Performance of a Microbial Fuel Cell–Based Biosensor for Online Monitoring in an Integrated System Combining Microbial Fuel Cell and Upflow Anaerobic Sludge Bed Reactor

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ABSTRACT

A hybrid system integrating a microbial fuel cell (MFC)-based biosensor with upflow anaerobic sludge blanket (UASB) was investigated for real-time online monitoring of the internal operation of the UASB reactor. The features concerned were its rapidity and steadiness with a constant operation condition. In addition, the signal feedback mechanism was examined by the relationship between voltage and time point of changed COD concentration. The sensitivity of different concentrations was explored by comparing the signal feedback time point between the voltage and pH. Results showed that the electrical signal feedback was more sensitive than pH and the thresholds of sensitivity were $S=3\times10^{-5}$V/(mg/L) and $S=8\times10^{-5}$V/(mg/L) in different concentration ranges, respectively. Although only 0.94% of the influent COD was translated into electricity and applied for biosensing, this integrated system indicated great potential without additional COD consumption for real-time monitoring.

Keywords：Upflow anaerobic sludge blanket; Microbial fuel cell; Biosensor; Signal feedback; sensitivity.

1. Introduction

Due to obvious advantages in removing pollutants and recovering energy, upflow anaerobic sludge blanket (UASB) reactors have became widely used for wastewater treatment in the last decade (Pant et al., 2010). The UASB reactor has some special characteristics in treating wastewater. Previous studies found that its ability to remove COD was superior to other biological treatment processes and the advantage of lower cost and less sludge production (Jing et al., 2013; Lu et al., 2015; Lu et al., 2016). So far, numerous studies have focused on the applicability of the UASB process to various industrial and municipal wastewaters (Onodera et al., 2014). However, the anaerobic microorganism acted as the core of operating. It was both complex and harsh for environmental conditions and sensitive to the changes of water quality and/or organic loading shock. Therefore, the absence of reliable dynamic information and complete online monitoring methods for anaerobic digestion are the current obstacles in applying the UASB process. Although the sensors are critical to provide stable and effective precaution strategies for the anaerobic reaction process, the biosensor performance is affected as the extension of running time, which cause inherent problems, such as equipment corrosion, signal fuzzy, and deviation for date analysis. To date, only very limited work has been undertaken to assess the UASB system's operating conditions.

Microbial fuel cell (MFC) is a type of biological electrochemical device subject to intensive investigation, and the last three decades have witnessed significant developments in the environmental and energy field (Li et al., 2011). MFCs are able to

convert chemical energy into electricity directly via bioprocesses catalyzed by exoelectrogenic microorganisms (Logan et al., 2006; Lovley, 2006; Srikanth & Venkata Mohan, 2012). Therefore，due to the possibility of wastewater treatment and utilization of the electrical signals for biological sensing simultaneously. MFCs are considered as prosperous applications for facilitating sustainability. Since voltage can be monitored easily online, MFC has the ability to function as an inexpensive on-line biosensor that operates in a steady way and is constructed with low-cost material (Seop et al., 2005; Kim et al., 2003) where microorganisms in the anode compartment act as biological recognition element whereas electrodes and proton exchange membrane (option) serve as a transducer (Kumlanghan et al., 2007). As a consequence such an on-line MFC-based biosensor measured an electrical voltage as the signal, does not need any mediation to convert it into a signal (Feng et al., 2013). Compared to other kinds of biosensors, the main advantages of MFC are miniaturization, portability and real-time monitoring (Di Lorenzo et al., 2009).

A number of researchers have used biosensor as a converter utilized by weak voltage for monitoring water quality in real time (Xu et al., 2015) and a comparative table (Table S1) showed the significance of the achievements in the light of already published literature. For instance, Dual-chamber microbial fuel cells were employed for energy valorization of an untested substrate, the liquid fraction of pressed municipal solid waste (LPW). It can be a useful, direct tool to access the impact of process disturbances though the time-profile of bioelectricity production monitoring in

