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Innovative operation of microbial fuel cell-based biosensor for selective monitoring of acetate during anaerobic digestion

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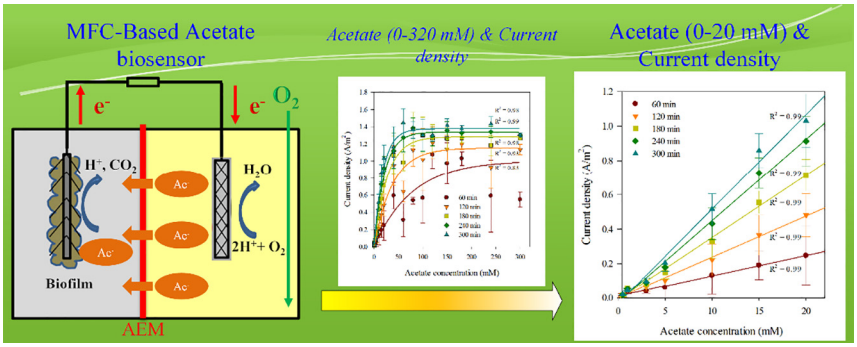
HIGHLIGHTS

- MFC based biosensor for acetate monitoring in AD process with high accuracy
- Linear detection range of 0.5–20 mM established within 5 hour response
- High selectivity to acetate and less interference from other VFAs
- Digestate with temperature of 37 °C had no significant influence on biosensor

GRAPHICAL ABSTRACT



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ABSTRACT

Volatile fatty acids (VFAs) especially acetate concentration have been proved to be a sensitive and reliable indicator for many anaerobic processes such as anaerobic digestion (AD). Microbial fuel cells (MFC) have been demonstrated as a promising VFAs sensor due to simple reactor design and operating conditions among microbial electrochemical biosensors. However, the conventional MFC biosensors may fail to distinguish between VFAs and other organics as real digestates containing complex organics and microbes are fed into anode directly. In the present study, an MFC based biosensor was developed and operated in a smart way for selective acetate detection. In the biosensor, acetate ions contained in the AD sample was first fed into the cathode, and then acetic ion transferred through the membrane from the cathode to anode chamber where it was further used as the sole substrate by pre-enriched electroactive biofilm for the current generation. A linear correlation between the current density and acetate concentrations (0.5–20 mM) at varied reaction time (1–5 h) was established. Then, the interference from propionate, butyrate, isobutyrate, and glucose on the performance of the biosensor was evaluated. Furthermore, the influence of sample temperatures (37 and 55 °C) was also studied. Finally, the VFAs content in real AD effluent with this biosensor was measured. The results corresponded well with gas chromatographic measurements. This simple, and reliable biosensor could serve as a promising alternative method for acetate detection in the AD process or any other acetate-rich fluids.

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1. Introduction

Anaerobic digestion (AD) technology has attracted worldwide attention due to its unique merits in organic wastes treatment and green energy production (Mao et al., 2015). AD processes are sensitive to variations of the operating parameters such as temperature, pH, feed amount and composition, and so on (Vanwonterghem et al., 2014). Thus, monitoring and control are essential for preventing the poor operational stability of the AD process. Therefore, reliable indicators are needed. Volatile fatty acids (VFAs) concentrations have been proven as a sensitive and reliable indicator for AD processes (Boe et al., 2010). Moreover, individual VFAs detection could give more specific information for process diagnosis (Ahring et al., 1995). Generally, acetate, usually constitute the biggest proportion of total VFAs in the AD process, is measured by gas or liquid chromatography that is expensive, time-consuming, mostly offline and usually not available at AD plants. Therefore, the development of a biological acetate sensing system with the potential of being online, accurate and cost-effective is an attractive alternative.

The application of microbial bioelectrochemical systems (BES) as a biosensor for water quality measurement (e.g., biochemical oxygen demand, toxic components, and acid rain detection) has attracted great interest in recent years (Jiang et al., 2015; Sun et al., 2015; Di Lorenzo et al., 2014; Zhang and Angelidaki, 2012; Stein et al., 2012; Zhang and Angelidaki, 2011; Zhang et al., 2011; Tront et al., 2008; Li et al., 2018). The BES sensors have great potential for onsite and real-time monitoring with rapid and simple testing (Chouler and Di Lorenzo, 2015). Moreover, BES sensors are based on anodic biofilm that oxidizes organic substrates as living and self-regenerating recognition element which could bring several advantages, such as cost-effective, long-time stable and environmental friendly (Jin et al., 2017; Kretzschmar et al., 2017). Recently, the feasibility of varied BES including microbial fuel cell (MFC), microbial desalination cell (MDC) and microbial electrolysis cell (MEC) have been demonstrated for online monitoring of VFAs during AD process (Jin et al., 2017; Kaur et al., 2014; Kaur et al., 2013; Jin et al., 2016). In particularly, the MFC sensor has more simple design and operating conditions among all BES biosensors for VFAs detection (Kaur et al., 2014; Kaur et al., 2013). However, MFC biosensors still have challenges that need to be addressed before commercialization. For instance, it may fail to distinguish between VFAs and other organics due to change of microbial communities when real digestates are fed into anode directly. It has been recently found that sending digestate into the middle of MDC or cathode of MEC could avoid the disturbance from other organics and microorganisms contained in the digestate (Jin et al., 2017; Jin et al., 2016). Extended detection range was also observed since VFAs need to transport from another side of the membrane into anode which creates the concentration gradient. We hypothesized that MFC biosensor could achieve the same goals by receiving digestate in cathode chamber instead of the anode, for which feasibility has never been explored. Furthermore, MFC sensors could also be developed for individual VFAs detection, by enrichment of anodic biofilm metabolizing a specific VFAs. If successful, MFC biosensors could greatly reduce the capital and operating costs (e.g., no need external power supply which is prerequisite for MEC sensor) on individual VFAs detection, compared to other BES sensors.

