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Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell

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HIGHLIGHTS

- Carbon-felt addition increased the methane production in anaerobic sludge digestion.
- Hydrolysis and acidification process was enhanced in the MEC reactor.
- A voltage on electrode further improved the performance of the anaerobic digestion.
- DIET between VFA-oxidizing bacteria and methanogens was promoted in MEC reactor.

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ABSTRACT

Increase of methanogenesis in methane-producing microbial electrolysis cells (MECs) is frequently believed as a result of cathodic reduction of CO_2. Recent studies indicated that this electromethanogenesis only accounted for a little part of methane production during anaerobic sludge digestion. Instead, direct interspecies electron transfer (DIET) possibly plays an important role in methane production. In this study, anaerobic digestion of sludge were investigated in a single-chamber MEC reactor, a carbon-felt supplemented reactor and a common anaerobic reactor to evaluate the effects of DIET on the sludge digestion. The results showed that adding carbon felt into the reactor increased 12.9% of methane production and 17.2% of sludge reduction. Imposing a voltage on the carbon felt further improved the digestion. Current calculation showed that the cathodic reduction only contributed to 27.5% of increased methane production. Microbial analysis indicated that DIET played an important role in the anaerobic sludge digestion in the MEC.

1. Introduction

Waste activated sludge (WAS) as a byproduct of municipal wastewater treatment process has been becoming increasingly important because of its huge production, environmental risks and high cost for disposal. Anaerobic digestion of waste activated sludge is an efficient and sustainable technology to stabilize sludge and produce methane (Wang et al., 2013). Anaerobic digestion of sludge undergoes three stages, e.g. (i) hydrolysis, (ii) acidification, (iii) methane generation (Lv et al., 2010). Sludge hydrolysis is commonly considered as the limiting step of whole digestion (Bougrier et al., 2006). To accelerate the hydrolysis, physical (Nah et al., 2000) or chemical (Chiu et al., 1997) methods have been applied to pretreat sludge. However, tedious procedures and expensive expenditure of the pretreatment process make it difficult for them to access the practice.

Microbial electrolysis cells (MECs) driven by exoelectrogenic bacteria under a small applied voltage have been widely reported to convert wastes to bio-products (Cheng et al., 2009; Logan et al., 2008). The methane-production MEC is considered one of the most promising technologies for bioenergy recovery. A pair of electrodes placed into an anaerobic digester to form a single-chamber MEC was reported to be capable of accelerating sludge hydrolysis and increasing methane production. The increase of methane production in MECs was mainly ascribed to the cathodic reduction of carbon dioxide to methane with hydrogenotrophic methanogens as biocathode ($CO_2 + 8H^+ + 8e^- = CH_4 + 2H_2O$, $E^0 \approx -0.44$ V vs [NHE]) (Cheng et al., 2009). The electrons used for the cathodic reduction of CO_2 are produced from anodic oxidation of substrates. Anodic oxidation is a process to transfer the electrons from organic matters to the electrode with exoelectrogens. From this opinion, the anodic oxidation may participate into the decomposition of organic matters of MEC. As a result, the

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sludge hydrolysis in MEC is accelerated compared with that in common anaerobic digester.

Geobacter, one of the most important exoelectrogens in bioelectrochemical systems, can utilize a broad of organics as substrates such as volatile fatty acids (VFAs), alcohols, phenols and benzene (Lovley et al., 2011). *Geobacter* species not only play a crucial role in transformation of minerals and natural organics in environment, but also have great potentials to participate into anaerobic digestion. Besides *Geobacter* species are likely to oxidize organics to accelerate anaerobic digestion, it can build a direct electron connection with other species, namely direct interspecies electron transfer (DIET). DIET has been documented in co-cultures of *Geobacter* species as well as in co-cultures of *Geobacter metallireducens* with *Methanosaeta* (Rotaru et al., 2014a) or *Methanosarcina* (Rotaru et al., 2014b) species. In these cultures electrically conductive pili and outer surface *c*-type cytochromes are important interspecies electron transfer components of the *Geobacter* species. Studies with a series of gene deletion mutants demonstrated that DIET in the presence of conductive carbon materials did not require the electrically conductive pili and associated *c*-type cytochrome involved in DIET (Liu et al., 2012). Conductive materials are considered to provide ecological advantages for users, because their investments in DIET can be reduced. Therefore it is reasonably proposed that the *Geobacter* is enriched in the presence of conductive materials. If so, DIET is likely enhanced to accelerate sludge digestion with presence of conductive materials added.

In our previous study, the methane production in MEC reactor was obviously higher than common anaerobic reactor. It was considered that the electrodes promoted the sludge decomposition through more efficient electron exchange between *Geobacter* and other microbes. However, whether the electrodes themselves as conductive materials induced DIET to enhance the sludge digestion maintained unknown. In this study, a pair of carbon electrodes (with no voltage supply) was placed into an anaerobic sludge digester to clarify its effects on a MEC reactor. Microbial community analysis via high-throughput 16S rRNA pyrosequencing was analyzed to further reveal the potential mechanism.

