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Assistant role of bioelectrode on methanogenic reactor under ammonia stress

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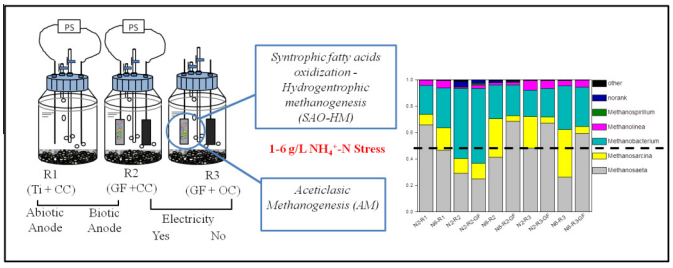
HIGHLIGHTS

- Performance of methanogenic reactors with abiotic or biotic electrode were compared.
- With the assistance of bioelectrode, VFAs degradations were enhanced by 10–70%.
- *Methanobacterium* significantly increased in reactors without electricity stimulation when suffered to NH_4^+-N stress.
- Acetoclastic *Methanosaeta* survived in MEC and preferred to enrich on bioelectrode under NH_4^+-N stress.

GRAPHICAL ABSTRACT



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ABSTRACT

To assess the role of abiotic/biotic electrode and electric field for enhancing methanogenesis under ammonia stress, three sets were conducted, i.e. R1 (titanium electrode + closed circuit), R2 (graphite felt + closed circuit), R3 (graphite felt + open circuit). Volatile fatty acids (VFAs) degradation and methane generation were gradually inhibited in all reactors when elevating NH_4^+-N to 4 g/L; nevertheless, butyrate and propionate degradation rates in R2 and R3 were enhanced by 10–70% as compared to R1. Under the extremely high stress of NH_4^+-N (6 g/L), insignificant difference was found among three tests and the methanogenesis were seriously hampered. Under ammonium stress, abundance of *Methanobacterium* significantly increased without electricity stimulation, however, acetoclastic *Methanosaeta* was found to survive and even increase in R2. Furthermore, *Methanosaeta* was enriched on graphite felt biofilm as compared to the suspended sludge, indicating the assistant role of bioelectrode for the methanogenesis under ammonium stress.

1. Introduction

Anaerobic digestion (AD) is a widely used technology for the treatment of high-strength organic wastewater and solid organic wastes. The rate-controlling step in the overall AD process is different against diverse treatment targets. For simple organics and degradable wastewater treatment, the rate-controlling steps of AD operation are related to the slow growth rate of methanogens and its high sensitivity to environmental conditions (Chen et al., 2008). Environmental stresses, such as the ammonium toxicity, organic overload, high salt concentrations and temperature variation have been frequently reported (Chen et al., 2008; Yenigün and Demirel, 2013). Although ammonium is a required nutrient for the bacterial growth involved in anaerobic digestion process, excess addition leads to inhibitory effects (Westerholm et al., 2012). However the specific threshold value for this inhibition varies depending on the non-acclimatized methanogenic cultures, which was in general deemed to be 3 g/L (Chen et al., 2008).

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To date, methanogenesis from acetate can follow two different pathways: acetoclastic and syntrophic acetate oxidation (SAO) pathway followed by hydrogenotrophic methanogenesis (Fotidis et al., 2013). Under ammonia stress, acetotrophic methanogenic pathway is usually considered to be more susceptible than the hydrogenotrophic ones (Fotidis et al., 2013; Angelidaki and Ahring, 1993). Acetoclastic methanogenesis is mainly carried out by *Methanosaeta* and *Methanosarcina*, while the hydrogenotrophic pathway is mainly performed by *Methanoculleus* sp. and *Methanobacterium* sp. (Fotidis et al., 2013; Hao et al., 2015). Lü et al. (2016) found that *Methanobacterium* was actively resistant to ammonium. Some researchers noticed the ammonia-tolerable co-culture of SAO and hydrogenotrophic methanogens was initiated by increasing ammonia concentration above 3 g/L of NH_4^+-N (Schnürer and Nordberg, 2008; Westerholm et al., 2012). Thereby the SAO pathway followed by hydrogenotrophic methanogenesis is more robust to ammonia toxicity. However, few conflicting results were also reported. Fujishima et al. (2000) reported that acetoclastic methanogenic bacteria were acclimated to high ammonia concentration. Fotidis et al. (2013) also found that when nonacclimatized thermophilic culture was exposed to high ammonia levels (7 g/L), acetoclastic *Methanosarcinaceae* spp. was found to be the dominant methanogen.

Recently, microbial electrolysis cell (MEC), a newly developed bioelectrochemical system (BES) has been attracting attentions because of its excellent performance on enhancing anaerobic digestion of organics and methane/hydrogen production (Sasaki et al., 2010). In MECs, a small voltage (0.2–0.8 V) was applied to drive the bioelectrochemical reactions. It is accepted that proper electric stimulation can promote microbial metabolism and bioactivity (Thrash and Coates, 2008). Until now, several bioelectrochemical technologies have been established and successfully applied to enhance anaerobic digestion in pilot-scale (Cui et al., 2014). The introduction of both anode and cathode of a BES in the anaerobic sludge bed resulted in enhanced methane production (Tartakovsky et al., 2011). Furthermore, De Vrieze et al. (2014) revealed a BES could remediate AD systems that exhibited process failure by introducing pre-inoculated electrodes in failing reactors.