In the present study, we propose an innovative bioelectrochemical sensor for acetate measurement composed by a two-chamber MFC, in which individual acetate ions in digestate move from cathode to anode for bacterial oxidation and current generation. To the best of our knowledge, such a way of running MFC for acetate detection has never been reported. To demonstrate the feasibility, the current response of the MFC biosensor to various acetate concentrations in the synthetic wastewater (simulating AD effluent) was evaluated. Subsequently, the interference of other VFAs such as propionate, butyrate, and isobutyrate, complex organic matter such as glucose, and the initial temperature of the sample on the performance of the sensor was investigated. Finally, effluent from a CSTR fed with cow manure was tested to verify the reliability of the biosensor. The simple biosensor showed promising potential in online monitoring of acetate concentrations. The outcomes would benefit the AD process monitoring and optimization and also expand the application of microbial electrochemical systems.

2. Material and methods

2.1. Biosensor setup and operation

A bio-electrochemical reactor consisting of two chambers, made of nonconductive polycarbonate plates, was used in this study. As shown in Fig. 1, two chambers with the equal dimensions (8 cm × 8 cm × 4 cm) were physically separated by an anion exchange membrane (AEM) (AMI 7001, Membrane international, NJ, 9 cm × 9 cm). The membrane was soaked overnight in 5% NaCl solution and then stored in deionized water before use. Eight stainless screws are used to assemble the plates which were sealed with rubber gaskets preventing leakage. Inserted rubber tubes were prepared for medium refilling, aeration, and sampling. The anode electrode was made of carbon fiber brush (5.0 cm in diameter, 7.0 cm in length, Mill-Rose, USA), which has been heat-treated in a muffle furnace at 450 °C for 30 min (Feng et al., 2010). The cathode electrode was a graphite plate (4.2 cm × 4.2 cm) coated with 0.5 mg cm^{-2} Pt. Two electrodes were connected through a 10 Ω external resistance except at the biofilm enrichment stage (use 1000 Ω external resistance).

To enrich exoelectrogenic biofilm on the electrode, the anode was first inoculated with domestic wastewater collected from primary clarifier (Lundtofte Wastewater Treatment Plant, Lyngby, Denmark). After twice refilling with wastewater, the anode chamber was filled with 15 mM acetate in buffer solution (pH = 7.25 ± 0.04) containing 50 mM phosphate buffer (Na2HPO4, 4.33 g L^{-1} and NaH2PO4, 2.03 g L^{-1}) and nutrient solution (NH4Cl, 0.31 g L^{-1}; KCl, 0.13 g L^{-1}; 12.5 mL mineral solution and 12.5 mL vitamin solution) (Kvesitadze et al., 2012) to acclimate the bacterial consortia. Ferricyanide solution (50 mM) was added in the cathode chamber as the electron acceptor. Instead, oxygen can be used in future to avoid chemical purchase and disposal. During this enrichment period, a 1000 Ω external resistance was used to connect the two electrodes. The solution in both chambers was replaced for every 8–10 days when the voltage became lower than 50 mV. After about three months of enrichment, the voltage from this system was stabilized at around 650 mV, which indicated that mature electrochemically active biofilm has attached to the surface of the anode and ready for the sensing experiment.

All experiments were conducted at batch mode. For each batch experiment, the anode chamber was filled with approximately 250 mL buffer solution (pH = 7.25 ± 0.04) containing 50 mM phosphate buffer and nutrient solution as previously described (Jin et al., 2016). The anolyte was renewed for each batch test to maintain the same initial condition. The cathode was filled with about 250 mL of synthetic wastewater which was prepared from a modified basic anaerobic (BA) medium (omitted the stock solutions C and E) containing varying concentrations of sodium acetate (Angelidaki et al., 1990). The anode was purged with N_2 for 10 min to maintain the anaerobic environment before each batch, while the cathode was continuously aerated with the flow rate around 50 mL min^{-1}. Every batch experiment was run for 5 h at room temperature (22 ± 2 °C).

2.2. Electrochemical analyses and calculations

Conductivity was measured using a CDM 83 conductivity meter (Radiometer) and pH was measured by a PHM 210 pH meter (Radiometer). VFAs concentrations were measured using a GC with FID detection (Thermo Scientific, TRACE1300). The voltage across the resistance was monitored using a virtual instrument based data logging system

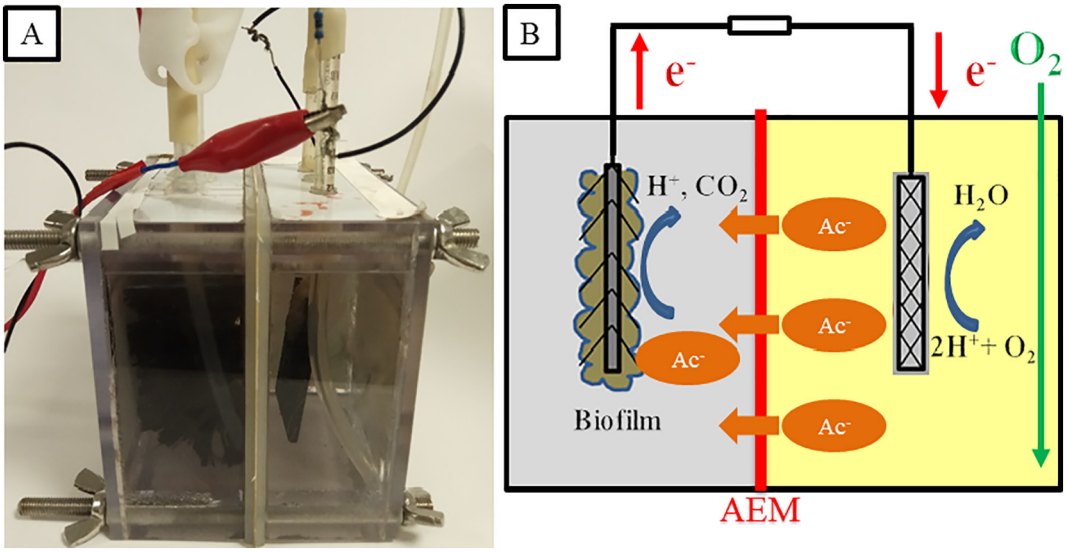

Fig. 1. Prototype (A) and schematic diagram (B) of the MFC based biosensor.

