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Innovative air-cathode bioelectrochemical sensor for monitoring of total volatile fatty acids during anaerobic digestion

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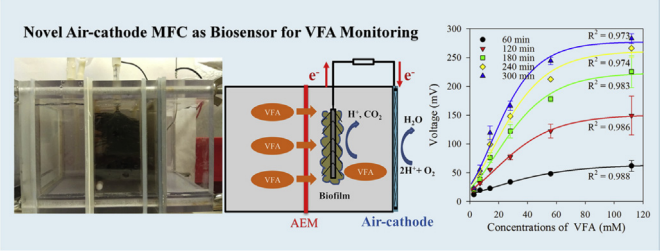
HIGHLIGHTS

- Air-cathode MFC based biosensor for VFA monitoring in the AD process was developed.
- Linear detection range below 14 mM was established within 2–5 h reaction.
- High concentrations of bicarbonate decreased the voltage output of the biosensor.
- Stirring accelerated and amplified the response but narrowed the detection range.
- Dominant microbiomes in the biofilm were identified by 16S rRNA gene sequencing.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioelectrochemical sensors have proven attractive as simple and low-cost methods with high potential for online monitoring of volatile fatty acids (VFA) in the anaerobic digestion (AD) process. Herein, an innovative dual-chamber air-cathode microbial fuel cell was developed as biosensor for VFA monitoring. The response of the biosensor was nonlinear and increased along with the concentration of VFA mixture increase (2.8–112 mM). Meanwhile, the relationship was linear with low VFA levels (<14 mM) within 2–5 h reaction. High concentrations of bicarbonate decreased the voltage. Stirring speeded up the response and amplified the signal but reduced the saturation concentration (approximately 30 mM) and therefore narrowed the detection range. The applicability of the biosensor was further validated with the effluents from an AD reactor during a start-up period. The VFA concentrations measured by the biosensor were well correlated with the gas chromatographic measurement. The results demonstrate that this biosensor with a novel design could be used for VFA monitoring during the AD process. Based on the 16S rRNA gene sequencing, the dominant microbiomes in the biofilm were identified as *Geobacter*, *Hydrogenophaga*, *Pelobacter*, *Chryseobacterium*, *Oryzomicrobium*, and *Dysgonomonas*.

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1. Introduction

Anaerobic digestion (AD) is considered a promising technology to recover renewable energy from organic wastes, which could bring both environmental and economic benefits to society (Lora Grando et al., 2017; Wainaina et al., 2019). Nevertheless, the AD process, involving a series of complicated biochemical reactions, suffers from the instability that is typically caused by disruptions of the microbial balance, due to unfavorable operation conditions, such as fluctuations in the feedstock, organic overload and inhibition by toxicants (Theuerl et al., 2019; Sun et al., 2019a). Volatile fatty acids (VFA) are intermediate metabolites in the AD process and thereby shown to be effective stability indicators for AD system (Boe et al., 2010; Falk et al., 2015). Technologies that are commonly adopted for VFA measurement include chromatography, titrations, spectroscopy, and biosensors (Wu et al., 2019). Among them, bioelectrochemical system-based biosensors have proven attractive as simple, with high potential for online monitoring, and low-cost methods, i.e., omitting expensive equipment and complex raw sample pretreatment such as filtration or centrifugation, which are necessary for other methods (Do et al., 2020). The design and development of bioelectrochemical sensors for VFA monitoring in the AD process have attracted increasing attention in recent years (Wu et al., 2019; Do et al., 2020). Biosensors usually use exoelectrogenic bacteria as catalysts to oxidize biodegradable substances and directly generate current in the external circuit (Lóránt et al., 2019). The microorganisms function as a biological recognition element, which can sense the variation of target analytes and give a response to its output electric signal proportionally (Su et al., 2011). Several bioelectrochemical biosensors have been developed for the measurement of total or individual VFA (Kaur et al., 2013; Jin et al., 2016, 2017; Jiang et al., 2019; Sun et al., 2019b, 2019c).

Based on the cyclic voltammetry method, current generation from a dual-chamber microbial fuel cell (MFC) coupled to VFA oxidation showed a linear increase along with the VFA concentrations (<80 mg L^{-1}) and therefore could be used for quantitative detection of VFA (Kaur et al., 2013, 2014). Biosensor built with a potentiostat-controlled three-electrode arrangement in a single-chamber flow cell was proposed for acetate measurement (0.5–5 mM) and its sensor parameters (cross-sensitivity, response dynamics, latency, and stability) have been studied with defined solutions and further evaluated by directly integrating into an AD reactor (Kretzschmar et al., 2016, 2017, 2018). The main challenge for their application was the change or loss of sensor functionality because of the gradual alteration of the microbial community in the pre-acclimated biofilm after a long-term operation with AD slurry that typically contained various microorganisms and organic compounds.

Separation of the anodic biofilm and the sample in two compartments by an anion exchange membrane (AEM) was an effective solution for stabilizing the microbial community and selectively monitoring of VFA. In this context, a total VFA biosensor with a large detection range (up to 200 mM) was developed based on a three-chamber microbial desalination cell (Jin et al., 2016). Subsequently, single or dual-chamber microbial electrolysis cell-based biosensors were proposed and proved robust with short response time (Jin et al., 2017; Jiang et al., 2019). However, the Ohmic resistance of the cells could be changed with the variations of ionic strength of the samples, which may result in measurement errors (Jin et al., 2017).