bioelectrochemical systems(Kook et al., 2016). Zhang and Angelidaki (2012) developed a submersible microbial fuel cell (SBMFC) as a biosensor for real-time monitoring of dissolved oxygen (DO) in water based on the electricity principle of MFC. Di Lorenzo et al. (2009) developed a Biochemical Oxygen Demand (BOD_5) biosensor fueled with artificial water. A linear relationship between MFC power output and BOD_5 concentration was established, indicating the applicability of real-time monitoring for BOD_{5b} (Di Lorenzo et al., 2009). A new wall-jet MFC sensor was designed by Liu et al. (2011) for the monitoring of anaerobic digestion process by the real-time detection of acetate based intermediates (Liu et al., 2011). A simple compact membrane MFC (MMFC) sensor by stacking two flat filter membranes without the proton exchange membrane(PEM) and paper reservoir was developed (Xu et al., 2015). The unique flat structure of MMFCs makes the direct installation on wastewater facilities and serves as an “on line sticker sensor” for in-situ real time wastewater quality monitoring with sensitivity and stability. Soon Bee Quek et al. (2015) developed an online low assimilable organic carbon (AOC) detection system for oxygen-saturated seawater by combining a suitably designed electrochemical oxygen removal cell with a MFC-biosensor. The coupling of an electrochemical cell with a MFC-biosensor can be effectively used as an online, rapid and inexpensive measure of AOC concentrations and as an indicator for biofouling potential of seawater (Quek et al., 2015).The subjects of variation of the current reflecting the toxic compounds in substrates were studied by other MFC-based biosensor studies (Jiang et al., 2015; Shen et al., 2013). However,

most analyses of MFC-based biosensor did not discuss the performance of biosensor in detail.

Based on the above issues, this research aimed to develop a novel monitoring system for the MFC-UASB process, which consisted of a single-chamber MFC where the UASB reactor worked as an anode chamber and the carbon cloth worked as an air-cathode. The objective of the series tests in this research is to investigate the performance of the MFC-based biosensor between different COD concentrations. Furthermore, optimum conditions are applied for studying the performance of the biosensor at various hydraulic retention times (HRTs) and external resistance to achieve a suitable condition for sensing. The development of a MFC-UASB system will provide a feasible method for UASB monitoring.

2. Material and methods

2.1 Integrated MFC–UASB system

The MFC-UASB was made of a plexiglass cylinder that consisted of an anode and cathode (Fig. S1). The UASB reactor worked as an anode chamber (internal diameter of 20 cm and height of 100 cm) with a working volume of 10 L. At the top of the reactor. A flow meter (LMF-1, Changchun Lvqingqi Co., Ltd., China) was connected to the gas outlet of the three-phase separator in the UASB. The MFC's structure was a single chamber without a proton exchange membrane (PEM) in which a piece of carbon felt that had an effective surface area of 64cm^2 (8cm×8cm) and 5-mm thick (Beijing Fengxiang Co., Ltd., China) served as an anode electrode and carbon cloth that had an

effective surface area of 50.24cm^2 with diameter was 8cm and 1.5-mm thick (HCP330N, Shanghai Hesen Co., Ltd., China) prepared as an air cathode electrode, respectively. Air was continuously sparged inside the cathode and functioned as the final electron acceptor. The MFC-UASB was operated by feeding the wastewater through the bottom of the UASB using a peristaltic pump (YZ1515, Tianjin Xieda Tech Co., Ltd., China). The electrode assembly was then submerged in the UASB reactor. The electrical circuit connected between the anode and cathode was fabricated by copper wires with a resistance box of 0-9999Ω and the voltage across resistor was collected by a multimeter.

The cathode was prepared according to the procedure that is briefly described below. Carbon cloth was placed in 5% polytetrafluoroethylene (PTFE) solution soaked for 10 min and then put in a muffle furnace (SX-GO5163, Tianjin Zhonghuan Co., Ltd., China) calcination for 30min at a temperature of 330□. A mixture of carbon powder (Shanghai Hesen Co., Ltd., China) and 40% PTFE solution were then applied to one side of the carbon cloth, air dried at room temperature for 2 hours, followed by heating at 330□ for 30 min. Additional brushing 60% PTFE solution onto the coating side, followed again by dying at room temperature and heating at 330□ for 30 min as Cheng described (Cheng et al., 2006a). Pt catalyst (HPT010, Shanghai Hesen Co., Ltd., China) was then applied to the other side of the carbon cloth, and this has been previously described using Nafion (D520, Shanghai Hesen Co., Ltd., China) as a binder (Cheng et al., 2006b).

2.2 Operating conditions

The anaerobic active sludge was taken from Jizhuangzi Wastewater Treatment Plant (Tianjin, China). It was then injected as an inoculum into the anode chamber after domestication that last for 30 days. To control the anode chamber in an anaerobic state, the nitrogen stripping method was used to eliminate all dissolved oxygen in the water throughout the whole experiment. Using soluble starch as the carbon source, urea served as the nitrogen source to synthesize simulative influent wastewater during the whole experiment. Phosphate buffer and trace metals were also employed in the experiment to ensure that the environment was suitable for growing bacteria (Lu et al., 2009). Each liter of the medium contained the following: $Al_2(SO4)_3 \cdot 18H_2O$, 0.0001 g; $MgSO_4 \cdot 7H_2O$, 0.003 g; $FeSO_4 \cdot 7H_2O$, 0.0035 g; $CaCl_2 \cdot 6H_2O$, 0.0003 g; $ZnSO_4 \cdot 7H_2O$, 0.0001 g; $CuCl_2 \cdot 2H_2O$, 0.0002 g; $MnSO_4 \cdot H_2O$, 0.0005 g, and the ratio of starch, urea, KH_2PO_4 was 100:7.2:1 as described by Punal (Punal et al., 2000). The pH value of the solution was adjusted to 6.8-7.2 with $NaHCO_3$ solutions. The DO level in the UASB reactor was continuously controlled below 0.2 mg/L. The MFC-UASB was operated in a temperature-controlled lab at (25±2) □.

