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Published in:
Environmental Science & Technology (Washington)

Link to article, DOI:
10.1021/acs.est.5b05267

Publication date:
2016

Document Version
Peer reviewed version

Article

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Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.5b05267 • Publication Date (Web): 30 Mar 2016

Microbial electrochemical monitoring of volatile fatty acids during anaerobic digestion

Xiangdan Jin, Irini Angelidaki, Yifeng Zhang*

Department of Environmental Engineering, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark

*Corresponding author:

Dr. Yifeng Zhang

Department of Environmental Engineering, Technical University of Denmark, Denmark

Tel: (+45) 45251410

Fax: (+45) 45933850

E-mail address: yifz@env.dtu.dk

Abstract

Volatile fatty acid (VFA) concentration is known as an important indicator to control and optimize anaerobic digestion (AD) process. In this study, an innovative VFA biosensor was developed based on the principle of a microbial desalination cell. The correlation between current densities and VFA concentrations was firstly evaluated with synthetic digestate. Two linear relationships were observed between current densities and VFA levels from 1 to 30 mM (0.04 to 8.50 mA/m^2, R^2=0.97) and then from 30 to 200 mM (8.50 to 10.80 mA/m^2, R^2=0.95). The detection range was much broader than that of other existing VFA biosensors. The biosensor had no response to protein and lipid which are frequently found along with VFAs in organic waste streams from AD, suggesting the selective detection of VFAs. The current displayed different responses to VFA levels when different ionic strengths and external resistances were applied, though linear relationships were always observed. Finally, the biosensor was further explored with real AD effluents and the results did not show significance differences with those measured by GC. The simple and efficient biosensor showed promising potential for online, inexpensive and reliable measurement of VFA levels during AD and other anaerobic processes.

Key word: Volatile fatty acids; Bioelectrochemical system; Biosensor; Anaerobic digestion; Online monitoring

Introduction

Countries across the world are devoting to sustainable society by setting ambitious goals for renewable energy supply. For example, Denmark's long-term energy goal is to become independent of fossil fuel utilization by 2050. Therefore, the increasing use of renewable biofuels such as biogas is inevitable for the future.[1] The objective of achieving optimum biogas production from anaerobic digestion (AD) is challenged by process instability.[2] Parameters like pH, alkalinity, the biogas producing volume and concentrations of methane and CO_2 are typical used as online indicators for the full-scale anaerobic reactor's monitoring. However, several investigations have pointed out disadvantages of these parameters as process indicators due to low sensitivity and reliability.[3, 4] Instead, volatile fatty acids (VFAs), products of the fermentation stage and substrates of the methanogenesis stage, have been widely accepted as sensitive and reliable indicators for the AD process, as they will accumulate and reflect the metabolic imbalance when operating parameters suddenly change or inhibitors occur. Indeed AD sensors that have been investigated the recent years, and have tried to correlate their signal to VFA concentrations, e.g. the sensor response to the gas phase concentration,[3] UV absorption,[5] electricity[6] and near infrared[4]. Traditional off line methods of the VFA quantitative measurement such as titration[7], gas chromatographic (GC)[8], HPLC[5] and mid-infrared spectroscopy[4] have been previously reported. However, most of off line methods are time consuming, inaccurate, require of complex equipment and skilled operations. There have been several attempts to develop online sensors via directly detecting VFA concentrations by GC analysis. However, due to difficult preparation of the samples for the GC analysis of the VFA, these methods have not been

easy to implement.[8, 9] Therefore, constructing a more simple, sensitive and accurate VFA sensing device is critical for the AD process monitoring and control.

Over the past decades, bioelectrochemical systems (BESs) such as microbial fuel cell (MFC) based biosensors have attracted great interest due to the unique advantages in the monitoring of water quality (e.g.; COD, BOD, DO and microbial activities)[10, 11, 12] and toxicants (e.g.; Ni^{2+} and Cu^{2+})[13, 14, 15]. These sensors exploited the microbial activities which converted chemical energy stored in organic matter to electrical signals. Comparing to conventional off line determining technologies, BES sensors have several advantages including no need of energy and chemicals, environmental-friendly andsustainable.[12] Recently, the feasibility of MFC as biosensor for monitoring VFAs has been demonstrated.[6, 16] The MFC biosensor responded to specific VFA, since the anodic biofilm was pre-acclimated using specific VFA as the sole substrates. The work indeed broadens the applications of MFC-based sensors. However, several challenges still need to be addressed before field application. Firstly, the microbial community in the pre-acclimated biofilm might be changed or lose its function during long-term operation with actual AD effluents which contain a large number of microorganisms. Secondly, actual AD effluents always contain complex substrates (e.g.; protein and lipid) in addition to VFAs, which might also change the microbial community and reduce the sensitivity of the sensor. Furthermore, the MFC biosensor might finally function as a sensor for total content of organic matter (e.g.; COD) instead of VFAs, since nearly all kinds of organic matter could be used as substrates in the anode.[17] Thus, a compact microbial electrochemical system that can avoid the aforementioned challenges should be pursued.