(National Instrument, LabVIEW 2012) combined with an analog signal acquisition card (USB 3252, ZLAD, China). The voltage signal was acquired every second and average voltage per minute was recorded. The current was calculated according to Ohm's law. The current density i (A m^{-2}) was calculated as follows:

$$i = I/A$$

where, I (A) is the current and A (m^{-2}) is the projected surface area of the cathode electrode.

3. Results and discussion

3.1. Response of current density to various acetate concentrations

The current density generated at different acetate concentrations was investigated. A typical current density time course at different acetate concentrations is shown in Fig. 2. Generally, the current density increased with time. This increase of current can be attributed to acetate. Acetate was transferred from cathode chamber to anode chamber where it was oxidized delivering electrons for electricity generation. No other substrates were present which could account for the electricity generation in the anode chamber. The current density increased gradually and reached a maximum value at the end of the batch test (5 h) when the initial acetate concentration was lower than 40 mM (Fig. 2A). The acetate transport through the membrane would be faster than its consumption by the biofilm, which leads to the accumulation of acetate in the anode chamber along with reaction time and initial acetate concentration. Then, the biosensor would approach saturated state due to the rising acetate compared to the limited oxidation capability of biofilm in the anode chamber (Liu et al., 2011).

The current density was higher at higher initial acetate concentration. The current increased sharply at the beginning of the experiments (Fig. 2B). The sharp initial increase could be explained by three reasons: firstly, due to acetate carry-over effect, which could be directly used by the biofilm; secondly, bacteria are able to store intracellularly compounds in the form of polymers (such as poly-β-hydroxybutyrate) and use them as carbon and energy sources during this period (Freguia et al., 2007); thirdly, the higher acetate transport rate through the membrane due to initial higher concentration gradient. In some

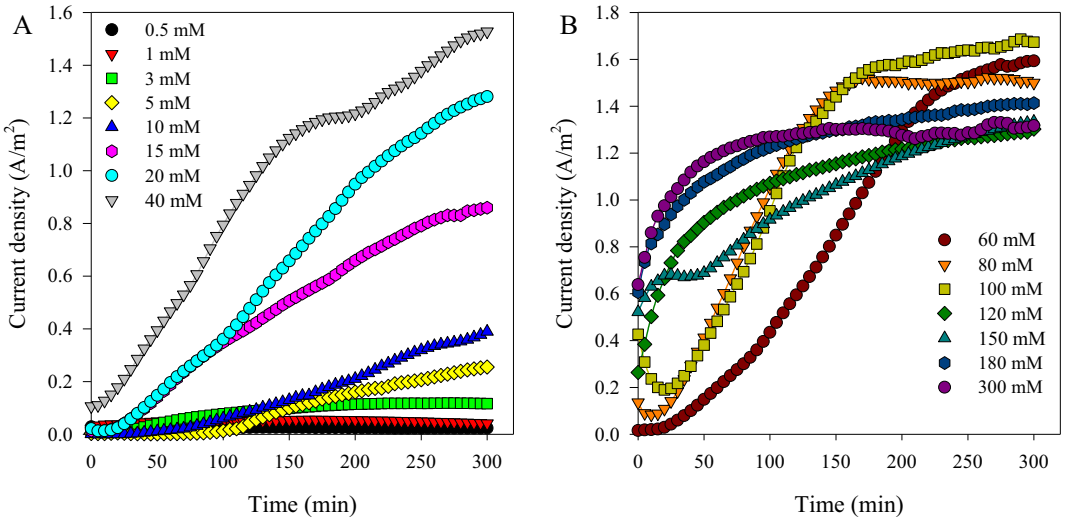

Fig. 2. Typical variation of current density of the bioreactor under different acetate concentration condition.

cases, a sudden drop of the current in the first few minutes of the batch experiment was observed. This sudden current drop might due to the capacitive property of exoelectrogens which store the electrons in the anode electrode from microbial oxidation of substrate during open circuit situations (refill solution of two chamber) and then release them during closed circuit (Lv et al., 2014; Deeke et al., 2012; Uría et al., 2011). The increase of current at the late stage of the batch experiment indicated that the biosensor was approaching saturation. This is in accordance with a previous study, in which current density increased more sharply and reached to maximum stable value in a shorter time at higher total VFAs concentrations compared to lower concentrations (Jin et al., 2016). The fluctuation of the saturation current may be due to the minor difference in the experimental conditions (such as the aeration rate, the electrode distance) in different batch experiments. Since 10 Ω external resistance was used in this biosensor, which was even lower than the internal resistance making the system sensitive to changes and resulting in relatively large deviations in the current (Jin et al., 2016).

The response of biosensor current to different acetate concentrations at specific reaction time (at 1, 2, 3, 4 and 5 h) are shown in Fig. 3A. The increments in current densities under high initial acetate concentrations were lower than that of relatively lower acetate concentrations at the same reaction time. A nonlinear relationship between the current densities and the initial acetate concentrations along with different reaction times were observed, which fit well with an exponential regression in function of $y = a * (1 - b\hat{}x)$. The coefficients of this nonlinear regression with different reaction time (1–5 h) are 0.83, 0.93, 0.98, 0.99, and 0.98, respectively. The nonlinear response might because of the biosensor is saturated due to the accumulation of acetate in the anode which would result in maximum current output even at relatively low acetate concentrations in the anode chamber (i.e., 2.3 mM and 3.2 mM) (Tront et al., 2008; Atci et al., 2016). More reliable and accurate results could be obtained from longer reaction time as the average relative standard deviation was 43.9%, 26.6%, 17.0%, 13.4%, and 11.7% at a reaction time of 1 to 5 h, respectively. However, linearity response is always being pursued from the aspect as a sensor.