Using traditional MFC-based biosensors could bring a unique advantage of no need for additional signal transducer or external power source, which is commonly required in other types of biosensors (Do et al., 2020). Thus, the potential of using a typical dual-chamber MFC as acetate biosensor was evaluated (Sun et al., 2019b). One main challenge would be the growth of aerobic bacteria on the surface of the cathode electrode, which may deteriorate the sensor stability. To overcome this disadvantage, a three-chamber MFC, including a sample receiving chamber which was distinctive from the anode chamber, was developed as acetate biosensor (Sun et al., 2019c). The interference from the variation of ionic strength of the sample on the output electrical signal that may also occur in traditional dual-chamber MFC biosensors can be avoided as well. Nevertheless, the three-chamber system, especially the cathode chamber, is still expensive for practical applications. Among the available configurations, air-cathode MFC has gained much attention in the past due to the simple design and easy operation (omitting aeration and catholyte) (Liu et al., 2005; Do et al., 2020). Thus, the air-cathode design could be adopted to simplify the structure of the three-chamber MFC biosensor and thereby reduce the capital and operating costs for VFA detection.

In this study, a novel dual-chamber air-cathode MFC composed of a single-chamber MFC and a sample chamber separated with AEM was developed and investigated to prove the technical viability of such design as a biosensor for VFA monitoring in the AD process. Similar to the previous three-chamber MFC design (Sun et al., 2019c), VFA will transfer from the sample chamber to the anode chamber, where they are oxidized by electroactive bacteria. The electrons are transferred from the anode to the cathode facing the air for electricity generation. Compared to the three-chamber MFC sensor, the new system is simpler with reduced capital (i.e., no need for cathode chamber) and operating costs (i.e., no need for aeration). Furthermore, the electrode distance is greatly reduced thereby resulting in lower internal resistance (or higher current density) and subsequently a more sensitive measurement. Even though the cathode electrode in the present design can be accessed by the bacteria from the anodic biofilm, the sensor performance would not be significantly affected. Because the digestate was not in direct contact with the cathode electrode. Besides, the microbial community of the cathodic biofilm was much simpler and the water matrix in the cell chamber was much cleaner, compared to that of the digestate. To assess the performance of the biosensor, its voltage response to different VFA species was studied. The influence of the bicarbonate, as well as the gentle stirring operation condition, on the VFA measurement, was analyzed. Furthermore, the biosensor was validated using real samples from the start-up period of an AD process. Finally, the microbial communities of the electroactive biofilm were characterized.

2. Materials and methods

2.1. Biosensor setup and operation

2.1.1. Biosensor setup

A dual-chamber air-cathode MFC was constructed by adding a sample chamber (8 cm × 8 cm × 4 cm) to the single-chamber (8 cm × 8 cm × 5 cm) air-cathode MFC (Fig. S1). Both chambers were rectangular compartments assembled from prefabricated parts made of nonconductive polycarbonate plates and separated with an anion exchange membrane (AMI 7001, Membrane international, NJ, 9 cm × 9 cm). Rubber gaskets and stainless screws were used to tighten the reactor and prevent leakage. The heat-treated (450 °C for 30 min (Feng et al., 2010)) carbon brush (Mill-Rose) (length: 7 cm, diameter: 5 cm) was used as the anode electrode. Carbon cloth (9 cm × 9 cm) with a gas diffusion layer (GDL-W1S1009, CeTech®) and coated with 0.5 mg cm^{-2} 20% Pt/C catalyst was used as the air-cathode electrode.

2.1.2. Inoculation

The inoculation and cultivation of electroactive biofilm were

conducted in a dual-chamber MFC with a cathode electrode made of graphite plate (5.2 cm × 5.2 cm) coated with 0.5 mg cm^{-2} 20% Pt/C. Domestic wastewater taken from the primary clarifier (Lundtofte Wastewater Treatment Plant, Denmark) was used as inoculum. The cultivation medium was 50 mM phosphate buffer solution (Na_2HPO_4, 4.33 g L^{-1} and NaH_2PO_4, 2.03 g L^{-1}) supplemented with nutrients (NH_4Cl, 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). 15 mM equimolar mixture of sodium acetate, sodium propionate, and sodium butyrate was used as the carbon source to acclimate the bacterial consortia. The cathode chamber was filled with 50 mM phosphate buffer solution and continuously aerated. Refreshing the cultivation medium in the anode chamber was performed every 7–10 days when the voltage across the 1000 Ω external resistance was lower than 50 mV. After the solution was renewed several times, the voltage was stabilized at around 650 mV, indicating a mature electroactive biofilm was attached on the surface of the anode electrode successfully. Then, the anode electrode with biofilm was transferred to the experimental reactor and conducted a one-month adaptation before the test.

2.1.3. Experimental operation

A 100 Ω external resistance was adopted to obtain a large sensing range and stable performance of the biosensor (Sun et al., 2019c). The tests were conducted in batch mode under non-sterile and non-agitated conditions, except the study on the effect of stirring (see section 3.3), and each lasted for 5 h at room temperature (23 ± 3 °C). The average value of duplicate tests for each setup was adopted for analysis. The synthetic wastewater containing varying VFA concentrations was prepared based on a basic anaerobic medium (as detailed in supplemental information) (Angelidaki et al., 1990). The sample was purged with mixed gas (80% N_2/20% CO_2) for 10 min before being tested. In each test, the anode chamber was filled with approximately 300 mL solution prepared with a 50 mM phosphate buffer solution containing nutrients (pH: 7.18 ± 0.03, EC: 6.56 ± 0.06 mS cm^{-1}), and purged with N_2 for 10 min. Followed by a starvation period of 1–3 h to maintain a low initial voltage (<15 mV). Then, the batch test was started by filling the sample chamber with synthetic wastewater (250 mL). At the end, the anode chamber was refreshed with the buffer solution containing nutrients and carbon source (an equimolar mixture of acetate, propionate and butyrate), while the sample chamber was renewed with deionized water.

