (Heng et al., 2009). Recently, removing microalgae from drinking water bodies has become a challenge that came along with the climate changes in Korea.

On the other hand, bioenergy production via mass culture of microalgae is being studied throughout the world, and efforts are being made to industrialize bioenergy production using them. Microalgae produce O_2 and effective products by consuming CO_2 and photo-energy which is spontaneously recycling. In addition, their photosynthesis efficiency (3–8%) is higher than that of terrestrial plants (0.5–2%), and yields a higher biomass production per a unit area (Vasudevan and Briggs, 2008). Moreover, specific conditions such as nitrogen depletion stimulate microalgae's ability to accumulate lipid in their cells unlike plants in which the condition is likely to result in plant diseases or no fruits (Hu et al., 2008). Thus, mass production of microalgae promotes reduction of CO_2 in the atmosphere and shows the unexplored potentials of microalgae to become a feedstock for biodiesel, a renewable energy for transportation fuel besides the utilization for food additives (Becker, 2007), health supplement foods (Benedetti et al., 2004), pigments (Wiltshire et al., 2000), livestock or fish farm feed (Roeck-Holtzhauer et al., 2009).

There are many ways to recover the photo-energy fixed in microalgal cells; thermochemical liquefaction (Sawayama et al., 1999; Huang et al., 2010), transesterificaion (Huang et al., 2010; Xu and

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Mi, 2011), gasification (Chakinala et al., 2010; Huang et al., 2010), thermo-hydrolysis (Alzate et al., 2012; Keymer et al., 2013) and pyrolysis (Huang et al., 2010). Methane production from microalgae biomass through anaerobic digestion is one of the methods which have been used for energy production from wet organic wastes to date.

In anaerobic digestion, microalgae can be used as a substrate for methane production. The main advantages of anaerobic digestion for energy recovery from microalgae are: (1) the microalgae harvested from water-based culture systems may directly act as a substrate, (2) the microalgae lipid-extracted, transesterified and co-digested with glycerol can be used for the substrate of anaerobic digestion (Keymer et al., 2013), (3) final residues remaining after anaerobic digestion can be used for fertilizers due to its high nitrogen contents as a disposal method, and (4) they can apply to microalgae harvested from various natural environments.

The compounds and structure of microalgae are various: no distinguishable cell wall, compounds that are mainly protein-originated, and cellulose or hemi-cellulose family compounds, other sugars and algal-resistant polymers. The pretreatment methods for effective energy recovery from organic matters can vary depending on the energy recovery process or energy forms; hydrothermal acid pretreatment for ethanol production (Nguyen et al., 2009; Harun and Danquah, 2011), a low temperature (Passos et al., 2013), thermal, alkali and ozone pretreatments for anaerobic digestion (Bougrier et al., 2006), decreasing temperature and increasing pressure for biodiesel production (Halim et al., 2011).

In this study, the energy recovery from microalgae biomass through anaerobic digestion were investigated, and evaluated the energy recovery efficiency of three pretreating methods, thermal-, ultrasonic-, and alkali-pretreatments, on microalgae for anaerobic digestion. The potential of anaerobic degradation was estimated by performing the biochemical methane potential (BMP) test after applying the pretreatments. The economic feasibility of energy recovery was assessed on the basis of balancing input and output energy. In addition, considering that microorganisms convert cells of microalgae into methane and carbon dioxide via organic acid on anaerobic digestion, the microbial community involved in the BMP test was investigated by means of the polymerase chain reaction and the denaturing gradient gel electrophoresis (PCR-DGGE) technique.

2. methods

2.1. Substrates and inoculum

A mixture of a *Chlorella* sp. (70% dry cell weight of total microalgae biomass weight, w w^{-1}) and a *Scenedesmus* sp. (30% dry cell weight of total microalgae biomass weight, w w^{-1}) was prepared as the substrate for anaerobic digestion in this study. Each biomass was harvested by sedimentation and centrifugation (5 min, 3000 rpm) after cultivation in cylindrical photo-bioreactors (the working volume is 22 L) under continuous illumination with fluorescent light (20 μmol m^{-2} s^{-1}) at 25 °C. A modified Bold's Basal Medium was used for the cultivation: K_2HPO_4 75 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ 251 mg L^{-1}, $CaCl_2 \cdot 2H_2O$ 25 mg L^{-1}, KH_2PO_4 175 mg L^{-1}, NaCl 25 mg L^{-1}, H_3BO_3 28 mg L^{-1}, $MnCl_2 \cdot 4H_2O$ 3.60 mg L^{-1}, $ZnSO_4 \cdot 7H_2O$ 22 mg L^{-1}, $CuSO_4 \cdot 5H_2O$ 3.92 mg L^{-1}, $Na_2MoO_4 \cdot 2H_2O$ 1.92×10^{-1} mg L^{-1}, $Na_2EDTA \cdot 2H_2O$ 49.9 mg L^{-1}, KOH 31 mg L^{-1}, $FeSO_4 \cdot 7H_2O$ 12.4 mg L^{-1}, H_2SO_4 1.86 mg L^{-1}, $CoCl_2 \cdot 6H_2O$ 7.99×10^{-1} mg L^{-1}, $NaNO_3$ 250 mg L^{-1}, $NaHCO_3$ 840 mg L^{-1}.

