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Methane production improvement and associated methanogenic assemblages in bioelectrochemically assisted anaerobic digestion

Shashikanth Gajaraj [a], Yuxi Huang [a], Ping Zheng [b], Zhiqiang Hu [a,*]

[a] Department of Civil and Environmental Engineering, University of Missouri, Columbia, MO 65211, United States
[b] Department of Environmental Engineering, College of Environmental and Resource Science, Zhejiang University, Hangzhou, China

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ABSTRACT

Microbial electrolysis cell (MEC) was incorporated into anaerobic digestion (AD) to evaluate the methane recovery from digestion of glucose and sludge and the pertinent methanogenic population. At the poise voltages of 0.3 and 0.6 V, the MEC-assisted AD systems increased the yields of methane from glucose degradation by 9.4 ± 0.4% and 9.4 ± 0.5%, respectively. The energy recovery efficiencies from the electricity use in the MEC-assisted systems were 41.6% and 33.7%, respectively. For waste sludge, methane yields increased by 8.1 ± 0.5% and 8.5 ± 0.6% at 0.3 and 0.6 V, respectively. qPCR studies of population dynamics, reveal that the composition of acetoclastic methanogens was not impacted by the MEC-assisted systems, but the population of hydrogenotrophic methanogens such as *Methanomicrobiales* and *Methanobacteriales* increased by up to 17.2 times. Our results suggest that applying a low voltage (0.3 V) to AD systems is beneficial to hydrogenotrophic methanogens, resulting in an increase in methane production.

1. Introduction

Energy consumption for plant operations and biosolids disposal are the major sources of recurring expenditure for a wastewater treatment plant [1]. However, given its intrinsic chemical energy of up to 17 kJ/g [2,3], there is a growing consensus that wastewater is a resource [4]. Anaerobic digestion (AD) has been commonly employed in wastewater treatment plants (WWTPs) to stabilize waste activated sludge [5,6] while generating methane, but the rate of digestion and efficiencies are rather low at a typical hydraulic retention time of 14–35 days [7,8]. Life cycle assessment analysis suggests that bioelectrochemical systems might be one of the technologies to turn around the economics of wastewater treatment including anaerobic digestion and provide significant environmental benefits [9]. Bioelectrochemical systems could potentially be used in WWTPs to increase the degradation of organic matter and recover more energy in the form of biogas from waste sludge.

A microbial electrolysis cell (MEC) is an archetype of bioelectrochemical systems where exoeletrogenic bacteria oxidize organic matter in the wastewater to CO_2 while releasing protons and electrons [10]. The electrons which are transferred to the anode travel through an external circuit to the cathode generating an electric current [11]. In the MEC systems, the cathode is deprived of oxygen similar to the anaerobic environment in an anaerobic digester, so the proton and electrons can combine to form hydrogen with the application of an electric current [12]. While in theory only about 0.14 V of external voltage is required to produce hydrogen, in reality because of over-potentials at the electrodes and electrolyte resistance, a voltage of 0.2 V or higher is required [13]. Nevertheless, since part of the energy for the reduction of protons is derived from bacterial activity, the electrical input is far lower than the typical 1.8–2.0 V required for water electrolysis in the absence of microbes. Meanwhile, microbes being deprived of electrons result in decreased growth yields [14]. Thus, MEC has the potential to produce valuable products such as hydrogen, methane and acetate [9] while treating wastewater with lower sludge yields [13,15].

Research on MECs related to electrode materials and catalysts [16,17], microbes involved [18–20], and process/reactor configuration – with membrane [21], membrane less [22], upflow [23], multi electrode [24] continues to improve the energy efficiency of such systems. To be considered significant for practical applications, MECs need to achieve a power density higher than 1000 W/m^3 reactor [25], whereas studies reporting power densities of that intensity have to use simple substrates, highly conductive synthetic media and very small sized reactors [9,26]. Although wastewater has been used as a substrate in pilot-scale systems, the setup was pH and temperature dependent [27] and the reactor configuration were rather complex and expensive [28]. Activated sludge can be used

* Corresponding author.
E-mail addresses: huzh@missouri.edu, zhh97001@yahoo.com (Z. Hu).

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as a substrate in an MEC [29], but sludge modification with mineral media (conductive media) and high applied voltages (>1.4 V) prohibit their practical use. Whether these results from milliliter scale MECs can be extrapolated to pilot or full-scale systems with higher energy recovery efficiency remains to be seen.

In the reported literature, MEC operation targeting an increased hydrogen production, favored the growth of hydrogenotrophic methanogens and has been considered as a limitation in hydrogen production using MECs [22,30,31]. An increased focus to generate high amounts of hydrogen in biogas has led underestimation of the value of methane being generated. It should be noted that methane could be a more favorable energy product despite its energy content per unit mass being lower than that of hydrogen. Methane is currently harvested from anaerobic sludge digestion, landfill, natural gas and coal gasification processes. Additionally, methane is relatively safer than hydrogen for storage, transportation and combustion. Methane production has been demonstrated to be more robust and consistent than hydrogen capture in the MEC [32]. It is therefore more appropriate to utilize MEC technology to increase the methane yield, while concomitantly stabilizing sludge and reducing the quantities for disposal.