2.2. Analytical methods

The electrical output of the biosensor was monitored by measuring the voltage across the external resistance and adopted as the analysis signal. A data logging system (LabVIEW, 2012; National Instrument) combined with an analog signal acquisition card (USB 3252, ZLAD) was used for recording average voltage per minute. Conductivity was monitored using a CDM 83 conductivity meter (Radiometer) and pH was measured by a PHM 210 pH meter. VFA concentrations were measured by using a gas chromatograph (GC) (TRACE 1300, Thermo Scientific) equipped with a flame ionization detector (Khoshnevisan et al., 2018). The bicarbonate concentration in AD effluent was estimated by a 5-pH point titration (Moosbrugger et al., 1993).

2.3. Microbial analysis

To elucidate the underlying mechanism of the biosensor, the microbial communities of the electroactive biofilm were characterized through the 16S rRNA method. The biofilm samples were collected in triplicate at different sites of the carbon brush electrode by using a sterilized scalpel at the end of the experiment. The PowerSoil DNA isolation kit (MoBio PowerSoil) was used to extract and purify DNA from the bio-samples (Zhu et al., 2017). The amplification of the total genomic DNA with the universal primers 515F (GTGYCAGCMGCCGCGGTAA)/806R (GGACTACNVGGGTWTCTAA) (on the V4 region) and the sequencing on the platform of Illumina MiSeq desktop sequencer were conducted by BMR Genomics® (Padova). Data analysis including raw data sequencing, multiple sequence comparison, abundant taxation selection, and the statistical tests for samples' comparison were the same as previously described (Wang et al., 2020). Raw data can be obtained from the database of Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA631912.

3. Results and discussion

3.1. Response to different VFA

To investigate the response of the proposed biosensor to different VFA species, synthetic wastewater containing individual VFA (7, 14, and 28 mM of acetate, propionate, or butyrate) was tested. The voltage of the biosensor increased with the reaction time and the initial VFA concentrations (Fig. 1A). All VFA could transport from the sample chamber through the AEM to the anode where they were metabolized to generate electricity by the biofilm, which was consistent with previous reports (Jin et al., 2016, 2017; Sun et al., 2019b). The increase in electricity generation was very slow at the beginning of the test. The duration of this phase was changed with the variation of concentrations and VFA species. At the end of the batch test, the voltage of the biosensor fed with 28 mM of acetate, propionate, or butyrate was 199.86 ± 10.52, 162.52 ± 3.24, and 32.55 ± 2.60 mV, respectively.

The different responses of the biosensor toward different VFA species is consistent with our previous observation of the three-chamber MFC-based biosensor (Sun et al., 2019c). The reasons might be different metabolic pathways in the electroactive biofilms and the varying transfer rates through the AEM (Table 1) (Lide, 2004). Acetate has been recognized as the most preferential substrate for electricity generation in MFCs producing the highest coulomb efficiency, while the propionate and butyrate were difficult to be degraded due to their less favorable thermodynamic properties (Chae et al., 2009; Kaur et al., 2013). Lower bacterial uptake of butyrate than that of acetate causing a lower current in single-chamber MFCs has been reported, i.e., the current densities from butyrate (0.77 A m^{-2}) were much lower than those using acetate (2.2 A m^{-2}) (Liu et al., 2005). Besides, the transfer of anions through the membrane follows Fick's law for the current biosensor design and operation conditions (Van der Bruggen, 2018). The transfer rate of a specific anion would be proportional to the concentration difference when the membrane area and thickness, and the diffusion coefficient were fixed (Dhar and Lee, 2013), and it would be relatively slower for anions with higher molecular weight and bigger molecular size, thus decreasing as the chain length among carboxylic acids increases (Zhang and Angelidaki, 2015). Therefore, the VFA accumulations in the anode chamber would be different, even with the same initial concentration and reaction time, thus influencing the response of the biosensor toward different VFA species.

To characterize the response of the biosensor to VFA mixtures, samples with a total VFA concentration of 14 mM containing a mixture of acetate, propionate, and butyrate were tested. The voltages from the samples with the same total VFA concentration but different VFA compositions were different (Fig. 2). Besides, the voltage obtained from the VFA mixture was close to the sum of the calculated values of each VFA according to their content in the

