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Optimization of sensing performance in an integrated dual sensors system combining microbial fuel cells and upflow anaerobic sludge bed reactor

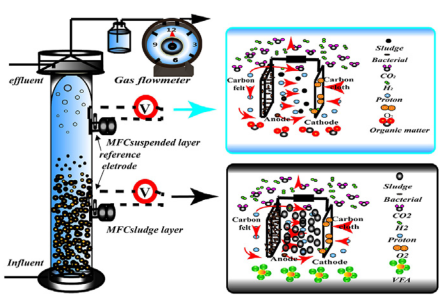
Hui Jia [a, b, **], Wenbin Liu [a, b], Jie Wang [a, b, *], Huu-Hao Ngo [c], Wenshan Guo [c], Hongwei Zhang [a, b]

[a] *State Key Laboratory of Separation Membranes and Membrane Process, Tianjin Polytechnic University, Tianjin 300387, China*
[b] *School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300387, China*
[c] *Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia*

HIGHLIGHTS

- A new type of UASB-MFC dual sensor system was developed for UASB operation performance online monitoring.
- The $MFC_{suspended\ layer}$ for effluent COD monitoring and the $MFC_{sludge\ layer}$ for TVFA monitoring.
- The adsorption saturation phenomenon of activated sludge was proved by three-dimensional fluorescence method.
- The sensor's characteristics were investigated based on the electrochemical method and microbial community analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

Two bio-cathode microbial fuel cells (MFCs) with Upflow anaerobic sludge blanket (UASB) are integrated to construct a UASB-MFC dual sensors system, developed to solve the problems of low accuracy and less information about the single sensor system. The bio-cathode MFC is developed as the biosensor for UASB operation performance and online monitoring. Owing to the biosorption of anaerobic microbes, the MFC in the suspended layer is more suitable for effluent chemical oxygen demand (COD) real-time monitoring, and the MFC in the sludge layer is more suitable for total volatile fatty acid (TVFA) real-time monitoring. Electrochemical analysis discovers that the lower electron transfer resistance determines the higher sensitivity of MFC in the suspended layer. The difference in species abundance indicates that TVFA has a stronger inhibitory effect on MFC in the sludge layer. The novel UASB-MFC dual sensors

* Corresponding author. State Key Laboratory of Separation Membranes and Membrane Process, Tianjin Polytechnic University, Tianjin 300387, China.
** Corresponding author. State Key Laboratory of Separation Membranes and Membrane Process, Tianjin Polytechnic University, Tianjin 300387, China.
E-mail address: wangjiemailbox@163.com (J. Wang).

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COD
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Electrochemical

system shows promising potential for COD and TVFA simultaneous online monitoring, which can enhance the reliability of anaerobic reaction.

1. Introduction

Anaerobic digestion has many significant advantages, such as low energy requirement, minimal sludge production, and possible energy recovery (Qian et al., 2016; De-Vrieze et al., 2015). Upflow anaerobic sludge blanket (UASB) reactors constitute a biological treatment process for anaerobic digestion of organic matter, because of their high rates of removal and due to changing temperatures and pH being well adapted to UASB reactors, which makes the UASB reactor widely used in the field of sewage treatment (Ahmad et al., 2011; Jing et al., 2013; Li and Sung, 2015). In spite of UASB reactors are developing rapidly, however, poor operational stability has become the main problem in its widely commercial application (Chen et al., 2008). During the UASB operation, acid forming and methane forming micro-organisms differ widely in terms of physiology, growth kinetics, nutritional needs, and sensitivity to environmental conditions (Wang et al., 2017). Failure to maintain the balance between these two groups of micro-organisms is the primary cause of reactor instability (Demirel and Yenigun, 2002). When the inhibitory substances are present in substantial concentrations in wastewater and sludge, it often cause the anaerobic reactor upset and failure. A wide variety of substances have been reported to inhibit the anaerobic digestion processes, such as the accumulation of organic acids and low pH. A material may be judged to be inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth. Therefore, the stability of the anaerobic reaction can be controlled by real-time monitoring the concentration of TVFA. (Lee et al., 2014). Meanwhile, the effluent COD concentration can intuitively reflect the treatment efficiency of the UASB reactor, but traditional methods cannot conduct online monitoring of the TVFA and COD.

Microbial fuel cell (MFC) is a new technology based on microorganisms. MFC can degrade biomass to produce electrical energy through microbial degradation (Liu et al., 2005; Zhang et al., 2014; Wang et al., 2015). However, MFC electricity production performance is relatively poor and the energy required for power is difficult to collect, so the electricity generated cannot be used effectively. However, use the characteristics of MFC can convert chemical energy into electrical energy, which has many potential applications, one of the most important being for electrical signals produced as a biosensor (Logan et al., 2006). Compared with other sensors, the feedback of MFC-based biosensor is directly by measuring the voltage as a signal, and no other signal conversion is required (Feng et al., 2013). The MFC-based biosensors have many advantages over conventional analytical techniques, such as working on-site, the possibility of portability and miniaturization. Furthermore, biosensors do not require consuming extra energy during sensing (Kaur et al., 2013). Previously, MFC-based biosensors have been widely used for water quality monitoring. For example, Wu developed a green MFC-based biosensor for in situ Chromium (VI) measurement in electroplating wastewater, and it can potentially help in preventing the violation of effluent regulations (Wu et al., 2017a, b). Jiang developed a novel MFC sensor with bio-cathode sensing element, and the biocathode sensing element made the MFC sensor directly applied to clean water body monitoring (Jiang et al., 2017). Yu developed a self-powered MFC-based biosensor for toxicity detection in water containing heavy metal ions (Yu et al., 2017). In the past decades, there are a variety of MFC coupling systems, such as MFC-MBR, MFC-Flocculation (Du et al. 2007), MFC-EAM (Wu et al., 2017a, b) and so on. Recently, a new system of UASB-MFC coupling has emerged, and many scholars have studied the system. Zhang developed a UASB-MFC-BAF integrated system for high strength molasses wastewater treatment and used the response surface methodology (RSM) for the investigation and optimization of the UASB-MFC integrated system for sulfate removal and bioelectricity generation (Zhang et al., 2009, 2012); Previously, Jia's study demonstrated that MFC as a biosensor for UASB reactor, its external parameters and energy distribution affect the sensing performance (Jia et al., 2016). However, many researches don't specifically mention when MFC as the sensor for integrated system, the position whether has an effect on the sensor's performance and the electricity generation or not. Moreover, compared with a single sensor system, the dual sensors system can obtain more reliable feedback information, which not only on-line monitor the comprehensive operation state of UASB reactor, but also improve the treatment efficiency of UASB reactor. Most importantly, the equilibrium state of anaerobic reaction can be judged by numerical calculation using the complete feedback signals.

