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Influence of power generation period on the oily sludge bio-electrical system

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ABSTRACT

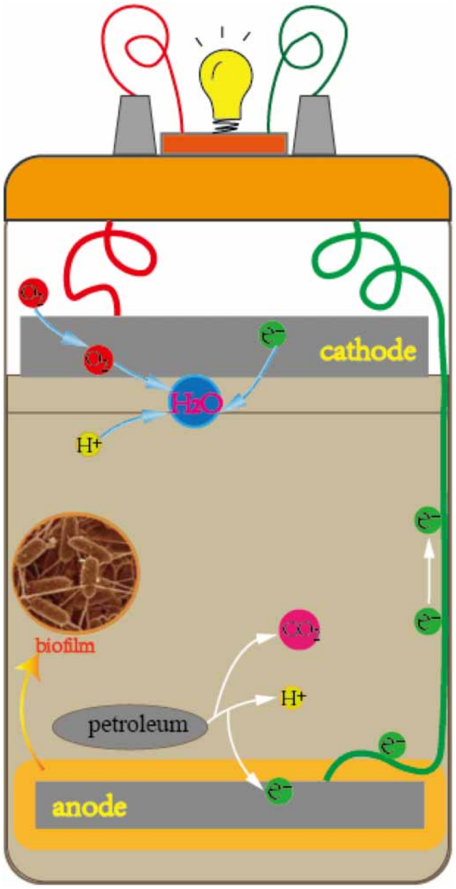
The single-chamber bio-electrical systems can degrade oily sludge in sediments while generating electricity from the microbial fuel cells (MFCs) and their characteristics in energy and environmental effects have attracted wide international attention in recent years. To explore the influence of the power generation period on the oily sludge bio-electrical system, an oily sludge bio-electrical system was constructed. The output voltage, polarization curve, power density curve, crude oil removal rate and microflora were detected during different power generation periods, respectively. The results of this study showed that under the stable power generation period, the power generation and oily sludge degradation performance of MFC are higher than the voltage rise period and voltage attenuation period. Besides, the oily sludge bio-electrical system during the stable period contained more electricity-producing bacteria than the other two periods. The voltage in the stable period of oily sludge bio-electrical system is about 280 mV, the electromotive force is 493.1 mV and the power density is 134.93 $mW \cdot m^{-3}$. It lays a foundation for the improvement of degradation of crude oil and power generation performance in oily sludge bio-electrical system.

Key words: crude oil removal rate, microbial flora, oily sludge, power generation period

HIGHLIGHTS

- We explore the influence of power generation periods on the performance of oily sludge SMFC.
- Oily sludge SMFC can effectively degrade crude oil and convert it into electricity.
- Power generation, oil degradation performance and bacterial community morphology, diversity, bacterial composition and relative abundance of oily sludge SMFC during different power generation periods were systematically compared.

GRAPHICAL ABSTRACT



1. INTRODUCTION

A microbial fuel cell (MFC) is a system that uses biological electrochemical oxidation of organic substances and microorganisms as biocatalysts and generates electricity. Electrons are transmitted directly or indirectly to the anode through microorganisms, and then from the external circuit to the cathode, thereby forming a current in the external circuit (Bullen *et al.* 2006; Hong *et al.* 2008; Logan 2010). Sediment microbial fuel cells (SMFCs) are special battery systems evolved from the traditional MFC structure, in which the anode material is embedded in the anaerobic sediment, the cathode electrode is suspended in the oxygen-enriched water body and is connected to an external circuit through wires (Cai *et al.* 2020; Guo *et al.* 2022). SMFCs can convert biomass energy into electricity, and at the same time decompose and metabolize sediments. In addition, SMFCs have a lower cost than ordinary MFCs (Song *et al.* 2017). Because SMFC has the advantages of wide substrate sources, low maintenance costs, cleanliness and environmental protection it is often used for the *in situ* restoration of the seabed or river sediments and has broad application prospects (Mohan & Chandrasekhar 2011; Hou *et al.* 2016). Due to the certain similarity between oily sludge and river sediments, SMFC is suitable for the treatment of oily sludge. The treatment of oily sludge not only generates electricity but also protects the environment, thus achieving a win–win situation of environmental protection and energy recovery.

