Computational and experimental analysis of organic degradation positively regulated by bioelectrochemistry in an anaerobic bioreactor system

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ARTICLE INFO

Article history:

Received 14 March 2017

Received in revised form

16 August 2017

Accepted 17 August 2017

Available online 30 August 2017

Keywords:

Methane

Bioelectrochemistry

Dynamic simulation

Microbial community

Electron balance analysis

ABSTRACT

Methane production was tested in membrane-less microbial electrolysis cells (MECs) under closed-circuit (R_{CC}) and open-circuit (R_{OC}) conditions, using glucose as a substrate, to understand the regulatory effects of bioelectrochemistry in anaerobic digestion systems. A dynamic model was built to simulate methane productions and microbial dynamics of functional populations, which were colonized in groups R_{CC} and R_{OC} during the start-up stage. The experiment results showed significantly greater methane production in R_{CC} than R_{OC} , the average methane production of R_{CC} was $0.131 \text{ m}^3/\text{m}^3/\text{d}$, which was 1.4 times higher than that of R_{OC} ($0.055 \text{ m}^3/\text{m}^3/\text{d}$). The simulation results revealed that bioelectrochemistry had a significant influence on the abundance of microorganisms involved in acidogenesis and methanogenesis. The abundance of glucose-uptaking microorganisms was 87% of the total biomass in R_{OC} without applied voltage, which was 20% higher than that in R_{CC} (67%) when external voltages were applied between the anode and cathode. The abundance of hydrogenotrophic methanogens in R_{CC} was 6% higher than that in R_{OC} . The simulation results were verified through 16S rDNA high-throughput sequencing analysis. An electron balance analysis revealed that alteration of the acidogenesis type led to more acetate and hydrogen production from glucose fermentation, compared with the situation without bioelectrochemistry. An additional pathway from acetate to hydrogen was introduced by bioelectrolysis. These two factors resulted in significant enhancement of methane production in R_{CC} . Bioelectrolysis process directly contributed to 26% of the total methane production after the start-up stage. When the applied voltages were cut down or decreased, R_{CC} could maintain considerable methane productions, because the microbial communities and electron transfer pathways were already formed. Starting-up with high voltage, but operating under low voltage, could be an energy-favorable strategy for accelerating biogas production in bioelectro-anaerobic bioreactors.

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1. Introduction

Anaerobic digestion (AD) technologies can reduce organic

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pollution from agricultural and industrial operations, while simultaneously offsetting the use of fossil fuels (Chen et al., 2008). AD can be divided into four major steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. With the cooperation of hydrolytic, fermentative bacteria and methanogens, complex organics are degraded and biogases (methane and carbon dioxide) are finally formed (Batstone and Jensen, 2011). AD technology has numerous advantages, such as low sludge production, low overall cost, and energy recovery (vanStarkenburg, 1997), however, poor operational stability caused by inhibitory factors prevents AD from being widely commercialized (Chen et al., 2008; Zhang and

Nomenclature

AD	Anaerobic digestion
ARB	Anode respiration bacteria
ADM1	Anaerobic Digestion Model No.1
PCR	Polymerase Chain Reaction
FID	Flame ionization detector
X_{su}	The concentration of glucose-uptaking microorganisms
X_{pro}	The concentration of propionate-uptaking microorganisms
X_{H_2}	The concentration of hydrogenotrophic methanogens
S_{su}	The concentration of soluble glucose
S_{pro}	The concentration of propionate
S_{H_2}	The concentration of hydrogen
S_{CO_2}	The concentration of soluble carbon dioxide
M_{red}	The concentration of reduced-form intracellular mediator in electricigenic microorganism
V_{H_2}	The volume of gaseous hydrogen
X_{max}	The maximum concentration of attainable biomass
f_{bu}	The proportion of butyrate produced from glucose degradation

f_{H_2}	The proportion of hydrogen produced from glucose degradation
MEC	Microbial electrolysis cell
BES	Bioelectrochemical systems
MFC	Microbial fuel cell
PBS	Phosphate buffer solution
COD	Chemical oxygen demand
X_{bu}	The concentration of butyrate-uptaking microorganisms
X_{ac}	The concentration of acetotrophic methanogens
X_e	The concentration of electricigenic microorganisms
S_{bu}	The concentration of butyrate
S_{ac}	The concentration of acetate
S_{CH_4}	The concentration of soluble methane
M_{ox}	The concentration of oxidized-form intracellular mediator in electricigenic microorganism
V_{CH_4}	The volume of gaseous methane
V_{CO_2}	The volume of gaseous carbon dioxide
f_{ac}	The proportion of acetate produced from glucose degradation
f_{pro}	The proportion of propionate produced from glucose degradation

Angelidaki, 2015a; 2015b, c). In an anaerobic bioreactor system, a sophisticated microbial structure and electron transfer paths spontaneously form in cells, which lack direct and effective regulatory approaches. Failure to maintain the balance among microorganisms is the primary cause of AD reactor instability (Demirel and Yenigün, 2002).