In this study, we proposed an innovative bioelectrochemical VFA biosensor on the basis of the microbial desalination cell (MDC). To date, the MDC-liked reactor has never been applied as

a VFA biosensor. The biosensor has three chambers and waste streams containing VFAs were dosed into the middle chamber. Ionized VFAs could transport into the anode chamber through an anion exchange membrane (AEM) which separates the anode and middle chambers and then be utilized by exoelectrogens on the anode electrode for producing electrons. Thus, the current generated might be only proportional to VFA concentrations in the solution of the middle chamber. The transportation of other complex organic matter such as lipid and protein to the anode could be avoided, since most of them are in non-ionic form, and thus, their interference with VFA monitoring could be eliminated. Furthermore, the microbial community in the anode will not be affected by the diverse microorganisms in the AD effluents, since they are separated in two chambers. The objective of this work is to demonstrate the feasibility of the biosensor as simple, sensitive and reliable sensor for monitoring of VFAs. The performance of the biosensor was evaluated in terms of the VFA detection range, sensitivity and reproducibility. The effect of different operating parameters (ionic strength, anion species and external resistance) on the performance of the biosensor was also investigated. Then the reliability of the biosensor was verified with actual AD effluents. The simple biosensor showed promising potential for direct, sensitive, reliable, inexpensive and online VFA monitoring. The outcomes offer a powerful tool for cost-effective monitoring and optimization of AD process and expand the application of microbial electrochemical system.

Material and methods

Biosensor configuration and operation. Two three-chamber bioelectrochemical reactors (made of nonconductive polycarbonate plates) were used in our present study. The rectangular

compartments of the fuel cell (anode, middle and cathode chambers) with equal dimension size (8 cm×8 cm×4 cm) were physically separated by an AEM (AMI 7001, Membrane international, NJ) and a cation exchange membrane (CEM, CMI 7000, Membrane international, NJ) (Fig. 1). Rubber gaskets and screws were used to tighten the reactors to avoid leakages. The anode electrode was made of a carbon brush 6.0 cm in diameter and 6.0 cm in length (Mill-Rose, USA). The cathode electrode was a stainless mesh with an area of 54.8 cm^2 (The Mesh Company, UK). Plastic tubes were inserted for medium filling and liquid sampling. The anode and cathode were connected through a 1000 Ω external resistance except as described.

Enrichment and sensing experiment. To enrich exoelectrogens, the anode electrodes were first inoculated in traditional two-chamber MFC reactors for about two months. During the enrichment period, 250 mL domestic wastewater collected from a primary clarifier (Lundtofte Wastewater Treatment Plant, Lyngby, Denmark) was used as inoculum. Acetate, butyrate, propionate and formate, each with concentrations of 4 mM were dosed as substrates to acclimate the bacterial consortia. In the cathode, 250 mL ferricyanide solution (50 mM) was used as the electron acceptor. Ferricyanide was only used in this enrichment period. Every 8 to 10 days the reactors were refilled when the voltage was lower than 50 mV. After two months enrichment, the anode electrodes produced maximum 500±50 mV in voltage reproducibly indicating the formation of mature electrochemically active biofilm on the surface of the anode, which was ready for the sensing experiment.

Before transferring the enriched anode electrode into the three-chamber biosensor, the anode was starved for about 2 days to consume the carbon storage in the bacterial cells (corresponding voltage was below 0.2 mV). The whole experiment was operated at room temperature (22±2 °C) in fed batch mode by conducting each batch test for 5 h. The anode chamber of the biosensor

was fed with a buffer solution containing (in g/L of distilled water): NH_4Cl, 0.31; $NaH_2PO_4 \cdot H_2O$, 2.69; Na_2HPO_4, 4.33; KCl, 0.13; 12.5 mL mineral solution and 12.5 mL vitamin solution[18]. The middle chamber was fed with the synthetic digestate containing the buffer solution and VFAs at different levels. VFAs were mainly composed of acetate, propionate, butyrate and formate at a concentration ratio of 10:2:2:1 to mimic the actual composition in the AD system. The cathode chamber was filled with 0.05 mM NaCl solution. At the beginning of each batch, the anode and middle chamber were purged with N_2 for 10 min to maintain an anaerobic condition. During the batch experiments, the cathode was continuously aerated while the anode chamber was mixed using the magnetic stirrer. All experiments were performed in duplicate.