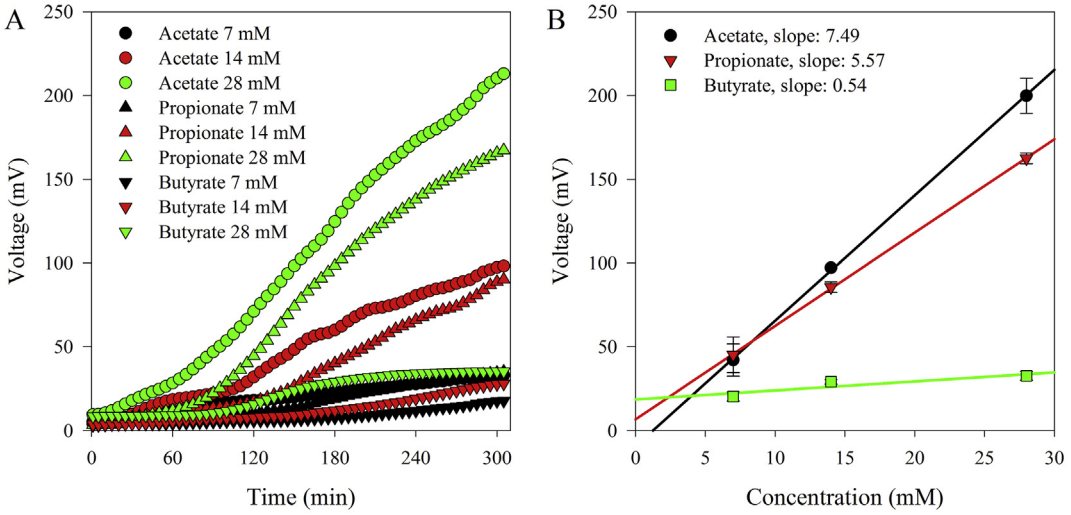

Fig. 1. Typical variation of voltage toward different VFA species (A) and the relationship between the voltage at 5 h and concentrations of different VFA (B) (The error bars refer to the maximum and minimum of the duplicate tests.).

Table 1
The diffusion coefficient of the anions at 25 °C (Lide, 2004).

Species	D (10^{-5} m^2/s)
CH_3COO^-	1.089
$C_2H_5COO^-$	0.953
$C_3H_7COO^-$	0.868
Cl^-	2.032
HCO_3^-	1.185

mixture using the slopes in Fig. 1B. This result indicated the voltage signal of the biosensor obtained from VFA mixture would be assumed according to the voltage response of the biosensor to individual VFA (Kretzschmar et al., 2017). It can be inferred that measurement error will occur in testing the sample containing VFA with different compositions from the calibration solution. Since the acetate and propionate are usually the most dominant VFA during the AD process (Ahring et al., 1995), the viable and similar response of the biosensor to both VFA species is important for the practical application of the current biosensor design. The electric signal of the present biosensor responding to propionate was close to that of acetate, which can reduce the influence of VFA compositions on the measurement accuracy to a certain extent. Therefore, the proposed biosensor does have a high potential for total VFA monitoring in the AD process.

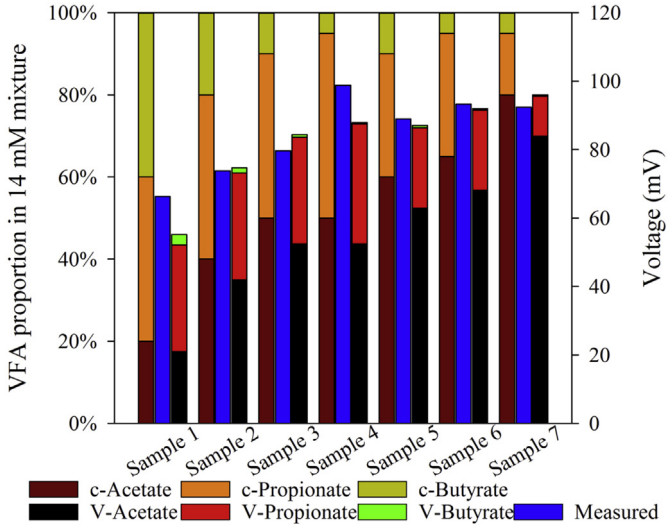

Fig. 2. The measured and calculated voltages toward 14 mM VFA mixture at different ratios (The "c-" refers to the proportion of VFA in the mixture, and the "V-" refers to the calculated voltage corresponding to VFA content in the mixture based on the slope in Fig. 1B).

3.2. Influence of bicarbonate

Separating the anodic biofilm and sample with an AEM (i.e., the transferred VFA would be the sole carbon source for the anode) was applied and proved effective for selectivity monitoring of VFA when the sample contains other nonionic or positive charged organic matter (Jin et al., 2016, 2017; Jiang et al., 2019; Sun et al., 2019b). However, the competitive transportation of other co-existed anions may cause a reduced amount of VFA transferred through the AEM and a negative effect on electricity production in current biosensor design (Jin et al., 2016). Therefore, it is necessary to evaluate the influence of bicarbonate on the VFA measurement, because the bicarbonate is usually presented in relatively high concentrations in AD reactors as a buffer to resist the pH variation. Samples containing 28 mM VFA mixture (acetate, propionate and butyrate at a ratio of 4: 4: 2) and supplied with different concentrations of sodium bicarbonate were tested.

As shown in Fig. 3A, compared to the sample without bicarbonate, the voltage obtained from the samples containing bicarbonate (30, 60, 90 and 150 mM) at the reaction time of 5 h was decreased by 30.62 ± 10.57%, 38.54 ± 1.80%, 45.83 ± 8.57%, and 52.97 ± 0.71%, respectively; and the voltage at different reaction times (2, 3, 4, and 5 h) from the sample containing 150 mM bicarbonate was decreased by 34.73 ± 7.32%, 45.10 ± 2.09%, 53.06 ± 1.69%, and 52.97 ± 0.71%, respectively. The interference of bicarbonate on electricity generation from the biosensor was getting stronger with the increase of reaction time and the bicarbonate concentrations. The voltage of the biosensor also decreased when 60 mM sodium chloride instead of sodium bicarbonate was added (Fig. 3B). The decrease of the voltage by 44.93 ± 1.48% at a reaction time of 5 h was even higher, as compared to the decrease of 38.54 ± 1.80% with the sample containing 60 mM bicarbonate.