Most MEC studies showed that hydrogenotrophic methanogens are primarily responsible for the methane generation (Lee and Rittmann, 2009). It was reported that hydrogenotrophic methanogens could use H_2 escaped from cathode to convert CO_2 into CH_4 in BES (Eq. (1)) (Cheng et al., 2009). Due to hydrogenotrophic methanogens were more tolerable to ammonia stress (Angelidaki and Ahring, 1993), we assumed a stable performance might prefer in MECs to counteract ammonia inhibition. It is believed that the microbial metabolism may be stimulated by an external electrochemical system to acclimate environment.

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (1)$$

Nevertheless, to the best of our knowledge, the inhibitory effects of NH_4^+-N on acetogenesis and methanogenesis in MEC have not yet been studies in detail. It is not clear how the methanogenic microbial communities respond to ammonia exposure within methane generating MECs. Therefore, to investigate the assistant role of biofilm and electricity alleviating ammonia inhibition for volatile fatty acids (VFAs) degradation in anaerobic digester, the performance of methane-generating MECs with biotic electrode and closed circuit was compared with the controlled reactors with abiotic electrode. Reactors were operated in parallel with elevating NH_4^+-N from 0 to 6 g/L. The microbial community enriched in suspended sludge and electrode under closed/open circuit operation were also investigated at ammonia stress by high-throughput 16S rRNA gene pyrosequencing.

2. Methods

2.1. Reactor setup

The single-chamber membrane-free MECs were made of blue cap glass bottle. The anode was an nitric acid treated graphite felt (3.0 × 7.0 cm; Beijing Sanye Carbon Co., Ltd.), and carbon paper containing a 0.5 mg/cm^2 Pt catalyst (3.0 × 7.0 cm; Shanghai Hesen electrical Co., Ltd.) serviced as cathode. Titanium wires were used to connect the electrodes to the external circuit. Electrode of Ag/AgCl (sat. KCl, 0.917 V vs. standard hydrogen electrode, SHE) was used as reference electrode. Reactors were inoculated with anaerobic sludge from a sewage sludge digester (Shanghai Quyang Wastewater Treatment Plant).

2.2. Experimental operation

In order to assess the role of abiotic/biotic electrode and electric field for ammonia stress in MECs, three sets were conducted in the presence or absence of graphite felt as electrodes (GF), in open circuit (OC, without applied voltage) or closed circuit (CC) conditions, i.e. R1 (Titanium electrode + CC), R2 (GF + CC), R3 (GF + OC). The electric current in R1 was quite small (less than 0 mA), which could be seen as conventional anaerobic digester. Each set had two parallel reactors and operated in fed-batch mode.

Before starting experiment, 25 mL of anaerobic sludge and 200 mL of medium were added to each reactor, and reactors were cultured with medium solution for about three months. The medium was prepared in a buffer (50 mM phosphate buffer solution, PBS, pH 7.0) and nutrient solution (0.31 g/L NH_4Cl; 0.1 g/L $MgCl_2$; 0.1 g/L $CaCl_2$; 0.13 g/L KCl; vitamin solution (5 mL/L) and trace element mixture (10 mL/L),) with 1.5 g/L mixed acids (acetic acid, propionic acid and butyric acid, each for 0.5 g/L).

During experiment, a gradient series (1, 2, 4, 6 g/L) of NH_4^+-N from NH_4Cl were added to all reactors (marked as N series: N1, N2, N4, N6) and compared with the control (marked as N0: with no extra adding of NH_4Cl). At stage N0, 0.31 g/L NH_4Cl was added to each reactor to guarantee the necessary nutrition for microorganism.

A battery test system (BTS-3008W-5V5mA-164, Neware) was connected to the circuit of R1 and R2 as a power supply to add fixed voltage of E_{ap} = 0.5 V. Those reactors were operated in fed-batch mode. The medium was replaced when current decreased below 20 mA. Each feeding cycle was repeated for three times, and results of display were the average values got from repeated experiments. All tests were conducted in a constant temperature (35 °C). Before starting experiment, reactors were purged with high purity nitrogen gas (99.998%) for 15 min to remove oxygen.

2.3. Analytical methods

2.3.1. Physiochemical analysis

Produced gas was collected and measured by disposable syringe. Gas composition in the headspace was analyzed at given time intervals with a gas chromatograph (GC9890B, Shanghai Linghua CO., China) equipped with a thermal conductivity detector (TCD) with argon as the carrier gas. VFAs (acetate, propionate, butyrate) were detected in high performance liquid chromatography (Waters 2695/2489, Waters, America). Current was recorded by a battery test system (BTS-3008W-5V5mA-164, Neware).

2.3.2. Samples prepared for microbial community analysis

Suspended sludge samples and anode graphite felt samples were collected at the end of each N feeding stage. The total DNA of all samples were extracted using the PowerSoil™ DNA isolation