Electrochemical analyses and calculations. Conductivity and pH were measured using a CDM 83 conductivity meter (Radiometer) and a PHM 210 pH meter (Radiometer), respectively. TS, VS and NH_4^+-N were measured according to the Standard Method.[19] Acetate, butyrate, propionate and formate were measured via a GC with FID detection (Agilent 6890). The voltage across the resistance was monitored using a digital multimeter (Model 2700, Keithley Instruments, Inc.; Cleveland, OH, USA) with 30 min intervals. Current was calculated according to ohm's law. Current density was calculated as $i=I/A$, where I (mA) is the current and A (m^2) is the project surface area of the cathode.

Results and Discussion

The response of current densities to various VFA concentrations. The feasibility of the biosensor was investigated under different VFA concentrations with an external resistance of 1000 Ω. The current density responses to different VFA concentrations along with the operation

time are shown in Figure 2a. The current density increased with the increasing of initial VFA concentrations. As no substrates were dosed in the anode, current increases could only be explained by the transportation of VFAs through the membrane from the middle chamber. When 1 and 5 mM VFAs were dosed, the current densities were close to the background level, which could be due to the limited VFA transportation to the anode. When the VFA levels were lower than 60 mM, the current densities increased gradually with time and reached to the maximum values (0.04-9.36 mA/m^2) at 5 h. Comparatively, the current density increased more sharply and reached to the maximum stable value in less than 3 hours when the initial VFA concentration was between 120 and 200 mM. Thus, the response time of the biosensor was chosen as 5 hours in the following tests. It was also observed that the differences among the maximum stable current densities turned to be lower when the initial VFA levels increased from 30 to 200 mM, which suggested the biosensor was approaching saturation at those concentrations of the fuels.

The correlation between the current density and the VFA level was established as shown in Figure 2b. Two linear relationships were observed between current densities and VFA levels. The current density first increased linearly with VFA levels from 1 to 30 mM (0.04±0.01 to 8.50±0.32 mA/m^2, R^2=0.97) and then from 30 to 200 mM (8.50±0.32 to 10.80±1.26 mA/m^2, R^2=0.95). During the first linear stage, the slope of the linear model was steep as large increments were achieved when enough VFAs were supplied. The current generation was primarily limited by the substrate concentration. During the second stage the slope of the line was quite flattened. The increments in current densities under high VFA concentrations were not as significant as those under lower VFA concentrations which suggested the effect of substrate on the sensor system was not dominant and the biosensor was nearly saturated at high VFA concentrations. The biosensor was further operated in two successive batches to explore its

response to dynamic changes of the VFA levels (e.g., increasing/decreasing the VFA concentration and undergoing a starvation period without VFAs present) (Figure S1). It was observed that the biosensor responded immediately to the VFA concentration changes without any lag phase. The biosensor also functioned well after a "starvation period". The above results indicate the feasibility of this biosensor for real time VFA monitoring in the range of 1 to 200 mM.

Changes in pH and conductivity. Figure 3a shows pH in the chambers under different VFA concentrations at the end of each batch. pH in the anode and middle chambers kept stable around 7.0 due to the high buffer capacity of the phosphate solution which benefited the microbial activities.[21] In the cathode, pH increased from 6.86±0.08 to 7.58±0.06 along with the VFA concentrations. High current densities under high VFA concentrations meant faster reaction rate, which might accelerate the cathode oxygen reduction with proton and lead to the increase of pH. However, the highest pH in the cathode chamber was 7.58±0.05 at a VFA concentration of 200 mM, which was much lower than those achieved in the cathode of MFC reactors.[22] This is because the batch period was short (5 h) which avoided a significant increase in pH. Stabilizing pH to desired values in the cathode is helpful to inhibit the potential of alkaline scale formation and membrane fouling.

The conductivity in the biosensor under various VFA concentrations at the end of each batch is shown in Figure 3b. The dosed medium in the anode and cathode chambers had stable composition and the conductivities kept at 5.15±0.12 and 4.52±0.23 mS/cm, respectively. At the end of each batch, the conductivity of the anolyte and catholyte increased a little ranging from 5.51±1.14 to 6.15±0.77 mS/cm and from 5.31±0.14 to 5.98±0.52 mS/cm, respectively due to ions migration and redox reaction. When the VFA concentration was lower than 30 mM, the

conductivities of the synthetic digestate in the middle chamber were below 7.2 mS/cm and the substrate concentration was more influential on the electricity generation. When the VFA concentrations were above 30 mM, the higher VFA concentration increased the substrate to the anode microbial community and the conductivity which together affected the current generation. The conductivity in the synthetic digestate increased from 7.12±0.89 (30 mM) to 14.45±1.81 mS/cm (200 mM). The internal resistance would be lowered a little with the minor conductivity increase.[23, 24] The current would be elevated further at higher VFA concentrations which was consistent with the results shown in Figure 2b, where the second positive linear relationship from VFA levels of 30 to 200 mM. Thus, the current generation of the biosensor was affected by both the substrate concentration and the solution conductivity.

