WATER RESEARCH 47 (2013) 5719–5728

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Effects of ferric iron on the anaerobic treatment and microbial biodiversity in a coupled microbial electrolysis cell (MEC) – Anaerobic reactor

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ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form
25 June 2013
Accepted 27 June 2013
Available online 6 July 2013

Keywords:
Anaerobic
Fe(III)
Microbial electrolysis cell
Pyrosequencing

ABSTRACT

Adding Fe(III) into a MEC – anaerobic reactor enhanced the degradation of organic matters. To clarify the respective effects of combining Fe(III) dosage and a MEC and Fe(III) dosage only on strengthening anaerobic digestion, three anaerobic reactors were operated in parallel: a MEC – anaerobic reactor with dosing $Fe(OH)_3$ (R1), an anaerobic reactor with dosing $Fe(OH)_3$ (R2) and a common anaerobic reactor (R3). With increasing influent COD from 1500 to 4000 mg/L, the COD removal in R1 was maintained at 88.3% under a voltage of 0.8 V, which was higher than that in reactor R2 and R3. When the power was cut off, the COD removal in R1 decreased by 5.9%. The addition of $Fe(OH)_3$ enhanced both anaerobic digestion and anodic oxidation, resulting in the effective mineralization of volatile fatty acids (VFAs). The reduced Fe(II) combined with electric field resulted more extracellular polymeric substances (EPS) production. Quantitative real – time PCR showed a higher abundance of bacteria in the anodic biofilm and R1. Pyrosequencing and denaturing gradient gel electrophoresis (DGGE) analysis revealed that the dominant bacteria and archaea communities were richer and more abundant in the anode biofilm and R1.

1. Introduction

Anaerobic digestion is a desirable technology for high-strength organic wastewater treatment, simultaneously with the generation of bioenergy resources. Anaerobic degradation of organic wastes consists of three steps: hydrolysis/fermentation, acetogenesis and methanogenesis, during which methanogenesis is considered to be a main rate – limiting step. Both the slow metabolism of methanogens and its sensitive characteristic to environmental perturbation are liable to cause the unbalance between acidogenesis and methanogenesis, thereby resulting in the accumulation of volatile fatty acids (VFAs), inhibition of methanogenesis and even failure of anaerobic process (Connaughton et al., 2006; Zhang et al., 2009). Therefore, how to improve the degradation of VFAs becomes a key problem for anaerobic system to maintain stable operation.

Recently, bio-electrochemical reactors (BERs) are attracting attention because of their excellent performance for the digestion of organics and methane/hydrogen production (Sasaki et al., 2011a; Tartakovsky et al., 2011; Liu et al., 2005). The addition of external electrochemical system in an anaerobic reactor can be used to enhance microbial metabolism and control the electric flow (Thrash and Coates, 2008; Sasaki et al., 2010). The reactor system has been conducted by other researchers for enhancing anaerobic digestion (Tartakovsky

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http://dx.doi.org/10.1016/j.watres.2013.06.056

et al., 2011). In our previous works, a pair of electrodes was inserted into an upflow anaerobic sludge blanket (UASB) reactor for enhancement of high salinity and azo dye wastewater treatment (Zhang et al., 2012a,b). This electric-anaerobic reactor could be considered as a coupling of UASB and Microbial electrolysis cell (MEC), and the COD removal was ascribed to the anaerobic digestion and anodic oxidation.

To further strengthen the treatment performance of the MEC – anaerobic reactor, the anaerobic digestion efficiency and/or anode oxidation rate should be improved. During the anaerobic digestion, electrons from organics are transferred to various acceptors (Stams et al., 2003). As the electron acceptor, the addition of Fe(III) can significantly enhance the effect of microbial Fe(III) reduction, which further accelerated the degradation of VFA and methane production during methane fermentation (Coates et al., 2005). In addition, the anode oxidation rate of MEC/MFC was mainly attributed to the members of exoelectrogenic bacteria on anode biofilm. It is accepted that most of exoelectrogenic bacteria on anode such as *Geobacter* belong to iron reducing bacteria (IRB) that can be enriched successfully by the addition of Fe(III) oxides (Bond and Lovley, 2003; Gralnick and Newman, 2007; Ringeisen et al., 2004). Thus, it was speculated that the addition of Fe(III) in the MEC – anaerobic reactor may accelerate the degradation of VFAs.