Based on the previously mentioned study, this research takes the air-cathode single chamber MFCs as the biosensors for UASB-MFC integrated system, constructs the UASB-MFC dual sensors system and compares the different sensing performance of MFCs. A series of experiments were undertaken with the objective of making the MFC sensor achieve real-time monitoring, establishing the relationship between different COD or TVFA concentrations and MFCs voltage. In addition, the different sensing performances of MFCs were compared using electrochemical method and microbiological analysis.

2. Material and methods

2.1. UASB-MFC dual sensors system

The UASB-MFC system is a cylindrical structure composed of plexiglass (Fig. S1). The whole device is 1.1 m high, and the water influent at the lower end is provided with a water tank through a peristaltic pump (Kosuno, Guangdong Shantou Kosuno Limited by Share Ltd, model KSN-203). Install the same air cathode single chamber MFC at 0.35 m (sludge layer) and 0.85 m (suspended layer) from the bottom of the UASB reactor, the effective volume of MFCs are 44 mL. The anodes of MFCs are located on the inner wall of the UASB reactor, and a part of the columnar structure is extended as the chamber of MFC, with the air-cathode being placed at the outermost side of the chamber. The anode material is carbon felt (PAN, Beijing Peak Xiang Co) with an area of 35 cm^2 (5 × 7 cm) and is 5 mm thick. The cathode material is carbon cloth (HCP330, Shanghai Hesen Electric Co Ltd) with an area of 38.5 cm^2 (radius 3.5 cm) and 1.5 mm thick. The electrical circuit connecting between the anode and cathode with a resistance box of 0–9999 Ω, and the voltage across the resistor was collected by a multi-meter. In the middle position of the anode and cathode inserted Ag/Agcl (3 M KCL, Agcl saturated, +0.210 V versus standard hydrogen electrode, 25 °C) reference electrode.

The electrode material needs to be simply pretreated before it is used, and it briefly described below. Carbon felt with respectively, 1 mol/L sodium hydroxide and 1 mol/L hydrochloric acid were immersed for 24 h, and then soaked in distilled water for 48 h. The carbon cloth is firstly coated with 5% polytetrafluoroethylene (PTFE) solution (Shanghai Hesen Co., Ltd., China), and then put it into a muffle furnace after being dried. The temperature of the muffle furnace is controlled at 330 °C and subjected to calcination for 30 min. A mixture of conductex (Shanghai Hesen Co., Ltd., China) and 40% PTFE solution was then applied to one side of the carbon cloth, keep it ventilation at room temperature for 2 h. This was followed by heating at 330 °C for 30 min with an additional brushing of 60% PTFE solution onto the coating side, and then repeated the operation until the PTFE solution was completely attached to the carbon cloth surface. Finally, the other side was coated with Pt catalyst (Cheng et al., 2006).

2.2. Synthetic wastewater and operating conditions

The inoculation seeds sludge was taken from the secondary settling tank of Municipal Sewage Treatment plant (Tianjin, China). Cleaning removes impurities and then added to the UASB reactor, the initial sludge concentration was 8400 mg/L. During the start-up period, the UASB reactor with the sludge volume was approximately 2 L, after 500 h initial operation the MFCs voltage attained steady and then discharged about 1 L sludge from the lower end of the sampling hole. Subsequently, the sludge layer height became slightly higher than the $MFC_{sludge\ layer}$. Hydraulic retention time (HRT) lasted 12 h, with the water flow rate being 0.058 mL/s. Synthetic wastewater (Table S1) was applied in our research, using soluble starch as the organics provide carbon sources for microbes, urea as the nitrogen source and adjust the appropriate of C/N proportion, using Phosphate as the buffer solution, and add some trace metals in the reactor to ensure the metabolism of microorganisms (Lu et al., 2009).

2.3. Analytical methods and data representation

The COD was analyzed according to the standard method of potassium permanganate. The concentrations of volatile fatty acids were analyzed using a gas chromatograph (GC-2018, Shimadzu), the oven temperature was programmed to be held at 100 °C for 4 min and then increased from 100 °C to 200 °C at a rate of 10 °C/min. Both the injector and detector temperatures were 250 °C. Nitrogen served as the carrier gas. Pretreated samples (1 μL) were injected into the GC.

The voltage and the electrode potential of the MFCs were taken at 30 min intervals throughout the whole experiment by a data acquisition instrument (34972A. Keysight, USA). Polarization tests for the MFCs were performed using variable resistors ranging from 10 Ω to 9999 Ω, and each resistor was applied for 15 min (Doherty et al., 2015). The EIS and CV curves are tested by an Electrochemical Workstation (CHI 660E, shanghai Chenhua, China). Power (P), which was calculated by $P = I \times V$ and normalized by the cathode area. Current (I) was calculated at a resistance (R) from the voltage (V) by $I = V/R$.

Fluorescence spectra were acquired on a Hitachi Fluorescence Spectrophotometer. To obtain fluorescent EEMs, excitation wavelengths were determined every 5 nm from 250 to 400 nm; emission wavelengths were determined from 300 to 450 nm at intervals of 5 nm (Osbum et al., 2012).

3. Results and discussion

3.1. MFC voltage response to COD concentration

During the whole experiment, the MFC-based biosensors were connected with external resistance of 1000 Ω. The UASB-MFC dual sensors system was continuously fed with different concentrations of influent until a steady voltage was generated and then the concentration of effluent COD was tested, and the COD degradation efficiency of $MFC_{suspended\ layer}$ is 42.3%–53.1%, and the $MFC_{sludge\ layer}$ is 67.4%–74.6%. Meanwhile, the flow path and energy distribution of COD in the system were analyzed (Fig. S2). Most of the COD had consumed by anaerobic digest process (71–72.5%) in UASB-MFC system, and some of COD out of the reactor in the form of biogases (1–1.5%). Exoelectrogens on the anode surface of MFCs can decompose a part of COD into electricity (1.8–2.5%). Some of the unmetabolized COD remained in the excess sludge (1–3%), and the residual COD was discharged with the effluent (16–24%). The same operation was run for 5 cycles, and each cycle change of effluent COD levels could reach a similarly steady voltage, indicating good sensor sensitivity and repeatability. Fig. S3A illustrates the dynamic response of the $MFC_{suspended\ layer}$ biosensor to different COD levels. The voltage increased stepwise as the effluent COD rose from 100 to 400 mg/L. The highest voltage of $MFC_{suspended\ layer}$ was achieved at 400 mg/L, which mainly due to the conditions suitable for the growing of exoelectrogens, and it can continuously decompose organic matter to produce electric energy. Therefore, the microbial metabolic activity was enhanced with the increase of effluent COD concentration. However, when the effluent COD concentration was more than 400 mg/L, the voltage of $MFC_{suspended\ layer}$ decreased and this was clearly observed. Meanwhile the effluent COD concentration continually increased, the VFAs also increased, for example formic acid, acetic acid and propionic acid (Yamada et al., 2015; Tampio et al., 2014). With subsequent studies showing this as shown in Fig. 2. However, the voltage of MFC began to decline, it is indicated that the balance of anaerobic reaction was destroyed, the accumulation of VFAs occurred in the system, resulting the decline of pH in the system and the activity of exoelectrogens decreased. In order to compare which MFC sensor is more suitable for COD online monitoring, under the same conditions the voltage of $MFC_{sludge\ layer}$ and the corresponding sludge layer COD concentration were measured simultaneously.

