A strategy for enhancing anaerobic digestion of waste activated sludge: Driving anodic oxidation by adding nitrate into microbial electrolysis cell

Hong Peng1,2, Zhiqiang Zhao1, Hong Xiao2, Yafei Yang1, Huimin Zhao1, Yaobin Zhang1,*

1. Key Laboratory of Industrial Ecology and Environmental Engineering, (Dalian University of Technology), Ministry of Education, School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China
2. Department of Environmental Science, College of Environmental Sciences, Sichuan Agricultural University-Chengdu Campus, Chengdu, Sichuan 611130, China

ARTICLE INFO

Article history:
Received 8 November 2018
Revised 9 February 2019
Accepted 11 February 2019
Available online 21 February 2019

Keywords:
Waste activated sludge
Microbial electrolysis cell
Nitrate
Anodic oxidation
Sludge reduction

ABSTRACT

Cathodic reduction of CO2 and anodic oxidation of organic matters are crucial to methane-producing microbial electrolysis cell (MEC) applied in anaerobic digestion of waste activated sludge. However, cathodic CO2 reduction is usually restrained by slow metabolism rates of H2-utilizing methanogens and low electron-capturing capacity of CO2, which consequently slows down the anodic oxidation that participates to sludge disintegration. Herein, a strategy with adding nitrate as electron acceptor to foster electronic transfer between the anode and cathode was proposed to improve anodic oxidation. Results showed that the average efficiency of anodic oxidation in the nitrate-added MEC increased by 55.9%. Accordingly, volatile suspended solid removal efficiency in the nitrate-added MEC was 21.9% higher than that of control MEC. Although the initial cumulative methane production in the nitrate-added MEC was lower than that of control MEC, the cumulative methane production in 24 days was 8.9% higher. Fourier transform infrared spectroscopy analysis indicated that anodic oxidation of MEC with nitrate accelerated the disintegration of sludge flocs and cell walls. Calculation on current signal further revealed that anodic oxidation driven by cathodic nitrate reduction was the main mechanism responsible for the improved sludge digestion.

© 2019 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

Published by Elsevier B.V.

Introduction

Waste activated sludge (WAS) produced from municipal wastewater treatment plants (MWTPs) is a problem with increasing importance due to its huge output and potential environmental risks (Feng et al., 2014; Yang et al., 2015). Anaerobic digestion (AD) is an attractive technology for the disposal of WAS and recovering energy from wastes (Zhen et al., 2017). Three successive processes are comprised in AD, i.e. hydrolysis; acidogenesis; and acetogenesis and methanogenesis (Zhao et al., 2016a; Zhen et al., 2017). Among them, hydrolysis is regarded as the rate-limiting process in sludge AD because cell walls of bacteria composed of peptidoglycan are resistant to be decomposed. Pretreatment methods such as chemical pretreatment (Devlin et al., 2011; Lin et al., 2009; Wu et al., 2014, 2015), mechanical pretreatment (Esikiciolu et al., 2009; Martín et al., 2015; Zhang et al., 2007), thermal hydrolysis (Noike, 1992) and their combination (Zhang et al., 2012) have demonstrated to...
improve the AD performance, which however remarkably increase the costs and complexity of operation. Therefore, exploiting new methods to accelerate the WAS hydrolysis during AD is of great significance. Therefore, exploiting new methods to accelerate the WAS hydrolysis during AD is of great significance.