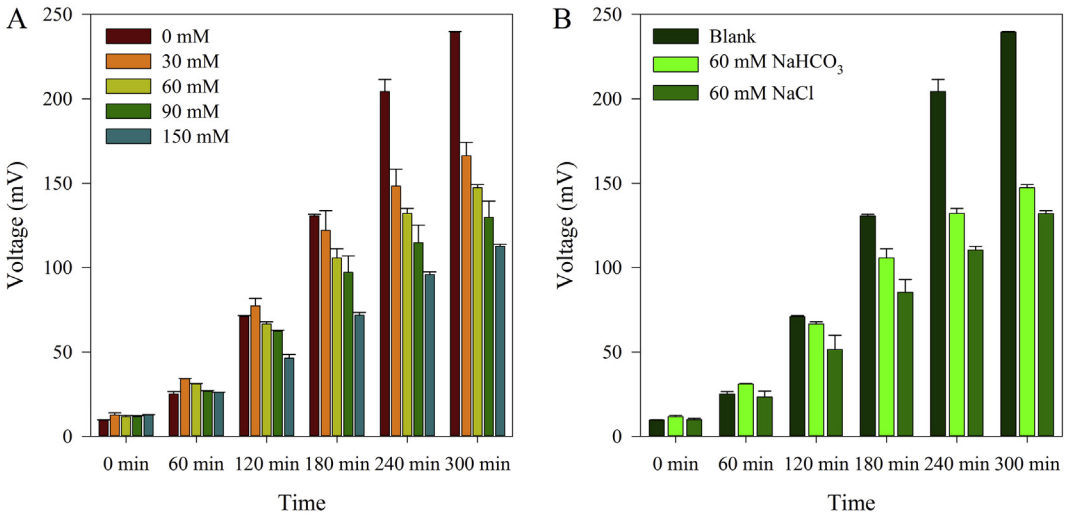

Fig. 3. The influence of bicarbonate on the biosensor measurement (A) and the comparison of the influence with that of sodium chloride (B) (The samples contained 28 mM mixture of acetate, propionate, and butyrate at a ratio of 4: 4: 2. The error bars refer to the maximum and minimum of the duplicate tests.).

These results showed the negative influence of the high concentrations of anions presented in the sample on the performance of biosensor. Since the introduction of small quantities of carbonate (50 mM) and bicarbonate (50 mM) only has a slight effect (<3%) on current generation in oxygen reduction reaction catalyzed by Pt (Vega and Mustain, 2010). The main reason for the significantly reduction of current generation should attributed to the nature of concentration gradient diffusion as the dominant parameter affecting anions transportation through the AEM to the anode in the current biosensor design. The amount of VFA transferred to the anode chamber would be reduced when other large amounts of anions co-existed. This is in line with the presence of high salts concentrations reducing the VFA accumulation in the anode chamber (Jin et al., 2016). The stronger impact of chloride ion compared to bicarbonate on reducing VFA transferred to the anode could be due to chloride's higher diffusion coefficient (Table 1) allowing a faster transfer rate through the AEM.

For accurate VFA measurement in a sample with a complex matrix, it is important to maintain a similar concentration of the bicarbonate or other co-existed anions with the biosensor calibration solution. Since a higher or lower concentration of bicarbonate in the sample would cause an underestimation or overestimation of the measurement, respectively. Besides, shortening the reaction time would be another effective solution to minimize the influence of anions on the performance of the biosensor. For the onsite application of the biosensor to monitor the AD process, it is recommended to calibrate the biosensor with the biogas slurry during the stable period of the specific biogas plant. In this case, the biosensor would generate higher output signal during the unstable AD process typically characterized by an increase of VFA and the resulting decrease of the bicarbonate (Sun et al., 2019a).

3.3. Effect of stirring

Continuous stirring of the anode is usually applied to avoid diffusion limitation in MFCs (Kretzschmar et al., 2016). A non-agitated operation strategy could simplify the onsite application of our proposed biosensor although it may lead to a poor mass transfer. The effect of stirring in the anode chamber on the performance of the biosensor was clarified in this section. For VFA concentrations over 7 mM (mixture of acetate, propionate, and butyrate at a ratio of 4: 4: 2), the voltage under a gentle stirring at 30 rpm was higher than that under the non-stir condition (Fig. 4A). A shortened lag time at the start period in the batch test was also observed. The improved electricity generation process with the help of the stirring operation was consistent with the previous report (Pham et al., 2008; Lu et al., 2013). This can be explained by the improved mass transfer in anode chamber under stirring condition, which facilitate the mixing of the microbes with the culture and increase the transfer rate of electrons (Pham et al., 2008; Lu et al., 2013). More VFA were available for biofilm and oxidized for electricity generation. Meanwhile, the voltage was lower in the test of 2.8 mM VFA mixture (Fig. 4A), which indicated the VFA consumption was relatively faster than their diffusion from the sample chamber, thereby causing a less accumulated VFA in the anode. A growth model (Gompertz) was used here to express the response of the biosensor to different VFA concentrations (Tjørve and Tjørve, 2017).

$$V = a^{*}\ e^{-e^{(-k^{*}(X-x_c))}} \quad (1)$$

where V is the voltage of the biosensor corresponding to the VFA concentrations (mV), X is the VFA concentration (mM), a represents the upper asymptote of curve (mV), k is a voltage growth-rate coefficient which affects the slope, and x_c represents the VFA concentration at inflection (mM).