It can be seen from Table 1 that with the increase of influent COD concentration (0–3000 mg/L), the COD concentration of sludge layer increased accordingly, and the corresponding voltage of $MFC_{sludge\ layer}$ also revealed an upward trend. However, when the influent COD concentration was in the 3000–5000 mg/L range, the sludge layer COD concentration to maintain the basic 2300 ± 200 mg/L, but the voltage of $MFC_{sludge\ layer}$ did decline over time. This is because the removal of organic compounds by anaerobic sludge is a process of biochemical adsorption and degradation. The process of biochemical adsorption is in accordance with the Langmuir adsorption isotherm, in that the adsorption amount increased as the matrix concentration also increased. This resulted in the adsorption saturation phenomenon (Duff et al., 1995; Pujol and Canler, 1992). When the adsorption saturation was reached, the COD concentration in the sludge layer changed very little, but owing to the accumulation of VFAs inhibited the activity of the bacteria, which resulted in the $MFC_{sludge\ layer}$ voltage decreasing rapidly.

According to the above study, the relationship between MFCs voltage and COD concentration were established as shown in Fig. 1. It is clearly shown that the relationship between $MFC_{suspended\ layer}$ voltage and effluent COD concentration (Fig. 1A; Fig. 1B). Meanwhile, the relationship between $MFC_{sludge\ layer}$ and COD

Table 1
The sludge layer voltage under different influent COD concentrations.

Flow rate (mL/s)	HRT (h)	Influent COD (mg/L)	Sludge layer COD (mg/L)	Voltage of $MFC_{sludge\ layer}$ (v)
0.058	12	300	110 ± 10	0.298 ± 0.002
0.058	12	800	320 ± 20	0.315 ± 0.004
0.058	12	1000	460 ± 20	0.331 ± 0.005
0.058	12	2000	1600 ± 50	0.348 ± 0.006
0.058	12	3000	2200 ± 100	0.317 ± 0.003
0.058	12	4000	2300 ± 100	0.284 ± 0.002
0.058	12	5000	2500 ± 100	0.247 ± 0.002

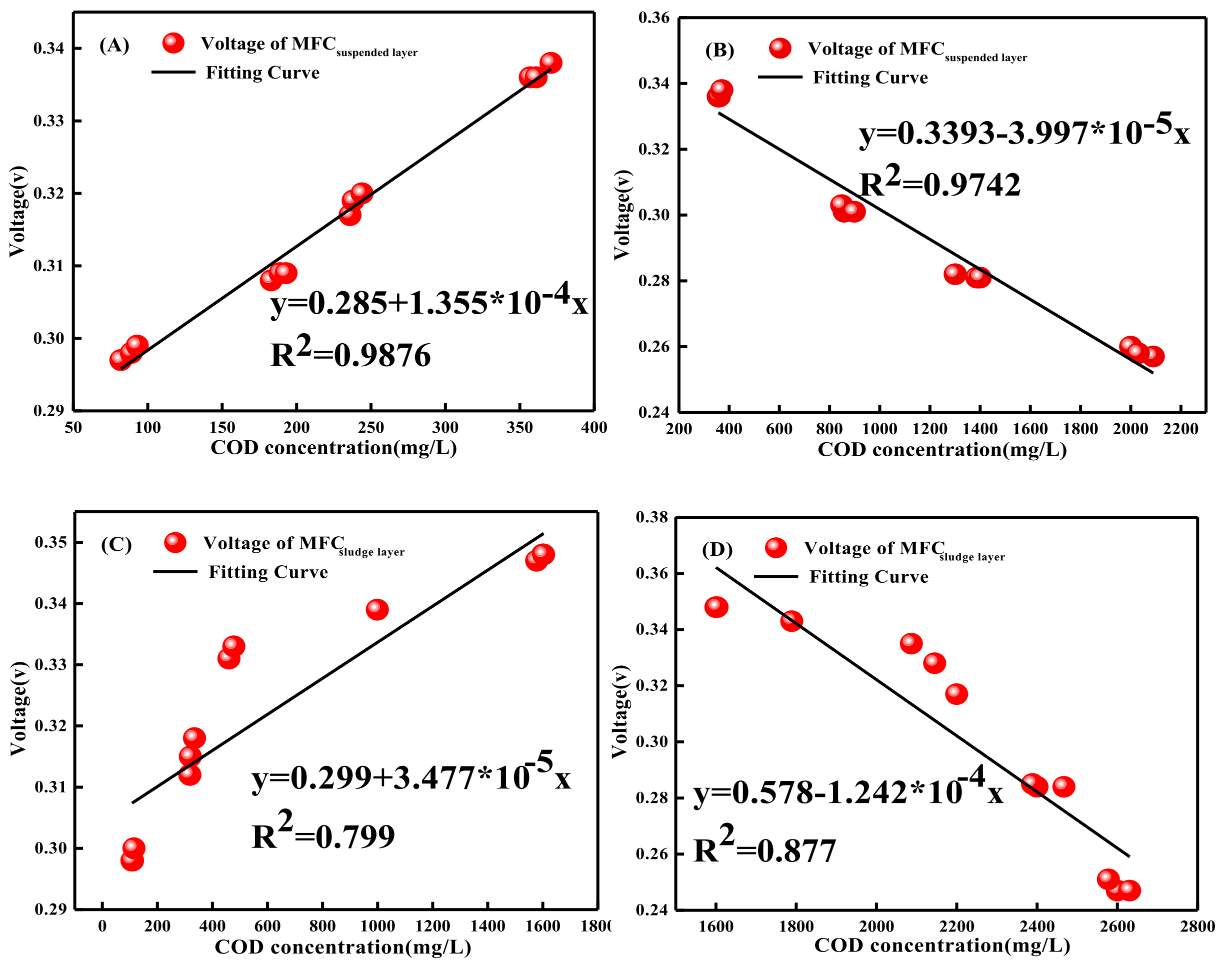

Fig. 1. The relationship between MFCs voltage and COD concentrations. (A.B) $MFC_{suspended\ layer}$ calibration curve; (C.D) $MFC_{sludge\ layer}$ calibration curve.

concentration was calculated (Fig. 1C; Fig. 1D). Compared the fitting curve regression coefficient (0.987 > 0.799, 0.974 > 0.877), the $MFC_{suspended\ layer}$ indicated a good linear relationship between voltage and effluent COD concentration. This further indicated that the MFC in suspended layer is more sensitive and accurate to the change of COD concentration. The above results suggest that the bio-cathode $MFC_{suspended\ layer}$ has the potential to be a simple and rapid COD biosensor and can be applied as a model to predict higher effluent COD concentrations. Compared with conventional COD measurement methods, the bio-cathode MFC-based biosensor constitutes an intelligent use of electrical energy. The voltage as a direct, real-time measure of COD can be easily measured (Wang et al., 2014).