In the past 20 years, the research of SMFC mainly focuses on factors that affect the electrical energy recovery and substrate degradation of sediments, such as electroactive microorganisms in the anode chamber, anode materials, electron acceptors in the cathode chamber, catalysts and so on (Guo *et al.* 2013; Cabezas *et al.* 2015; Zhu *et al.* 2016; Zafar 2019). In recent years, the use of SMFCs to process oily sludge and simultaneous production capacity has become a hot topic for MFC research (Guo *et al.* 2022). The previous research showed that when the MFC was used to repair submarine oil pollutants for 64 days, the oil degradation efficiency was 18.7 times than under natural degradation conditions (Meng *et al.* 2015). The studies show that

MFC can effectively accelerate the degradation of petroleum hydrocarbons (Guo *et al.* 2020; Huang *et al.* 2020). What affects the crude oil removal capacity? Is there a big impact on the power generation capacity and the crude oil removal capacity in different power generation periods? Research has shown that there are great differences in the pollution control and electricity generation of MFC during the process of MFC power generation, and there are also great differences in the microbial community structure (Li *et al.* 2017a, 2017b; Hou *et al.* 2019; Liu *et al.* 2021). However, there are few systematic studies on the effects of different power generation periods on power generation, crude oil removal performance and microbial community structure.

Therefore, in order to fill the gap of systematically studying the effects of different power generation cycles on power generation, crude oil removal performance and microbial community structure, we will investigate the effects of different power generation cycles on power generation, crude oil removal performance and microbial community structure. In this paper, a series of SMFCs were constructed using oily sludge as an anode substrate, and the power generation periods' effect on SMFC's power generation performance and oil sludge degradation were systematically studied. Furthermore, the changes in diversity and composition of the microbial community in oily sludge SMFC during different power generation periods were also discussed. It also provides basic research for the improvement strategy of oily sludge performance and the mechanism of pollution control and electricity generation.

2. MATERIALS AND METHODS

2.1. Construction and operation of oily sludge bio-electrical system

The experiment uses a single-chamber bio-electrical system without a membrane, and the usable volume of the device is 2 L (Figure 1). The oily sludge was taken from Shengli Oilfield, and the oil content and water content are, respectively, 40.71 and 10.70%. After the oily sludge and the culture medium ($MgSO_4{\cdot}7H_2O$ 250 mg/L, $(NH4)_2SO_4$ 1,000 mg/L, K_2HPO_4 10,000 mg/L, NaCl 5,000 mg/L, $MgCl_2$ 180 mg/L, NH_4Cl 500 mg/L) are evenly mixed at a ratio of 9:1, 1 L is deposited on the bottom of the battery, the upper part is the catholyte and the volume is 1 L (Li *et al.* 2017a). Both anode and cathode electrodes are made of pretreated round graphite felt (thickness 10 mm, diameter 80 mm, fixed carbon $\geq$ 98.5%, ash content $\leq$ 0.05%, tensile strength: 0.15Mpa, purchased from Shanghai carbon factory store), the anode is buried in oily sludge, the cathode is floating on the catholyte surface and in contact with the air, the distance between the two electrodes is 12.5 cm. The pre-treatment methods of carbon-based materials are as follows: the electrode material was ultrasonically cleaned in absolute ethanol for 0.5 h to remove oil stains on the surface, and then heated in a 30% hydrogen peroxide water bath for 1 h. When