The nonlinear regression between the voltages at the specific reaction time and VFA concentrations showed a relatively low saturation concentration of the biosensor (around 30 mM) under a stirring condition (Fig. 4B), which indicates a narrowed measuring range as compared to that of the three-chamber biosensor reported previously (Sun et al., 2019c). This is the result of improved mass transfer in the anode that bring more VFA available to the biofilm which can reach its maximum current output even at relatively low substrate concentration (i.e., 2.3 mM and 3.2 mM of acetate) (Tront et al., 2008; Atci et al., 2016). Above all, the stirring operation could accelerate the response and amplify the signal of the biosensor, but narrow the measuring range.

3.4. Validation in the monitoring of AD start-up process

The AD system can be suboptimal or unstable during the start-up period due to the slow growth rate and the need to establish a balanced microbial composition which reflects the specific

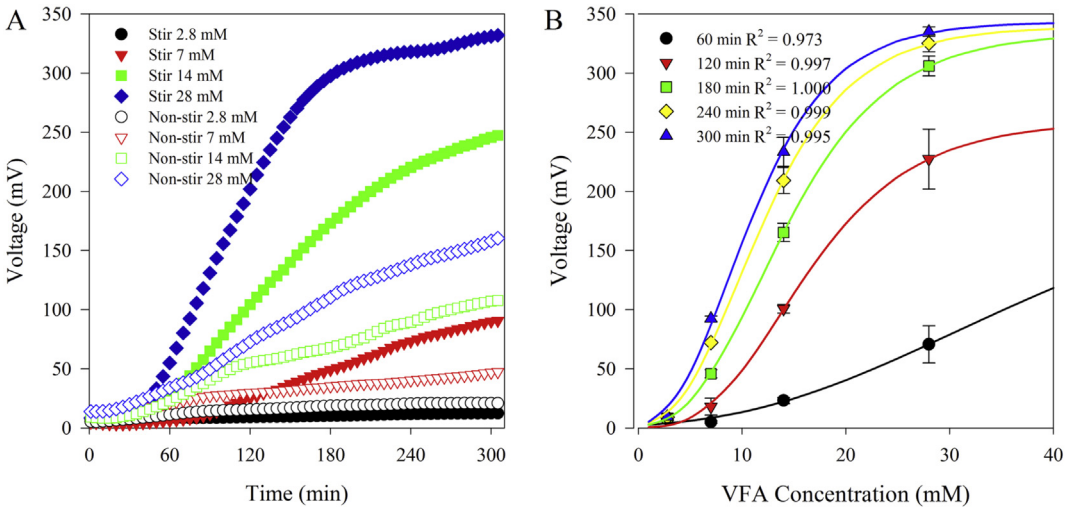

Fig. 4. Typical variation of voltage with and without stirring (A), and the relationship between the voltage at specific reaction time and VFA concentrations under stirring condition (B) (The samples contained a mixture of acetate, propionate, and butyrate at a ratio of 4: 4: 2. The error bars refer to the maximum and minimum of the duplicate tests.).

feedstock (Ahmed and Rodríguez, 2020). Start-up of AD processes requires efficient monitoring since the process is in the transition to establish its balanced steady state. By monitoring, this transition period can be significantly reduced and fatal process failures can be avoided. Samples taken from an AD reactor during the start-up period were tested to validate the applicability of the biosensor for real digestate measurement.

Aqueous VFA solutions at various concentrations of 2.8−112 mM (mixture of acetate, propionate, and butyrate at a ratio of 4: 4: 2) were tested to build the calibration curves. The typical voltage-time course to different VFA concentrations is shown in Fig. S2. The VFA concentrations and the voltages at specific reaction time can be nonlinearly regressed with Eq. (1) (Fig. S3), which agreed with the observations of the MFC-based biosensors (Sun et al., 2019b, 2019c). Linear regression was also applicable for low VFA concentrations (<14 mM) (Fig. 5A). These linear regression equations were used here to convert the electric signal to VFA concentration.

Fivefold diluted effluent from the CSTR was tested with the biosensor. The voltage-time course corresponding to different samples is shown in Fig. S4. In comparison with GC results, the average relative difference of the VFA concentrations obtained at different operation time (2, 3, 4, and 5 h) with the biosensor was (−29.1 ± 46.2)%, (3.9 ± 48.7)%, (17.3 ± 35.8)%, and (26.8 ± 21.8)%. The deviation between the biosensor and the GC measurements was relatively high. However, the dynamic change of the VFA results measured by two methods was basically synchronized (Fig. 5B). The correlation coefficient between the measured results by GC and the biosensor at different reaction time (2, 3, 4, and 5 h) was 0.93, 0.93, 0.95, and 0.98. These results indicates the VFA measurement by the biosensor can reflect the dynamic variation of the VFA in the AD process and thus be capable of routine monitoring of the AD process.

The main reason for the low accuracy of the biosensor in testing the real digestate was the different characteristics of the calibration solution and real digestate. The calibration curves were based on simple aqueous solutions with a VFA mixture at a fixed ratio. However, the real digestate has a more complex composition which may vary during operation. For instance, the total VFA increased from 1.12 ± 0.07 mM at initial and reached a maximum of 36.19 ± 2.09 mM at day 12 followed by a rapid decrease to 3.61 ± 0.37 mM (Fig. S5). Acetate and propionate were the most dominant VFA in this period. Based on the previous experiments, the presence of butyrate can only generate a very low level of the voltage. Thus, there would be an overestimation (up to 25%) in the calculation when the AD effluents had an extremely low level of butyrate (<0.51 ± 0.17 mM) while it occupied 20% of total VFA in the calibration solutions. Moreover, the bicarbonate concentrations decreased from an initial of 214.88 ± 3.70 mM to as low as 89.67 ± 2.88 mM at the second hydraulic retention time (Fig. S6). The bicarbonate concentrations in the tested samples would range from 17.93 to 42.99 mM, which would lead to a negative or positive interference of the VFA measurement by using the biosensor calibrated with solutions containing 30 mM of bicarbonate. Although the biosensor is promising for onsite VFA monitoring in the AD process, more efforts are still needed to increase its accuracy.