Microbial electrochemical cells (MECs) are promising technologies for anaerobic wastewater treatment and energy recovery (Zhang and Angelidaki, 2014). In MECs, electrochemical active bacteria oxidize the substrate and extracellularly transfer electrons to the anode. With the drive of a small voltage (0.2–0.8 V), electrons travel through an external circuit to the cathode, which can be used for hydrogen production (Liu et al., 2005). The bioelectrochemical process is more easily controllable than typical AD processes. External voltage acts like a “pump”, which can adjust the direction and velocity of electron flows to achieve optimal energy efficiency. Introducing bioelectrochemistry into an AD system as a special metabolic pathway may regulate the establishment of microbial structures and electron transfer paths, and increase overall energy efficiency and stability. Currently, attempts to introduce MEC electrodes into AD reactors have been reported to be practically effective in promoting the efficiency and stability of the entire system (Cerrillo et al., 2016; Gajaraj et al., 2017; Liu et al., 2016; Zhang et al., 2013), however, the role of bioelectrochemistry in an anaerobic bioreactor system has not been thoroughly investigated, and it is essential for the further development of MEC-AD coupling processes.

Methane-production in an AD system relies on coexistence and cooperation between various anaerobic microbial populations, while bioelectrochemical processes are closely related to the metabolism of electrochemically active bacteria (Liu et al., 2012). When electrochemical conditions are applied in an AD system, cooperation and competition between anaerobic-digestion populations and electrochemically active bacteria will be key factors affecting overall efficiency. The start-up stage is the key period for the establishment of microbial structures (Ruiz et al., 2014),

therefore, this study will focus on the effect of bioelectrochemical processes on the enrichment of functional populations during the start-up stage.

Tracking population enrichment dynamics or analyzing electron flow in a complex system requires an effective tool, such as dynamic simulation. A few studies have reported on the modeling of bioelectrochemical systems (BESs). Wang et al. simulated CO_2 utilization and fuel production in a microfluidic electrochemical cell (Wang et al., 2013). Pinto et al. proposed a multi-population MEC model (Pinto et al., 2011a) and a unified dynamic model describing MFC and MEC in 2011 (Pinto et al., 2011b), but existing models have failed in representing anaerobic digestion processes in a MEC system. It is, therefore, necessary to modify MEC models with comprehensive AD models, such as the Anaerobic Digestion Model No.1 (ADM1), which has been widely recognized as a platform for anaerobic digestion simulation since it was first published by the International Water Association (IWA) in 2001 (Batstone et al., 2002).

In this study, membrane-less MEC reactors fed with glucose were started up under both closed- and open-circuit conditions (R_{CC} and R_{OC}) to represent the mode of anaerobic bioreactors with or without bioelectrochemical regulation. A multi-population dynamic model for the glucose digestion process was built to simulate the start-up processes in R_{CC} and R_{OC} . The effects of bioelectrochemistry on the colonization of the main functional microbial populations, particularly those related with acidogenesis fermentation, were analyzed based on the simulation results and 16S rDNA high-throughput sequencing results of biomass samples. Electron balance analysis was used to evaluate the regulatory effect of bioelectrochemistry on electron transfer paths in anaerobic bioreactor systems. The contribution of the bioelectrolysis process to methane production was evaluated through simulation and verified by decreasing or cutting down applied voltage. The perspective of introducing bioelectrochemistry to an anaerobic system for enhancing methane production in practice was also discussed.

2. Materials and methods

2.1. Membrane-less MEC configuration

Six membrane-less MEC reactors were used in this study, the schematics for which are shown in Fig. 1. The reactor comprised of a glass cylinder, with a liquid volume of 700 mL, and a headspace of 200 mL. The anodes were fabricated from a fiber graphite brush with a spatial volume of 78.5 cm³ (5 cm in diameter and 4 cm in length). The cathodes were assembled from five layers of circular stainless steel mesh (40-mesh, 5 cm in diameter) with a surface area of 194 cm² and an interspace of 5 mm. The distance between each anode and cathode was 3 cm. Each reactor was equipped with a magnetic rotor and placed on a six-position magnetic stirrer (84-1, Shanghai Meiyongpu Instruments Co., Ltd., China) to create a homogeneous mixture. External voltage was supplied by a switching power source (FDPS-150, Fudan tianxin Inc, China). All reactors were kept in a thermostatic incubator, with the temperature maintained at 35 ± 1 °C.