During the test the mixed liquor suspended solids (MLSS) concentration of the anode compartment was 9600 mg/L at set-up. It was then increased step by step due to the cultivation of the sludge. To retain the stability of the sludge concentration, the mixed sludge liquor was charged from anode compartment regularly.

2.3 Experimental overview

The specific strategy was to retrieve the variation of slope produced by the

injection of different concentrations of water samples into the biosensor. For each range of COD concentration, the quantitative feedback metrics were analyzed to differentiate the signal feedback time point. This included the acceleration rate (AR, i.e. rate of voltage increase) and the subsidence rate (SR, i.e. rate of voltage decrease) (see Fig. S2).

2.4 Electrochemical and chemical analysis

The MFC was continuously monitored using a data acquisition system. The voltage and pH were recorded automatically at 5 min intervals using a digital electronic multimeter (VICTOR 86E, Shenzhen Shengli Electronic Tech Co., Ltd., China) and precise pH meter (PHG-210, Bohai Zhiyuan Tech Co., Ltd., China), respectively. Internal resistance was calculated by employing the polarization slope method as described by Fan (Fan et al., 2008). The COD was analyzed according to the standard method using chromate as the oxidant as described by Bullock et al. (1996) and the total COD removal (△COD，mg/L) was defined as the difference between the input and output COD values. DO was measured using a portable DO meter (HACH HQ30D, USA).

3. Results and discussion

3.1 Performance of signal feedback

The integrated system was incubated with synthetic wastewater in a continuous-flow mode at a COD concentration of 2000mg/L and HRT for 24h. With the active of microorganism gradually improved the voltage output of MFC became stable

at 0.36±0.01V and a COD removal rate at 85±2% occurred within a short start-up period (about 30 days). The feedback time worked as an important parameter for studying the feedback mechanism of the MFC biosensor. In the scenario where external resistance was 1000 Ω and HRT was 12 h, the processes of voltage change with different concentrations are shown in Fig. 1.

(Insert Figure 1 here)

Increasing concentration occurred at nearly 24 h, while voltage varied at around 30 h when new concentrations were added over a period of time (Fig. 1a-c). The voltage increased or decreased after a few hours interval rather than the changes occurring immediately when the influent concentration changed. The anode biofilm contacted organisms after a certain time. Consequently, the time needed from the concentration changed to a significantly changes in the voltage was defined as the feedback time.

Each substrate was injected into the reactor initially until the voltage reached a steady state and then switched to another concentration. A voltage date was recorded at every hour interval and the signal feedback time point was judged by the slope of voltage changed. A summary of the metrics generated by the process of varying different samples is shown in Fig. 1(d). For instance, the voltage varied slightly up to the first hour from the moment that concentration changed. No matter whether the voltage increased or decreased in different ranges, the value of SR and AR were around 0.001. This was mainly resulted from the large capacity of the reactor, any changes in the solution concentration were lower than the threshold value of sensor changed, so

that the voltage feedback changed slightly after mixing different concentration solution. In the first few hours, the variable quantities were tiny at every hour interval and AR or SR increased significantly until running for 6 h. The reactor solution was updated constantly with the continuous injection, and the change of concentration increased gradually. The variation in AR or SR was conspicuous as a consequence. In other words, the slope altered obviously after the biosensor felt the variation of COD over a period of time. Therefore, the point of feedback time was estimated according to the rate of voltage variation in unit time.

3.1.1 Effect of HRT

Due to the electrodes being located in the suspended layer of the UASB reactor, the quicker organic matter came into contact with the electrode passing through the sludge layer, the faster the signal feedback on substrate concentration occurred. Thus, the sensing property of the MFC biosensor would have suffered damage with extended feedback time caused by HRT. Table 1 reports the changes in voltage and feedback time response when the artificial water flow rate decreased as HRT increased.

