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Ultrasound pretreatment of filamentous algal biomass for enhanced biogas production

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ABSTRACT

The filamentous alga *Hydrodictyon reticulatum* harvested from a bench-scale wastewater treatment pond was used to evaluate biogas production after ultrasound pretreatment. The effects of ultrasound pretreatment at a range of 10–5000 J/mL were tested with harvested *H. reticulatum*. Cell disruption by ultrasound was successful and showed a higher degree of disintegration at a higher applied energy. The range of 10–5000 J/mL ultrasound was able to disintegrated *H. reticulatum* and the soluble COD was increased from 250 mg/L to 1000 mg/L at 2500 J/mL. The disintegrated algal biomass was digested for biogas production in batch experiments. Both cumulative gas generation and volatile solids reduction data were obtained during the digestion. Cell disintegration due to ultrasound pretreatment increased the specific biogas production and degradation rates. Using the ultrasound approach, the specific methane production at a dose of 40 J/mL increased up to 384 mL/g-VS fed that was 2.3 times higher than the untreated sample. For disintegrated samples, the volatile solids reduction was greater with increased energy input, and the degradation increased slightly to 67% at a dose of 50 J/mL. The results also indicate that disintegration of the algal cells is the essential step for efficient anaerobic digestion of algal biomass.

1. Introduction

The exhaustion of fossil fuels and the global warming situation are strongly motivating research in alternative energies (Berndes et al., 2003). Many countries are interested in renewable energy sources, especially in sustainable forms of energy, i.e., geothermal power, wind power, small-scale hydropower, solar energy, biomass energy, tidal power, and wave power. Biomass energy is gaining increasing importance because of its environmentally sound and energy-saving production methods (Zheng et al., 2012). Various biomasses derived from the carbonaceous waste of human and natural activities could be utilized as renewable energy resources. Algae have been identified as a promising biomass feedstock because of their high biomass productivity and their non-food-source properties (Mandal and Mallick, 2009).

Algae have attracted increasing attention as a sustainable process component for nutrient removal and biofuel production, as well as for mitigation of excessive CO_2 production. Biofuel generated from algal treatment of wastewater is a more sustainable fuel while using significantly less energy (Rawat et al., 2011; Satyanarayana et al., 2011; Ehimen et al., 2013). Biogas production from algal biomass by anaerobic digestion is one of the most environmentally beneficial technologies, based on both total produced biomass and the residuals remaining after conversion to biofuel. Anaerobic digestion is a process wherein anaerobic bacteria convert organic matter into biogas. Biogas is a mixture of methane gas (CH_4) and carbon dioxide gas (CO_2). Natural gas consists of approximately 90–95% methane, but biogas is composed of approximately 50–65% methane, signifying a low-grade natural gas. This biogas can be used as a fuel for heating, in gas engines for electricity and heating, or for upgrading to natural gas quality. Thus, biogas production is an interesting alternative energy because it contributes to not only energy production but also to reducing organic wastes.

Methane production from algal biomass has been discussed in the literature, with an emphasis on anaerobic digestion of single-celled species such as *Chlorella* spp. (Oswald and Goluke, 1960). However, the practicality of energy generation from algae has been limited because of the economic and energy costs associated with cultivating and harvesting unicellular microalgae species (Sialve et al., 2009). Filamentous algae are easier and less expensive to harvest compared with unicellular algae because of their physical characteristics. Low-cost filtration methods could be used to harvest filamentous algae strains described high rate algae by microstrainer to retain larger cells and washing out smaller non-filamentous algae (Logan and Ronald, 2011). Thus, the use of filamentous algae could potentially improve the economics of energy generation from algae. There are only a few reports on CH_4 production using filamentous algae (Samson and LeDuy, 1983). *H. reticulatum* is a diverse filamentous alga that can be found in

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almost all aquatic environments, and this alga is usually observed as green, thread-like, mat-forming structures floating close to the surface of non-turbulent water bodies (Flory and Hawley, 1994). Owing to its widespread availability, *H. reticulatum* was used as the biomass in this investigation.

In the case of methane fermentation of solid organic materials such as microbial cells, the methane yield is significantly affected by the mass transfer of each biological step as well as by food availability (Izumi et al., 2010). Up to date, however, the literature on anaerobic digestion of microalgae is limited (Passos et al., 2013a). Anaerobic biogas production from algal biomass is impeded by the hard cell wall of microalgae (Chen and Oswald, 1998). Golueke et al. (1957) reported that digested sludge provides a noticeable green color during anaerobic digestion because of the persistence of chlorophyll, which is an intracellular material. This observation suggests that cellular lysis was not completed during digestion, thereby reducing the total biogas production. To improve the overall substrate degradability, pretreatment or disintegration of the algal biomass is required. Various disintegration techniques, including ultrasonography, have been successfully applied as pretreatment methods to enhance anaerobic digestibility (Nickel and Neis, 2007). Biomass disintegration improves the solubility of the sludge particles by disrupting the sludge/flocs in the aqueous phase. The dissolved components can be readily degraded and utilized as substrates in the biological process, thus resulting in increased bioavailability (Park et al., 2004; Lee et al., 2005). Recently, sonication was applied to break down unicellular algal biomass and improve methane production, achieving up to a 60% increase in methane yield (González-Fernández et al., 2012; Alzate et al., 2012). Passos et al., (2013a, 2013b) employed microwave irradiation and thermal treatment to enhance the disintegration and digestibility of microalgae. However, little information is available regarding filamentous algal biomass-disintegrating methods for anaerobic digestion.