As shown in Figs. 3B and S2, the current densities of biosensor showed a linear relationship with the initial acetate concentration (≤20 mM) at reaction time from 1 to 5 h ($R^2 = 0.99$). Moreover, the linearity range of this biosensor could extend to 40 mM at a reaction time of 1 and 2 h ($R^2 = 0.99$, 1.00, respectively) (Fig. S1). The average relative standard deviation with the acetate concentration from 0.5 to 40 mM at different reaction time (from 1 to 5 h) was 57.4%, 36.5%, 24.4%, 19.0%, and 16.7%, respectively. The linear growth of the current density could be maintained under the condition that the acetate accumulated in the anode chamber was far below the saturated concentration, for instance, under low initial concentration (<20 mM) condition. Longer reaction time would accumulate more acetate in the anode chamber assuming that the diffusion rate through the membrane is higher than the reaction rate for degradation of acetate in the anode. Moreover, higher initial concentration also promotes acetate accumulation. Therefore, at long reaction time and at high initial acetate concentrations, the possibility of exceeding acetate saturation limits in the anode was high. However, the minimum relative standard deviation was obtained at the longest reaction time (5 h), which means longer reaction time improve the accuracy of this biosensor. Therefore, 5 h reaction time was suggested, even the upper limit for a linear response range would be lowered to 20 mM.

The results demonstrate the feasibility of this biosensor for accurate acetate measurement in the range from 0.5 to 20 mM with 5 hour response time. For practical applications however, 5 hour measuring time would be adequate for acetate monitoring in the AD processes which often have hydraulic retention times in the range of 15–30 days. Therefore, monitoring of acetate a few times per day would be enough for adequate process monitoring. Even though, a quick response is always being expected from a sensor. The requirement of reaction time from 3 to 5 h to ensure satisfactory measurement result appears to be rather slow compared to small-scale continuous flow MFC sensors. The main reason is the fact that the sample was added in cathode chamber that takes time for acetate transfer through the membrane and being utilized for the current generation. It might also be related to the "large scaled" MFC reactor (about 500 mL) which contains two chambers with equal dimensions (8 cm × 8 cm × 4 cm). Therefore, more efforts are needed in the future to accelerate the response of the biosensor.

Acetate concentration in AD process could easily over 20 mM (Kretzschmar et al., 2016). Thus, detection of acetate at a larger range is still needed. Dilution of samples can be seen as one alternative solution to extend the biosensor detection range. Moreover, with such a sensor structure, the acetate can be consumed by

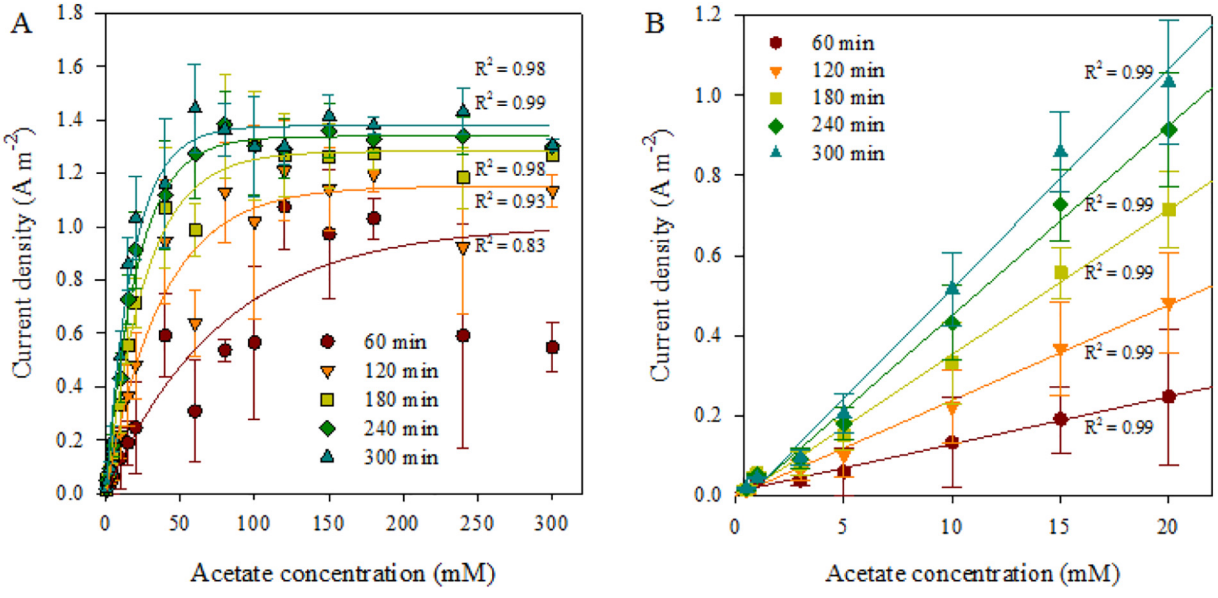

Fig. 3. The relationship between current density of the bioreactor at a specific reaction time corresponding to different acetate concentration in the artificial AD effluent.

aerobes in the cathode chamber. Obvious growth of aerobes in cathode has been observed after more than two weeks. Thus, the cathode chamber was cleaned per week to minimize the growth of internal aerobes. Furthermore, this issue could also be avoided in short test time. Therefore, further efforts are needed to obtain an accurate measurement within a short time.