Coupling microbial electrolysis cells (MECs) producing methane with anaerobic digesters is also gaining ground (Aryal et al., 2018). Electromethanogenesis operated in the microbial electrolysis cells (MECs) has been developed to enhance the conversion of organic wastes into methane (Ahn et al., 2017; Cheng et al., 2009; Clauwaert and Verstraete, 2009; Feng et al., 2015; Zhao et al., 2014, 2016b). In this system, organic matters are initially oxidized by exoelectrogenic bacteria in the anode to produce H+ and electrons (Ahn et al., 2017; Zhao et al., 2016a). Electrons are transferred to cathode from anode through an external circuit, combined with CO2 and H+ to form CH4 by hydrogenotrophic methanogens based on the reduction reaction of CO2 + 8H+ + 8e− = CH4 + 2H2O (Cheng et al., 2009; Fant et al., 2012; Park et al., 2018; Zhao et al., 2016a). The combination of cathodic reduction of CO2 and anodic oxidation of organic matters is an alternative to decomposing substrates and producing methane, which is different with the traditional AD. It was reported that anodic exoelectrogenic bacteria could utilize diverse substrates as electron donors, such as short-chain fatty acids (SCFAs), glucose, aromatic hydrocarbons, chlorinated benzenes and chlorinated benzenes, etc. Although no evidence to confirm that exoelectrogenic bacteria may directly utilize protein and polysaccharide which are the two main components of the sludge, it had been extensively reported that the disintegration of sludge were accelerated in MECs (Chaudhuri and Lovley, 2003; Lovley et al., 2011; Zhao et al., 2016b).

The cathodic CO2 reduction in methanogenic MEC with H2-utilizing methanogens is fragile and weak. Methanogens are sensitive to environment and have slow metabolism rates, which may affect the MEC efficiency especially under unfavorable conditions. Additionally, low oxidation-reduction potential (ORP) of CO2 is inferior to capture the electrons in the cathode, which may delay the overall electrode reactions. Comparatively, denitrifying bacteria have rapid metabolism rates and adapt easily to changes of the environment. In recent years, nitrate removal in a bioelectrochemical system (BES) has been extensively studied and nitrate has been proved to be an ideal electron acceptor (Sevda et al., 2018). Compared with CO2, NO3− has a stronger capacity to capture electrons (E(NO3/NO2) = 0.74 V vs E(CO2/CH4) = −0.44 V, SHE, pH = 7) and therefore better drives the anodic reaction.

Until now, adding nitrate to drive anodic oxidation of WAS in methane-producing MECs has not been reported. To demonstrate the effect of this process and clarify the underlying mechanism, chemical analysis, Fourier transform infrared spectroscopy (FT-IR), fluorescence in situ hybridization (FISH) and current signal calculation were conducted.

### 1. Materials and methods

#### 1.1. Substrates and inoculum sludge

Waste active sludge (WAS) collected from a municipal wastewater treatment plant (Dalian, China) was used as the substrate in this study. The collected sludge was stored at 4°C for use. Inoculum seed sludge was collected from an anaerobic digester of an industrial wastewater treatment plant of Dalian (China). The seed sludge was cultured in the laboratory with a batch anaerobic digester (5 L working volume) before experiment. The operating conditions were consistent with previously described by Zhao et al. (2016a). The sludge mixture for digestion consisted of seed sludge and WAS with a volume ratio of 1:9. The main characteristics of raw sludge, seed sludge and sludge mixture are listed in Table 1.

#### 1.2. Experimental setup and operation

The schematic diagram of experimental equipment digester is shown in Fig. 1. Experiments were conducted in four serum bottles (250 mL) used as digesters. 200 mL of sludge mixture was added into every bottle digester. The first digester was installed with a pair of graphite pillar electrode (410 mm × 48 mm, anode and cathode, respectively) with a 40 mm distance. A DC power source (Atten, APS3005D, China) was applied on these two electrodes to form a single-chamber MEC digester, the applied voltage of which was 0.8 V according to our previous work (Zhao et al., 2016b). The second digester was the same as the MEC digester, but additionally adding NaN03 to form a NO3−-added MEC (with the initial NO3− of 1000 mg/L). The third digester was the same as NO3−-added MEC but without two graphite electrodes, named as NO3−-added digester. The fourth digester was a control anaerobic digester with no electrodes installed and no addition of nitrate. These four-group digesters were capped with silica gel stoppers, thereafter subjected to 15 min of N2 flushing to remove oxygen from the digesters. Silica gel stopper was drilled with two holes, one for connecting with a gas collecting bag and another for sludge samples, respectively. These four-group digesters were placed in an air-bath shaker (160 r/min) at 35 ± 1°C for 24 days. All the experiments were replicated in triplicate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw sludge</th>
<th>Seed sludge</th>
<th>Sludge mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.2 ± 0.1</td>
<td>7.5 ± 0.1</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>54,042 ± 874</td>
<td>57,667 ± 623</td>
<td>55,235 ± 989</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>32,166 ± 542</td>
<td>32,947 ± 658</td>
<td>32,532 ± 793</td>
</tr>
<tr>
<td>TCOD (mg/L)</td>
<td>44,525 ± 1028</td>
<td>46,610 ± 921</td>
<td>44,894 ± 1489</td>
</tr>
<tr>
<td>sCOD (mg/L)</td>
<td>610 ± 81</td>
<td>1011 ± 98</td>
<td>667 ± 48</td>
</tr>
<tr>
<td>Total protein (mg/L COD)</td>
<td>2908 ± 284</td>
<td>3208 ± 401</td>
<td>3005 ± 378</td>
</tr>
<tr>
<td>Total polysaccharide (mg/L COD)</td>
<td>1483 ± 172</td>
<td>1729 ± 196</td>
<td>1569 ± 148</td>
</tr>
</tbody>
</table>