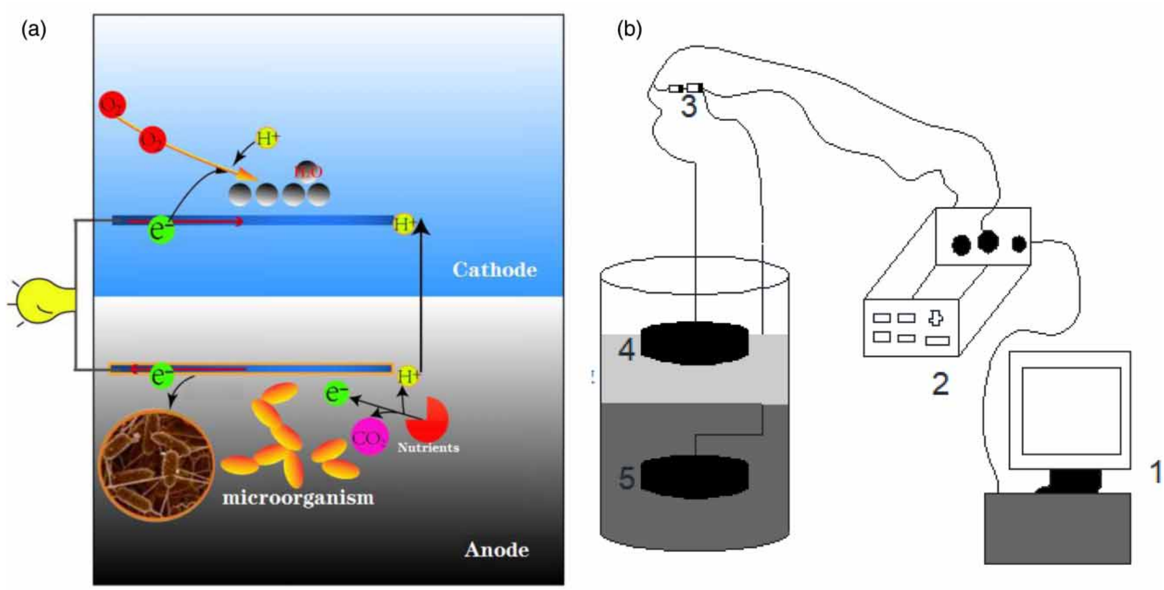


Figure 1 | Schematic diagram of the SMFC device :(a) Schematic diagram of the reactor, (b) the reactor connection data collector (1 – Computer, 2 – data collector, 3 – external resistance; 4 – cathode, 5 – anode).

heating, it was turned over properly to further remove stains and then rinsed repeatedly with flowing pure water to remove the residues inside the material of hydrogen peroxide. The anode and the cathode were connected to an external resistance (500 Ω) through a copper wire, and the two ends of the external resistance were connected to the data acquisition system, which was connected to the computer to export data, and the data acquisition device recorded every 10 min. It was carried out in a constant temperature incubator (31 ± 1 °C) and cathode buffer was added regularly during battery operation.

2.2. Electricity production performance test

The output voltage of the oily sludge bio-electrical system was recorded in real time by the data collector and uploaded to the computer. The power density curve and polarization curve of the 3rd, 12th and 19th oily sludge bio-electrical system through steady-state discharge were obtained, and the apparent internal resistance calculated by fitting the polarization curve (Elmekawy *et al.* 2013; Song *et al.* 2017). The power density calculation formula of the oily sludge bio-electrical system is as follows:

$$P = UI/V \tag{1}$$

where P is the power density, mW/m^3; U is the voltage, mV; I is the current, mA; V is the volume of the anode chamber, m^3.

The output voltage, current density and power density were used to evaluate the electrical performance of MFC. The output voltage can be obtained directly from the data collector (Agilent, 34970A). Current density and power density can be calculated from $I = U/R \times V$ and $P = U \times I/V$, respectively, where I is the current density (mA/m^3), U is the output voltage (mV), R is the external resistance, P is the anode power density (mW/m^3), and V is the effective volume of the anode chamber (m^3). The external resistance was gradually changed in the range of 0–9,000 Ω as follows: when the resistance was in the range of 0–10 Ω, it was changed with a step of 1 Ω; when it was at 10–100 Ω, the changing step was 10 Ω when it was in the range of 100–900, the changing step was 100 Ω; whereas at the final range of 1,000–9,000 Ω, the resistance was changed by a step of 1,000 Ω. Data were recorded after 10 min of stabilization of MFC for each interval. The measured data were used to calculate the current, current density and power density, and to draw the polarization and power density curves.

