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Enriching functional microbes with electrode to accelerate the decomposition of complex substrates during anaerobic digestion of municipal sludge

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ABSTRACT

Methane-production microbial electrolysis cells (MECs) have been widely reported as an efficient strategy to enhance anaerobic digestion of waste activated sludge (WAS). However, the primary mechanism for accelerating the decomposition of complex substrates contained in WAS remains unclear as so far. In this study anaerobic sludge digestion operated in a single-chamber methane-production MEC was investigated. It was found that the decomposition rate of proteins and carbohydrates were significantly accelerated in MEC, which resulted in the improvement of methane production as compared with the common anaerobic sludge digester. The energy income from the increased methane production was equivalent to 13.4 times as more as the electric energy supply. Further bacterial community analysis showed that anaerobic fermentative bacteria were largely enriched in MEC especially its anodic biofilm. Together with anodic exoelectrogenic bacteria (mainly *Geobacter* species) accounting for the dominant part of bacterial community in the anodic biofilm, it was suggested that the potential for syntrophic interaction between anaerobic fermentative bacteria and anodic exoelectrogenic bacteria enriched might be the important reason for accelerating the decomposition of complex substrates contained in WAS, which further resulted in the high-efficiency methane production as well as energy recovery.

1. Introduction

Waste activated sludge (WAS) produced from biological wastewater treatment is an important problem needing to be addressed since its huge production, environmental risk and high disposal cost [1,2]. Anaerobic digestion producing bioenergy i.e., methane is considered an ideal option for municipal sludge disposal [1,3]). A major factor limiting the effectiveness during anaerobic sludge digestion is the relatively slow decomposition of fermentative or hydrolytic products, such as proteins and carbohydrates [1]. Accelerating the decomposition of proteins and carbohydrates is expected to improve better methanogenesis during anaerobic sludge digestion.

Microbial electrolysis cells (MECs) driven by exoelectrogenic bacteria have been widely reported to convert organic waste to bioproduct, such as hydrogen [4,5], methane [6,7] and other organic compounds [8,9], with a small applied voltage. Among them, the methane-production MEC has shown the great potential for bioenergy recovery in practice. Apart from the free of precious metal as the cathodic catalyst [5], this MEC can be carried out under a lower applied voltage as compared with the hydrogen-production MEC (−0.24 V for 'electromethanogenesis' vs −0.41 V for 'electrohydrogenesis', normal hydrogen electrode [NHE], pH 7.0) [6]. Furthermore, 'electromethanogenesis' can be conveniently established in a common anaerobic system. Only placing a pair of electrodes into the anaerobic reactor with an applied voltage can form a single-chamber methane-production MEC. In this system, methane can be produced both from the traditional methanogenic pathway (aceticlastic methanogenesis and hydrogenotrophic methanogenesis) and cathodic reduction of carbon dioxide [10]. As a result, a growing number of researches have fixed their attention on using this MEC to enhance anaerobic digestion for various substrates [10–13], especially for WAS [14–17].

The increase of methane production in the MECs for sludge digestion should be primarily ascribed to the potential for the cathodic reduction of carbon dioxide to methane by hydrogenotrophic methanogens [6]. The electrons for the cathodic

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reduction are produced from the oxidation of substrates by anodic exoelectrogenic bacteria. The main complex substrates contained in WAS, accounting for 50–70% of total organic compounds, are proteins and carbohydrates [3]. Even though a few exoelectrogenic bacteria (e.g., *Rhodoferax ferrireducens*, *Klebsiella pneumoniae* and *Aeromonas hydrophila*) can directly oxidize some complex substrates for electricity generation in pure-culture MECs [18], there is no evidence to demonstrate that the exoelectrogenic bacteria detected in mixed-culture MECs can directly utilize proteins and carbohydrates. Especially, *Geobacter* species, the predominant exoelectrogenic bacteria usually detected in the anodic biofilm of MECs or MFCs, can only utilize hydrogen, short-chain fatty acids and alcohols as substrates [19]. Furthermore, conversion of proteins and carbohydrates to simples such as short-chain fatty acids or hydrogen is the business of anaerobic fermentative bacteria, which however are low performance in the initial stage of sludge digestion since the low amount of inoculum (10–20% inoculant sludge) [20,21]. If raising the ratio of inoculum sludge, it will inevitably decrease the sludge loading to result in a low efficiency of digester.

Multiple lines of evidence suggested that the interspecies syntrophic interaction between anaerobic fermentative bacteria and anodic exoelectrogenic bacteria is the major route to drive the degradation of complex substrates such as glucose for current production in mixed-culture MFCs or MECs [22–25]. Glucose is first oxidized to organic acids or hydrogen by anaerobic fermentative bacteria, followed by consumption of fermentation products by anodic exoelectrogenic bacteria, which eliminates feedback inhibition of glucose fermentation [24,25]. However, this syntrophic metabolism for the decomposition of complex substrates in MECs during anaerobic sludge digestion is as yet known. The purpose of this study reported here was to investigate the potential for syntrophic metabolism that accelerated the decomposition of complex substrates contained in WAS. The performance of the decomposition of proteins and carbohydrates as well as methane production in the MEC reactor and control reactor was compared. 454 pyrosequencing of 16S rRNA gene was used to analyze the microbial community structure and the potential syntrophic interactions.