3.2. Changes in pH and conductivity

The variations of pH and conductivity in both chambers during each batch test under varies initial acetate concentrations are shown in Fig. 4. In the anode chamber, pH slightly decreased from 7.25 ± 0.04 to 7.22 ± 0.07 during all the batch runs (Fig. 4A), which would not affect the performance of the biosensor. The decrease of pH mainly due to the proton generated from the microbial activities, but the high buffer capacity of the phosphate solution minimized pH change and maintained a suitable environment for exoelectrogens. As shown in Fig. 4C, pH in the cathode chamber increased from 8.22 ± 0.13 to 8.79 ± 0.21 on average, which was mainly due to the cathodic oxygen reduction. However, in this short period (5 h), this increase of pH would have no significant effect on biosensor performance (Mehanna et al., 2010; Rozendal et al., 2008). As reported, MFC performance would improve with the increase of conductivity of the solution (ionic strength) which leads to lower internal resistance (Liu et al., 2011; Hong et al., 2009; Feng et al., 2008). The variation of conductivity in the anode chamber is shown in Fig. 4B. It varied from 6.68 ± 0.06 to 6.66 ± 0.15 mS cm $^{-1}$ in all batches. The conductivity in the cathode chamber increased from 5.42 ± 0.05 to 12.91 ± 0.16 mS cm $^{-1}$ along with increasing initial acetate concentrations (0.5 to 120 mM) in the synthetic AD effluent (Fig. 4D). After 5 h of operation, the conductivity of catholyte decreased to 5.27 ± 0.76% on average. In this case, the conductivity of the solutions would no significantly influence the biosensor performance.

3.3. Effect of propionate, butyrate, isobutyrate and glucose on the biosensor performance

In this study, the biofilm on the anode was expected to react specifically to acetate since it was enriched with acetate as a sole carbon source for about three months. Meanwhile, a wide range of soluble or dissolved complex organic matter has been demonstrated to be used as substrates in MFC reactors (Pant et al., 2010). VFAs and other organic matter such as lipids and proteins, commonly present in AD effluents, might be used as potential substrates by the anode biofilm and create noise to the sensor signal. Thus, it is of particular interest to examine the effect of these interfering components on the biosensor performance for establishing the selectivity of this biosensor (Kretzschmar et al., 2017).

To test the selectivity of the biosensor, we dosed other organic compounds (glucose, propionate, butyrate, isobutyrate propionate, butyrate, isobutyrate) instead of acetate into the cathode chamber. As shown in Fig. 5A, almost no electricity was produced during the 5 hour reaction, when 20 mM glucose was added in the cathode

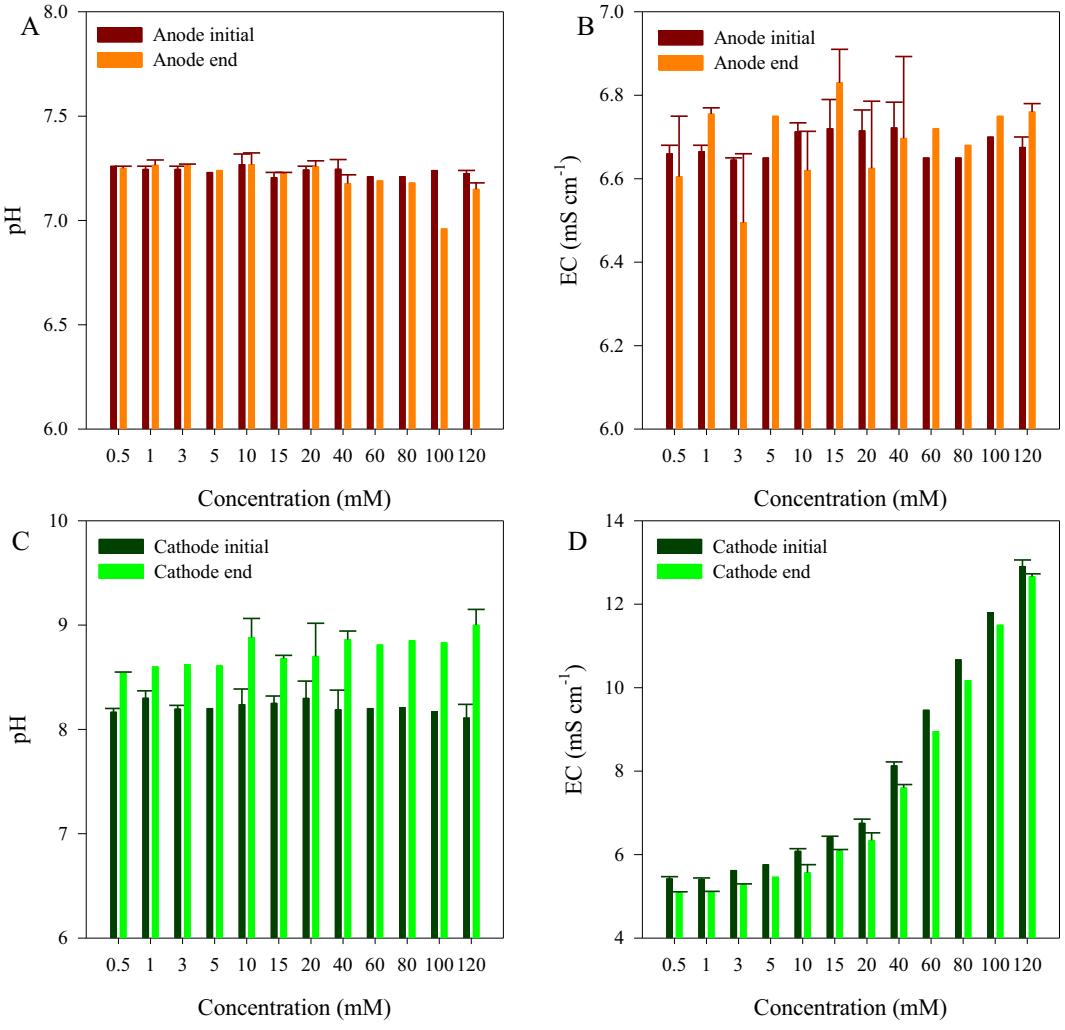

Fig. 4. Conductivity and pH variation in bioreactor after 5 h with various acetate concentrations.