3.2. MFC voltage response to TVFA concentration

VFAs are important mid-products in the production of methane, and their concentrations affect the efficiency of fermentation. Under the condition of overloading and in the presence of inhibitors, methanogenic activity cannot remove hydrogen and VFAs as quickly as they are produced. High VFAs concentrations in the system cause methanogenesis to be curtailed (Huang et al., 2015; Feng et al., 2015). Therefore, the monitoring of VFAs is critical in the operation of the UASB system. The accumulation of VFAs is mainly caused by the increase of organic load, result in the destruction of the dynamic balance of anaerobic digestion and even the stagnation of the whole digestive process. Firstly, the ratio of VFAs and total concentration under different COD levels were measured. It is

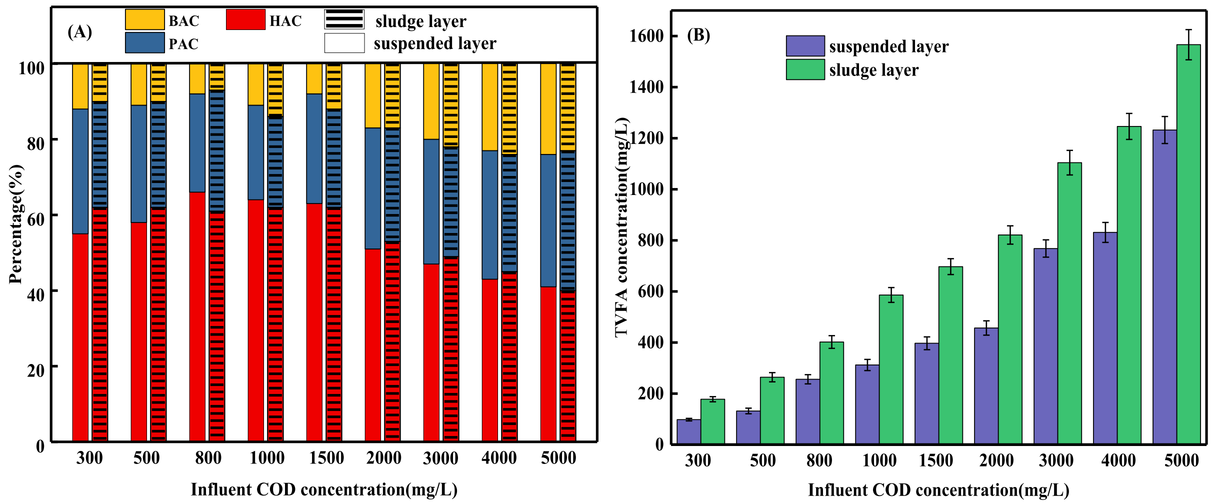

Fig. 2. Suspended layer and Sludge layer TVFA concentrations under different inlet COD. (A) The percentage of VFAs; (B) the TVFA concentration.

clearly evident in Fig. 2B, with the increase of the influent load, the concentration of VFAs in the UASB reactor also increases. When the concentration of COD was smaller than 2000 mg/L, the ratio of VFA was similar, but when the concentration of COD was larger than 2000 mg/L, the ratio of acetic acid began to decrease, and the ratio of propionic acid and butyric acid began to rise (Fig. 2A). As a whole, the concentration of TVFA in sludge layer is higher than that in suspended layer (Fig. 2B), which owing to the substrate concentration in suspended layer is lower, and the VFA will have a dilution and diffusion in the process of rising (Appels et al., 2011.; Zhang et al., 2013). On the other hand, the microbes in sludge layer has a certain biological adsorption on long-chain fatty acids (Lin et al., 2013; Sun et al., 2017). Therefore, the sludge layer can more accurately respond to the accumulation of TVFA in the UASB reactor.

Secondly, the UASB running performance (Fig. S3) and the MFC voltage corresponding to different concentrations of TVFA were tested (Fig. 2), the aim is to make MFC produce an electrical signal so that the concentration of VFAs can be monitored online. By changing the different influent load so that the VFAs in sludge layer and suspended layer is changed, the corresponding voltage of MFCs can be determined. In Fig. S3B, it can be clearly seen that at lower TVFA concentrations the voltage of the $MFC_{sludge\ layer}$ increased with the increase of TVFA concentrations. When the concentration of TVFA in the sludge layer was about 800 mg/L, the maximum $MFC_{sludge\ layer}$ voltage attained was approximately 0.35 V (Fig. 3C), which indicated that the exoelectrogens were very activity in this scenario. However, when the TVFA concentration rose to more than 800 mg/L, it was found that the voltage began to decline. This was due to the increase of influent concentration disrupt the balance of the anaerobic reaction, led to the accumulation of VFAs, and caused the decline of pH in the system. He et al. (2008) showed that pH of 8–10 is the most suitable environment for exoelectrogens growth and metabolic. Therefore, the amount accumulation of VFAs leads to the decrease of metabolic activity of exoelectrogens. The experiment repeated 5 cycles under the same conditions and obtained the same results, confirming that the $MFC_{sludge\ layer}$-based biosensor for TVFA online monitoring had a good sensitivity and recoverability (Fig. S3). We use the same equation model ($Y = a+bx$) to estimate the linear fit between the voltage of MFCs and TVFA concentrations. In Fig. 3, the goodness of fit between $MFC_{suspended\ layer}$ and TVFA is not good (Fig. 3A; Fig. 3B). The main reason can be summarized that the substrate concentration in suspended layer is lower and has a certain dilution effect on the VFAs. Therefore, the accumulation of VFAs in suspended layer cannot be accurately measured. There is a high degree of the fitting curve between $MFC_{sludge\ layer}$ and TVFA (Fig. 3C; Fig. 3D). This is ascribed to the anode carbon felt of the $MFC_{sludge\ layer}$ being completely immersed in sludge layer, so the exoelectrogens could quickly and accurately sense the accumulation of VFA in the UASB reactor and produce a feedback signal by changing the voltage. High detection sensitivity and high accuracy determined it has the potential to industrialize and broad application prospects (Brzeka et al., 2004).