TSS: total suspended solids; VSS: volatile suspended solids; TCOD: total chemical oxygen demand; sCOD: soluble chemical oxygen demand. Average data and standard deviation obtained from triplicate tests.
1.3. Chemical analysis

Sludge samples taken from the digesters were centrifuged at 8000 r/min for 10 min and immediately filtered through a cellulose membrane with pore size of 0.45 μm for measure of soluble COD (sCOD), soluble protein, soluble polysaccharide and volatile fatty acids (VFAs). Total suspended solid (TSS), volatile suspended solid (VSS) and total chemical oxygen demand (TCOD) (include total COD and solute COD) were measured according to standard methods for the examination of water and wastewater. Methane content in the gas collecting bags was analyzed by a gas chromatograph (Tianmei, GC-7900P/TCD, China). VFAs (acetate, propionate, butyrate and valerate) were measured by a gas chromatograph (Tianmei, GC-7900P/FID, China) with GC-flame ionization detector, a 30 m × 0.25 mm fused-silica capillary column (DB-FFAP). Proteins (include total protein and solute protein) were determined with Lowry’s method (Frolund et al., 1995) using bovine serum albumin as a standard solution. Carbohydrates (include total carbohydrates and solute carbohydrates) were analyzed with phenol-sulfuric acid method using glucose as a standard solution (Masuko et al., 2005). The equivalent relationship between COD and substrate were as follows: 1.07 g COD/g acetate, 1.51 g COD/g propionate, 1.82 g COD/g butyrate, 2.04 g COD/g valerate, 1.5 g COD/g protein and 1.06 g COD/g carbohydrate (Lu et al., 2012). Data acquisition card (Hongge, PCI-821H, China) was used to record electrical current data between the two electrodes (Wang et al., 2010).

The electron transfer of the MEC and of NO$_3^-$-added MEC was evaluated in terms of anodic coulombic efficiency ($C_e$), current density ($I_s$, A/m$^2$) and total available electrons of cathodic reduction ($N$) according to the following Eqs. (1)–(3):

$$C_e = I \times t \times 100\% / (nF(C_{in}-C_{out})V/M)$$  

(1)

$I$ (A) is the measured current, $t$ (sec) is the fermentation time, $n$ is the electron amount ($n = 4$), $F$ is faradays constant (96,485 C/mol), $M$ is the molecular weight of oxygen (32× 10$^3$ mg/mol), and $C_{in}$ (mg/L) and $C_{out}$ (mg/L) are the total COD concentration in the influent and the effluent, respectively.

$$I_s = I/S$$  

(2)

$S$ (m$^2$) is the electrode working surface of MEC.

$$N = It/F$$  

(3)