Theoretically, $Fe(OH)_3$ could directly mineralize VFAs via microbial Fe(III) reduction process during anaerobic digestion. Further, it assumed that the addition of $Fe(OH)_3$ might be favorable for improving anode oxidation process through enriching exoelectrogenic bacteria. However, the effect of $Fe(OH)_3$ on the degradation of organics in the MEC – anaerobic reactor was not clarified until now. The response relationship among dosing $Fe(OH)_3$, electrochemical system and microbial communities (bacteria and archaea) was not established to date. To clarify these issues, $Fe(OH)_3$ was dosed into a MEC – anaerobic reactor for enhancement of high organic wastewater treatment in this study. The effect of $Fe(OH)_3$ on anaerobic digestion and anode oxidation of organics was investigated respectively. The changes of the bacterial community structure and archaeal community structure in the bio-electrochemical system were studied firstly by the 454 GS – FLX pyrosequencing technology and the denaturing gradient gel electrophoresis (DGGE). The absolute quantity of bacteria was determined using real-time PCR.

2. Materials and methods

2.1. Experimental setup

A couple of carbon felt electrodes (60 mm width × 60 mm length) were placed into an acrylic plastic up-flow anaerobic blanket reactor (UASB) (280 mm length × 100 mm width × 100 mm height) accompanied with the addition of $Fe(OH)_3$ powder (30 g, analytical reagent). The anode felt was located in the anaerobic sludge phase and placing the cathode in the surface of settling section to form a MEC combined UASB reactor (hereafter referred to as R1). The electrodes were connected with a regulated DC power source through an electric wire. The working volume of the reactor was 2 L. The control experiment was conducted in a common UASB reactor that was the same as R1 but without the electrodes (hereafter referred to as R2). The other control experiment were conducted in a common UASB reactor that was the same as R1 but without electrodes and $Fe(OH)_3$ dosing (hereafter referred to as R3).

After being seeded, these three reactors were operated with a hydraulic retention time (HRT) of 24 h at 35 ± 1 °C under a continuous mode. During the operation, the three reactors were conducted in parallel with increasing influent COD gradually from 1500 to 4000 mg/L. For the first 71 days, the reactor R1 was operated at a fixed voltage of 0.8 V with the increasing addition of COD. To clarify the effect of electric field, the voltage (0.8 V) was cut off from day 72 to day 91. The working voltage is significantly lower than the theoretical value of electrolysis of water (1.23 V).

2.2. Inoculum and synthetic wastewater

Seed sludge was obtained from a laboratory-scale UASB reactor in our laboratory. The ratio of volatile suspended sludge to total suspended sludge (VSS/TSS) was 0.75 with initial TSS of 14.4 g/L.

These three reactors were fed with synthetic wastewater. Sucrose, NH_4Cl and KH_2PO_4 were added in the Synthetic wastewater as the carbon, nitrogen, and phosphorus sources, respectively, to give a COD:N:P ratio of 200:5:1. The trace elements were added according to the following composition: 1 mL/L of a trace element solution containing Zn at 0.37 mmol/L, Mn at 2.5 mmol/L, Cu at 0.14 mmol/L, Co at 8.4 mmol/L, Ni at 0.25 mmol/L, H_3BO_3 at 0.8 mmol/L and EDTA at 3.4 mmol/L. The pH of the influent wastewater was adjusted to 7.5 using $NaHCO_3$ solution.

2.3. Analysis

COD was determined according to the standard methods (APHA, 2005). After sampling from the effluent of reactors, a few drops of dilute hydrochloric was added into the effluent samples to prevent the oxidation of Fe(II). Then, these samples were centrifuged at 8000 g for 10 min to remove impurity. The supernatant was used to determine the concentration of Fe (II) ions using *ortho* phenanthroline spectrophotometer at an absorbance of 510 nm (Techcomp, UV-2301, Shanghai, China). Sludge sample was taken from the bottom of the reactors, which was added a few drops of dilute hydrochloric to prevent the oxidation of Fe(II) and then centrifuged at 8000 g for 10 min. After removing supernatant, the bottom sludge was washed three times by dilute hydrochloric acid (pH2) to dissolve remnant ferric hydroxide and then granular sludge was selected by a filter screen (bore diameter 0.5 mm). In this way, pure granular sludge was almost separated from remnant ferric hydroxide and most of Fe(II) ion in the sludge was reserved. Finally, these granular sludge were destructed with agua regia (mixture of 2.5 ml 65% HNO_3 and 7.5 ml 37% HCl) for the roughly determination of Fe element i.e. Fe(II) in the sludge phase. The pH was recorded using a pH analyzer (Sartorius PB-20, Germany). MLSS and MLVSS were determined based on the weighing method after being dried at 103–105 °C and burnt to ash at 550 °C. Total VFAs including