2.3. Determination of crude oil removal rate

Before and after the operation of the oily sludge bio-electrical system in different power generation periods, the anode substrate was sampled. Then it was naturally dried at room temperature, the crude oil was separated from the sample by Soxhlet extraction method, and the weight was calculated (Zhang 2019). The oil mass fraction and crude oil removal rate are calculated as shown in the following formula. Each sample was tested three times, and the results were averaged and standard deviation (SD) calculated.

$$R_1 = m_1/m_2 \tag{2}$$

$$R_d = (R_0 - R_t)/R_0 \tag{3}$$

where R_1 is the mass fraction of oil, mg/kg; m_1 is the mass of crude oil in the sample, mg; m_2 is the mass of the sample after dehydration, kg; R_d is the crude oil removal rate, %; R_0 is the mass fraction of oil before treatment, mg/kg; R_t is the treatment after oil content, mg/kg.

2.4. Determination of the removal rate of normal alkanes

Gas chromatography–mass spectrometer (Agilent 7890A + 5975C, America, GC–MS) was used to test various normal alkanes in petroleum. GC–MS analysis conditions: the chromatographic column was a DB-1 capillary column (60 m × 0.32 mm i.d. × 0.25 μm), the injection temperature was 290 °C, the carrier gas was He (flow rate 1.2 mL/min). Mass spectrometry conditions: electron bombardment ion source, electron beam energy 70 eV. Ion source temperature 260 °C, transmission line temperature 280 °C, mass scanning range m/z50-650, scanning period 100 ms. The peak area of each series of compounds was integrated into relative percentage content, and the absolute residual amount of each series of compounds was calculated by using the absolute content of the added internal standard substance (44-deuterolite icosane). The calculation formula for the degradation rate R_2 of normal alkanes in oil hydrocarbons is as follows:

$$R_2 = (m_0 - m_i)/m_i \tag{4}$$

where m_0 is the mass of each substance in the oily sludge before treatment, μg; m_i is the mass of each substance in the oily sludge after treatment, μg (Song *et al.* 2017).

2.5. Microbial flora characteristics

(1) The scanning electron microscope (SEM) of microbial flora

SEM (S4800) was used to observe the morphology of microorganisms in different power generation periods. A small piece of oily sludge was taken out from near the anode of the sludge MFC during different power generation periods. It is based on the method provided in the literature (Jiang *et al.* 2016a), dried and gold-plated pre-treatment, and SEM observation.

(2) Composition and abundance of microbial flora

① DNA extraction: the biological metagenome classification and sequencing method was used to analyze the microbial flora structure in oily sludge MFC during different power generation periods. The DNA of oily sludge in MFC was extracted separately during different power generation periods.

② PCR amplification: the extracted DNA was amplified by PCR, and the V4–V5 region was selected as the amplification primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′).

③ Obtain the operation classification unit: the sequence was measured on the Illumina Miseq™ platform. After splicing and screening, the data were aggregated into operational taxonomic units (OTU) at a coincidence of 97%.

④ Analysis of the composition and abundance of biological communities: the obtained OTU was searched and classified by the classifer program in the RDP database (http://rdp.cme.msu.edu/), and the composition and relative abundance of the community were analyzed according to the analysis method in the literature (Liu *et al.* 2019).

3. RESULTS AND DISCUSSION

3.1. Effect of power generation period on electricity generation of oily sludge bio-electrical system

3.1.1. Effect of power generation period on the voltage of oily sludge bio-electrical system

As shown in Figure 2, the output voltage of the oily sludge bio-electrical system during different power generation periods is different. The power generation period is mainly divided into three stages: voltage rise period, voltage stabilization period and voltage decay period. In the voltage rise period (0–7 days), the voltage is increased and relatively high (up to 323.70 mV), but is unstable. That is because the natural electromotive force (EMF) between the deposited sludge and the floating catholyte is large, so the system voltage is relatively large (Wu *et al.* 2011). The bacteria that are beneficial for electricity production gather at the anode, thereby gradually increasing the voltage of the system (Rezaei *et al.* 2007). The better the growth of electricity-generating bacteria, the greater the electricity-generating performance of the oily sludge bio-electrical system. However, the voltage will drop due to the unsuitable environment of the electricity-generating bacteria, and so during the stable power