2.2. Startup and operation of semi-continuous MEC

All MECs were inoculated with 50 mL of effluent solution from a glucose-feeding MEC (Cui et al., 2016) and 5 mL of waste activated sludge (Taiping sewage treatment plant, Harbin, China). The operational processes of MECs were divided into four stages, and the conditions for each stage are listed in Table 1. In the pre-startup stage, stage 1, and stage 2, six reactors (R1–R6) were divided into two groups (R_{CC} and R_{OC}) and each group had three reactors as replicates. R_{CC} and R_{OC} were operated under closed (0.8 V) and open circuits, respectively.

Following normal practice from related studies (Wang et al., 2014), MECs first underwent a pre-startup stage for ARB enrichment. The medium used in this stage was 2000 mg/L sodium acetate, a 50 mM pH 7.0 phosphate buffer solution (PBS), 0.31 g/L NH₄Cl, 0.13 g/L KCl, and trace elements (Wolin et al., 1963). All reactors were operated under a batch mode for 8 days until the anode potentials in the R_{CC} group were below −400 mV, versus the reference electrode (saturated calomel electrode, +247 mV vs.

standard hydrogen electrode, model-217, Shanghai Precise Sci. Instru. Co., Ltd. China), which indicated successful acclimation of the bioanodes.

The carbon source was then changed to 2000 mg/L glucose as the fermentative substrate, and reactors were operated in a semi-continuous mode. To perform model calibration and validation, stages 1 and 2 were operated under different batch times. The data obtained in stage 1, with a batch time of 24 h, were used for parameter (f_{ac} , f_{bu} , f_{pro} and f_{H2}) estimation, and the data from stage 2, with a batch time of 48 h, were used for model verification.

In stage 3, the applied voltages of three reactors (R1, R2, and R3) in group R_{CC} were adjusted to different values to evaluate the effects of applied voltage on system stability after the start-up stage. The voltage of R1 was fixed at 0.8 V, while the voltage of R2 was reduced to 0.5 V, and no voltage was provided to R3 (i.e. open-circuit condition).

2.3. Model description

A dynamic model of a single-chamber MEC fed with glucose was established based on ADM1 (Batstone et al., 2002) and a basic MEC model proposed by Pinto et al., (2011a). The new model consisted of four parts, including biochemical and bioelectrochemical processes, liquid-gas exchanges, and biofilm retention and detachment, which can be elaborated as follows:

2.3.1. Biochemical process

The glucose degradation process is mainly considered in this model. The disintegration of composites (dead biomass) and subsequent protein degradation processes are neglected due to low amounts of dead biomass and short batch periods. Five functional populations are expected to be involved in glucose fermentation:

- I Glucose-uptaking microorganisms (X_{su}), which are capable of degrading glucose (S_{su}) and producing soluble butyrate (S_{bu}), propionate (S_{pro}), acetate (S_{ac}) and hydrogen (S_{H2}) as fermentation products.
- II Butyrate-uptaking microorganisms (X_{bu}), which are capable of degrading butyrate and producing acetate and hydrogen.

Fig. 1. MEC reactor configuration and model structure description.

Table 1
Operation conditions for MEC reactors.

Condition			Stage			
			Pre-startup (8 days)	Stage 1 (Day 0–5)	Stage 2 (Day 6–19)	Stage 3 (Day 20–30)
Voltage	R _{CC}	R1	0.8 V	0.8 V	0.8 V	0.8 V
		R2				0.5 V
	R _{OC}	R3	0 V	0 V	0 V	0 V
		R4–R6				Stop operating
Cycle time			Batch	24 h	48 h	48 h
Substrate			Acetate	Glucose		

III Propionate-uptaking microorganisms (X_{pro}), which are capable of degrading propionate and producing acetate and hydrogen.

IV Acetotrophic methanogens (X_{ac}), which are capable of producing methane with acetate as a substrate.

V Hydrogenotrophic methanogens (X_{H_2}), which are capable of using hydrogen as an electron donor to reduce carbon dioxide to methane.

The combined process is described in Fig. 1. All state variables, kinetic rate equations, and biochemical rate coefficients were obtained from ADM1, and were listed in Supporting information S1–S3, respectively.

