Renewable Energy 91 (2016) 334–339

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Microbial electrolysis contribution to anaerobic digestion of waste activated sludge, leading to accelerated methane production

Wenzong Liu [a], Weiwei Cai [b], Zechong Guo [b], Ling Wang [b], Chunxue Yang [b], Cristiano Varrone [c], Aijie Wang [a, b, *]

[a] Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, China
[c] Technical University of Denmark, Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Lyngby, Denmark

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
26 December 2015
Accepted 25 January 2016
Available online 2 February 2016

Keywords:
Microbial electrolysis AD reactor
Waste activated sludge
Energy recovery
Bio-electron
Methanogenesis

ABSTRACT

Methane production rate (MPR) in waste activated sludge (WAS) digestion processes is typically limited by the initial steps of complex organic matter degradation, leading to a limited MPR due to sludge fermentation speed of solid particles. In this study, a novel microbial electrolysis AD reactor (ME-AD) was used to accelerate methane production for energy recovery from WAS. Carbon bioconversion was accelerated by ME producing H_2 at the cathode. MPR was enhanced to 91.8 gCH_4/m^3 reactor/d in the microbial electrolysis ME-AD reactor, thus improving the rate by 3 times compared to control conditions (30.6 gCH_4/m^3 reactor/d in AD). The methane production yield reached 116.2 mg/g VSS in the ME-AD reactor. According to balance calculation on electron transfer and methane yield, the increased methane production was mostly dependent on electron contribution through the ME system. Thus, the use of the novel ME-AD reactor allowed to significantly enhance carbon degradation and methane production from WAS.

1. Introduction

The large amount of activated sludge generated during wastewater treatment poses a critical threat (when not properly disposed) to ecological systems [1], while proper treatment and disposal of excess sludge is quite expensive (Wei et al., 2003). On the other hand, anaerobic digestion (AD) is widely used for sludge reduction as an energy saving and recovering method [2]. However, AD rate is substantially limited by the first two steps (hydrolysis and acidogenesis) to convert complex organic compounds into suitable substrates for methanogenesis, in raw sludge [3–5]. Commonly, it takes from 20 to 30 days to degrade 30–50% of the total COD or volatile solids (VS) of raw WAS, under mild environmental conditions [6]. The pressure of rapid human population growth and increasing energy demand have thus promoted further research on development and improvement of an rate-accelerating AD process, in order to enhance biogas production and achieve faster degradation rate from WAS [7,8].

Recently, some researchers pointed out that bioelectrochemical systems have the ability to promote carbon oxidation on anode and in-site CO_2 capture and reduction on cathode, thus providing additional CH_4 formation in an integrated AD system [9,10]. Recently a direct interspecies electron transfer for methanogenesis has been proved between *Geobacter* and *Methanosaeta* [11]. However, few efforts have been made to better understand bioelectrochemical contributions to organic conversion or methane promotion, which is very important to achieve viable reactor operations in the future. Lately, microbial electrolysis cells (MECs) have been tested for their ability to convert waste organic compounds from sludge fermentative liquid (SFL) to electrons and hydrogen, showing high efficiencies [12–14]. It seems thus possible to achieve a faster conversion of complex substrates and fermentation end-products into H_2, under an external voltage [15]. It is well-known that methane is synthesized by hydrogenotrophic and acetoclastic methanogenesis from simple carbon sources, including CO_2-type substrate, methyl-type and acid-type substrate (acetate) [16,17]. More complex substrates can usually not be quickly (or directly) converted to methane. However, recovery products on cathode would trigger AD process in different energy-flow

* Corresponding author. Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
E-mail address: ajwang@rcees.ac.cn (A. Wang).

http://dx.doi.org/10.1016/j.renene.2016.01.082
0960-1481/

pathways, leading to methane production from CO_2 reduction [11]. Therefore, it is possible to stimulate a fast methane production with the contribution of microbial electrolysis process. On the other hand, the exact contribution of microbial electrolysis system in AD for sludge treatment still needs to be well understood, both in terms of its contribution to enhanced substrate degradation, as well as enhancement of methane production rate.

Therefore, in this study, a coupled system was tested, by putting a microbial electrolysis (ME) system into an AD system, for raw waste activated sludge treatment at mild environmental conditions. The microbial electrolysis system was enriched in MECs and the anodic biofilm was subsequently used to set up the ME-AD reactor. The performance of the methane production rate was evaluated, based on current electrons in the circuit of the ME-AD system. Moreover, functional communities (on key positions) were analyzed by means of high throughput sequencing, to illustrate microbial electrolysis stimulation.