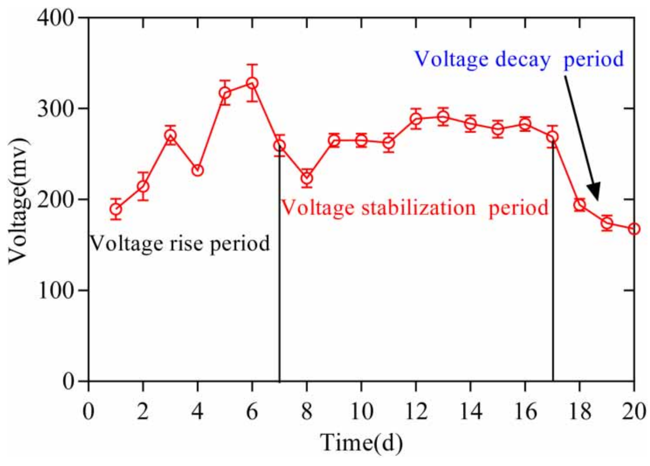


Figure 2 | Voltage of oily sludge bio-electrical system in different power generation period.

generation period (8–17 days), the voltage is lower than the voltage rise period, and the stable voltage is about 280 mV. During the voltage decay period (18–20 days), the voltage gradually decreases, and the lowest is about 170 mV. That may mean that oily sludge can be used as a carbon source for microorganisms in the bio-electrical system and provide nutrients for the metabolism of microorganisms, and that the growth of electricity-producing bacteria directly determines the electricity-generating performance of the system.

3.1.2. Effect of power generation period on polarization curve and power density curve of the oily sludge bio-electrical system

The polarization curve (Figure 3(a)) of the oily sludge bio-electrical system shows that the EMFs of the three periods are 248.8, 493.1 and 259.6 mV, relatively. The maximum power densities of the oily sludge electrochemical system are 51.94, 134.93 and 51.94 mW/m^3, respectively (Figure 3(b)). Although the voltage and the EMF of the electrochemical system of oily sludge in the period of rising electricity production are higher than that in the stationary period, the power density and the EMF are lower than that in the stable period. It shows that the oily sludge bio-electrical system has the highest electrochemical activity during the stable power generation period. It may be because the electricity-producing bacteria are more active during the stable electricity generation period of the system (Mocali *et al.* 2013).

3.2. Effect of power generation period on oil removal performance of oily sludge bio-electrical system

3.2.1. Effect of power generation period on oil removal rate of oily sludge bio-electrical system

It can be seen from Figure 4 that the oil removal rates of the three periods of the oily sludge electrochemical system are 15.61, 43.73 and 32.75%, respectively. The highest oil removal rate is during the stable power generation period. It can be seen that the oily sludge bio-electrical system can significantly reduce the oil content of oily sludge during the stable power generation period. This is because oily sludge provides rich nutrients for degrading microorganisms, speeds up their growth and metabolism and improves the degradation effect of crude oil (Zhang 2017).

3.2.2. Effect of power generation period on *n*-alkanes removal rate of oily sludge bio-electrical system

In order to facilitate the study of the degradation of *n*-alkanes by the oily sludge bio-electrochemical system, the oily sludge can be divided, according to the carbon number, into low-carbon short-chain alkanes (<C20), medium-carbon medium-chain alkanes (C21–C26) and high carbon number alkanes (Manaswini *et al.* 2010). The effect of long-chain alkanes with carbon number (>C27) and oil-bearing sludge bio-electrochemical system on the degradation rate of normal alkanes with different numbers of carbon segments is shown in Figure 5. It can be seen that the average degradation rate of short, medium and long-chain alkanes during the stable power generation period of the oily sludge bio-electrochemical system are 5.37, 37.5 and 50.10%. The average degradation rate of long-chain alkanes is the highest, perhaps the bio-electrical system of oily sludge is selective for the removal rate of long-chain *n*-alkanes. This further shows that crude oil (especially long-chain *n*-alkanes) in oily sludge provides rich nutrients for degrading microorganisms.