(Insert Table 1 here)

Synthetic water was fed into the UASB reactor at different flow rates, and the voltages were monitored. The influent COD and external resistance were fixed at 3000 mg/L and 1000 Ω, respectively. Six different flow rate i.e. 0.42, 0.5, 0.65, 0.85, 1.2, and 2.5 L h^{-1} were tested for the anodic compartment. The substrate and resistor were the same as that for the optimization of anodic flow rate. By decreasing the flow rate and

increasing the HRT, the feedback time increased. For the first step, the feedback time was 2.5 h and both steady-state voltage and COD removal rate were lower. This illustrated that flow rate have double effects on MFC signal due to mass transfer and stability of biofilms. The system not being able to degrade the organic matter rapidly when there was a higher loading rate. The acid-base imbalance from the inside led to the microorganisms' activity being restrained, and the electron transport was delayed due to the accumulated VFA. With a longer feedback time and lower voltage, a worse COD removal rate was observed. The loading rate was suitable while HRT was 11.8 h. Metabolized microorganisms from the sludge layer were supplied by organic matter, and the activity of exoelectrogen which adhered to the anodic biofilm enhanced simultaneously. Consequently, the voltage and COD removal rate almost achieved the appropriate peak and feedback time, respectively. When HRT increased continuously, it was found that higher HRT resulted in longer feedback time, indicating that the feedback time prolonged with slower interaction between organics and electrodes.

3.1.2 Effect of external resistance

External resistance is another important parameter influencing the feedback time, which can not only delay the time of achieved peak voltage but also affect the rate of electronic mass transport and restrict the feedback time of the biosensor. Table 2 describes the changes in steady-state voltage, COD removal rate and feedback time for each external resistance.

(Insert Table 2 here)

Different external resistances had serious impacts on electricity generation in the MFC. According to the previous study, the system achieved better performance while HRT was 11.8h. Therefore, the tests were therefore done in which the external resistance varied from 100Ω to 1000Ω at a constant inlet COD concentration of 3000 mg/L and HRT 11.8 h to explore the effect of external resistance on the sensor of performance. The voltage stable peak increased by 143% from 0.134V to 0.325V when external resistance raised from 100Ω to 1000Ω. The feedback time increased with each step in external resistance, doubling from 2.1 h for a resistance of 100 Ω to 4.2 h for a resistance of 1000 Ω. Yet the COD removal rate hardly changed at all. Based on the experiments, it became clear that changes in external resistance might be the result of a serious change in voltage but the effect of feedback time was shorter than that of HRT. The sensitivity of MFC-based biosensor can be optimized by adjusting the values of flow rate and resistance.

3.2 Performance of signal feedback sensitivity

Sensitivity of the biosensor refers to the ratio between the changes in output values compared to the changes in measured values. The thresholds of sensitivity were S=K and S=2Kx across the entire measuring range for the biosensors which had a relationship of $y=Kx+b$ and $y=Kx^2+b$, respectively. Obviously, the values of each point differed since the sensitivity was in constant flux. The detection sensitivity was one of the basic performance indicators for the biosensor. However, high detection sensitivity does mean high accuracy, and this determined whether it could be used widely (Brzeska

et al., 2004).

The air-cathode MFC biosensor was tested with external resistance of 1000 Ω and HRT of 12 h, and the reactor was continuously fed with different concentrations of influent until a steady voltage was generated at approx. pH 7.2. Some different gradients of COD concentration were prepared in the 500-5000mg/L range, according to the steady-state voltage generated to detect the influent concentration. Each influent concentration recorded at least three replicates; the average voltage was calculated by three times. Fig. S3 shows the variation in steady voltage with COD concentration. In the whole tested range, sensor signal had a Monod type equation correlation with substrate concentration. Highest power was achieved at 3000 mg/L with the substrate concentration ranging from 500 to 5000 mg/L. This was mainly due to most of the substrates being consumed by the sludge for microorganism metabolism and methanogenesis as the influent concentration increased.

(Insert Figure 2 here)

There were still more surplus substrates decomposed by electricity-producing bacteria that generated electricity. Thus, the microbial metabolic reaction rate was enhanced. When the reactor was fed with a COD concentration of more than 3000mg/L, a decline in the voltage was obviously observed. It was indicated that due to the increased influent concentration, the accumulation of VFA occurred in the MFC-UASB, for example formic acid, acetic acid and propionic acid. MFC-UASB acidification resulted in decreased activity of electricity-producing bacteria. The correlation between

voltage and COD concentration was established and is shown in Fig. 2 (a) and (b). It is clear that the voltage (0.26±0.02-0.32±0.01V) increased linearly (regression correlation，R^2=0.9368) with COD up to 3000 mg/L from 500 mg/L and voltage (0.32 ±0.01-0.17±0.01V) decreased linearly (regression correlation，R^2=0.9326) with COD from 3000 mg/L to 5000 mg/L, respectively. The voltages were almost inversely proportional to the COD concentration when it reached a high level. According to S=K of the relationship y=Kx+b，the biosensor's sensitivity threshold was $S=3\times10^{-5}$V/(mg/L) while the COD concentration was 500-3000 mg/L and $S=8\times10^{-5}$V/(mg/L). Meanwhile the COD concentration was 3000-5000 mg/L. This indicated that the MFC-based biosensor had good sensitivity in different COD concentrations, but the value of sensitivity was not the higher the better. The measured values will be affected and become unstable if the value extends beyond a particular range.