2.3.2. Bioelectrochemical reactions

The liquid in the reactor was mixed completely and surrounded the electrodes evenly. The bioelectrochemical process is described by several equations proposed by Pinto (Pinto et al., 2011a), in which acetate is assumed to be consumed by electricigenic microorganisms (X_e) and degraded into carbon dioxide, and the oxidized intracellular mediator (M_{ox}) in electricigenic microorganisms get electrons and become reduced (M_{red}) (Equation (1)). Electrons, carried by M_{red} to the anode surface, and intracellular mediators return to an oxidized form (Equation (2)). The electrons transfer to the cathode, driven by applied voltage; and finally, electrons are released from the cathode and bond with H^+ to produce hydrogen (Equation (3)). This process can be evaluated by currents (I) through the external circuit.

The process of acetate-uptake by electricigenic microorganisms was calculated by the Monod equation, and current was calculated by the ohm's law, the Nernst equation and the Butler-Volmer equation, which are described in Supporting information S4.

2.3.3. Liquid-gas transfer process

Three gas components (methane, hydrogen and carbon dioxide) are produced in the liquid phase and are transferred into the gas phase. The gas components are collected under a constant pressure (1 atm) at 35 °C in the experiments, and the volumes of methane (V_{CH_4}), hydrogen (V_{H_2}), and carbon dioxide (V_{CO_2}) was calculated by gas-liquid mass transfer equations, which are listed in Supporting information S5.

2.3.4. Biofilm retention and detachment

All biomass is assumed to attach onto the electrodes in the MECs, and the biomass of each population is calculated for the

whole reactor, without differentiating between anodes or cathodes. Biofilm retention on the electrode surface is controlled by the maximum attainable biomass (X_{max}). Biofilm does not detach until it reaches maximum value, after which biomass begins to detach from the electrode surface at the same rate as biomass growth. This is described in the model by adding a dynamic detachment process, which is explained in Supporting information S6.

The following hypothesis simplifies the semi-continuous operational mode of MEC: at the start of each batch, the biomass of each population in the biofilm continues from the last batch, and the concentration of liquid components returns to its initial values.

2.4. Analytical and calculation methods

Voltage across the resistor (10 Ω) and electrode potentials were measured using a multimeter/data acquisition system (Model 2700, Keithley Instruments). Gas composition (methane, hydrogen, and carbon dioxide) was analyzed using a gas chromatograph (Agilent 7890, USA), equipped with a flame ionization detector (FID). Liquid samples were filtered through a 0.45 μm filter (Tianjin Jinteng Experiment Equipment Co., Ltd., China). Chemical oxygen demand (COD) was analyzed according to standard methods (Potassium Dichromate Method). Volatile acids were measured by a gas chromatography (Agilent 7890, USA), as mentioned in a previous study (Zhou et al., 2014).

The integration of model equations was performed using Aquasim software (version 2.1d). Efficiency indexes of MEC including coulombic and electrochemical contribution efficiency, and net energy profit, were calculated following references (Guo et al., 2016), and are listed in Supporting information S7.

2.5. Biomass sampling, DNA extraction and high-throughput sequencing

The biomass in each MEC reactor was sampled at the end of the experiment for microbial community structure analysis. The sampling procedure was as follows: the reactor was half filled with sterilized water, then shocked by a vortex mixer (XH-C, Huacheng, China) to force the biofilms to detach from the electrodes, and the resulting liquid was collected in a sterilized bottle. This procedure was repeated twice to maximize biomass collection from the electrodes. All of the liquid was collected together and concentrated by centrifugation (8000 rpm). The supernatant was discarded and the precipitate was stored at –20 °C for analysis.

The total genomic DNA of each sample was extracted with the Soil DNA Isolation Kit (Sangon Biotech Co., Ltd.), following the manufacturer's instructions. The quantity and quality of the extracted DNA were assessed by measuring its absorbance at 260 and 280 nm using a spectrophotometer. PCR amplification and pyrosequencing analyses were performed following the previous references (Guo et al., 2015).

Fig. 2. Experimental and simulated methane production performances of R_{CC} and R_{OC} .

3. Results and discussion

3.1. Methane-producing performance

Methane production rates in both groups increased gradually in the first 8 days, and then reached stable state (coefficient variations <10%, Fig.S8), which was noticeably higher in R_{CC} with applied voltage than that of R_{OC} in open circuit during the whole operation period (Fig. 2). The average methane production of R_{CC} in the stable period was $0.131 \text{ m}^3/\text{m}^3/\text{d}$, which was 1.4 times higher than that of R_{OC} ($0.055 \text{ m}^3/\text{m}^3/\text{d}$). Methane content, COD removal efficiency, and methane recovery efficiency of R_{CC} in the stable stage were also greater than those of R_{OC} (Table 2). Starting-up under closed-circuit conditions significantly enhanced ($p < 0.05$) methane production and biodegradation performances of MECs.