1.4. FISH

FISH was used to determine the abundance of hydrogenotrophic methanogens in archaea and the abundance of denitrifying bacteria in bacteria (Virdis et al., 2011; Wu et al., 2001). The attached sludge of cathodic surface (2 mL) in NO$_3^-$-added MEC digester was collected after centrifugation (10,000 r/min for 15 min at 4°C). Two genus-specific probes for total archaea (ARC915, 5′-GTGCTCCCCGCAAATTCCCT-3′) and hydrogenotrophic methanogens (MB1174, 5′-TACCGTGGTCCGCTGCC-3′) (Raskin et al., 1994) were used in this study. A cyanine 3 (cy3)-labeled Pae997 probe with a sequence of 5′-TCTGAAAAAGTTCAGCA-3′ and Par1457 probe with a sequence of 5′-CTACCGTTGGTCCGCTGCC-3′ were used for characterization of denitrifying bacteria (red), while a fluorescein isothiocyanate (FITC)-labeled EUB338 probe with a sequence of 5′-GCTGCCTCCGAGTAAGTG-3′ was used for characterization of bacteria (green) (Virdis et al., 2011). FISH was conducted according to the method described by Wu et al. (2001). The sludge hybridized was viewed by confocal laser scanning microscopy (Leica-SP2, Heidelberg, Germany). The FISH images obtained were imported to Image-Pro plus 6.0 for analysis of the relative abundance of the microorganisms.

1.5. FT-IR analysis

To study the physicochemical change of the hydrolysis of large organic molecules under the applied electric field, FT-IR (VERTEX 70, Bruker, Germany) was used to analyze the

![Fig. 1 – Schematic diagram of experimental equipment.](image-url)
suspended sludge samples (Martínez et al., 2012; Rakotonirainy et al., 2015). The procedures of FT-IR analysis were conducted according to the description by Gröbel and Machnicka (2014).

2. Results and discussion

2.1. Methane production

From Fig. 2, more methane production was observed in the NO3\textsuperscript{−}-added digester and in the MEC than in the control. In the NO3\textsuperscript{−}-added MEC, the methane production further increased. As compared with the control, the accumulative methane production increased by 4.3% for the NO3\textsuperscript{−}-added digester, by 15.3% for the MEC, and by 25.6% for the NO3\textsuperscript{−}-added MEC, respectively. It was reported that MEC increased the methane production from the following two aspects. Exoelectrogenic bacteria enriched in the anode of MEC participated in the degradation of complicated organic matters of sludge to release available substrates for methaogenesis. In addition, the cathodic reduction of CO\textsubscript{2} could produce methane with hydrogenotrophic methanogens as biocatalyst based on Eqs. (4) and (5) (Cheng et al., 2009; Zhao et al., 2014).

Anode: \[ \text{CH}_3\text{COO}^- + 2\text{H}_2\text{O} = 2\text{CO}_2 + 7\text{H}^+ + 8\text{e}^- \approx -0.28 \text{V (SHE, pH = 7)} \] (4)

Cathode: \[ \text{CO}_2 + 8\text{H}^+ + 8\text{e}^- = \text{CH}_4 + 2\text{H}_2\text{O} \approx -0.44 \text{V(SHE, pH = 7)} \] (5)

\[ 2\text{NO}_3^- + 10\text{H}^+ + 8\text{e}^- = \text{N}_2 + 8\text{H}_2\text{O} \approx 0.74 \text{V(SHE, pH = 7)} \] (6)

Adding nitrate in MEC further enhanced the methane production. From Fig. 2, the accumulative methane production in the NO3\textsuperscript{−}-added MEC was up to 1601.3 ± 40 mL (mean ± standard deviation), which was 8.9% higher than that without nitrate. As compared with MEC, the improvement of methane production in the NO3\textsuperscript{−}-added MEC was ascribed to the acceleration of anodic oxidation due to the cathodic reduction of NO3\textsuperscript{−}. Nitrate could be bio-electrochemically reduced using electrons from the anode oxidation (Eq. (6)) (Hussain et al., 2017; Park et al., 2005, 2017; Virdis et al., 2008). Compared with CO\textsubscript{2} used as cathodic electron acceptor, NO3\textsuperscript{−} had a higher potential to serve as electron acceptor to drive anode oxidation. As a result, the enhanced anode oxidation further forwarded the decomposition of complicated organic matters of sludge to produce small-molecule substrates available for methanogenesis. Besides, addition of nitrate in AD also helped disintegrate the sludge flocs and cell walls of sludge through denitrification (Wang et al., 2016), accelerating the sludge hydrolysis and methanogenesis.