Effect of protein and lipid on the biosensor performance. The anodic biofilm in BESs is able to metabolize a variety of organic compounds after a period of adaptation.[23] Protein and lipid are common organic matter in AD reactors which can also be utilized as substrates by exoelectrogens on the anode.[25] The current generation of existing MFC-based VFA sensors would be proportional to all the degradable substrates and the result would be overestimated since samples were dosed into the anode chamber and bacteria could not differentiate between VFAs and other assimilable organic matter. In this study with our BES biosensor, we hypothesize that only anionic substances such as VFAs can transport through the AEM while other non-ionic complex organic matter would be retained. Then the overestimation that was caused due to protein and lipid and other organic molecules on the estimation of the VFA by the biosensor could be eliminated. To prove such hypothesis, 2 g/L gelatin and 9.2 g/L glyceryle trioleate (GTO), as model of protein and lipid, respectively, were added in the synthetic digestate in the middle chamber of the BES (R1). In a similar reactor, synthetic digestate containing 0.2

g/L gelatin and 0.92 g/L GTO was dosed into the anode chamber (C1). The current density of the two BES reactors is shown in Figure 4. The current density in R1 kept below 0.2 mA/m^2 during 24 h operation while that in C1 increased above 8.0 mA/m^2 after 5 h. After 24 h operation, the detected ammonia nitrogen in C1 was 0.12 g-N/L suggesting the protein was digested when it was available to the bacteria. The low current density in R1 indicated that gelatin and GTO was retained by AEM effectively. In contrast, ionized VFAs could easily pass through the AEM and were utilized to produce electricity. Overall, the BES biosensor could effectively eliminate the interference of protein and lipid and could monitor the VFA concentrations correctly.

Effect of ionic strength and external resistance. To investigate the influence of inorganic ionic substances on the VFA biosensor performance, different concentrations of NaCl and Na_2SO_4 (10, 30 and 100 mM) were dosed into the synthetic digestate. The conductivity in the middle chamber, the current density and the accumulated VFA concentrations in the anode chamber after 5 h, as the function to the varying VFA concentrations in the synthetic digestate are displayed in Figure 5. High salinity increased the conductivity and the anion concentration which may compete with VFA species on the transportation via the AEM. Compared to the synthetic digestate without an additional salt, the average increases in conductivity were 45%, 78% and 149% by adding 10, 30 and 100 mM NaCl, respectively. When 10, 30 and 100 mM of Na_2SO_4 were dosed, the conductivity increased by 48%, 108% and 196% in average, (Figure 5a and 5b). In Figure 5c and 5d, the smallest current densities at the lowest conductivity demonstrated that the increased ionic strength was advantageous for the bioelectricity production. High ionic strength benefited the system by reducing the internal resistance. As shown in Figure 5e and 5f, accumulated VFAs in the anode decreased along with salt concentrations. High salt concentrations led to a more competitive migration of Cl^- and SO_4^{2-} to balance the charge.

Relatively less ionized VFAs passed through the membrane which played a negative effect on electricity production. Current density increased linearly from 0.06±0.03 to 9.37±0.69 mA/m^2 (1-30 mM VFAs) and from 9.37±0.69 to 11.15±0.37 mA/m^2 (30-200 mM VFAs) at 10 mM NaCl. When 30 mM NaCl was added, the current density increased further from 0.14±0.08 to 12.25±1.92 mA/m^2 (1-30 mM VFAs) and from 12.25±1.92 to 13.48±1.32 mA/m^2 (30-200 mM VFAs). This is because the effect of conductivity was more significant than the competition between inorganic anions and ionized VFA on the biosensor performance. When the salt concentration was further increased to 100 mM, the current densities decreased lower than those at 30 mM salt concentration. It could be due to that the high conductivity could not lower the internal resistance much further while the competition on the anionic transportation was enhanced.

