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Biomethane recovery from *Egeria densa* in a microbial electrolysis cell-assisted anaerobic system: Performance and stability assessment

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HIGHLIGHTS

- A microbial electrolysis cells (MECs)-assisted anaerobic system was designed.
- Use of MECs stabilized *E. densa* fermentation and upgraded overall performance.
- Stabilizing effect was quantitatively demonstrated by three statistical analysis methods.
- Win–win interactions between fermenting and electroactive bacteria promoted process stability.

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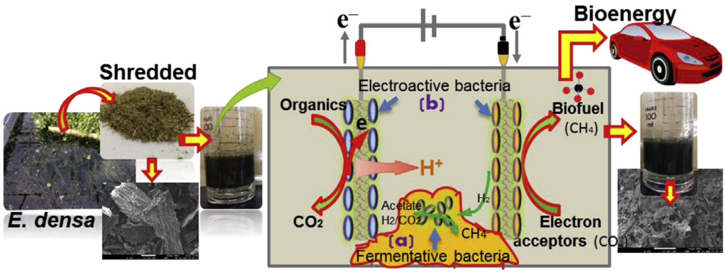
GRAPHICAL ABSTRACT



ABSTRACT

Renewable energy recovery from submerged aquatic plants such as *Egeria densa* (*E. densa*) via continuous anaerobic digestion (AD) represents a bottleneck because of process instability. Here, a single-chamber membrane-free microbial electrolysis cell (MEC) equipped with a pair of Ti/RuO_2 mesh electrodes (i.e. the combined MEC-AD system) was implemented at different applied voltages (0–1.0 V) to evaluate the potential effects of bioelectrochemical stimulation on methane production and process stability of *E. densa* fermentation. The application of MEC effectively stabilized *E. densa* fermentation and upgraded overall process performance, especially solid matters removal. *E. densa* AD process was operated steadily throughout bioelectrochemical process without any signs of imbalance. The solubilization-removal of solid matters and methane conversion efficiency gradually increased with increasing applied voltage, with an average methane yield of approximately 248.2 ± 21.0 mL L^{-1} d^{-1} at 1.0 V. Whereas, the stability of the process became worse immediately once the external power was removed, with weaken solid matters removal along with methane output, evidencing the favorable and indispensable role in maintaining process stability. The stabilizing effect was further quantitatively demonstrated by statistical analysis using standard deviation (SD), coefficient of variance (CV) and box-plots. The syntrophic and win–win interactions between fermenting bacteria and electroactive bacteria might have contributed to the improved process stability and bioenergy recovery.

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1. Introduction

In response to the water body pollution and eutrophication, various purification techniques have been developed and studied over the past few decades, including natural/constructed wetlands (DeBerry and Perry, 2015), chemical adsorption/precipitation (de Vicente et al., 2008), macrophytes restoration (Gao et al., 2009), etc. Among them, the water purification using submerged aquatic plants has aroused intensive attention. This process can not only remove nutrients from water and sediments to stabilize clear-water conditions (Sachse et al., 2014), but also efficiently capture solar energy into biomass. Conflictingly, the uncontrolled, rapid growth of aquatic plants has also brought other environmental threats such as water stagnation, foul odor, interference with navigation, deoxygenation of water, and increasing mosquito breeding sites (O'Sullivan et al., 2010; Koyama et al., 2014). Moreover, decaying macrophytes may be a potential phosphorus source; if the dead plants are not cleared timely, the conserved phosphorus could be released again to water body (Zhou et al., 2000). Approximately 1200 tons of aquatic plants are removed every year from Lake Biwa, the biggest lake in Japan, with the total harvesting cost reaching up to USD 0.36 million (Kobayashi et al., 2015). The disposal and recycling of the harvested plants has been thus becoming an issue facing environmental researchers.

Anaerobic digestion (AD) is a well-developed bioenergy recovery technology, and has been extensively employed for the disposal of a great variety of organic waste streams (Zhen et al., 2014; Fernández-Rodríguez et al., 2015; Weinrich and Nelles, 2015; Zhen et al., 2015a). Yet, increasing attention has been drawn on fermentation of aquatic plants for bio-methane production. For instance, O'Sullivan et al. (2010) explored the potentials of three aquatic weeds, *water hyacinth*, *cabomba*, and *salvinia*, as substrates for anaerobic digestion. In the case of Koyama et al. (2014), the authors selected five submerged macrophyte species (*Ceratophyllum demersum*, *Egeria densa*, *Elodea nuttallii*, *Potamogeton maackianus* and *Potamogeton malaianuswere*) as a substrate for anaerobic digestion to investigate the chemical composition and the anaerobic digestibility. However, plant materials show high resistance to enzymatic attach/hydrolysis because of its complex components (i.e. lignin, cellulose, and hemicellulose) and the strong protection offered by the recalcitrant lignin (Zhao et al., 2009; Nizami et al., 2010), leading to significantly low anaerobic conversion efficiencies and poor long-term stability of AD facilities. This is why most of anaerobic systems set up before were operated mainly at a batch (Koyama et al., 2014) but not semi-/continuous modes (Kobayashi et al., 2015). In order to optimize energy conversion efficiency and operational stability of aquatic plants fermentation, various strategies, e.g. NaOH/cellulase pretreatment (Cheng et al., 2010), co-digestion with other biomass (Zhen et al., 2015b), etc. Thus have been proposed in literature.