On the other hand, nitrate competing with CO\textsubscript{2} for electron might reduce cathodic methanogenesis which could be observed in the initial operation (Fig. 2). However, the cathodic methanogenesis was generally not the mainstream of methane production due to low coulombic efficiency of anodic and cathodic reaction, and then the competition might have less influence on the methane production. Instead, adding nitrate accelerated the sludge decomposition rate via heterotrophic denitrification and autotrophic denitrification that was resulted from the combination of cathodic nitrate reduction and anodic oxidation to increase the overall methane production.

2.2. Organic matters removal

The removal of organic matters greatly increased in all of the digesters with the addition of MEC and/or nitrate. As shown in Fig. 3a, the organic matters removal in NO3\textsuperscript{−}-added MEC digester was the maximum, followed by NO3\textsuperscript{−}-added digester, MEC, and control digester. After 24 days of experiment, the effluent TSS, VSS and TCOD concentrations of the control digester were 44.4, 23.6 and 30.1 g/L, respectively. Accordingly, the removal ratio of TSS, VSS and TCOD of NO3\textsuperscript{−}-added digester were 29.3%, 37.6% and 41.4%, respectively, compared with 25.2%, 33.8% and 38.4% in the MEC, respectively, and 32.2%, 42.7% and 47.9% in the NO3\textsuperscript{−}-added MEC, respectively. Organic matters removal in the NO3\textsuperscript{−}-added MEC was resulted from AD, anodic oxidation and denitrification. Addition of nitrate in the MEC could at least improve heterotrophic denitrification and autotrophic denitrification to induce complicated substrate decomposition and subsequent methane production. Besides, combination of a bioanode with a biocathode may boost anodic oxidation to remove organic matter. The performance of bioanode for organic matter removal in BES (MEC or microbial fuel cell (MFC)) is of great concern. Pasupuleti et al. (2016) achieved a COD removal rate of 96.27% in a dual-chamber microbial fuel cell (MFC) with an air cathode and a carbon-cloth anode. The MFC operated with 0.01 mol/L acetate in PBS as electrolyte under batch mode. Pant et al. (2016) reported COD removal rates of 32.8%, 71.4%, 83.1% and 90.4% in MFCs with an air cathode and a carbon-felt anode for treating wastewaters from chemical industry, milk industry, soya industry and laundry, respectively. Obviously, the COD removal rates of their studies were different from ours. The reasons behind this difference are related to electrode characteristics, substrate properties, operating modes, etc.
increased initially and then declined to a stable level. Average anodic Coulombic efficiencies in the MEC and NO3-added MEC were 43.8% ± 2.5% and 68.3% ± 5.8%, respectively. The average anodic Coulombic efficiency calculated in the NO3-added MEC increased by 55.9%. It indicated that adding nitrate in MEC could significantly improve the efficiency of anodic oxidation.

The current between anode and cathode is recorded (Fig. 6). The total available electrons for the cathodic reduction of CO2 in the MEC based on the current density calculated were 0.034 mol (=19.2 A/m² [accumulative current density] × 0.002 m² [electrode working surface] × (24 × 3600) sec/96,485 C/mol). Assumed that the electrons were completely used for cathodic methanogenesis in this nitrate-free MEC, the cathodic methanogenesis contributed to 108 mL of methane production (=0.034 mol/8 [electron of per-mol methane production] × 25.3 × 10³ mL/mol [the molar volume of gas at 35°C]), which accounted for 7.3% (=108 mL/1470 mL × 100%) of the total methane production of MEC. Thus, the methane production via AD (mainly like acetotrophic methanogenesis) was calculated as 1362 mL (=1470-108 mL), 6.8% higher than the methane production in the control digester (1275 mL). Actually, it was impossible that the cathodic methanogens totally accepted the electrons transferred from the anode. Thus, the methane production via AD was actually higher than the above production. In other words, the increased methane production from AD might be much higher than the electricity calculation above (namely 6.8%). This indirectly confirmed that the combination of anodic oxidation and cathodic reduction accelerated the sludge disintegration to improve AD.

