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Enhanced decomposition of waste activated sludge via anodic oxidation for methane production and bioenergy recovery

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ABSTRACT

Anaerobic digestion operated in microbial electrolysis cells (MECs) may have a higher methane production since the potential cathodic reduction of carbon dioxide. However, the complicated organic components in municipal sludge retard the sludge hydrolysis and limit the efficiency of methanogenesis. Sludge hydrolysis and its effects on methanogenesis and organic matter removal in a single-chamber MEC were investigated in this study. As compared with the control reactor without electric field, total chemical oxygen demand (TCOD) removal and methane production in MEC with applied voltage of 0.8 V increased by 26% and 28%, respectively. Energy income from the increased methane was about five folds of the electric energy supply. Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM) and transmission electron microscope (TEM) analysis indicated that anodic oxidation of MEC significantly improved the disintegration of sludge flocs and cell walls. Anodic Coulombic efficiency and current density further revealed that anodic oxidation coupled with cathodic reduction of carbon dioxide was the predominant mechanism in the improvement of sludge decomposition and methane production during the initial fermentation, which hereby accelerated the rate of sludge hydrolysis.

1. Introduction

Waste activated sludge (WAS) has been widely treated via anaerobic digestion process to reduce volume, kill pathogens and recycle bioenergy by producing methane (Appels et al., 2008; Kelessidis and Stasinakis, 2012). A major factor limiting the effectiveness of anaerobic sludge digestion is the relatively slow hydrolysis that disintegrates the sludge flocs and cell walls (Bolzonella et al., 2007), which is also the important reason resulting in the long solid retention time and low methane production of anaerobic sludge digestion (Appels et al., 2008). Therefore, accelerating the rate of sludge hydrolysis is expected to promote better methanogenesis during anaerobic sludge digestion.

Microbial electrolysis cells (MECs), converting organic waste to bioenergy with a small applied voltage, have been widely studied recently (Call and Logan, 2008; Cheng and Logan, 2007; Cheng et al., 2009; Logan et al., 2008). In MECs, organic matters are firstly oxidized by anodic exoelectrogenic bacteria like *Geobacter* or *Shewanella* species that transfer the electrons produced from the oxidation of organic substrates to electrode (Bond and Lovley, 2003; Chaudhuri and Lovley, 2003; Lovley, 2008). It has been reported that exoelectrogenic bacteria are capable of utilizing a broad type of substrates as electron donors such as short-chain fatty acids (SCFAs), glucose, aromatic hydrocarbons, halogenated solvents and chlorinated benzenes et al. (Chaudhuri and Lovley, 2003; Lovley et al., 2011). It means that anodic oxidation of MEC driven by exoelectrogenic bacteria is likely to utilize complicated organic matters as substrates, such as municipal sludge. Based on this consideration, a growing number of researches have fixed their attention on using the single-chamber MEC to enhance anaerobic sludge digestion for improving methane production (Guo et al., 2013; Kargi et al., 2011; Liu et al., 2012; Lu et al., 2012a,b; Sasaki et al., 2013). In these researches, the predominant mechanism for the improvement of methane production is ascribed to the potential cathodic reduction of carbon dioxide to methane by hydrogenotrophic methanogens as biocathode (Cheng et al., 2009; Villano et al., 2010). However, few reports have focused on the sludge hydrolysis in MECs as so far.

Multiple lines of evidence suggested that proteins and carbohydrates that were the two dominant organic components constituting sludge cell walls could be utilized be exoelectrogenic

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bacteria in MECs (Lu et al., 2012a,b; Lu et al., 2009; Lu et al., 2010; Selembo et al., 2009). Therefore, anodic oxidation of MEC might help decompose proteins and carbohydrates to disintegrate the sludge flocs and cell walls and then accelerate the rate of sludge hydrolysis. Furthermore, the electrons produced from the sludge decomposition might be transferred from the anode to cathode for cathodic methanogenesis. The destroyed sludge in the bio-electrochemical process might be more accessible for traditional anaerobic methanogenesis especially like aceticlastic methanogenesis. To clarify this, this study was conducted to investigate the effects of bioelectrochemical process on the sludge hydrolysis as well as methane production in a single-chamber MEC.

2. Material and methods

2.1. Waste activated sludge and anaerobic inoculum sludge

Waste activated sludge (WAS) used in this study was collected from a secondary sedimentation tank of the municipal wastewater treatment plant in Dalian, China, which was stored at 4 °C before use. The inoculum sludge was obtained from a waste sludge treatment plant in Dalian, China. Before the experiments of anaerobic sludge digestion, the WAS was mixed with the inoculum with a ratio of 9:1. 400 mL of this mixed sludge was added to the reactors for anaerobic sludge digestion. The main characteristics of this mixed WAS are displayed in Table S1.