The MFC-UASB biosensor sensitivity was investigated in a continuous mode with alkalinity of artificial water being 2000 mg/L (Lin et al., 2015). The MFC biosensor's sensitivity was reflected with the response time of voltage and pH at different operated COD concentrations. The experiments used influent concentrations that were changed every 80 h, and they operated for 160 h in each stage. As shown in Fig. 3(a) the artificial water concentration was 1000 mg/L for the first 80 hours. The change in pH was the same as voltage in that it rose in a stable way and slowly, then turned the concentration to 2000mg/L in the 80th hour. The voltage was increased significantly since the 83rd hour and remained steady at 0.27V. It indicated that the biosensor responded to different

solution concentrations without delay when it felt the variation. However, pH and voltage increased after the concentration changed for 10 h and 3 h, respectively. It can thus be seen that the feedback time which pH compared to voltage was 7 h later, indicating a response by the MFC-UASB system in terms of electric signal being more sensitive than pH. This was due to biodegradable organic matter could be directly converted to electricity via MFC, and MFC itself is an integration of signal generator and transducer, which reduces the consumption for external transducers and shortens the time necessary to switch from one transducer to another.

(Insert Figure 3 here)

Figures 3(b), 3(c), and 3(d) show a similar phenomenon in that the variation time where pH was compared to the electric signal (same as Fig. 3(a)). When the solution concentration changed to 4000 mg/L, pH declined steadily to 6.0 (Fig. 3(c)). This was mainly due to the substrate concentration being too high to make anaerobic microorganisms could not consume the organics totally in the degradation process of fermentation. The organic matter's failure to disintegrate resulted in anaerobic fermentation staying in the hydrolysis fermentation stage and pH declined. However, the intermediates such as volatile fatty acid produced by anaerobic fermentation were consumed gradually, and the buffering capacity of the inner system was strengthened when $NaHCO_3$ buffer solution was added to the aqueous solution. The decrease in pH was limited. A similar outcome was shown in a concentration of 5000mg/L (Fig. 3d). Therefore, no matter if it involved a high concentration (5000mg/L) or low

concentration (1000mg/L), the MFC-based biosensor through the electric signal feedback on the concentration was more sensitive than by pH and achieved an average of 8h in advance. The operation of the MFC-UASB system can be monitored online quickly and conveniently in future experiments.

3.3 Performance of reproducibility

Good reproducibility was necessary if the biosensor could be applied for a long time. The operating principle of the MFC-based biosensor was that the metabolic state in the process of anaerobic digestion was feedback according to the respiration in the anodic biofilm. Nevertheless, the microbial communities of the anodic biofilm will affect the recoverability of the biosensor. In addition, proton exchange membrane worked as another impact factor affected the electric voltage. There were proton exchange membranes adopted between anode and cathode in the microbial fuel cell conventional. Operating over a period of time, PEM will become fouled and electric voltage generated will be affected seriously (Kumlanghan et al., 2007). The MFC-based biosensor studied was single chamber without a proton exchange membrane, so the biosensor remained stable in the absence of membrane fouling.

(Insert Figure 4 here)

The experiments were tested with external resistance of 1000Ω and HRT of 12h, and the reactor was continuously fed with different concentrations of influent (1000 mg/L, 2000 mg/L, 3000 mg/L, etc.). The dynamic response of the MFC reactor voltage with COD concentration of 1000-5000 mg/L was investigated and depicted in Fig. 4.

The biosensor maintained a relatively stable response for voltage when the influent concentrations were identical. For instance, the steady-state voltages were almost 0.27V at three times of 1000 mg/L concentration. The relative standard deviation in the value of the calibration curve (Fig. 2b) was only 1.8%. When the influent concentration changed constantly and repeatedly, the deviation in each same concentration steady-state voltage was less. In addition, with the COD concentration increased to 5000mg/L, the steady-state voltage in stages 1 and 2 was 0.20V and 0.17V, respectively. The relative standard deviation was more than 15%. Considering the above, the low values of relative standard deviation for three replicate measurements (n=3) proved that the proposed biosensor could be potentially used as a sensitive and precise device for diagnosis applications with acceptable reproducibility and remained stable within an appropriate range of influent concentrations (1000-4000 mg/L). On the other hand, the property of reproducibility will be affected slightly when the concentration increased and beyond the limitation. The main reason was that acidification emerged in the UASB reactor, and the operation of the integrated system was compromised as a result. The MFC biosensor as a whole enjoyed stable and higher reproducibility for different variations of wastewater.