The current density (I) obtained in the NO3-added MEC was higher than that in the MEC. This was because NO3 as cathodic electron acceptor to replace CO2 of MEC could drive a more efficient cathodic reaction due to its higher potential. In the NO3-added MEC, if the electrons of currency were totally used for cathodic methanogenesis and denitrification, the total electrons available for the two cathodic reactions in the NO3-added MEC were calculated as 0.076 mol (=42.4 A/m² [accumulative current density] × 0.002 m² [electrode working surface] × (24 × 3600) sec/96,485 C/mol). Assumed that the ratio of cathodic methane production to total methane production of NO3-added MEC was the same as that of MEC, the cathodic methane production in MEC-NO3 would be 116.9 mL (=1601 mL × 7.3%), equivalent to 0.034 mol of electron consumed by cathode methane production. Thus, the electrons consumed by the cathodic denitrification was 0.042 mol (=0.076-0.034 mol), equivalent to 368 mg/L (= 0.042 mol × 14 g/mol/8 [electron of per-mol nitrate consumption]/0.2 L × 10³) of nitrate that was denitrified in the cathode. Considering that NO3 was completely removed (from initial 1000 mg/L addition) in the system after the treatment, the autotrophic denitrification contributed to 36.8% of total denitrification. Actually, the ratio of cathodic methanogenesis to total methanogenesis of NO3-added MEC was less than that of NO3 free MEC because NO3 preferentially accepted the electron to thereby decrease the cathodic CO2 reduction for methanogenesis. It implied that the cathodic/autotrophic denitrification actually contributed to a more portion of total denitrification. The higher ratio of cathodic denitrification meant that combination of cathodic NO3 reduction and anodic oxidation worked well to forward anodic oxidation.

The raw sludge comprised of 3.0 g/L protein and 1.6 g/L polysaccharide. From Fig. 3b, after 24 days of AD, the removal of total proteins of NO3-added MEC, NO3-added MEC and the control digesters were 63.1%, 58.4% and 47.9%, respectively. Removal of total polysaccharides of NO3-added, MEC, NO3-added MEC and the control digesters were 44.1%, 36.9%, 52.8% and 21.1%, respectively. Removal of total protein and polysaccharide in four digesters was in accordance with the sludge reduction.

From Fig. 4, the VFAs concentration of four digesters showed a tendency to rise firstly and then drop. The rate of consuming VFAs in the control was relatively slow during the entire digestion. Comparatively, the total VFAs concentration in the MEC increased obviously. Adding NO3 further increased total VFAs concentration. For NO3-added MEC, total VFAs concentration were higher than that in the MEC, but lower than that in the NO3-added digester, in agreement with sludge decomposition promoted in the NO3-added MEC.

2.3. Current signals analysis

The anodic Coulombic efficiency (Cc) is an indicator to assess the fraction of electrons available from the substrates that ends up as electrical current in the system. From Fig. 5, anodic Coulombic efficiency in the MEC and NO3-added MEC
As a result, the sludge disintegration was accelerated to enhance sludge digestion. Due to the parts of electrons from organics decomposition utilizing for denitrification, the increase of methane production was lower than the improvement of sludge reduction in NO$_3^-$-added MEC.

2.4. FISH and FT-IR analysis

From Fig. 7, the relative abundance of autohydrogenotrophic denitrifying bacteria (Pae997-CY3 and Par1457-CY3, red) on the cathode surface of NO$_3^-$-added MEC on 4th, 11th and 22nd day was 38.4% ± 2.2%, 28.4% ± 1.7% and 15.7% ± 1.3%, respectively. However, it was almost undetectable on the cathode surface of MEC. These results indicated that adding nitrate could enrich the autohydrogenotrophic denitrifying bacteria on the cathode surface, which induced cathodic reduction of NO$_3^-$ to forward the anodic oxidation of sludge. The reason for the decreasing relative abundance is that NO$_3^-$ concentration was falling under the sequential batch mode in present study. On the other hand, the anodic Coulombic efficiency in the NO$_3^-$-added MEC
increased from 26.6% ± 3.4% on 4th day to 88.0% ± 3.3% on 11th day. Therefore, although denitrification was weakened over time, the current signal increased significantly, suggesting that cathodic methanogenesis was enhanced. From Fig. 7, the relative abundance of hydrogenotrophic methanogens (MB1174-CY3, red) on the cathode surface of NO$_3^-$-added MEC digester on 4th day (d), 8th day (e) and 22th day (f), cathodic biofilm of MEC-NO$_3^-$ digester on 4th day (a), 8th day (b) and 22th day (c), cathodic biofilm of MEC-NO$_3^-$ hybridized with specific probes for total archaea (ARC915-FITC, green), hydrogenotrophic methanogens (MB1174-CY5, red); FISH images of denitrifying bacteria of cathodic biofilm in MEC-NO$_3^-$ digester on 4th day (d), 8th day (e) and 22th day (f), cathodic biofilm of MEC-NO$_3^-$ hybridized with specific probes for total bacteria archaea (labeled EUB338- FITC, green), denitrifying bacteria ((Pae997-CY3, red) and (Par1457-CY3, red)).