The above results suggest that the $MFC_{sludge\ layer}$ is more suitable for TVFA concentration online monitoring. At the same time, the $MFC_{suspended\ layer}$ can monitor the COD concentration, and the ratio of TVFA/COD can be used for express the methane production of the anaerobic digestion process (Carlos et al., 2017). With the increase of methane production, the accumulation of VFA will be reduced, and the ratio of TVFA/COD decreased. Methane production is a symbol of metabolic balance in anaerobic digestion, which can reflect the process of anaerobic reaction. In this study, when the value of TVFA/COD is greater than 2.0, which means that the equilibrium of anaerobic reaction was destroyed and amount accumulation of VFAs in the system. The dual sensors system is an innovative way of monitoring the operator states of UASB reactor. These features make the UASB a much more attractive and promising technology.

3.3. Comparing the performance of MFCs

3.3.1. Three-dimensional fluorescence analysis

The initial adsorption of organic matter by anaerobic activated sludge is mainly the process of biosorption. The influent concentration wields a great influence on the adsorption capacity, and the adsorption capacity increases as influent concentration increases. However, when the substrate concentration increases to a certain level, the adsorption of organic matters by anaerobic sludge reach saturation. In order to prove that the adsorption of anaerobic sludge reached saturation, the three-dimensional method was used, which determined the protein-like fluorescence intensity of suspended layer and sludge layer under different COD levels. The method of three-dimensional fluorescence is more sensitive and accuracy compared with the traditional ultraviolet method (Chen et al., 2018; Song et al., 2018). In addition, the protein-like fluorescence intensity is often used to express the concentration of

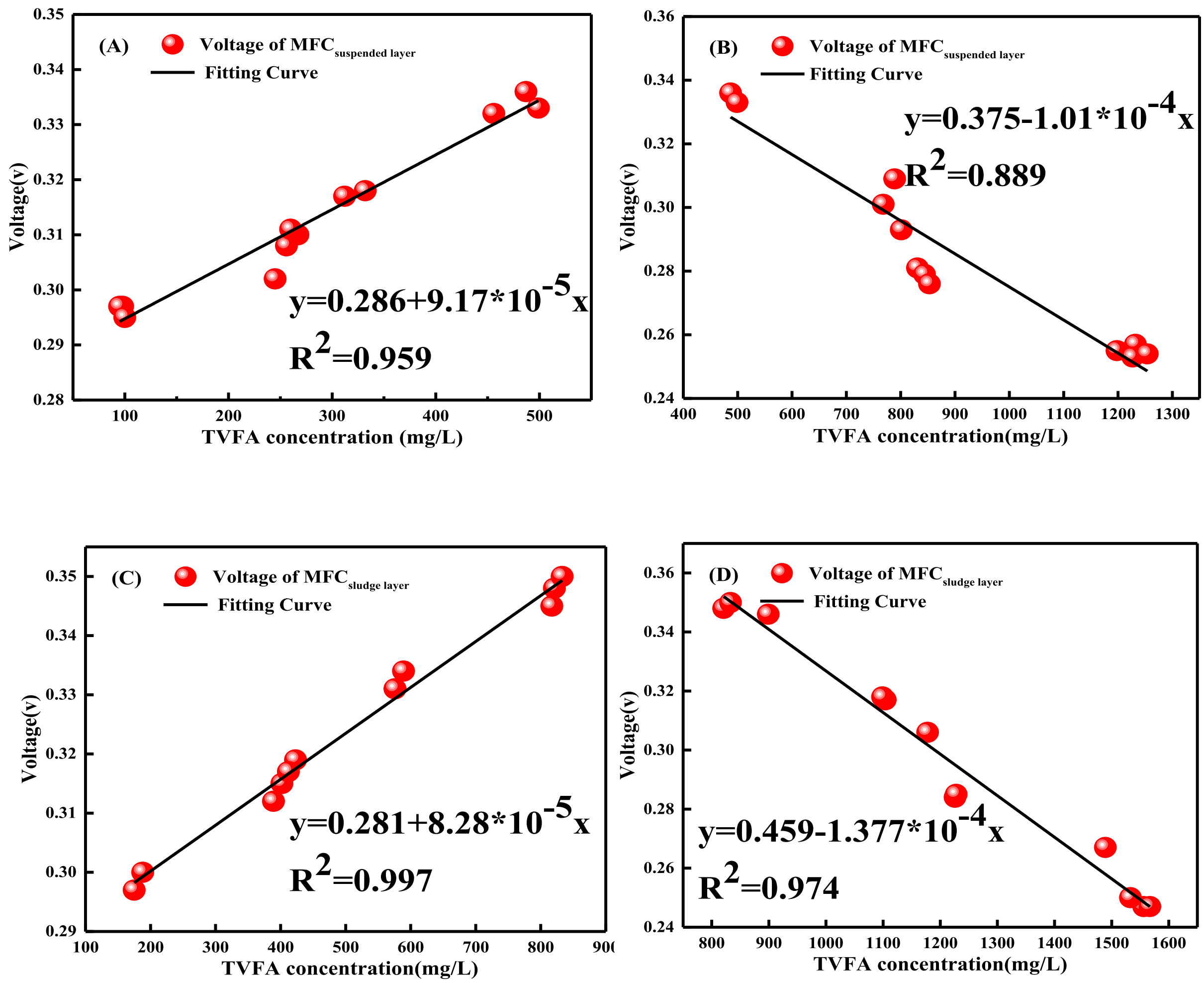

Fig. 3. The relationship between MFCs voltage and TVFA concentrations. (A.B) $MFC_{suspended\ layer}$ calibration curve; (C.D) $MFC_{sludge\ layer}$ calibration curve.