Simulations on methane production in both closed-circuit (simulated R_{CC}) and open-circuit conditions (simulated R_{OC}) were carried out with a dynamic model. The simulation results showed the same trends as the experimental data (Fig. 2). The simulated methane production rate in the stable stage was $0.118 \text{ m}^3/\text{m}^3/\text{d}$ in R_{CC} and $0.060 \text{ m}^3/\text{m}^3/\text{d}$ in R_{OC} , which were close to the experimental data. The average errors between the simulated results and experimental data in R_{CC} and R_{OC} were 18.6% and 23.2%, respectively, which indicated that simulations fit the methane-production measurements of MEC well.

3.2. Effect of bioelectrochemistry on functional community colonization

The start-up stage in biofilm-based reactors (e.g. MEC) can be considered as a competitive process for the valid space of supporters among functional populations (Zhou et al., 2013), and it was found that applying voltage significantly influenced on this interactive microbial process. The enrichment of the main functional microbial populations in R_{CC} and R_{OC} were simulated over the whole start-up stage (Fig. 3). The enrichment process can be

divided into three periods: the slow-growth period (first 3 days), rapid-growth period (3–13 days), and biomass-saturation period (13–19 days). In the slow-growth period, the biomass of all populations was very low and grew slowly, after which the biomass of the populations grew rapidly. Glucose-uptaking microorganisms (X_{su}) grew rapidly and became the dominant population in both R_{CC} and R_{OC} ; electricigenic microorganisms (X_e) were the second most abundant population in R_{CC} ; hydrogenotrophic methanogens (X_{H_2}) became abundant in both groups, and the biomass of X_{H_2} in R_{CC} was higher than that in R_{OC} , because additional substrates (hydrogen) were provided by the bioelectrolysis process. The biomass of butyrate-uptaking microorganisms (X_{bu}), propionate-uptaking microorganisms (X_{pro}), and acetotrophic methanogens (X_{ac}), however, was still low due to low growth rates. In the biomass-saturation period, all of the valid areas (or spaces) on electrodes for the colonization of microorganisms had been occupied, so the colonization of each population on electrodes began to be restricted and the microbial community structure became stabilized. The biomasses of X_{su} , X_e , and X_{H_2} were maintained or decreased slightly, but the biomasses of other populations continued to increase, with lower growth rates.

The main differences between R_{CC} and R_{OC} in the simulated functional population were the following: there was considerable content of X_e and more X_{H_2} in R_{CC} than in R_{OC} , but there was less X_{su} in R_{CC} . This was probably attributed to competition between X_e and X_{H_2} . The biomass of other populations, including X_{bu} , X_{pro} and X_{ac} , were still low (<1%) in R_{OC} , but higher than in R_{CC} .

The microbial community structures of R_{CC} and R_{OC} were also experimentally analyzed using high-throughput sequencing technology. The abundance of dominant genera (relative abundance >1%) are summarized based on their metabolic characteristics in Fig. 4. The sequencing analysis results provided verification for the reliability of dynamic simulations of functional population enrichment. Electricigenic microorganisms in R_{CC} , including the *Geobacter* and *Desulfovibrio* genera (Liu et al., 2012), had the second largest abundances (26% of the total bacterial sequences), which was consistent with the simulation results. The distribution of archaea genera also corroborated the simulation result. Hydrogenotrophic methanogens were the dominant population in both R_{CC} and R_{OC} (over 90% of the total archaea sequences). More hydrogenotrophic methanogens (including *Methanobacterium*, *Methanobrevibacter*, and *Methanospirillum*) (Lino et al., 2010; Oren and Garrity, 2015; Rea et al., 2007; Shlimon et al., 2004) but fewer acetotrophic methanogens (*Methanosarcina*) (Shimizu et al., 2015) were enriched in R_{CC} . Acidogenic bacteria were dominant in bacterial communities of both R_{CC} and R_{OC} . *Lactococcus* and *Enterococcus* (Lucena-Padros et al., 2014; Yan Yang et al., 2016), lactic acid producing bacterial genera, were most abundant in the bacteria, and their relative abundance in R_{OC} (~70%) was much higher than that in R_{CC} (~50%), which also showed similar trends to the simulation results.

Glucose-uptaking microorganisms (X_{su}) in the model corresponded to the total abundance of lactate, acetate, propionate, butyrate and ethanol-producing bacteria in the experimental results (Fig. 4). Since lactate-producing bacteria were the largest group in X_{su} , the effect of electrolysis on the abundance of lactate-

Table 2
Performances of R_{CC} and R_{OC} .