2.2. Experimental setup

Anaerobic sludge digestion coupled with MEC was conducted using a cylindrical anaerobic reactor (Ø80 mm × 100 mm) with a working volume of 500 mL. A graphite-brush anode (Ø25 mm × 80 mm, surface areas: 17,671 mm^2) and a graphite-rod cathode (Ø7 mm × 80 mm, surface areas 1759.2 mm^2) with 50 mm distance were installed into the reactor (hereafter referred to as R2). A DC power source (Zhaoxin, RXN-305D, China) was connected with the electrodes to provide the voltage supply. Another reactor with the same working volume as R2 but without addition of electrodes was used as a control (hereafter referred to as R1). Biogas was collected by a gas sampling bag and sludge samples were taken from a sampling port of the reactor. The reactors were placed in a shaker (140–150 rpm, 37.0 ± 2.0 °C) for digestion. The MEC reactor with applied voltage of 0.8 V (R2) and control reactor (R1) were operated continuously for 24 days experiments. Another two groups of parallel experiments were operated simultaneously.

Another four MEC reactors as same as R2 imposed respectively with applied voltage of 0.6 (A2), 0.8 (A3), 1.0 (A4) and 1.2 V (A5) and another control reactor the same as R1 (A1) were operated for 24 days experiments in the batch mode. The experimental settings and applied voltages are summarized in Table S2. Another two groups of parallel experiments were operated simultaneously.

2.3. Analysis

Total suspended solid (TSS), volatile suspended solid (VSS) and chemical oxygen demand (COD) (include total COD and solute COD) were measured according to standard methods for the examination of water and wastewater. Biogas collected from all the reactors was used a gas collecting bag and the content of methane were analyzed by a gas chromatograph (Tianmei, GC-7900P/TCD, China). Proteins were measured with Lowry's method (Frolund et al., 1995) using bovine serum albumin as a standard solution. Carbohydrates were measured with phenol-sulfuric acid method using glucose as a standard solution (Masuko et al., 2005). The concentrations of short-chain fatty acids (SCFAs) in the sludge were analyzed by another gas chromatograph (Tianmei, GC-7900P/FID, China) (Jiang et al., 2007). The pH was recorded using a pH analyzer (Sartorius PB-20, Germany). Electrical current data between the electrodes was collected by data acquisition card (Hongge, PCI-821H, China) (Wang et al., 2010).

2.4. Fluorescence in situ hybridization

Fluorescence *in situ* hybridization (FISH) was used to determine the abundance of aceticlastic and hydrogenotrophic methanogens in archaea microbial community (Wu et al., 2001). The suspended sludge samples (5 mL) were taken from the bottom of each reactor and harvested by centrifugation (110 × 100 *g* for 15 min at 4 °C). Three genus-specific probes for total archaea (ARC915, GTGCTCCCCCGCCAATTCCT), aceticlastic methanogens (MX825, TCGCACCGTGGCCGACACCTAGC) and hydrogenotrophic methanogens (MB1174, TACCGTCGTCCACTCCTTCCTC) (Raskin et al., 1994) were used in this study. The sludge samples were gently washed three times with phosphate-buffered saline (PBS; 0.13 M NaCl and 10 mM Na_2HPO_4 at pH 7.2), fixed in PBS containing 4% (w/v) paraformaldehyde at 4 °C overnight, and washed three times with PBS. To improve the penetration efficiency of oligonucleotide probes, the granule was subjected to at least five cycles of freeze-and-thaw (−80 °C to +60 °C) after fixation. The fixed sample was then dehydrated in a 50% ethanol solution at 4 °C overnight, and washed with a series of ethanol/water solutions (50%, 80% and 96%, 3 min of each), then with an ethanol/xylene mixture (50:50, v/v), and finally with 100% xylene. The xylene solution was gradually replaced with an equal volume of paraffin/xylene mixture with the paraffin content varying from 25%, 50%, 75%–100% at 62 °C (12 h for each replacement). *In situ* hybridization was conducted according to protocols previously described (Wu et al., 2001). Initially, 10 uL hybridization buffer (0.9 M NaCl, 1% SDS, 100 mM Tris/HCl, pH 7.2) containing 50 ng of each labelled probe was added to each well on the slide and hybridized at 45 °C for at least 12 h. The hybridization stringency was controlled by adding different amounts of formamide to the hybridization buffer (20% for MX825 and 35% for ARC915 and MB1174). For washing, the slide was briefly rinsed with double-distilled water and incubated in the same hybridization solution without the addition of probes for 30 min at 48 °C. The slide was briefly rinsed with milli-Q water and air-dried. Then the sludge samples were observed under a confocal laser scanning microscope (Leica SP2, Heidelberger, Germany). The FISH images obtained were imported to Image-Pro Plus 6.0 for analysis of the relative abundance of microorganisms. The morphology of the sludge cells was examined using a scanning electron microscope (SEM) (Hitachi; S-4800, Japan).

2.5. Electron microscope analysis

For SEM observation, the suspended sludge was immobilized in a 2.5% glutaraldehyde solution, dehydrated in graded water-ethanol solutions, then lyophilized and sputter-coated with gold (Liu et al., 2011). The structure of the sludge cells was observed by a transmission electron microscopy (TEM; JEM-2000EX, Japan). The procedures of TEM analysis were performed according to the description by Zhang et al. (2012). To study the physicochemical changes of cellular matter and the hydrolysis of large organic molecules under the applied electric field, Fourier transform infrared spectroscopy (FTIR) (Bruker; VERTEX 70; Germany) was used to analyze the suspended sludge samples (Martínez et al., 2012; Rakotonirainy et al., 2015). The procedures of FTIR analysis were performed according to the description by Grűbel and Machnicka (2014).