In our previous study, a bio-electrolytic sensor has been stably operated for over 5 months without any maintenance service (Jin et al., 2017). Though the biosensor has a different configuration from the previous system, they did use the same electrode and membrane materials. Thus, the previous study could demonstrate the long-term stability of a similar type of biosensors. The long-term stability of the current design could be further explored in future studies.

3.5. Microbiological analysis

Microorganisms in the biofilm are the core engine of the present biosensor, as they can oxidize organic compounds and transfer electrons to anode electrode (Reguera et al., 2006; Logan et al., 2019). For a better understanding of the role of microorganisms, the microbial community in the biofilm was analyzed (Fig. 6 and Table S1). At the phylum level, microorganisms in the biofilm were dominated by *Proteobacteria* (74.61%), followed by *Bacteroidetes* (17.25%), *Firmicutes* (4.65%), *Synergistetes* (2.02%), and *Actinobacteria* (0.73%) (Fig. 6A). The predominance of *Proteobacteria* and *Bacteroidetes* was consistent with the previous report of air-cathode MFCs (Shehab et al., 2013). The microbial community distribution of the biofilm at the class level is presented in Fig. 6B. *Deltaproteobacteria* (35.99%) and *Betaproteobacteria* (30.83%) were the dominant class, followed by *Bacteroidia* (10.28%), *Alphaproteobacteria* (6.78%), *Flavobacteriia* (6.46%), *Clostridia* (4.65%), and *Synergistia* (2.02%).

The genus-level identification could present more detailed information about the microbial distribution of the biofilm. The most

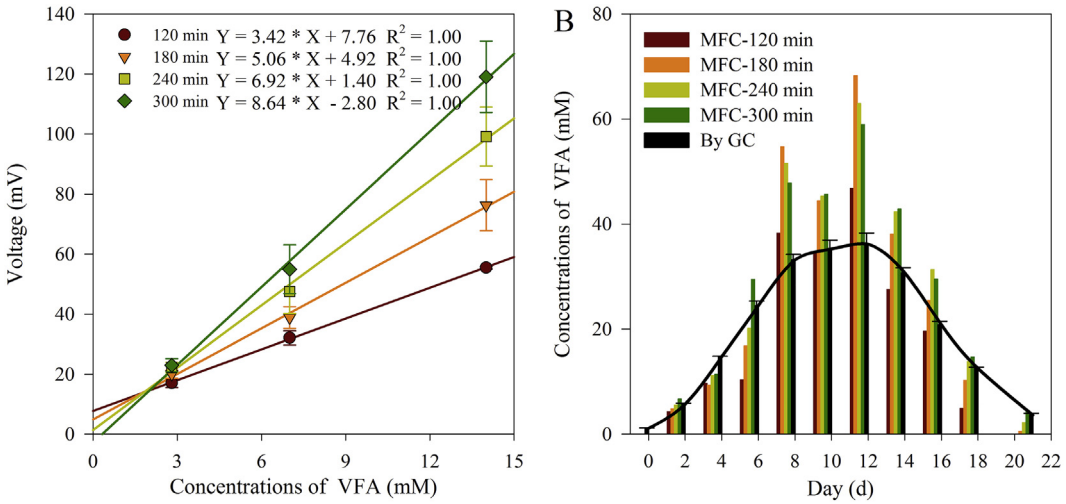

Fig. 5. Linear relationship between the voltage and VFA concentrations (A), and the comparison of the biosensor measurement with the GC results (B) (The samples tested in (A) contained a mixture of acetate, propionate, and butyrate at a ratio of 4: 4: 2; the samples tested in (B) were five-time diluted biogas slurry from an AD reactor during the start-up period. The error bars refer to the maximum and minimum of the duplicate tests.).