	Methane production rate ($\text{m}^3/\text{m}^3/\text{d}$)	Methane content (%)	COD removal (%)	Methane recovery (mL/gCOD)	Average current (mA)	Coulombic efficiency (%)
R_{CC}	0.131 ± 0.005	43.7 ± 5.0	70.1 ± 8.3	138.1 ± 19.8	13.3	17.8
R_{OC}	0.055 ± 0.008	29.1 ± 4.9	59.3 ± 9.2	58.3 ± 7.7	—	—

Fig. 3. Simulated biomass of functional populations in R_{CC} and R_{OC} .

producing bacteria represented the effect of electrolysis on the enrichment of X_{su} .

Lactate was a common intermediate product of the glucose acidification process, which could rapidly degrade into other products (acetate, propionate, butyrate and ethanol) without accumulation. Additionally, glucose and lactate have the same stoichiometric relationship (Equation (4)), and there were no other compounds involved in this reaction, so neglecting lactate-related processes would not significantly affect the simulation results.

3.3. Effect of bioelectrochemistry on acidogenesis pathway

Acidogenesis included several fermentation-types, according to the different composition of fermentation end products, i.e. butyric-acid type (the end products of butyrate, acetate, and hydrogen), propionic-acid type (the end products of propionate and acetate), and ethanol type (the end products of ethanol, acetate, and hydrogen). It was supposed in the model that three reactions occurred in glucose acidogenesis, and the proportion of four fermentation end products – acetate (f_{ac}), butyrate (f_{bu}), propionate (f_{pro}), and hydrogen (f_{H2}), were determined by the proportions of reactions 1, 2 and 3:

Reaction 1 (producing acetate):

Reaction 2 (producing acetate and propionate):

Reaction 3 (producing butyrate):

The parameters f_{ac} , f_{bu} , f_{pro} , and f_{H2} were estimated according to the experimental data from R_{CC} and were set as the default values (proposed by ADM1) for R_{OC} ; all values are listed in Table 3. More glucose was degraded to acetate (reaction 1) in R_{CC} than in R_{OC} , resulting in an increase in the proportions of acetate and hydrogen in total fermentation products and a decrease of the proportions of butyrate and propionate. This suggested that the applied voltage

Fig. 4. Experimental relative abundance of functional populations in R_{CC} and R_{OC} .

Table 3

Stoichiometric coefficients of glucose-uptaking process in MECs under closed-circuit and open-circuit conditions.

	Reaction 1	Reaction 2	Reaction 3	f_{ac}	f_{bu}	f_{pro}	f_{H2}
R_{CC}	75%	18%	7%	0.54	0.06	0.14	0.26
R_{OC}	50%	30%	20%	0.41	0.13	0.27	0.19

had a substantial influence on glucose fermentation type.

The high-throughput sequencing results also support this finding (Fig. 4). The abundance of genera which could produce butyrate (such as *Eubacterium*) (Wallace et al., 2003) and propionate (such as *Anaerobaculum*) (Strömpl and Lünsdorf, 2015) as fermentation end products were lower in R_{CC} than in R_{OC} , however, ethanol-producing genera (such as *Klebsiella*) (Imhoff, 2005) were enriched in R_{CC} . Ethanol was more likely to be converted into acetate and hydrogen compared to butyrate and propionate in the thermodynamic point of view (see equations (8)–(10)), so considering ethanol as an intermediate product of the degradation process of glucose into acetate (reaction 1, equation (5)) made little difference. The decrease of butyrate and propionate-producing bacteria and increase of ethanol-producing bacteria explains the alteration of fermentation type.

Propionic-acid type fermentation often takes place in an AD system which is affected by sudden changes in environmental conditions, and it was generally considered to be an indicator of a collapse in anaerobic bioreactors. The application of voltage presented a positive influence on preventing the propionic acid type pathway, which improved the stability of the anaerobic bioreactor system.

3.4. Electron balance analysis for digestion process

To provide a complete picture of the role of bioelectrolysis reactions in the glucose digestion process, an electron-balance analysis was conducted, based on the simulation. In an operational batch, electrons obtained from complete oxidation of glucose in the influence were considered as total electrons at 100%. The proportion of electrons transferred through each reaction or stored in each product were calculated and presented in Fig. 5.