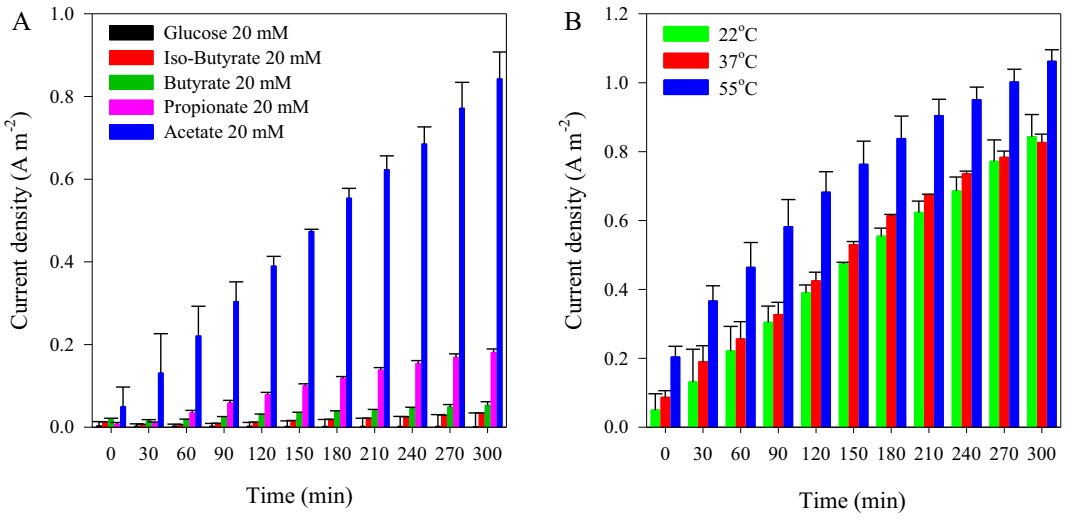

Fig. 5. Current density variation along with time from the biosensor corresponding to other substrates (A) and different initial temperature (B).

chamber. This could be explained by the nonionic characteristics of glucose which doesn't permit its transport through AEM (Jin et al., 2017). Similarly, we can assume that other nonionic complex organic matter (such as lipid and protein) would not result in any response (Jin et al., 2017; Jin et al., 2016).

To investigate the effect of propionate, iso-butyrate and butyrate on the biosensor performance, 20 mM of these compounds, were used as substrates in the cathode chamber. In this batch test, the current density with the 20 mM of acetate was 0.843 ± 0.065 A m^{-2} (Fig. 5), which was lower than that of the average value (1.032 ± 0.155 A m^{-2}) for the whole experiment period (Fig. 3). The possible explanation could be the variation of the biofilm activity due to the temperature change (from summer to autumn) and membrane fouling after long-term operation. Experiment data from the same conditions were chosen for comparison. The current densities of the biosensor increased slightly during the batch experiment with the other VFAs and achieved 0.181 ± 0.008 A m^{-2} (propionate), 0.033 ± 0.001 A m^{-2} (iso-butyrate), and 0.053 ± 0.009 A m^{-2} (butyrate) after 5 hour reaction time (Fig. 5A). The current densities at the end of the batch experiment with 20 mM of propionate, iso-butyrate, and butyrate were 21.52%, 3.60%, and 6.31% compared to the current density from same concentration of acetate. This is consistent with previously reported by Kretzschmar et al. (2017), in which, butyrate and propionate resulted in a slight response (Kretzschmar et al., 2017). The other VFAs were probably also transferred through the membrane to the anode and were used as a substrate for the current generation. However, the diffusion rate of other VFAs might lower than acetate, since lighter weight molecules will transport faster than heavier molecules. Even though the biosensor could still respond to other VFAs but nothing like as sensitive as it is to acetate. The application of the suitable nanofiltration membrane might be an alternative solution to further improve the specificity of the acetate biosensor (Baruah and Hazarika, 2014). For practical situation, propionate, iso-butyrate and butyrate are often present at much lower in digested slurry concentrations than the ones tested in the present study, which will further decrease the possible overestimation caused by the other VFAs. Although the selectivity of the biosensor to acetate is not perfect, it is still feasible for the purposes of AD process monitoring.

Nevertheless, propionate, iso-butyrate and butyrate are also able to be used by exoelectrogens after acclimation. Long-term exposure of propionate, iso-butyrate and butyrate to the anode might induce a higher current. Therefore, efforts in maintaining the stability of the biofilm are needed, such as shorten the reaction time and using a selective membrane. On the other hand, the development of a biosensor for monitoring other substrates could be an interesting topic in the future.

3.4. Effect of initial sample temperature on the biosensor performance

It is well known that bacterial activities are affected by temperature variation. Moreover, mass transfer and oxygen reaction rates catalyzed by Pt on the cathode are also affected by temperature (Liu et al., 2005). Obviously, changes in temperature could influence MFC performance, especially for monitoring samples taken from thermophilic or mesophilic AD reactors. Therefore, the effect of sample temperature on the biosensor performance was investigated by adding a solution containing 20 mM acetate with different initial temperatures in the cathode chamber, while maintaining the other condition same as the other batch assays. Initial temperatures of 37 °C and 55 °C were chosen for simulating AD effluents from mesophilic and thermophilic conditions. There was no temperature control in both chambers. The effect of sample temperature on the anodic biofilm could be neglected. Firstly, though the samples had an initial temperature of 37 or 55 °C, the actual temperature in the cathode would be much lower and would quickly drop to ambient temperature. Secondly, 55 °C much blows the pasteurization temperature (72 °C). Thirdly, biofilm cells are more resistant to high temperature than planktonic cells.

As shown in Fig. 5B, at the initial temperature of 55 °C, the current generation increased during the batch experiment. The final current density after 5 h was approximately 25.95% higher compared to the current density at 22 °C. Meanwhile, no obvious improvement of the current production was observed when the temperature was 37 °C. Since the solution we tested was added into the cathode chamber, and therefore the temperature at the anode chamber and the anodic biofilm activity would not be affected significantly. This is in accordance with the previous reporting that the reactor performance was similar when the temperature was increased from 30 °C to 37 °C (Liu et al., 2005; Min et al., 2008). Another aspect to consider is the improved mass transfer in the cathode chamber at 55 °C, which might have affected the current density. Thus, there would be no significant effect on the

Table 1
Characteristics of the anaerobic digested slurry.