2. Materials and methods

2.1. Reactor design

Studies were conducted in three batch-flow anaerobic reactors each of which had a working volume of 500 mL (Ø80 mm × 100 mm). One of the three reactors was used as the single-chamber MEC reactor with a graphite-brush anode (Ø25 mm × 80 mm, surface areas 17671 mm^2) and a graphite-rod cathode (Ø7 mm × 80 mm, surface areas 1759.2 mm^2) installed into (hereafter referred as the MEC reactor). There was a 50 mm distance between these two electrodes. A DC power source (Zhaoxin, RXN-305D, China) connected with the electrodes was used as the electric supply of this MEC reactor. The applied voltage throughout the whole experiments was kept at 0.6 V. A gas sampling bag of 1000 mL was connected with the top of the MEC reactor for gas collection. Another two reactors operated in parallel were used as the control reactors. One was a common anaerobic sludge digester (hereafter referred to as the control reactor 1). The other was as same as the MEC reactor with electrodes but without applied voltage (hereafter referred to as the control reactor 2). Another two groups of parallel experiments were operated simultaneously. All the reactors were operated at 37.0 ± 1.0 °C and placed in a shaker at 140–150 rpm.

2.2. Waste activated sludge and anaerobic inoculum sludge

Waste activated sludge (WAS) used in this study was obtained from a secondary sedimentation tank of a local municipal wastewater treatment plant using the activated sludge process in Dalian, China. The collected sludge was stored at 4 °C before use. The main characteristics of WAS are displayed in Table S1. Anaerobic inoculum sludge (TSS: 77,300 ± 210 mg/L, VSS: 28,100 ± 433 mg/L) was obtained from the refluxed liquid of anaerobic fermentative sludge of a waste sludge treatment plant in Dalian, China.

2.3. Pretreating WAS and mixing with the inoculum sludge

The WAS were pretreated at pH 10 for 8 days in a cylindrical anaerobic reactor with a working volume of 5 L (Ø200 mm × 160 mm) based on the method reported by Zhang et al. [20]. The reactor was operated in a room temperature (22.0 ± 2.0 °C) equipped with mechanical stirrer at a speed of 80 rpm. During pretreatment the fermentation pH was maintained at 10.0 ± 0.2 by 4 M sodium hydroxide (NaOH). After 8 days the fermentative pH was adjusted to 7.0 used 4 M hydrochloric acid (HCl) immediately and then the pretreated WAS were mixed with the inoculum sludge with a ratio of 9:1. The main characteristics of the mixed WAS are displayed in Table S2. Before sludge digestion the mixed sludge of 400 mL was added to each of reactors.

2.4. Analysis

Total suspended solid (TSS), volatile suspended solid (VSS) and chemical oxygen demand (COD) were measured based on Standard Methods for the Examination of Water and Wastewater. Proteins were measured with Lowry's method [26] using bovine serum albumin as a standard solution. Carbohydrates were measured by phenol-sulfuric acid method using glucose as a standard solution [27]. VFAs (volatile fatty acids) were measured by a gas chromatograph (Tianmei, GC-7900P/FID, China) [28]. The equivalent relationship between COD and substrates are as follows: 1.5 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 [29]. The biogas volume in the gas sampling bag was measured by a glass syringe of 100 mL every day. The composition of biogas was analyzed with a gas chromatograph with a flame ionization detector (Tianmei, GC-7900P/TCD, China) [29]. pH was recorded using a pH analyzer (Sartorius PB-20, Germany). Electric current of external electric circuit was record by a multimeter/data acquisition system (Hongge, PCI-821H, China) with high-precision resistor (10 Ω) [30].

2.5. DNA extraction, PCR amplification and 454 pyrosequencing

After 51 days experiments 454 pyrosequencing of 16S rRNA gene was used to analysis the bacterial richness and community of the suspended sludge in the MEC reactor and control reactor 1, anodic biofilm of MEC reactor and initial mixed sludge, respectively [31]. The suspended sludge of 10 mL was taken from the bottom of the MEC reactor and control reactor 1. Anodic biofilm was collected from the graphite-brush anode of MEC with a knife. All the four sludge samples were rinsed twice by phosphate-buffered saline (PBS; 0.13 M NaCl and 10 mM Na_2HPO_4 at pH 7.2) and then centrifugated (110 × 100 g for 10 min at 4 °C). The genomic DNA of the sludge samples was extracted using an extraction kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions. The quality of the extracted DNA was checked by measuring its absorbance at 260 and 280 nm using a Beckman DU800 spectrophotometer.