The characteristic functional group of the sludge samples collected from different digesters was analyzed with FT-IR (Fig. 8). The intensity of absorption peaks of functional group got weakened, showing that most of large-molecule organic matters were decomposed. The peaks around 2930 and 2852 cm$^{-1}$ were attributed to asymmetric and symmetric aliphatic methylene, respectively. Aliphatic methylene groups are the parts of many organic molecules, the absorption peaks of which became weak for the sludge sample of NO$_3^-$-added MEC digester as compared with that of the control. Peaks at 1657 and 1546 cm$^{-1}$ corresponded to N–H bending band groups. The peak around 1047 cm$^{-1}$ was attributed to P–O–C stretching groups. The two functional groups were related to amino acid and phospholipid acid derived from microbial cell walls. Compared to these adsorption peaks of control digester, the adsorption peaks obviously decreased in the NO$_3^-$-added and MEC digester, indicating that addition of NO$_3$/MEC facilitated the decomposition of complicated molecules, thereby enhancing sludge decomposition. For the NO$_3^-$-added MEC digester, the characteristic peaks of the functional groups were weakest, which agreed with the profile of soluble protein and polysaccharide. Those demonstrated that using both MEC and/or NO$_3^-$-added could further expedite the disintegration of sludge.

The characteristic functional group of the sludge samples collected from different digesters was analyzed with FT-IR (Fig. 8).
2.5. Application prospect

Experimental results showed that adding nitrate in MEC promoted sludge reduction and methane production of MEC-based anaerobic sludge digestion system, demonstrating its good technical feasibility. As regards its economic feasibility, there is no need to add NO$_3^-$ in the form of a chemical agent, which accounts for extra cost. In fact, NO$_3^-$ used in this process can be produced from AD liquor that generally contains high-strength ammonium nitrogen (500–1500 mg N/L). After ammonium nitrogen being converted to nitrate in a separate aerobic nitrification process (NH$_4^+$-$N \rightarrow NO_3^--N$), it may be recycled into MEC-based AD to enhance sludge digestion (Fig. 9). In addition, the needed NO$_3^-$ could also be provided by nitrified wastewater containing suitable ammonium nitrogen. It is worth mentioning that NO$_3^-$ concentration may have a significant impact on the effect of the experiment. In present study, an initial NO$_3^-$ concentration of 1000 mg/L was tested. Optimization of NO$_3^-$ concentration deserves further study. Overall, this technology has a good application prospect in anaerobic digestion of WAS. However, challenges still existed considering its upscaling and real implementations. For example, the development of new electrode materials with high activity, conductivity and stability requires ongoing efforts. Also, the enrichment and proliferation of electroactive biofilm remains a huge barrier. A pilot study is urgently needed to advance this technology into practicality.

3. Conclusions

Adding nitrate into MEC could be an effective approach to promote sludge reduction and methane production in MEC-based sludge AD system. Nitrate besides carbon dioxide as electron acceptor effectively enhanced the anodic oxidation, consequently accelerated sludge hydrolysis and improved methanogenesis via traditional AD. On the other hand, adding nitrate could improve denitrification to induce the decomposition of sludge flocs and cell walls, more intracellular substance was therefore released, contributed to more methane production.

Acknowledgments

This work was supported by the National Natural Scientific Foundation of China (No. 51578105).

REFERENCES