When the VFA concentration in the synthetic digestate was less than 30 mM, the differences in current densities (Figure 5c and 5d) and accumulated VFA concentrations in the anode (Figure 5e and 5f) under different salt concentrations were not significant. This is because the substrate was the main limiting factor for current generation compared to ionic strength. The results indicate the biosensor's applicability to the AD processes with varied salinity at VFA levels below 30 mM. When enough substrates were supplied, the difference of accumulated VFAs and current densities under varying ionic strength were obvious because ionic strength played a main role. Thus, a calibration would be needed for the samples with high salinity at high VFA levels. In addition to the sensor calibration, the effluent from AD plants treating high salinity wastewaters could be diluted to avoid such interference. At the same concentration, Na_2SO_4 rather than NaCl contributed more in the conductivity increase, which was in line with the steeper slopes in correlation curves between the current densities and VFA concentrations. Less

VFA were accumulated in the anode when Na_2SO_4 was added in the synthetic digestate instead of NaCl. It could be due to that more charges can be balanced when the same amount of double-valent SO_4^{2-} migrated through the membrane compared to monovalent Cl^-, with respect to the constant ion exchange capacity of the membrane. In previous report[24], the high NaCl concentration was proved to be inhibitory for the anaerobic microorganisms. In our work, the sample was separated from the anode with the AEM which improved the biosensor's ability to resist high salinity. Thus, the biosensor has the potential to monitor AD processes treating high salinity wastewaters. Cations such as ammonium which is one of the important substances in AD reactors were not studies here, as they would transfer toward the cathode and not compete with VFA species on the transportation.

Apart from the ionic strength, the external resistance is another factor that has an influence on the electron flow rate, microbial communities and the sensor performance.[26] Thus, different external resistances (10, 180, 518 and 1000 Ω) were applied in the system to evaluate the impact. The current densities at 5 h against different VFA concentrations are displayed in Figure 6a. The application of a lower external resistance allowed a relatively higher current density. Two linear relationships were observed between the current density and the VFA concentration for all the tested external resistances. The difference in the current density under different external resistances was much bigger at higher VFA concentrations than that at lower VFA concentrations. This could be due to that the substrate concentration was the main limiting factor for current production at low VFA concentration, while the external resistance became the main limiting parameter at higher VFA concentrations. Lower concentrations of VFA accumulated in the anode at low external resistance as shown in figure 6b. As the same ionic strength and species were applied under all the external resistances, there were no big differences in the

amount of migrated ionized VFAs. However, high current outputs with low external resistances demonstrated fast rates of respiration and more substrate consumption.[27] So less VFA accumulation was obtained. The results suggested that the external resistance was important for the sensor's sensitivity. Changes in current related to different substrate concentrations will be amplified at low external resistance. However, relatively larger deviations in the current generation were observed with the low external resistance. This might be because the external resistance is close to the internal resistance or even lower than the internal resistance. In this case the internal resistance played a more important role in the system and easily fluctuated with respect to the environmental conditions. Thus, the sensor subjected to environmental changes will be more robust and stable at high external resistance in the long run.

Application in real AD effluent. The BES biosensor was then tested with real AD effluents to verify its applicability. Five samples were taken from four lab-scale AD reactors. The determination of the VFA composition and the operational data of the reactors were given in Table S1 and S2 (Supporting Information). The samples were stored at 4°C before used except sample 3 which was stored in the incubator at 55.3°C to deplete the substrate. The results obtained from the biosensor (Figure S1) and GC are summarized in Table 1 along with some characteristics of the samples. The values obtained from the biosensor were close to those measured by GC despite the samples with varied VFA compositions and reactor operating conditions. Anova analysis showed negligible difference between the data achieved by our biosensor and GC ($F=0.90>F_{(5,\ 4)}=0.16$, $P<0.05$) which demonstrated the accuracy of the biosensor. As shown in Figure S2, our biosensor ran stably and sensitively even the samples were fed successively. It can be concluded the biosensor has shown reliable results for detecting the VFA concentration of AD effluents.

306 **Perspectives**. The present work for the first time demonstrated the applicability of the
307 innovative three-chambered bioelectrochemical sensor for online VFA monitoring during AD
308 processes. Compared with traditional off line sensing technologies, the novel bioelectrochemical
309 biosensor developed here has several advantages. First, no external power is needed since the
310 reactor can power itself from substrate oxidation, which suggests the suitability for in situ and
311 long-term monitoring. Secondly, there is no need of a signal transducer because the current
312 produced can be directly used as a measurement of the VFA concentrations. Compared to the
313 MFC-based biosensors, the biosensor still has its own merits. The biosensor separated the
314 biofilm and the bulk substrate innovatively which can distinguish VFAs from other complex
315 organic matter; therefore results will be much more reliable. Moreover, the detection range was
316 widened significantly as the bulk solution was dosed in the middle chamber. Thirdly, the AEM
317 can also protect the anodic microbial community from high salinity, inhibitors (e.g.; NH_4^+) and
318 toxicants (e.g.; metal ions) presented in the AD effluents.
319 After 7 months operation, reproducible and stable current was achieved without membranes
320 cleaning or replacement which demonstrates the robustness of the biosensor. Moreover, the
321 response of 5 h is adequate for frequent VFA monitoring in AD reactor systems with relatively
322 long hydraulic retention times which are usually more than 10 days. The response time of our
323 biosensor at present stage is comparable with other described VFA sensors. Further reduction of
324 the response time below 5 hours which is the current response time in our biosensor, could be
325 advantageous in some applications. However, for conventional biogas processes, the response
326 time is more than adequate. Some suggestions for increasing the frequency for VFA monitoring
327 could be to increase the electrical field of the anode and cathode electrodes to accelerate the VFA
328 migration and enhance the voltage output further. Thus, faster equilibrium state could be