Indeed, a recent study has shown that the VSS and COD removal is consistently higher in the bio-electrochemical anaerobic digesters by 5–10% compared to the control digester at $22.5 \pm 0.5\,^{\circ}C$ [33]. As for the microbes responsible for methane production, hydrogenotrophic methanogens may benefit from the induced bioelectrochemical hydrogen production, while acetoclastic methanogens are susceptible to inhibition at high hydrogen concentrations [34]. MEC enhanced methanogenesis has been reported, in which the direct electron transfer between electrode and cathodic biofilm is considered to be important for the enhancement [35]. More recent studies indicate that biomass retention on electrodes rather than electrochemical interaction with the electrodes enhances stability in anaerobic digestion [36]. Questions remaining to be answered include 'How will MEC operation at different voltages affect methanogenic assemblages in anaerobic digestion?' The objectives of this study were therefore to determine the energy recovery efficiency in MEC-assisted anaerobic digestion and to determine the effect of MEC operation on methanogenic assemblages.

2. Materials and methods

2.1. Bioreactor design and operation

Six single chamber cylindrical reactors were designed from Plexiglas tubes with a 10.2 cm diameter and 15.2 cm height to accommodate 0.8 L of working volume with a headspace of 0.4 L as depicted in Supporting information (Fig. S1). Reticulated vitreous carbon (RVC) (ERG, Oakland, CA) was used as the electrodes (7.5 cm × 7.5 cm × 0.6 cm, distanced by 2.5 cm) due to its high mechanical strength, high electrical conductivity, and high surface area (20 pores per linear inch). The headspace gas was channeled through an AER-200 respirometer system (Challenge Technology, AK) to measure the cumulative gas volume with time. The batch anaerobic bioreactors were equipped with sludge and headspace sampling ports. Digested sludge from a two-stage mesophilic digester (operated at a hydraulic retention time of 20–30 days) from the Columbia WWTP (Columbia, MO) served as a seed source. The sludge was filtered through a steel mesh (1.6 mm opening) to remove large particles, and purged with pure nitrogen gas for 5 min to remove dissolved residual biogas before use. At the time of seeding, each bioreactor had seed sludge with a COD concentration of about 3000 mg/L.

All of the reactors were initially provided with a simple substrate: acetate at concentrations of 500 mg/L (Stage 1: Acclimation, Table 1) being dozed every other day. A DC power supply (Circuits Specialists, Tempe, AZ) was used to apply a fixed (poise) voltage of 0.2 V for about 30 days for reactor acclimation. Afterwards, the applied voltage increased in steps of 0.1 V every day to a final poise voltage of 0.3 or 0.6 V between anode and cathode. The bioreactors were operated in duplicate under three test conditions (control, 0.3 V, and 0.6 V). All anaerobic glucose degradation and sludge digestion tests were carried out in batch mode at $35 \pm 1\,^{\circ}C$ in a constant temperature room. Two different substrates glucose (Stage 2–anaerobic glucose degradation) and waste activated sludge (Stage 3–sludge digestion) were tested and each was evaluated in batch digestion experiments. For anaerobic glucose degradation studies, an aliquot of glucose was added to digested sludge (~800 mL) at a final concentration of 1000 mg/L of glucose. This concentration was chosen because it was unlikely to cause accumulation of long chain fatty acids (VFAs) or high H_2 partial pressures that could inhibit methanogenesis [37]. In anaerobic sludge digestion studies, 100 mL centrifuged activated sludge obtained from the Columbia WWTP was mixed with 700 mL of digested sludge, resulting in a total biomass COD concentration of $10{,}000 \pm 440$ mg/L. The headspaces of digesters were purged with pure nitrogen gas for 5 min before being sealed. The resulting mixture in each bioreactor was stirred at 300 rpm by a multi-position magnetic stirrer. The headspace gas was channeled through the AER-200 respirometer system and the cumulative gas volume was recorded.

Finally, in separate batch experiments, to elucidate the role of methanogenesis during anaerobic digestion in the conversion of the bioelectrochemical hydrogen to methane, sodium 2-bromoethanesulfonate (BES), a coenzyme M analog that inhibits methanogenesis [38], was added to each sludge sample at a final concentration of 50 mM to ensure complete methanogenesis inhibition [39,40]. Glucose at 1000 mg/L concentration was used as the substrate during this experiment (Stage 4). The headspace gas was again channeled through the AER-200 respirometer system and the cumulative gas volume was recorded.

2.2. Gas composition, chemical and statistical analysis

Cumulative biogas volumes were recorded every 10 min on the respirometer for the duration of the experiment. For direct measurements of cumulative methane volume, experimental runs were repeated and the headspace gas was bubbled through a CO_2 trap (10 M KOH solution) before the gas (methane) volume was recorded by the respirometer. At predetermined time intervals, 100 μL aliquots of biogas were drawn directly from the headspace (approximately 400 mL) for gas composition analysis. Hydrogen, carbon dioxide and methane concentrations in the biogas were determined by gas chromatography (GC-2014, Shimadzu). A thermal conductivity detector (TCD) in series with a flame ionization detector (FID) coupled with a ShinCarbon ST 80/100 Column (Restek, PA) was used for constituent separation with helium as the carrier gas. The GC operating parameters were as follows: injection temperature, 100 °C; flow rate, 10 mL/min; column temperature, held at 40 °C for 3 min, then increased to 150 °C at 10 °C/min and held for 1 min while the TCD and FID temperatures was held at 200 °C and 230 °C, respectively (Table 2).

For chemical analysis, aliquots of sludge slurry (10 mL) were withdrawn at different time intervals from the bioreactors and centrifuged at 3000 rpm for 5 min. The supernatant was then filtered through a 0.45 μm syringe filter prior to soluble COD (SCOD) and pH measurements [41]. Volatile suspended solids (VSS) were also measured according to the standard method [41]. To avoid oxygen exposure, all sampling work was conducted in an anaer-