3.4 Evaluation of energy-distribution

For further insight into the allocations of energy for electricity production in MFC, the mass balances of COD were analyzed. When the artificial water COD concentration of 3000 mg/L was fed into the UASB reactor, the procedures of mass balance in the

hybrid system are shown in Fig. S4. The TCOD in the influent was calculated as 59760 mg/d. After anaerobic digestion and metabolization by microbes in the sludge layer, the COD was left in the MFC. The COD consumption in the MFC may include anaerobic digestion and process of generating electricity, and COD abatement related to electricity generation was calculated from the electrical charge extracted from the MFC (Wang et al., 2016):

$$e\Delta COD_{exp} = (32/RbFV) \times \sum_{i=1}^{n} Uiti$$

Where: 32 is the molar mass of oxygen (g/mol), R is the external resistance of the reactor (Ω), F=96485 is the Faraday constant (C/mol), V is the solution volume (m^3), b=4 is the number of electrons exchange per mol of oxygen oxidized (mol^{-1}), U is the voltage (V) and t is the time (s). Based on the above equation, the electricity generation process in MFC could be calculated as 568 mg/d, and it could be inferred that only about 0.94% energy of the sewage in the integrated system was turned into electricity for biosensing. A small proportion of COD consumption was related to the electricity generating process and played a significant role in biosensing.

The above results suggest that the air-cathode MFC has the potential to be a precise and rapid COD sensor. Compared to other conventional COD sensor or measurement methods, the air-cathode MFC-based biosensor for UASB exhibited many improvements and difference. Firstly, in principle, the electrical voltage as a direct real time measure of COD could be easily measured. With regards to other sensors based on the electrochemical or physical principles, the MFC biosensor based on the biochemical

reactions although current biofilm MFC has the performance of long-term stability due to the nature of biofilm. Secondly, it could rapidly respond better to the changes in COD concentration with voltage compared to other parameters, which had to be accumulated for process monitoring. Thirdly and lastly, the biosensor system remained stable and had superior reproducibility. It had strong adaptability in different conditions and the development in the air-cathode MFC integrated with the UASB system also provided an opportunity to widen the application of the MFC-based biosensor. The MFC-UASB system has the potential to integrate well with wastewater treatment, energy recovery, biosensing and real-time online monitoring.

Further works are expected by the use of online real time MFC biosensor. The integrated system can also be validated with a more complex influent with a better approximation to a typical industrial waste and leaving the UASB running for longer times. On the other hand, combined analysis based on different sensors had been proved to provide crucial support for the process diagnosis and control of anaerobic digestion. MFC biosensor can be coupled with other measurements, for instance gas flow, VFAs, gas content to justify and evaluate the performance of MFC and operation of UASB.

4. Conclusion

This study evaluated the performance and feasibility of air-cathode MFC used as a biosensor for monitoring the operation of UASB which utilized electrical signal. Compared to pH for the load variation, the electrical signal feedback had higher sensitivity due to MFC itself as an integration of signal generator and transducer, which

reduced the consumption for external transducers. During the optimal procedure that studied, the thresholds of sensitivity expressed by voltage in different ranges recorded were highly reproducible. This research has provided valuable information that can assist in the future development of COD monitoring.

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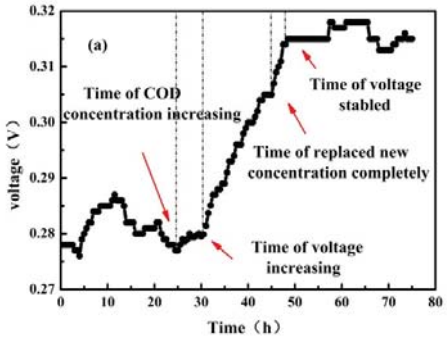
Figure Captions

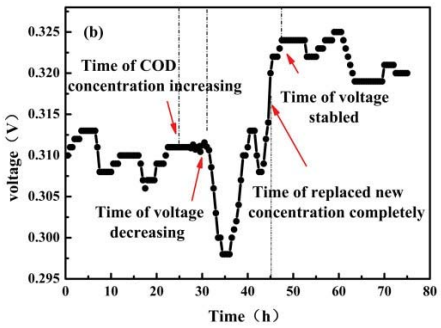
Fig.1. The variation of voltage in different influent COD concentrations. (a). COD concentration up to 2000mg/L from 1000mg/L; (b). COD concentration up to 3000mg/L from 2000mg/L; (c). COD concentration up to 4000mg/L from 3000mg/L; (d).The summary of metrics with different concentration. External resistance：1000Ω. HRT：12h.