329 achieved with a shorter response time. The response time could be shortened through system
330 optimization (e.g., a new reactor configuration and the electrode modification). This could be
331 needed for AD processes operating at HRTs much lower than a couple of days, which is however,
332 very seldom the case. To further consolidate the application of the biosensor to other anaerobic
333 processes, it would also be interesting to test the biosensor with more different VFA
334 compositions. Besides, the anode solution could be operated in a mode with regular refilling with
335 anodic solution to eliminate the effect of the VFA accumulation on the detection during
336 continuous monitoring. Therefore, supplying an external voltage, minimizing the architecture,
337 modifying the anode and cathode materials, continuous monitoring with different anaerobic
338 processes, shortening the response time and promoting the practical application of the VFA
339 biosensor will be the focus in future works.
340

341 **Acknowledgement**

342 The authors would like to acknowledge financial support from the China Scholarship
343 Council and the technical assistance by Hector Gracia with analytical measurements. The authors
344 also thank Xiaohu Li for advice on experiment and thank Ilaria Bassani, Xinyu Zhu and
345 Temesgen Mathewos Fitamo for supplying AD effluents from their reactors. This research was
346 supported financially by The Danish Council for Independent Research (DFF-1335-00142).

347 **Supporting Information Available**

348 Table S1, Table S2, Figure S1 and Figure S2 as noted in the text. This material is available
349 free of charge via the Internet at http://pubs.acs.org/
350

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437 **Table 1**. Determination of VFAs in real AD effluents by the BES sensor and GC and characteristics of
438 the samples

Sample	VFAs[a] (mM)	VFAs[b] (mM)	pH	Conductivity (mS/cm)	TS (g/L)	VS (g/L)
1	3.86±0.73	3.02±0.06	7.76±0.1	12.98±0.24	16.65±0.03	10.09±0.02
2	9.32±0.35	8.83±0.89	9.01±0.2	17.16±0.17	15.16±0.04	8.01±0.04
3	0±0	0.04±0.02	8.07±0.2	6.44±0.12	3.02±0.04	0.58±0.03
4	6.25±0.58	6.67±0.54	8.55±0.1	15.09±0.15	10.27±0.04	3.76±0.03
5	35.30±0.92	36.82±0.65	7.73±0.1	9.73±0.13	16.18±0.12	11.91±0.07

439 [a]Measured by GC method.

440 [b]Measured by the biosensor.

441

442

443 **Figure Caption**

444 **Figure 1**. Sensor prototype (a) and schematic diagram (b). A, the anode chamber; M, the middle chamber;
445 C, the cathode chamber; AEM, the anion exchange membrane; CEM, the cation exchange
446 membrane.

447 **Figure 2**. Typical current generation from the biosensor during the batch mode experiment (a) and the
448 relationship between current density and VFA concentrations at 5 h (b).

449 **Figure 3**. pH (a) and conductivity (b) in the bioreactor at 5 h with various VFA concentrations. Error bars
450 represent the range of duplicate tests.

451 **Figure 4**. Current density from the biosensor. R1: 2.0 g/L gelatin and 9.2 g/L GTO were dosed in the
452 middle chamber; C1: 0.20 g/L gelatin and 0.92 g/L GTO were dosed in the anode chamber.

453 **Figure 5.** The synthetic digestate conductivity in the middle chamber (a, b), the correlation curves
454 between VFA levels and current density (c, d) and the accumulated VFAs in the anode chamber
455 (e, f) at 5 h under different NaCl and Na_2SO_4 concentrations.

456 **Figure 6**. The correlation curves between VFA levels and current density (a) and the accumulated VFAs
457 in the anode chamber (b) at 5 h through different external resistances.