An alternative and promising option to anaerobic digestion can be microbial electrolysis cell (MEC), derived from microbial fuel cell (MFC), which is a recent emerging technique for methane production via electromethanogenesis. In a MEC process, exoelectrogenic bacteria in anode consume organic matters anaerobically while releasing electrons to the anode and protons into solution; with a small voltage input, the electrochemically active microorganisms (i.e. electrotrophs) in cathode (Schröder et al., 2015) can accept electrons transferring to cathode through the external circuit (Selembo et al., 2009; Montpart et al., 2015), or alternatively use cathodic H_2 as electron carriers to drive methane formation (Cheng et al., 2009; Nevin et al., 2010; Villano et al., 2010; Zhen et al., 2015c). Moreover, quite superior to conventional dark fermentative bacteria, the exoelectrogens can further use the fermentation dead-end products such as acetate to produce electrons (Liu et al., 2005; Cheng and Logan, 2007; Lu et al., 2012). Because of the favorable role in enrichment of unique microbes, several studies have already attempted to couple MEC with AD systems (MEC-AD) for methane production; indeed, the relatively positive results have been observed. The direct use of MEC in the digesters can substantially enhance the production of methane (Sasaki et al., 2011; Pant et al., 2012; Guo et al., 2013; Sun et al., 2015), and even remediate AD systems that exhibit process failure (Vrieze et al., 2014). In consideration of hard and complex structure of submerged aquatic plants, if MEC process is introduced into an aquatic plant-fed AD system, the upgraded process stability and methane yield might be expected. To date, several works have been investigated on different substrates (Table 1S in Supplementary information), there are still no reports about the feasibility of the combined MEC-AD system on the continuous submerged aquatic plant fermentation.

Therefore, the purpose of this study was to evaluate whether or not/how the application of MEC can advance the long-term process stability of aquatic plant (*E. densa*) digestion and methane production. To accomplish this goal, a single-chamber MEC equipped with a pair of Ti/RuO$_2$ mesh plates was operated at different voltages (0–1.0 V) for 150 d to evaluate the process stability and optimize electromethanogenesis process. Three statistical analysis techniques including the standard deviation (SD), coefficient of variance (CV) and box-plots were employed to identify the exact roles of MEC in the anaerobic digestion stability of aquatic plant and methane conversion efficiency with great accuracy. Scanning electron microscope (SEM) was used to visualize the microstructure of grass *E. densa* before and after fermentation; fluorescence in situ hybridization (FISH) was employed to analyze the effects of bioelectrochemical stimulation on microbial communities.

2. Materials and methods

2.1. Substrate and inoculum

E. densa used in this study is harvested from a testing flume of water purification (5 m × 20 m), which is semi-continuously fed with the mixture of domestic wastewater and water from Lake Kasumigaura in Ibaraki Prefecture, Japan (36.09°N, 39.55°E) (Zhen et al., 2015b). After harvesting, the aquatic plants were drained, dried at 65 °C for 24 h and shredded into the size below 2.0 mm using an electric blender. The main characteristics of *E. densa* have been reported in our previous research: protein 294 mg g^{-1} TS, lipid 29.1 mg g^{-1} TS cellulose 202 mg g^{-1} TS, hemicellulose 0 mg g^{-1} TS, and lignin 50 mg g^{-1} TS (Kobayashi et al., 2015). The plant powder was diluted with tap water to obtain the final total solids (TS) of 50.0 g L^{-1}, which was then used as feedstock for the semi-continuous anaerobic experiment. For each cycle, the feedstock was prepared freshly prior to use. Inoculum was collected from a mesophilic continuously stirred tank reactor (CSTR) treating kitchen waste in our lab. Main properties of the inoculum used were as follows: pH 7.7 ± 0.0, total solids (TS) 11.0 ± 0.2 g L^{-1}, volatile solids (VS) 6.3 ± 0.1 g L^{-1}, alkalinity 10.8 ± 0.6 g CaCO$_3$ L^{-1}, NH$_4^+$ 2430.5 ± 13.4 mg L^{-1} and PO$_4^{3-}$ 128.5 ± 126.6 mg L^{-1}.

2.2. Single-chamber membrane-free MEC-AD system construction and operation

A single-chamber MEC-AD system was constructed using Plexiglass, with a working volume of 0.8 L (Fig. 1S in Supplementary information). The anode and cathode were a pair of Ti/RuO$_2$ mesh plates (4 cm × 7 cm). The electrodes were fixed in parallel at a distance of 5 cm and then connected through a copper wire to a regulated digital DC power supply (AD-8735, A&D Co., Japan) for