This technical study aimed at providing preliminary information on methane yields from the anaerobic digestion of the filamentous alga *H. reticulatum*. An additional aim of this work was to study the effects of different ultrasound doses to algal feed on anaerobic digestion of the filamentous alga *H. reticulatum*.

2. Materials and methods

2.1. Biomass sources

The filamentous alga used in this study, *H. reticulatum*, was supplied by the Korean Research Institute of Chemical Technology, Daejeon, Korea, and was cultivated in secondary effluent of treated wastewater collected from the municipal wastewater treatment plant in Ansan, Korea (Table 1).

H. reticulatum was cultivated in a bench-scale raceway pond with secondary wastewater (Fig. 1). The capacity of the raceway pond was 100 L (width, 0.4 m; length, 1.4 m; depth, 0.5 m) and the pond was constructed and operated under indoor conditions with agitation using a stainless steel paddle wheel. An artificially illuminated raceway reactor was used, and light sources were positioned on both sides of the reactor. Illumination was provided by a light-emitting diode (LED) array. The incident average light intensity was $120 \pm 20\ \mu mol\ m^2\ s$ with a 12-h light/dark cycle. The growth was manually harvested once daily ($7.25\ g/m^2\ d$) using a sieve. Although the harvested samples contained non-*H. reticulatum* biomass because of the wastewater cultivation conditions, the *H. reticulatum* biomass accounted for more than 99% of the total biomass dry weight due to sifting out of small organisms. The harvested samples were initially used in the pretreatment process without cleaning. Prior to their use in the digestion process, the concentration of total solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), soluble COD, and ammonia nitrogen were analyzed (see Fig. 2).

2.2. Pretreatment

Blended *H. reticulatum* samples were further subjected to mechanical disruption using a low frequency ultrasound homogenizer (STH-750S; Sonitopia, Korea). A constant frequency of 20 kHz and an ultrasonic power of 150 W were used. The homogenizer was equipped with a horn (20×123 mm in diameter). The ultrasound dose is related to the amount of energy supplied per unit volume of substrate (expressed in J/mL). However, the dose does not depend on the TS concentration. The ultrasound dose cannot be used to compare substrates with different TS contents. When the TS content remains constant, the ultrasound density is a practical method of expressing the energy input for the disintegration of algal cells on a volume basis. The different ultrasound doses applied to 100 mL of *H. reticulatum* (10,000 mg/L TS) were 10 J/mL, 20 J/mL, 30 J/mL, 40 J/mL, 50 J/mL, 500 J/mL, 1000 J/mL, 2500 J/mL, and 5000 J/mL.

Soluble COD release was used as a direct measurement of *H. reticulatum* cell disintegration. When the *H. reticulatum* cells were sonicated, the intracellular contents were released into the aqueous phase. Increased soluble COD after ultrasonic disintegration was an indicator of the cell disintegration efficiency. Samples were filtered through a 0.45 μm membrane and then used for soluble COD measurements.

2.3. Biochemical methane potential (BMP)

Batch digestion was performed in a series of BMP assays by incubating algal biomass inoculated with anaerobic bacteria. The active anaerobic inoculum operating at methophilic conditions was obtained from the anaerobic digester at the Ansan municipal wastewater treatment plant in Korea. Feed sludge was also taken to compare digestibility with algal biomass. The inoculum was then filtered by stainless steel filter mesh to prevent inorganic foreign materials, mixed thoroughly, and used for the digestion trials. A nutrient/mineral/buffer (NMB) medium prepared according to Young and Tabak (1993). *H. reticulatum* samples thawed to room temperature were used in the BMP assays. The BMP assays were performed using sealed 160-mL serum bottles at 35 °C. The produced biogas was measured and was used to represent the BMP. Duplicate units of the digestion setup were used for all pretreatment schemes in this study. Biogas production from the inoculum and medium was recorded and used as the blank. Inoculum and substrate were used at a ratio of 1:1 using the TS mass. Nutrients required for the growth of anaerobic microorganisms were added to each BMP serum bottle (NMB medium) at a volume that was

Table 1
Secondary effluent composition as an algae growth medium.

	COD	T-N	Nitrate N	Ammonia N	Nitrite N	T-P	Ortho-phosphate
Concentration (mg/L)	12.8 (1.9)*	7.7 (0.4)*	3.7 (0.5)*	0.2 (0.1)*	0.04 (0.02)*	1.1 (0.5)*	0.8 (0.2)*

* Standard deviation.