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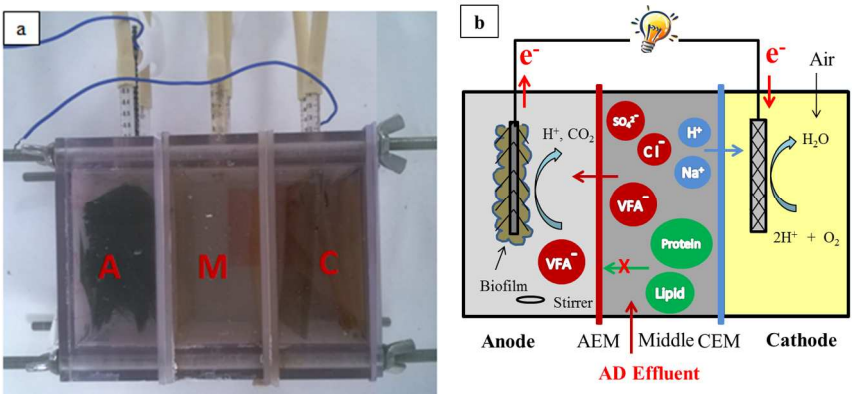

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460

461 **Figure 1**

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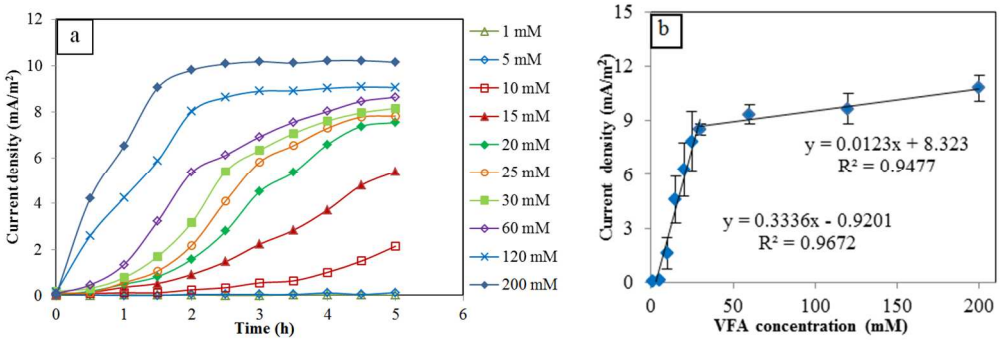

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Figure 2

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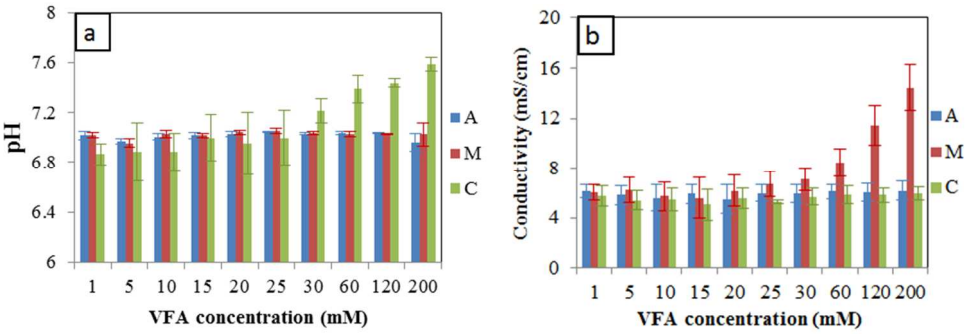

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469 **Figure 3**

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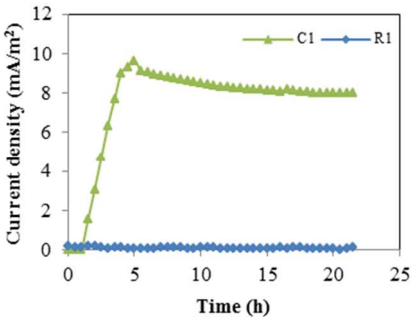