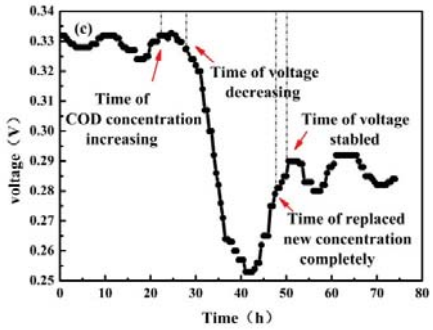
Fig.2. MFC–UASB response to COD concentration. (a) calibration curve (500-3000mg/L); (b) calibration curve (3000-5000mg/L).

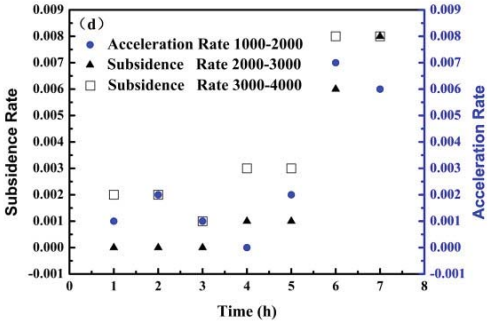
Fig.3. The variation of voltage and pH in different COD concentrations. (a). the variation in COD concentration of 1000-2000mg/L; (b). the variation in COD concentration of 2000-3000mg/L; (c). the variation in COD concentration of 3000-4000mg/L; (d). the variation in COD concentration of 4000-5000mg/L.

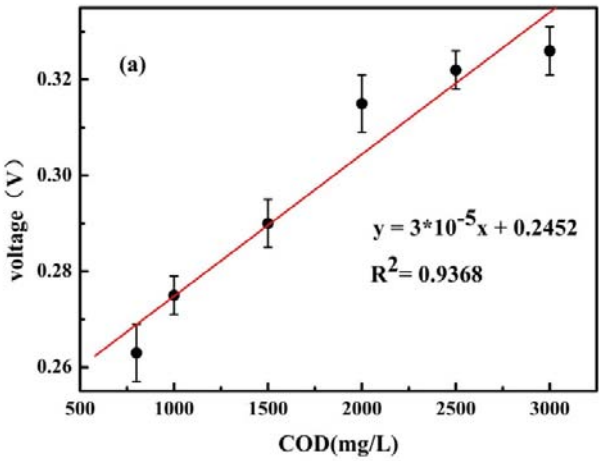
Fig.4. The voltage response to different inlet COD concentration of the MFC-based biosensor