Parameter	Data	Parameter	Data
TS (%)	2.072 ± 0.003	Acetic acid (mg L^{-1})	278.0 ± 10.5
VS (%)	1.262 ± 0.008	Propionic acid (mg L^{-1})	17.9 ± 0.4
pH	8.54	Isobutyric acid (mg L^{-1})	<5
EC (mS cm^{-1})	17.22	Butyric acid (mg L^{-1})	42.8 ± 6.8
NH_4^+ (mg L^{-1})	2139.3 ± 13.8	Isovaleric acid (mg L^{-1})	10.2 ± 0.4

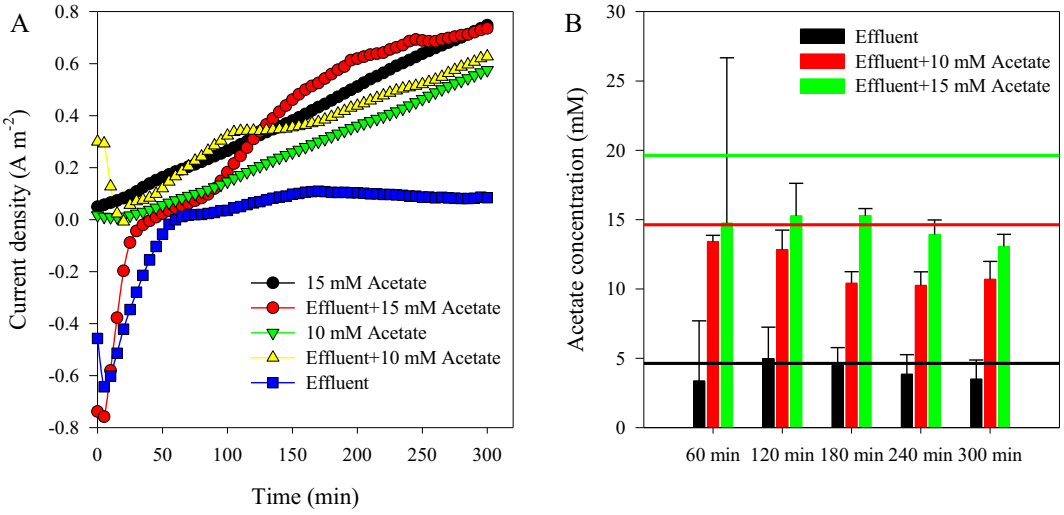

Fig. 6. Variation of the current density of the bioreactor with AD effluent (A) and the acetate concentration in AD effluent measured by the biosensor (B).

performance of this biosensor system when the digestates of 37 °C are used in practical applications. However, the temperature should be lowered before analysis when the effluent is from thermophilic reactors, as this is necessary to obtain an accurate result. For practical application, the time of cooling is not really an issue in a 5-hour assay. Moreover, the high temperature can be lowered by simply mixing the sample with other liquid before adding to the reactor.

3.5. Verification of the biosensor with AD effluent

The acetate biosensor was finally tested with AD effluent from a 10 L CSTR fed with cow manure to verify its applicability. Three samples were used in this test; one is original AD effluent (Table 1) while the other two were same AD effluent amended with extra 10 and 15 mM acetate. The variation of current density along with experiment time was shown in Fig. 6A. A reverse of current direction at the beginning of the experiment was observed. This was probably due to the microbial activity of the added AD effluent at the cathode (Kaur et al., 2013; Kim et al., 2005). The average value from duplicate tests is shown in Fig. 6B. The acetate concentration in the AD effluent was compared to GC measurements. The sensor measured acetate concentration of the original AD effluent was in good agreement with the GC measured value, while the samples with added acetate were a bit lower.

Meanwhile, another similar biosensor system with a slightly larger size of the cathode electrode (5.2 cm × 5.2 cm) was operated to investigate the long-term stability for AD effluent measurement. A typical current density time course, at different acetate concentrations, is shown in Fig. 7. As shown in Fig. 7A, after about two weeks test with the AD effluent, biofilm growth on the surface of the cathode electrode was observed (Fig. S3) which prevented current generation in the following test (An et al., 2017). However, the sensor performance recovered when the cathodic biofilm was removed (Fig. 7A). This indicates that routine maintenance of the biosensor system, such as cleaning every two weeks, would be necessary to guarantee the performance. Alternatively, the growth of cathodic biofilm could be avoided if using a three compartment MFC (cathode, anode, and sample chamber) with the same type of anionic

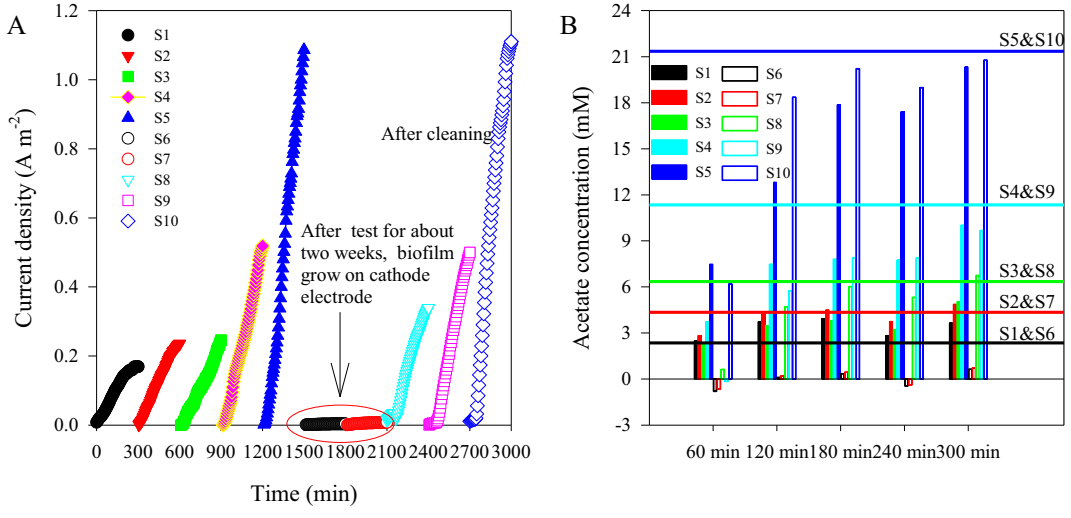

Fig. 7. Acetate concentration measurement by bioreactor, current density variation (A) and results (B). The sample was 5 times diluted biogas slurry form a CSTR reactor fed with cow manure amended with different concentration of acetate.