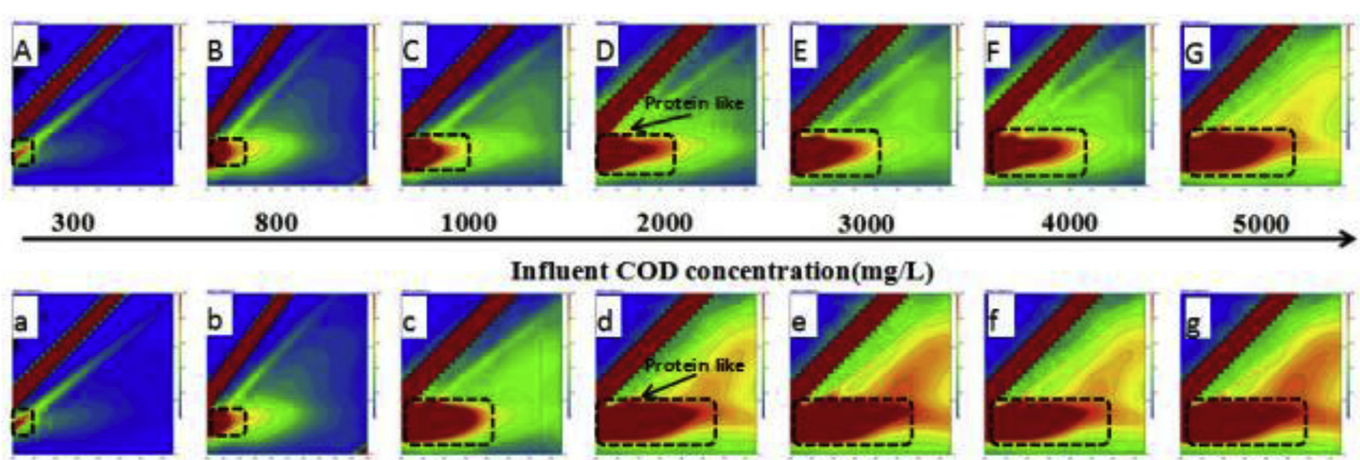

Fig. 4. The protein-like fluorescence intensity under different influent COD concentra-tions (suspended layer, A-G; sludge layer, a-g).

dissolved organic matter, and there is a close linear relationship between them (Yu et al., 2013; Massimiliano et al., 2017). In Fig. 4, the size of the red peak represents the intensity of fluorescence of protein-like. It can be clearly seen that the protein-like fluorescence intensity in the suspended layer increased stepwise as the influent COD increased from 300 mg/L to 5000 mg/L (Fig. 5A). Similarly, the protein-like fluorescence intensity in the sludge layer increased stepwise as the influent COD increased from 300 mg/L to 2000 mg/L, but when the influent COD concentration of more than 2000 mg/L occurred, only a small change in the fluorescence intensity was evident (Fig. 5B). This somewhat proved the adsorption saturation of the anaerobic sludge.

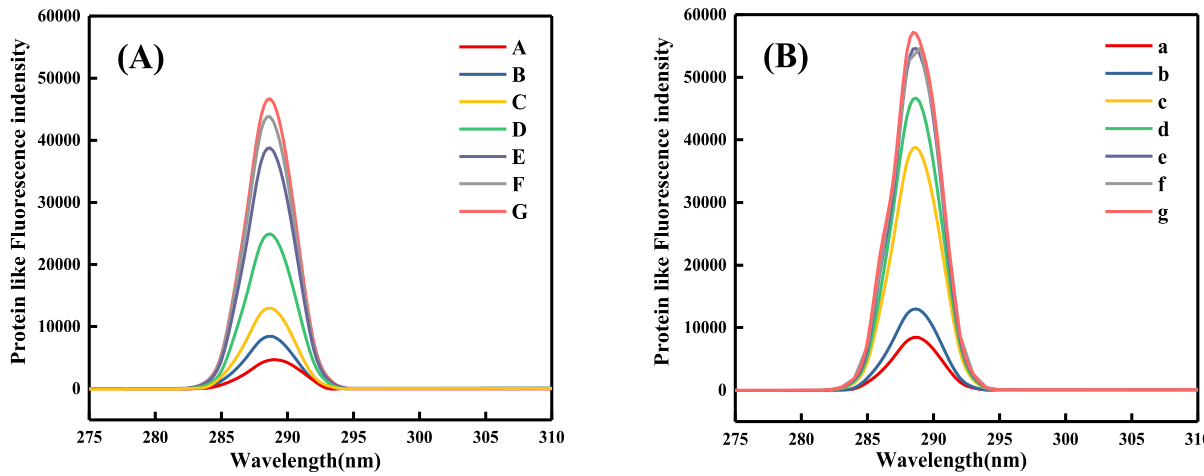

Fig. 5. Synchronous fluorescence spectra of UASB-MFC system. (A) The protein-like fluorescence intensity in suspended layer; (B) The protein-like fluorescence intensity in sludge layer.

When the anaerobic sludge reached adsorption saturation, the concentration of organic matter in the sludge layer changed slightly. However, the voltage of $MFC_{sludge\ layer}$ was affected by the metabolic capacity of micro-organisms and subsequently changed. It generated a weak linear correlation between MFC voltage and COD concentration in the sludge layer. Therefore, the $MFC_{suspended\ layer}$ proved to be more suitable for COD online monitoring. Meanwhile, the adsorption of VFA by current producing bacteria enabled the $MFC_{sludge\ layer}$ to establish a good linear relationship between voltage and TVFA concentration.

3.3.2. Electrical performance

In the UASB-MFC dual sensors system, MFCs as biosensors have the potential to provide quick feedback signals on COD and TVFA, monitoring the operating conditions of the UASB reactor by a simple voltage signal, so that improve the stability of the anaerobic reaction. Compared with conventional sensors or measurement methods, the bio-cathode MFC-based biosensor for UASB has its own advantages. There is no need for external power sources. MFC can recover energy from the wastewater while degrading organic matter, therefore, MFC-based biosensor is an effective way to use electricity. Moreover, the weak voltage is a direct, real-time measure of COD and TVFA can be easily measured. In order to make the sensor suitable for long-term monitoring and improving the stability of the MFC-based biosensor, the performance of MFCs were tested and further insights into this method of electricity generation were conducted. In Fig. 6A, it can be clearly seen that when the influent concentration is 500 mg/L, the maximum power density of $MFC_{suspended\ layer}$ (MFC_1) is 0.02 W/m^2. When the influent concentration is 1000 mg/L, the maximum power density of $MFC_{suspended\ layer}$ is 0.036 W/m^2, and when the influent concentration is 2000 mg/L, the maximum power density of $MFC_{suspended\ layer}$ is 0.054 W/m^2. The power output achieved in the test was comparable with other MFCs (Shao et al., 2006; Zhang et al., 2011) or MFC integrated with anaerobic systems (Ghosh-Raya and Ghangrekar, 2015). The maximum power density of $MFC_{sludge\ layer}$ (MFC_2) shows the same trend with the change of influent concentration in Fig. 6B. When the substrate concentration increases from 500 mg/L to 2000 mg/L, the maximum power density increased from 0.05 W/m^2 to 0.07 W/m^2. However, compared with $MFC_{suspended\ layer}$ (0.02 W/m^2-0.054 W/m^2), Under the lower VFAs concentrations, VFAs are important substrates for electricity generation by MFC, and the metabolism of exoelectrogens rises with the increase in substrate concentration, which leads to a gradual increase in maximum power density. This shows that MFC is normally operating, and the difference in maximum power density may be due to the different resistance levels of electron transfer. Thus the experiment on MFCs impedance data tested by the electrochemical workstation with the MFC three electrode system (Hou et al., 2013). The Nyquist plots from EIS measurement revealed properties of the electrodes in the electrolyte. The diameter of the high frequency region indicated the charge-transfer resistance (Zhen and Mansfeld, 2009). It can be seen from Fig. 6C that when the influent concentration is 500–2000 mg/L, the electron transfer resistance of $MFC_{suspended\ layer}$ is less than $MFC_{sludge\ layer}$. Therefore, the response of $MFC_{suspended\ layer}$ to the change in substrate concentration is more sensitive in normal operation. When the influent concentration increased from 2000 mg/L to 3000 mg/L, the maximum power density of $MFC_{suspended\ layer}$ decreased from 0.054 W/m^2 to 0.03 W/m^2, and the maximum power density of $MFC_{sludge\ layer}$ fell from 0.07 W/m^2 to 0.035 W/m^2. Compared with $MFC_{suspended\ layer}$, the maximum power density of $MFC_{sludge\ layer}$ decreased greatly. This indicated that acidification occurred in the system. In the process of anaerobic digestion, it is known that VFAs are important intermediary compounds in the metabolic pathways of methane fermentation and cause microbial stress if present in high concentrations, resulting in a decrease of pH, and ultimately leading to failure of the digester (Wang et al., 1999). Meanwhile, owing to the exoelectrogens are more sensitive to pH, and lower pH inhibited the metabolism activities of exoelectrogens which adhere on the anode surface of MFCs. Therefore, the higher VFAs concentrations lead to a decline of the MFC_S performance (Zhang et al., 2017). It can be clearly seen from the Fig. 6D that when the influent concentration increased from 2000 to 3000 mg/L, the redox properties of $MFC_{suspended\ layer}$ and $MFC_{sludge\ layer}$ are weakened, with $MFC_{sludge\ layer}$ perhaps more obviously. This is attribute to the accumulation of VFAs in the sludge layer and the inhibition of exoelectrogens metabolism on the surface of the $MFC_{sludge\ layer}$ anode is more powerful.