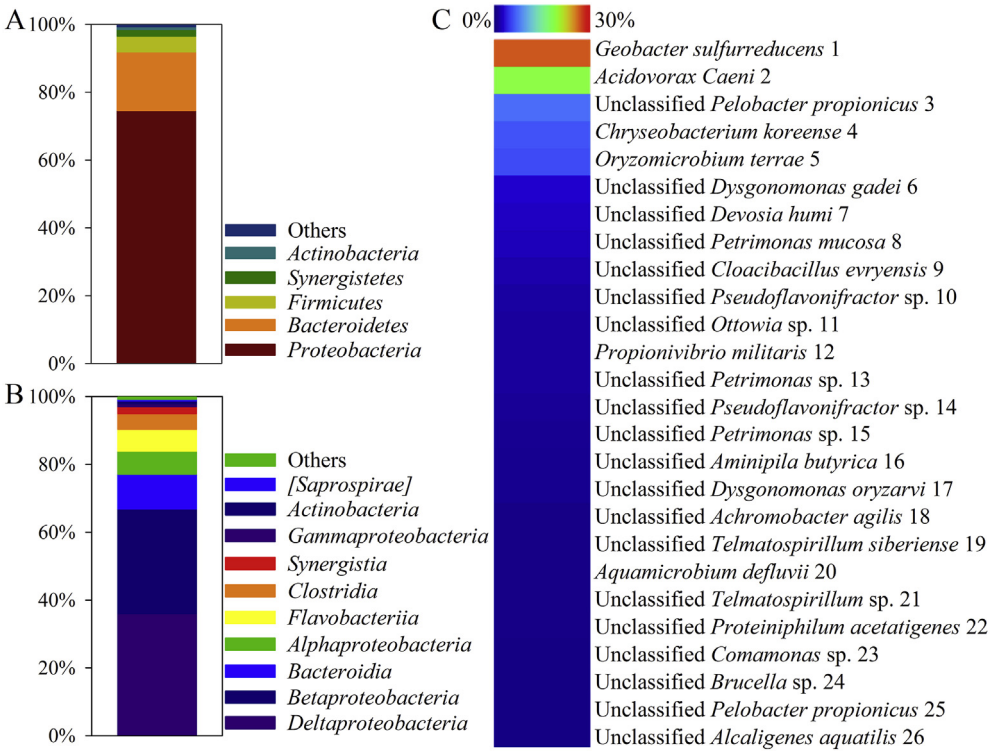

Fig. 6. The relative abundance of microorganisms reads retrieved from the biofilm of the biosensor classified at the phylum (A) and class (B) level (Phyla and classes that represent less than 0.5% of the total microbial community composition are classified as "others".), and heat map of the species with a relative abundances over 0.5% (C).

abundant genera were *Geobacter* (28.46%). The *Hydrogenophaga* (19.09%) was the second abundant genera, followed by *Pelobacter* (7.36%), *Chryseobacterium* (6.30%), *Oryzomicrobium* (5.77%) and *Dysgonomonas* (4.01%). The abundance of the *Geobacter* in the present study was relatively low compared to the previous study with 72% *Geobacter* as the dominant genus in the anodic biofilm of an acetate-fed MFC (Shehab et al., 2013). The main reason could be the different carbon sources used in these two studies. Based on BLAST comparison, the most frequently identified sequences were most closely related to strains of *Geobacter sulfurreducens* (26.70%), *Acidovorax caeni* (19.04%), *Pelobacter propionicus* (7.36%), *Chryseobacterium koreense* (6.21%), *Oryzomicrobium terrae* (5.77%), and *Dysgonomonas gadei* (3.08%) with a similarity of 100%, 100%, 98%, 100%, 100%, and 96%, respectively (Fig. 6C). *Geobacter sulfurreducens* are the most reported exoelectrogenic bacteria, oxidizing acetate and performing direct electron transfer to the anode electrode in MFCs (Reguera et al., 2006). *Acidovorax caeni* could use the propionate and butyrate as carbon sources for anaerobic respiration and growth through denitrification (Heylen et al., 2008). *Pelobacter propionicus* was speculated to generate sulfide with iron reduction,

but incapable of transferring electrons to the anode of an MFC due to lacking cytochromes such as MacA and OmcB (Freguia et al., 2010; Yan and Wang, 2019), even though there might be mutualistic relationship between *Pelobacter* sp. (fermenting initial substrates to acetate) and *Geobacter* sp. (exporting electrons and thereby generating current) in an MFC (Kiely et al., 2011). *Chryseobacterium koreense* can assimilate acetate and propionate (Kampfer et al., 2009). *Oryzomicrobium terrae* has been reported to oxidize propionate (Liu et al., 2017). *Dysgonomonas gadei* is associated with glucose fermentation under microaerophilic and strictly anaerobic conditions with the production of acid but no gas (Hofstad et al., 2000). Its presence might be due to the complex syntrophic interactions that existed in biofilm (Lu et al., 2012). The complex composition of microbial communities in the biofilm enables it to degrade different VFA species, which consisted of the basis for sensing of the current biosensor design. The predominance of *Geobacter* sp. in the biofilm may associate with the experimental observation that a higher current was produced with the acetate than the same concentration of propionate or butyrate. Moreover, the voltage from propionate or butyrate was relatively high in comparison with a biosensor with a mixed-species *Geobacter* sp.-dominated (75%–88%) biofilm that only generated a baseline signal in testing propionate or butyrate (Kretzschmar et al., 2017). This indicated the important role of other microorganisms in the biofilm for degradation of propionate or butyrate. Only a small population of microorganisms are capable of using the butyrate, which may also be responsible for the low electric signal generated by the biosensor in testing butyrate. The selectivity of the biosensor toward different VFA depended on the interactions between species in its mixed-culture electroactive biofilm to a large extent (Prévoteau and Rabaey, 2017). A deep insight into the role of the microorganisms in the biofilm is still needed for exploring a more proper consortium that can oxidize different VFA with a similar electric signal produced, thus to further optimize the VFA biosensor.

4. Conclusions

The novel air-cathode MFC-based biosensor responded to different VFA species and showed a linear response to VFA concentrations below 14 mM. The presence of anions would reduce the transportation of VFA through AEM and thus influence the output signal. Stirring could accelerate the response and amplify the signal, but narrow the detection range. The monitored VFA was synchronously varied with the measurement by GC for real digestate. This is a proof of concept of a simple biosensor that can serve as a promising alternative method for online VFA monitoring in the AD process or any other VFA-rich fluids, after further optimization.

Author statement

Hao Sun: Conceptualization, Investigation, Formal analysis, Writing – original draft, Mingyi Xu: Investigation, Writing – original draft, Shubiao Wu: Writing – review & editing. Renjie Dong: Writing – review & editing. Irini Angelidaki: Writing – review & editing. Yifeng Zhang: Conceptualization, Writing – review & editing, Supervision, Funding acquisition

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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