In the acidogenesis process, without applied voltage, more electrons were transferred and stored in propionate and butyrate (34.9% in R_{OC}), but this was lower with bioelectrochemistry regulation (17.9% in R_{CC}); more electrons in R_{CC} were transferred from glucose to hydrogen (23.4%) and acetate (48.6%), while the values for these were only 17.1% and 36.9%, respectively, in R_{OC} . In the acetogenesis process, the conversion of propionate and butyrate to acetate and hydrogen was limited by the low biomasses of X_{bu} and X_{pro} in both R_{CC} and R_{OC} (<1%). As a result, most of the propionate and butyrate remained in the effluent. In R_{CC} , 16.1% of the electrons were transferred from acetate into the circuit current through the bioelectrochemical process, and 10.6% of the total electrons were transferred into hydrogen, during which about 3% of the electrons were lost in circuits. In the methanogenesis process, electrons from hydrogen were totally transferred into methane in both R_{OC} (16.3%) and R_{CC} (32.0%), due to the considerable proportion of X_{H_2} . Acetate cannot be completely converted into methane due to relatively low amounts of X_{ac} . Only 3.6% of the total electrons were transferred from acetate to methane in R_{OC} , and 5.6% in R_{CC} . In general, the acetogenesis and acetotrophic methanogenesis processes were the rate-limiting steps in both R_{OC} and R_{CC} . The bioelectrolysis reaction in R_{CC} created an additional pathway between acetate and hydrogen, which enhanced electron transfer to methane. Additionally, the alteration of acidogenesis type under applied voltage pushed more electron flow into acetate and hydrogen, instead of butyrate and propionate. These effects led to a significant enhancement of methane production (Fig. 2).

According to the electron balance results, the contribution of the circuit current to methane production was not satisfying. Electrons transferred via the circuit only accounted for 10% of all of the electrons from glucose, and they only contributed 26% of the total methane production in R_{CC} (Fig. 5). To verify the direct contribution of bioelectrochemistry to methane production, three reactors (R1, R2 and R3) in group R_{CC} were operated under different voltages for several batches after 20 days of operation. After the start-up stage, the electrodes were saturated with biofilm and the communities were relatively stable, so changes of voltage reflected the direct contribution of the electrolysis reaction. About 8% decrease ($p = 0.02$) in the methane production rate was caused by reducing the applied voltage from 0.8 V (R1) to 0.5 V (R2), and a 15% decrease ($p < 0.01$) by removing the applied voltage (R3), which was in consistent with the results of the electron balance analysis. The methane producing performances of R2 and R3 were simulated by

Fig. 5. Electron balance analysis in R_{CC} and R_{OC} after the start-up stage.

Fig. 6. Experimental and simulated methane production performances of R1, R2 and R3.

adjusting the E_{applied} (applied voltage of MEC) parameter in the model to 0.5 V and 0 V, respectively (Fig. 6). The methane production rates that were predicted by the model were consistent with the experimental results (relative error lower than 10%), which further proved the reliability of computational analysis.

3.5. Effect evaluation of bioelectrochemistry on anaerobic digestion

It was a pity that energy recovery was not substantially contributed by bioelectrochemical process in this study. A considerable amount of acetate, as well as other byproducts, remained in the effluent, instead of rapidly converting to electrons (hydrogen) by the electrolysis process in a 48 h batch operation. This was mainly caused by a relatively low current density of $18.6 \text{ A/m}^3_{\text{reactor}}$ in this study. Thus, there is still potential to increase the ratio of bioelectrochemical reactions in the total electron flow by increasing current density. The methods for reducing internal resistance and increasing current density included optimizing the reactor configuration (Kadier et al., 2016; Logan et al., 2015), increasing electrode areas (Freguia et al., 2007; Guo et al., 2016; Haeger et al., 2014; Jia et al., 2013), and screening the efficient materials for the anode and cathode (Sangeetha et al., 2016), etc. For example, Hou et al., (2015) obtained a current density of 132 A/m^3 , and increased methane production rate ($0.17 \text{ m}^3/\text{m}^3/\text{d}$) using high-surface-area spiral wound electrodes. Cai et al., (2016) achieved a current density of $300 \text{ A/m}^3_{\text{reactor}}$ under a voltage of 0.8 V by using a 3D nickel foam-graphene cathode.

Besides current density, substrates also played important roles in determining the effects of bioelectrochemistry on anaerobic digestion processes. Previous studies indicated that acetate was the most efficient electron donor for electrode-respiring bacteria (Liu et al., 2010; Wang et al., 2014). Supplying acetate as a substrate was beneficial for establishing an efficient anode biofilm and having good electrochemical performance (Liu et al., 2005; Mehanna

et al., 2010). The contribution of electrochemistry was, therefore, generally significant in bioelectrochemical systems fed with acetate (Wang et al., 2009). When using glucose or wastewater as carbon substrates, however, the biofilm communities and anaerobic digestion processes became more complex (Varrone et al., 2014). The relative abundance of electrode-respiring bacteria and the electron transfer efficiency were generally lower than those in the systems using acetate (Zhang et al., 2011), which might weaken the effects of electrochemistry on the anaerobic digestion process.