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473 **Figure 4**

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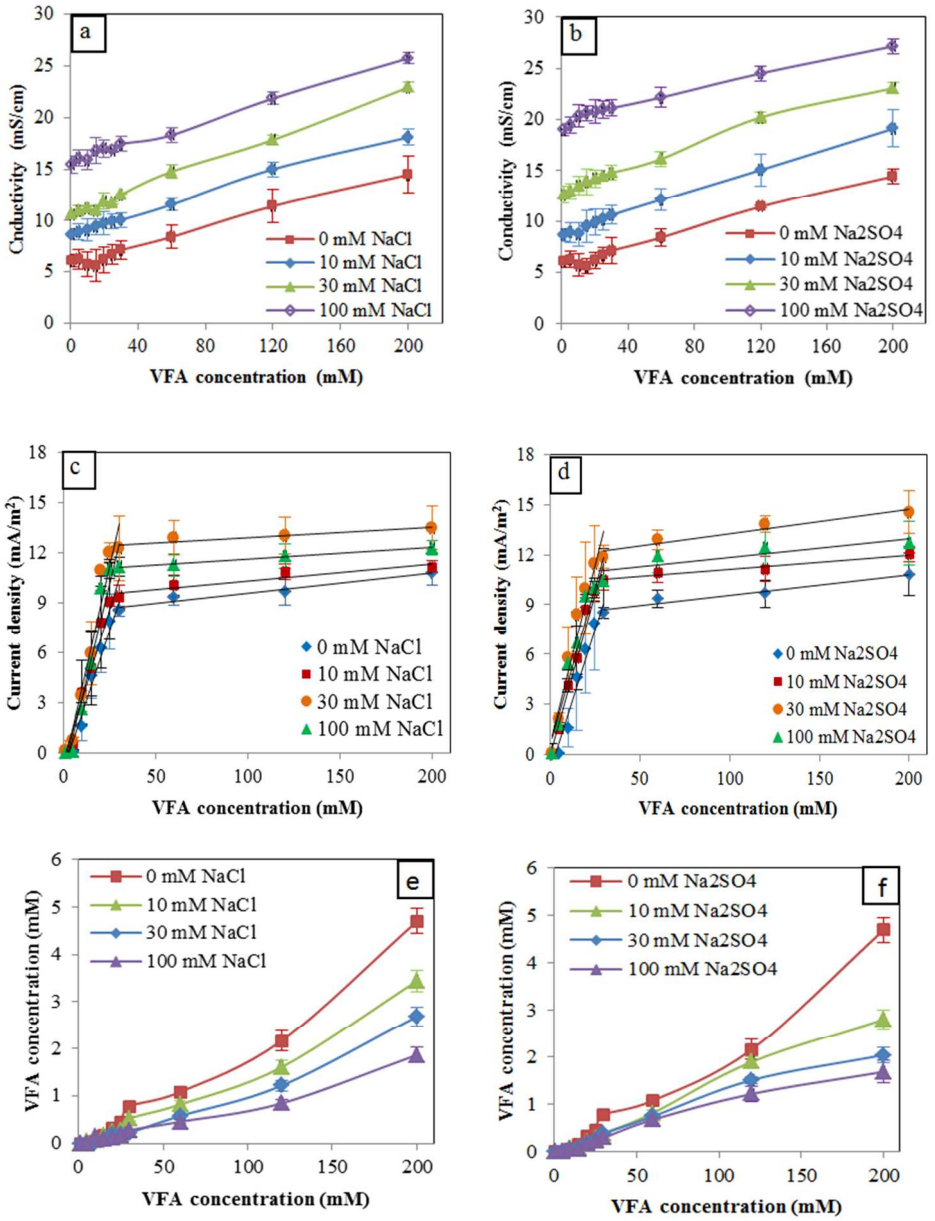

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479 **Figure 5**

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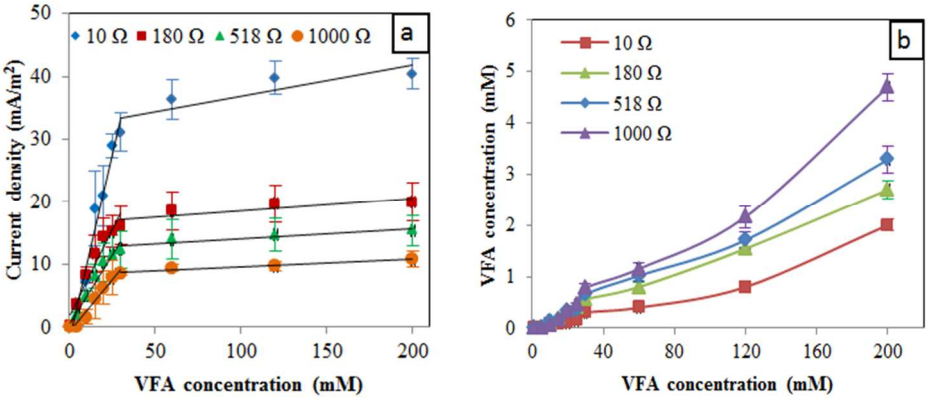
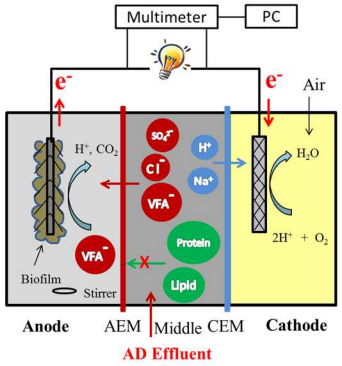

a
10 Ω
180 Ω
518 Ω
1000 Ω
Current density (mA/m²)
VFA concentration (mM)
b
10 Ω
180 Ω
518 Ω
1000 Ω
VFA concentration (mM)
VFA concentration (mM)

Figure 6

496 **TOC art**



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