2. Material and methods

2.1. Microbial electrolysis system setup

Microbial electrolysis cells were set up to enrich functional anodic communities, using single chamber reactors made of polycarbonate (45 mm diameter, 80 mm length; volume 130 mL) [18]. The anode was a graphite brush (40 mm diameter, 80 mm length; 1.01 m^2 surface area). The cathode was made from carbon cloth (40 mm diameter, YW-50 YiBang; China), covered with a Pt catalyst layer (0.5 mg Pt/cm^2 inner side). Eight single-chamber MEC reactors were inoculated, using aeration tank effluent from the Wenchang municipal WWTP in Harbin, China. All reactors were started up as replicates, at a fixed applied voltage of 0.8 V (FDPS-150, Fudan Tianxin Inc. China). Acetate (1500 mg L^{-1}) was used as carbon source in a phosphate buffer medium (50 mM; pH = 7.0) [15]. The replicates were operated in 48 h batches, until reaching stable (and similar) performance. Subsequently, three MEC reactors were randomly taken from the replicates, and kept running, using sludge fermentative liquid as carbon source, to test the biodegradation and energy recovery. Four anode brushes with functional biofilms were taken out from the remaining replicates and used as bioanode to set up hybrid ME-AD reactors.

2.2. ME-AD reactor operation and performance test

The novel ME-AD reactor consisted of a glass cylinder of 70 mm inner diameter x 180 mm height, with an effective volume of 650 mL. The anode brush with its biofilm (previously enriched in the MECs) and a new cathode were put into the cylinder. The distance between downside cathode and upside anode brush was 1 cm. The working volume was ~500 mL, with a headspace of ~150 mL, when the ME-AD reactor was operated in batch mode with 0.8 V external voltage at the beginning (Fig. S1). Current of electron transfer was measured over a 10 Ω resister in series connection with reactor using a multimeter (model 2700; Keithley Instruments). The bioelectrochemical system can work well for hydrogen harvest if the current went up over 0.5 mA [12,19]. Two ME-AD reactors were set as replicates. Two AD reactors were also operated as control reactor, without anode brush. 500 mL pretreated waste activated sludge was thus put into the ME-AD reactor for anaerobic digestion at room temperature (20–25 °C). Batch operations were monitored over 45 days, and six microbial community samples were taken at different time points.

2.3. Characteristics of waste activated sludge

Waste sludge was collected from the secondary sedimentation tank of the same local WWTP. The sludge was concentrated by settling for 24 h and washing away the water layer. The large particles were separated by means of a 40 mesh sieve before used as feedstock. The main characteristics of concentrated WAS are reported in Table S1. Bi-frequency ultrasonic pretreatment was performed with 28 + 40 kHz ultrasonicator (Ningbo Scientz Biotechnology Co., China), by applying an ultrasonic energy density of 0.5 kW/L for 10 min, before addition to the ME-AD reactors. Ultrasonic-pretreated WAS was hydrolyzed and acidified in bench-scale batch experiments for 4 days, at room temperature of 20–25 °C [12]. The sludge fermentative liquid was centrifuged and collected for single chamber MEC tests.

2.4. Sample collection, DNA extraction and 16S rRNA gene pyrosequencing

Biofilm samples were taken from graphite fibers, which were cut from anodes or cathode cloth and fragmented, using sterile scissors. Biofilm samples were taken in three different locations of the targeted electrode and combined together for DNA extraction. Before DNA extraction, fibers were gently rinsed with deionized water to remove the residual sludge [13]. Liquid samples were taken and centrifuged at 8000 g to remove supernatant; approximately 0.25 g pellet were used for DNA extraction. A rapid soil DNA isolation Kit (SK8234, Sangon Biotech, Shanghai) was used to extract DNA, according to the manufacturer's instructions. DNA was quantified by Qubit 2.0 DNA Kit for PCR amplification. PCR amplicons were visualized by using gel electrophoresis to confirm amplification of properly-sized products. Purified PCR products were quantified as described for the DNA extracts, then stored at −20 °C before pooling for sequencing.

Miseq sequencing was constructed for Illumina, using bacterial primers 341F: CCTACACGACGCTCTTCCGATCTN (barcode) CCTACGGGNGGCWGCAG and 805R: GACTGGAGTTCC TTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC for the V3–V4 region of the 16S rRNA gene. Raw sequencing data obtained from this study were deposited in the NCBI Sequence Read Archive. To minimize the effects of random sequencing errors, low-quality sequences were removed, by eliminating those without an exact match with the forward primer, those without a recognizable reverse primer, length shorter than 200 nucleotides, or containing any ambiguous base calls (Ns).

2.5. Analysis and calculation method

Voltages were measured over a 10 Ω resister in each circuit, using a multimeter (model 2700; Keithley Instruments). The electron production and coulombic contribution were calculated in order to characterize the performance of the ME system [20]. The gas was collected in a gas bag (500 mL; Cali5-Bond; Calibrated Instrument Inc) and the volume measured by means of a glass syringe. Gas composition (methane, hydrogen, carbon dioxide) was analyzed by a gas chromatograph (Fuli, GC9790; Zhengjiang instrument Inc, China), with a packed column [12] (TDX-01; 2 m length) and a TCD detector. VFAs were analyzed by a gas chromatograph (Agilent, 4890; J&W Scientific, USA), with a capillary column (19095N-123HP-INNOWAX; 30 × 0.530 mm × 1.00 μm; J&W Scientific, USA) [20], equipped with an FID. Liquid samples were centrifuged at 10,000 rpm min^{-1} and filtered through 0.45 μm membrane filters, before GC analysis. The sludge was characterized according to standard methods, including TSS, VSS [21].

The coulombic efficiency were calculated to characterize the