The electron transfer resistance of $MFC_{suspended\ layer}$ is smaller than $MFC_{sludge\ layer}$, which makes it sensitive to any changes in matrix concentration. Therefore, $MFC_{suspended\ layer}$ is more suitable for monitoring of COD. When acidification occurred in the system, the accumulation of VFAs in sludge layer was higher, which made the inhibition of $MFC_{sludge\ layer}$ more powerful, and the performance of $MFC_{sludge\ layer}$ decreased more significantly, so the $MFC_{sludge\ layer}$ proved to be more suitable for monitoring TVFA.

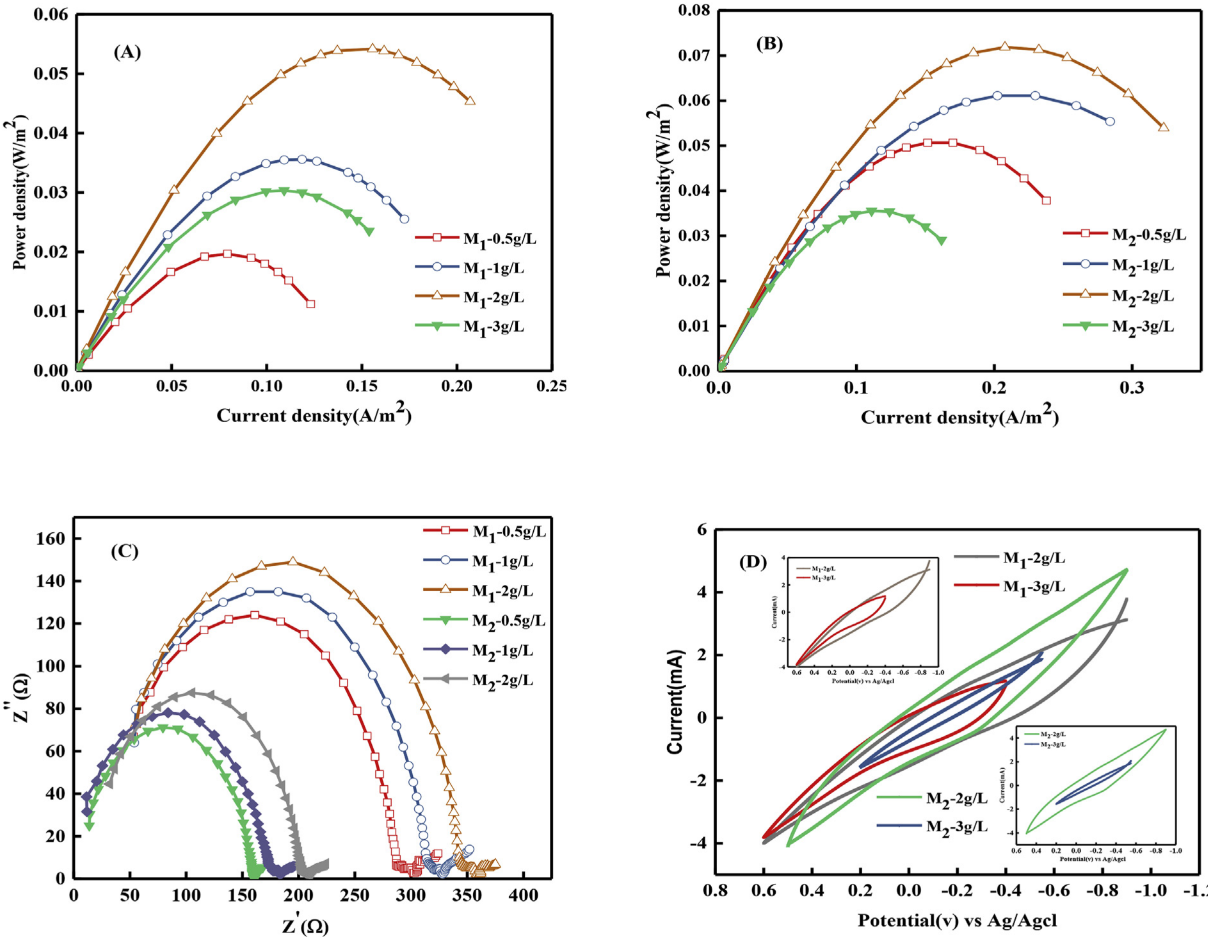

Fig. 6. MFCs electrical performance. (A) P-I curves for the $MFC_{suspended\ layer}$; (B) P-I curves for the $MFC_{sludge\ layer}$; (C) AC impedance diagram for the MFC_S; (D) Cyclic voltammograms of anodic biofilm for the $MFC_{S.}$

3.3.3. Microbial community dynamics

The microbial composition and metabolic activity on the surface of the anode are the main cause of MFCs varying levels of performance efficiency, and the electroac-tive species plays a critical role in transferring electrons from the substrate to the anode (Tommasi et al., 2016). In order to better understand the species abundance and community composition of the MFCs anode surface, high output sequencing of micro-organisms on the anode surface were carried out, and the test results were compared and analyzed. MFC exoelectrogens were widely distributed in a variety of micro-organisms, the main ones being *Geobacter*, *Shewanella*, *Pseudomonas* and other metal-reducing bacteria (Kim and Jung, 2007).