The potential effects of bioelectrochemistry on anaerobic microbial community establishment are worth noting. Both experimental and computational results in section 3.2 about functional population enrichment indicated that external voltage, as a positive growth condition or selective pressure, had substantial regulatory effects on microbial community structures. Applied voltage acts as a pressure, regulating community shift through microbial competition or growth conditions (Liu et al., 2010; Zhou et al., 2013). In principle, applying voltage can create a suitable habitat for anaerobic and facultative populations by reducing electrode potential (Liu et al., 2010; Rabaey and Verstraete, 2005), and boost hydrogenotrophic methanogens by providing additional hydrogen (Wang et al., 2009). In the voltage changing experiment, it was found that the methane production performance in R3 (voltage reduced from 0.8 V to 0 V) was noticeably higher than those in group R_{OC} (started under open-circuit conditions) (Fig. 6). This phenomenon confirmed the stimulating effect of voltages on the methane-production functions of microbial communities. Considering the positive influences on microbial community structure and function, introducing a bioelectrolysis process can be a feasible strategy for accelerating the start-up stage of an anaerobic digestion system.

Additional energy consumption is an unavoidable issue associated with introducing a bioelectrolysis process into an AD system, so energy-conserving strategies are favored in the research of bioelectrolysis-assisted AD systems. Reactors that are started-up with voltage maintained considerable methane producing performances after switching to open-circuit conditions (Fig. 6), which provides an indication of an energy saving strategy. The net energy profits of R1, R2, R3, and R_{OC} were calculated and are listed in Table 4. There was no energy input, but the net energy profit in R_{OC} was the lowest (only 18.6 mW) due to the lowest methane production. In group R_{CC} , the net energy profit of R1 was 31.9 mW at a fixed 0.8 V, while net energy profit increased when the applied voltages were decreased to 0.5 V (R2) or removed completely (R3), which were 35.8 mW and 37.2 mW, respectively. This resulted from decreased energy input, but methane production was still steady. Judging from the current results, it would be more energy-favorable if applied voltage was only applied during the start-up stage.

Considering the long-term performance of electrolysis-assisted AD systems, however, it would be necessary to apply a low or intermittent voltage after the start-up stage. Compared with other extreme changes in conditions (such as organic load impact, great pH change, or existence of poisonous factors), variations in external voltage is a mild irritation for microbial metabolism, which is

Table 4
Energy balances in R1, R2, R3 and R_{OC} .

Group	Voltage (V)	Current (mA)	Energy input (mW)	Methane production (mL/d)	Raw energy income (mW)	Net energy profit (mW)
R_{OC}	0	0	—	40.4	18.6	18.6
R1	0.8	13.0	10.4	91.7	42.3	31.9
R2	0.5	3.0	1.5	84.0	38.7	37.2
R3	0	0	0.0	77.7	35.8	35.8

Note: the energy input and net energy income was focus on the electrical energy, which was calculated without considering temperature or other factors.

unlikely to cause a noticeable impact on a steady microbial community. Without the selective pressure of voltage, however, functional microbial communities established under closed-circuit conditions may slowly shift because of microbial competition, after a long-period of operation under open-circuit conditions, thus decreasing the initial benefits of applied voltage. Accelerating the start-up stage of an anaerobic digestion system by introducing a bioelectrochemical process and acclimatizing electrodes using high voltage, but operating them at a low or intermittent voltage may be a more feasible strategy.

4. Conclusions

Applying voltage has a significant influence on functional community enrichment and anaerobic digestion pathways formation in the start-up stage of an AD system. Application of external voltage led to a decrease in glucose fermentation microorganisms, but an increase in electricigenic microorganisms and hydrogenotrophic methanogens. Bioelectrochemical reactions changed acidogenesis fermentation type of glucose positively to produce more acetate but less butyrate and propionate. The bioelectrolysis process created an additional pathway for electrons moving from acetate to hydrogen, resulting in a significant enhancement of methane production in a closed-circuit system. The direct contribution of bioelectrochemical processes to energy recovery was limited when the current density of the whole system was low, but accelerating the start-up stage of an anaerobic digestion system by introducing a bioelectrochemical process, acclimatizing electrodes with a high voltage, and operating them at a low voltage could be an energy-efficient strategy.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgements

This research was supported by National Natural Science Foundation of China (No. 51578534), HIT Environment and Ecology Innovation Special Funds (No. HSCJ201621) and Youth Innovation Promotion Association CAS. Youth Innovation Promotion Association CAS (No. 2017062).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2017.08.039>.

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