The anaerobic sludge obtained from an anaerobic digester at Su-yeong WWTP in Busan, Korea was used as the inoculum to decompose the microalgae mixture in the following BMP test. Each concentration of the substrate and the anaerobic sludge was adjusted to ca. 5 g-VS (Volatile Solid) L^{-1}, respectively, by using distilled water, and then BMP experiments were conducted after mixing them.

2.2. Pretreatments

Several pretreatment methods such as thermal-, ultrasonic-, and alkali-treatment were selected from the preliminary study and literature review (Bougrier et al., 2006; López Torres and Espinosa Lloréns, 2008), and they were applied to enhance anaerobic digestion of the mixed-microalgae biomass by partial destruction of cells. However, the pretreatments stronger than the strengths applied in this study were not attempted because of high input energy costs.

Thermal-pretreatment was conducted for 30 min at 50 °C and 80 °C with a water bath (CW-10G, Jeio Tech), and at 120 °C with an autoclave (HAC-045, LK LabKorea). Ultrasonic pretreatments was conducted for 30, 90, and 180 s on an ultrasonicator (VCX-130, Vibra-Cell) of 130 W. Alkali pretreatment was adjusted to pH 9, 11, and 13 by 5 N NaOH. The efficiency of solubilization by the pretreatment methods was calculated by the following equation expressed as a function of changes in chemical oxygen demand (COD_{cr}).

$$S\ (\%) = (SCOD_f - SCOD_i) \times 100/(TCOD_i - SCOD_i)$$

where *S*, SCOD, and TCOD refer to solubilization (%), soluble chemical oxygen demand (SCOD, f: final, i: initial) (m L^{-1}) and total chemical oxygen demand (TCOD, mg L^{-1}) in that order.

2.3. Biochemical methane potential (BMP) test

A BMP test provides the optimum condition for anaerobic digestion and has an advantage of simple measurement of biogas and methane produced. Hence, the test has been widely used to evaluate the maximum potential of methane production which is obtainable from certain substrates in a batch experiment of anaerobic digestion (Angelidaki et al., 2009; Alzate et al., 2012). The BMP test was conducted in 100 mL glass serum bottles sealed with Teflon rubber septum in this study. The substrate and inoculums (1 to 1, g g^{-1}) adjusted to 10 g-VS L^{-1} were mixed, and the total working volume was set at 60 mL. The pH was adjusted to pH 7 after the mixing, and the headspace of the bottles was flushed with N_2 gas to maintain an anaerobic condition. The gas and methane production obtained from each pretreated mixed-microalgae biomass on the BMP tests were compared with those obtained from non-pretreated mixed-microalgae biomass.

The BMP tests were carried out in duplicates for each sample. The bottles were placed in a shaking incubator of 80 rpm at 37 °C (B-201S, Hanbaek Scientific Co.). Samples were collected periodically for analysis. Biogas and methane production were measured throughout the incubation period of 23 days at appropriate time interval. During the initial 7 days after the incubation, the biogas and methane production were measured with the interval of 1 day and with the interval of 2 or 3 days after the initial 7 days. The biogas production was measured after sampling with a glass syringe, and the content of methane produced was determined by a gas chromatography (GC5890, Hewlett Packard) equipped with a thermal conductivity detector. The biogas production was corrected for STP condition (Angelidaki et al., 2009). At the end of the BMP tests, the final $SCOD_{Cr}$ concentrations in each sample were measured to estimate the $SCOD_{Cr}$ removal efficiency.

2.4. Effect of initial solubilization by pretreatments on BMP tests

Two samples thermal-pretreated at the temperatures of 50 °C and 120 °C for 30 min were selected for this experiment because the solubilization of the biomass thermal-pretreated by the