In Fig. 7A, the MFCs anode surfaces have a similar species composition, demonstrating that the sensors meet the basic sensing requirements. However, in Fig. 7B, compared with $MFC_{sludge\ layer}$, the abundance of *Geobacter* species on the anode surface of $MFC_{suspended\ layer}$ is relatively low. The *Geobacter* can transmit electrons by decomposing organic matter, and then converted into electrical signals for feedback. Owing to the MFCs voltage reveals little difference under the same conditions, it was demonstrated that the activity of *Geobacter* on the $MFC_{suspended\ layer}$ was stronger. Therefore, the MFC in the suspended layer is more sensitive to the change in COD concentration. Besides the different abundance of *Geobacter* on the anode surface of the MFCs, we also found that the abundance of *Methanosarcina* in $MFC_{suspended\ layer}$ is higher than $MFC_{sludge\ layer}$. Owing to the fact that *Methanosarcina* can decompose VFA to produce methane. When a small amount of VFAs was accumulated, *Methanosarcina* could slow down the inhibitory effect of VFAs on *Geobacter* to some extent. However, the abundance of *Methanosarcina* in the $MFC_{sludge\ layer}$ is lower, and this lead to the rapid inhibitory effect of VFA on *Geobacter* with a decline in the number of electrical signals. Therefore, the MFC in the sludge layer is more sensitive to the accumulation of VFAs.

Differences in microbial abundance on the surface of the anode lead to different sensing properties of MFCs. In future studies the specific substances can be detected by inoculating pure bacteria, and further broadening the application of biosensors.

4. Conclusion

A novel UASB-MFC dual sensors system was developed for UASB operating states and specifically online monitoring. Owing to the biosorption of microbes in UASB-MFC system, the $MFC_{suspended\ layer}$ evidenced a better linear relationship between voltage and COD ($y = 0.285 + 1.355 \times 10^{-4}x$, $R^2 = 0.987$; $y = 0.339–3.997 \times 10^{-5}x$, $R^2 = 0.974$). Meanwhile, the $MFC_{sludge\ layer}$ had a better linear relationship between voltage and TVFA ($y = 0.281 + 8.28 \times 10^{-5}x$, $R^2 = 0.997$; $y = 0.459–1.377 \times 10^{-4}x$, $R^2 = 0.974$).

Electrochemical analysis demonstrated that the reduced electron transfer resistance determines the higher sensitivity of the $MFC_{suspended\ layer}$. The difference in species abundance indicated that TVFA had a stronger inhibitory effect on the $MFC_{sludge\ layer}$. The dual sensors system based on MFC can effectively monitoring the COD and TVFA in the UASB reactor. The acid balance and anaerobic

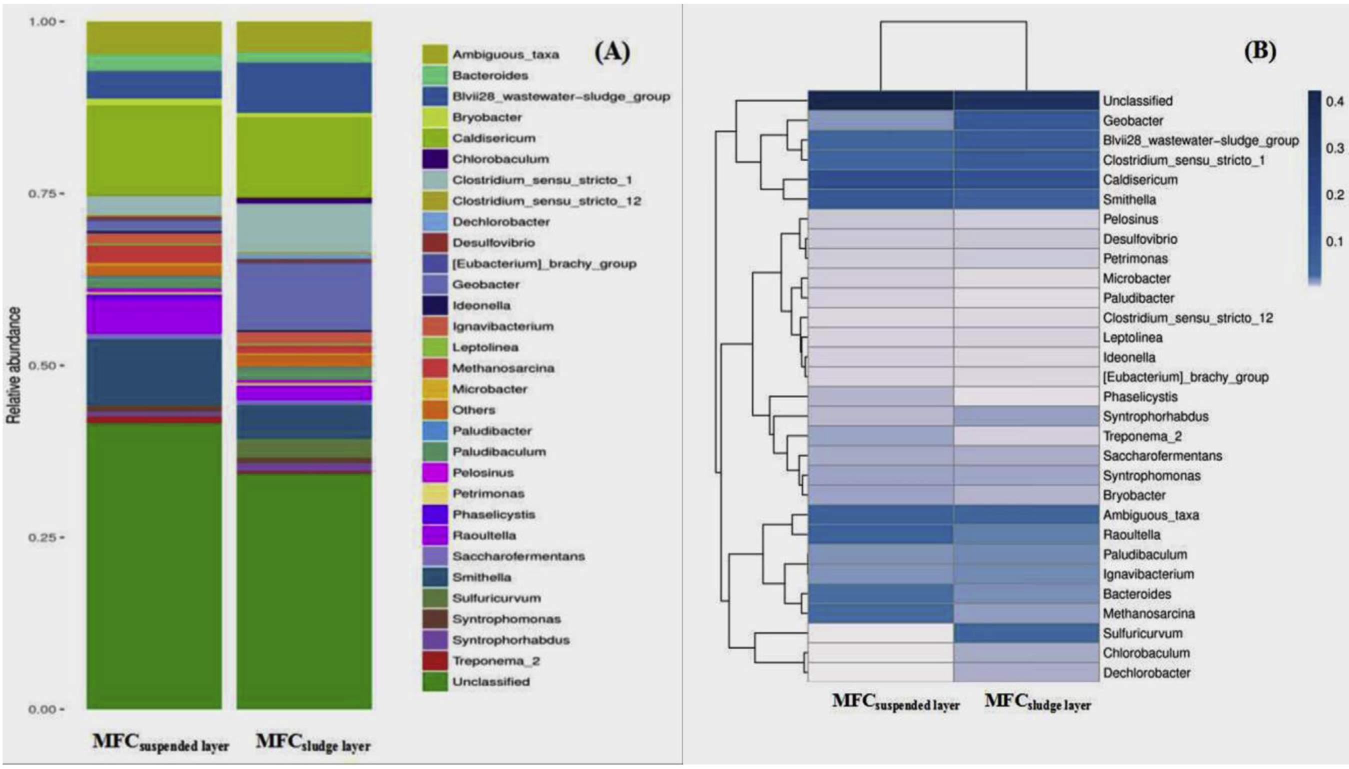

Fig. 7. Microbial analysis chart. (A) Thermal clustering map of species distribution; (B) Species distribution histogram.

digestion process of the reactor can be evaluated by the relationship of TVFA/COD. Finally, this research provided an opportunity to widen the application range of MFC-based biosensor and improve the stability of UASB reactor at the same time. In the future, the long-term treatment efficiency and sensing effect should pay more attention, and further optimizational work on different structures of dual sensors UASB-MFC coupling system should be developed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.07.119.

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