2. Methods

2.1. Substrates and inoculum

Waste activated sludge collected from a municipal wastewater treatment plant (Dalian, China) was used as the substrate in this study. Before digestion the collected sludge was stored at 4 °C. The main characteristics (mean ± standard deviation) of the WAS are as follows: pH 7.14 ± 0.02, total suspended solids (TSS) 44,700.0 ± 317.6 mg/L, volatile suspended solids (VSS) 29,584.0 ± 162.1 mg/L, total chemical oxygen demand (TCOD) 36,691.2 ± 1008.3 mg COD/L, soluble chemical oxygen demand (SCOD) 9340.8 ± 1142.4 mg/L, total carbohydrate 1219.9 ± 162.0 mg COD/L, soluble carbohydrate 239.7 ± 15.6 mg COD/L, total protein 1154.8 ± 117.5 mg COD/L, soluble protein 216.3 ± 48.4 mg COD/L, total short-chain fatty acids (SCFAs) 397.5 ± 58.7 mg COD/L.

Inoculant sludge was collected from an anaerobic digester of a waste sludge treatment plant of Dalian (China). Before experiment, the inoculums was cultured in a batch anaerobic reactor (Φ200 × 320 mm, 10 L working volume) that was operated at a room temperature (22.0 ± 2.0 °C) and a hydrolytic retention time (HRT) of 24 h. 1000 mg/L glucose and NH_4Cl and KH_2PO_4 with a ratio of COD:N:P 200:5:1 were added as the substrate (COD: 1000 mg/L), nitrogen sources and phosphorus sources respectively. Trace elements were added according to Zhao et al. (2015a). In this experiment mixture of the WAS and inoculums with a ratio of 9:1 was added into the reactors for digestion.

2.2. Batch experiment

Three anaerobic reactors (Φ80 × 120 mm) were operated in parallel. The first was a MEC-anaerobic reactor (hereafter referred to as R1). A pair of carbon felt tube electrode (Φ80 × 110 mm, anode) and graphite pillar electrode (Φ8 × 110 mm, cathode) was placed into the reactor, in which the graphite pillar electrode was located at the axes of carbon felt tube electrode, and the pair of electrodes was imposed with a voltage of 0.6 V. The second reactor was same as R1 but no voltage supply on the electrodes (namely 'opened' MEC reactor, hereafter referred to as R2). The third reactor was a common anaerobic digestion, the structure of which was the same with R1 but using the plastic rods and plastic plate instead of carbon materials added into reactor (hereafter referred to as R3).

Before the digestion, nitrogen gas was used to remove the oxygen from the headspace of the reactors for 10 min, and then the reactors were sealed. The cap of the reactor was drilled a hole to connect with gasbag through a silica tube. The biogas produced was collected by a gas sampling bag and drawn out by a syringe every day to measure the biogas volume and the component. The reactors were operated with a batch mode and each batch was lasted for 24 days. The reactors were stirred at 120 rpm with magnetic stirrers during the fermentation and were operated at 35 °C. The experiments were repeated in triplicate.

2.3. Analyses

Two mL sludge was taken out every day to measure short-chain fatty acids. During the digestion the current between the two electrodes of MEC reactor (R1) was recorded every 30 min by a multimeter/data acquisition system through measurement of the voltage across a high-precision resistor (10 Ω). At the end of the experiment total and soluble chemical oxygen demand (COD), carbohydrate, protein, total suspended solid (TSS), volatile suspended solid (VSS) were measured. TSS, VSS, TCOD and SCOD were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Protein was analyzed with Lowry's method using bovine serum albumin as a standard solution (Frolund et al., 1995). Polysaccharide was measured with phenol–sulfuric acid method using glucose as a standard solution (Fr et al., 1995). The equivalent relationships between COD and substrates were as follows: 1.50 g-COD/g protein, 1.06 g-COD/g carbohydrate, 1.07 g-COD/g acetate, 1.51 g-COD/g propionate, 1.82 g-COD/g butyrate and 2.04 g-COD/g valerate (Lu et al., 2012). Cytochrome *c* was measured with a Cytochrome *c* ELISA kit. The conductivity of the sludge was analyzed according to reference Li et al. (2014).

The methane content in the biogas was analyzed with a gas chromatograph (Shimadzu, GC-14C) equipped with a thermal conductivity detector and a 1.5 m stainless-steel column (Molecular Sieve, 80/100 mesh). The temperatures of injector, detector and column were kept at 100, 105 and 60 °C according to reference (Zhao and Yu, 2008). Nitrogen was used as the carrier gas provided at a flow rate of 30 mL/min. VFAs (acetate, propionate, butyrate and valerate) were measured with another gas chromatograph (Shimadzu, GC2010) with GC-flame ionization detector, FID (Shimadzu, Model 14B) and a 30 m × 0.25 mm × 0.25 μm fused silica capillary column (DB-FFAP). Temperature for the injection port and the FID was 170 °C. Temperature in the oven was gradually increased from 100 to 130 °C at a rate of 5 °C/min according to reference (Fan et al., 2006). Nitrogen as the carrier gas was provided with a flow rate of 30 mL/min.

Anodic Coulombic efficiency is an index to assess the fraction of electrons available in the substrate that ends up as electrical current in the system. Coulombic efficiency of the MEC reactor was