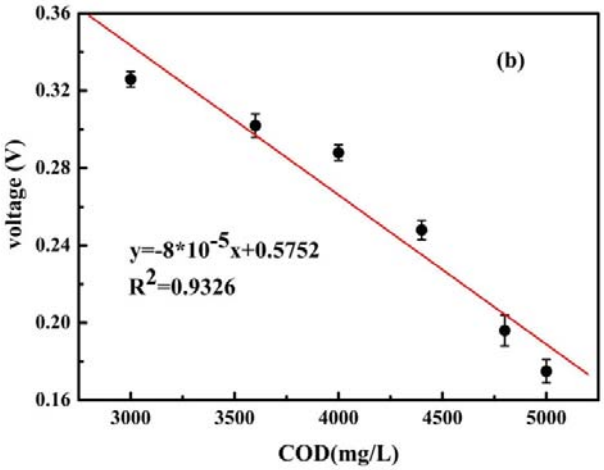


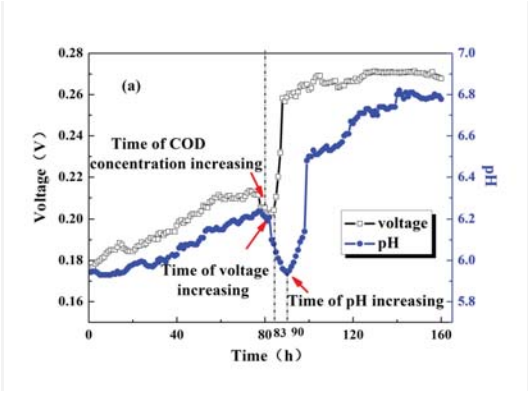


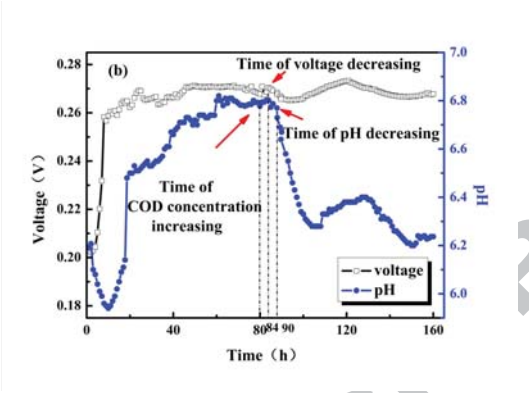


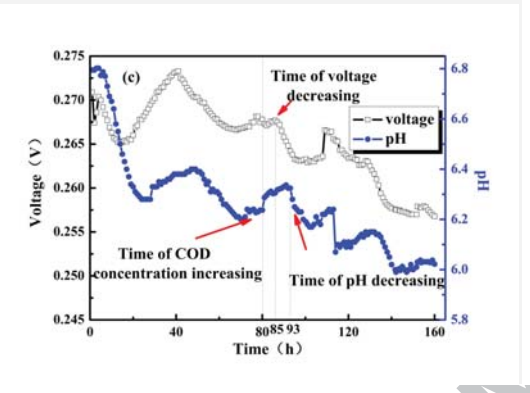


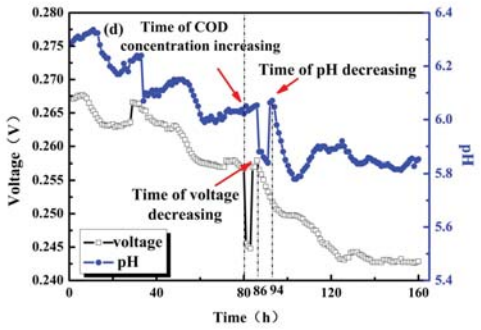












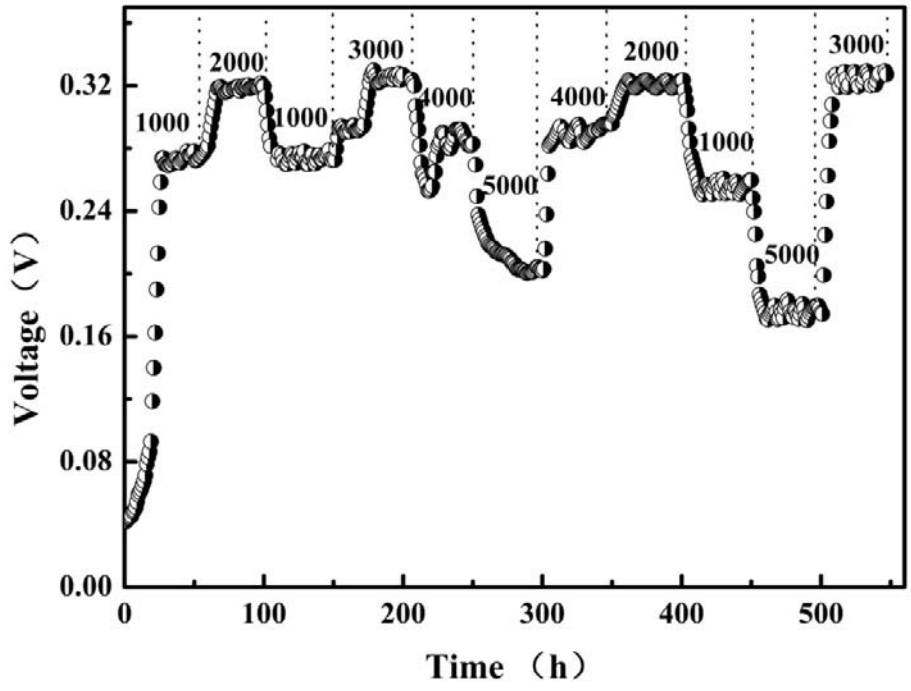


Table 1
Effect of HRT on voltage generated and feedback time

Flow rate (L/h)	HRT (h)	Steady-state voltage (V)	COD removal rate (%)	Feedback time(h)
2.5	4	0.239±0.008	69±3	2.5±0.2
1.2	8.3	0.286±0.005	78±1	3.2±0.1
0.85	11.8	0.325±0.01	86±2	4.2±0.3
0.65	15.4	0.335±0.004	88±1	5.5±0.3
0.5	20	0.306±0.004	85±2	6.3±0.2
0.42	24	0.262±0.006	82±2	8.6±0.4

Table 2
Effect of external resistance on voltage generated and feedback time

External resistance (Ω)	Steady-state voltage (V)	COD removal rate (%)	Feedback time (h)
1000	0.325±0.01	86±2	4.2±0.4
500	0.275±0.008	88±1	3.6±0.2
300	0.236±0.01	84±3	2.8±0.2
200	0.184±0.007	83±2	2.4±0.1
100	0.134±0.008	85±1	2.1±0.2

HIGHLIGHTS

A novel MFC-UASB system was established for real-time online monitoring rapidly and steady.

Better signal feedback sensitivity and reproducibility were achieved when COD concentration changed.

The MFC-based biosensor through the electric signal feedback on the concentration was more sensitivity than by pH.

Graphical Abstract