Table 2
Summary and comparison of biosensors for VFAs detection.

Structure	Memb	Electrode			ET (Ω)	RT	Performance	Stir	T(°C)	EM	Ref.
		Anode	Cathode	Reference							
H type MFC	CEM	Carbon paper with biofilm	Carbon paper coated Pt	Ag/AgCl	1000	1–2 min	Acetate, propionate, and butyrate	Magnetic stirring 5–40 mg L^{-1}	30	CV	(Kaur et al., 2013)
		Carbon paper modified by polypyrrole with biofilm						0–60 mg L^{-1}			(Kaur et al., 2014)
Single chamber	\	SPEs (DRP C110 and DRP C220AT)	\	Ag	\	<2 min	Acetate: DRP C110: 100–400 mM DRP C220AT in KCl: 50–300 mM	\	Room	CV SWV	(Ndiaye et al., 2016)
A disposable borosilicate glass pasteur pipette	\	Carbon fiber with biofilm	\	Ag/AgCl	\	<2 min	Acetate: LDR: 0.05–1.6 mM	\	30	Current	(Atci et al., 2016)
Single chamber	\	Graphite rods with biofilm	Graphite rods	Ag/AgCl	\	CV: 1.3 h CA: 180 s	Acetate: 0.5–5 mM	Magnetic stirring	38	CV CA	(Kretzschmar et al., 2017; Kretzschmar et al., 2016)
Three chamber MDC	AEM CEM	Carbon brush with biofilm	Stainless mesh	\	1000	5 h	Total VFAs: 1–200 mM	Magnetic stirring in anode	22 ± 2	Current	(Jin et al., 2016)
Double chamber MEC	AEM		Titanium woven wire mesh coated Pt	\	10	1 h	Total VFAs: 0–100 mM				(Jin et al., 2017)
Double-chamber MFC	AEM	Carbon brush with biofilm	Graphite plate coated Pt	\	10	1–5 h	Acetate: 0–20 mM	\	22 ± 2	Current	Present study

AEM: anion exchange membrane; CEM: cation exchange membrane; CV: cyclic voltammetry; CA: chronoamperometry; EM: electrochemical measurement; ER: external resistance; LDR: linear dynamic range; MDC: microbial desalination cell; MEC: microbial electrolysis cell; MFC: microbial fuel cell; Memb: membrane; RT: response time; SWV: square wave voltammetry; SPEs: screen-printed electrodes; T: temperature; VFAs: volatile fatty acids.

membrane to separate the anode and sample chamber. From Fig. 7B, it can be concluded that calculated acetate concentrations based on 5 h reaction times are concentrations closest to the reference value, which indicates that longer reaction times are increasing the biosensors accuracy. As shown in Fig. S4, the linear relationship between the current densities and initial acetate concentration at 3, 4 and 5 h could also be established. It can be deduced that this biosensor is a quite promising alternative way for acetate detection in AD effluent.

3.6. Perspectives

BES based biosensors have attracted increasing interest in recent years. A brief summary of biosensors for VFAs monitoring is shown in Table 2. In comparison to these biosensors, the main advantages of the proposed MFC biosensor can be listed as: a, wider linear dynamic range of the current densities corresponding to the acetate concentration has been obtained; b, simple structure consist of two chambers separated by AEM which could reduce capital investment and avoid the influence on anode from the microorganisms; c, only the voltage across the external resistance was monitored as sensor signal; d, simple operation by adopt the strategy that running reactor with non-sterile, non-agitated, and under room temperature condition. On the contrary, disadvantages could not be ignored: minor interference from other VFAs still cannot be eliminated; aerobic bacteria which could consume acetate would grow in cathode chamber without proper maintenance; the chemical requirement; and the detection range also needs to be extended. Nevertheless, despite the remaining challenges MFC based biosensor, seem to be promising and could serve as a simple tool for acetate detection and further optimized the AD process for organic waste treatment and bioenergy production. The feasibility of MFC based biosensor for acetate monitoring with the sample added to the cathode chamber has been demonstrated in current work. Further improvements before its onsite application can be conducted from the flowing aspects: shortening the reaction time, reducing reactor volume and the chemical requirement, eliminating the requirements of cathode platinum catalyst. Moreover, continuous operation and using nanoporous (anionic) membrane that only allows acetate to pass through could be effective ways to address the challenges in response time and sensor selectivity.

4. Conclusions

The present work demonstrated the feasibility of MFC-typed sensor for acetate detection in the AD process. The sensor is constituted by a traditional double-chamber MFC reactor with AEM. The key sensing element is a matured electroactive biofilm enriched with the acetate as the sole carbon source. A linear relationship between current densities and initial acetate concentrations (0.5 to 20 mM) was obtained. The biosensor showed high selectivity since only the anions could transport through the AEM and then be utilized for the current generation. The interference from other VFAs was very limited. Sample temperature of 37 °C would not significantly affect the biosensor performance, but the temperature should be lowered before analysis when the effluent is from thermophilic reactors. The proposed biosensor does have a potential in AD process monitoring, but a further improvement to extend detection range, reduce the maintenance and be more robust is still needed to fit the practical application situation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.336.

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