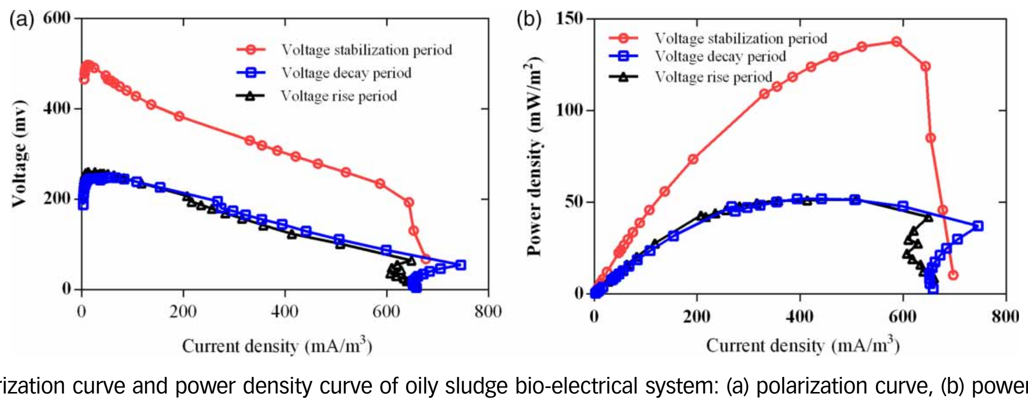


Figure 3 | Polarization curve and power density curve of oily sludge bio-electrical system: (a) polarization curve, (b) power density curve.

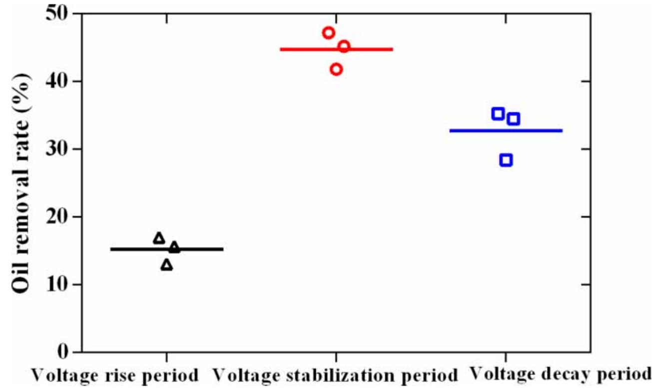


Figure 4 | Oil removal rate of oily sludge bio-electrical system.

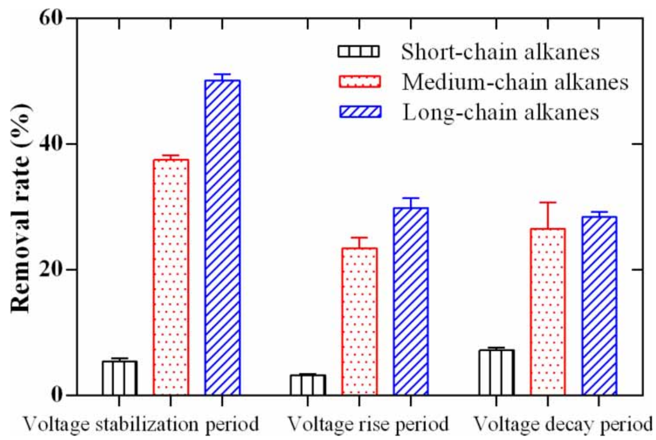


Figure 5 | The removal rate of normal alkanes in oily sludge bio-electrical system.

3.3. Effect of power generation period on microflora of oily sludge bio-electrical system

3.3.1. Effect of power generation period on microflora SEM of oily sludge bio-electrical system

Figure 6 shows the different period SEM of oily sludge bio-electrical system. It can be seen from Figure 6(a) that the SEM in the period of rising electricity production is about 1.0 μm. The SEM in the period of voltage stabilization is rough and there are attachments (Figure 6(b)). It can be seen from Figure 6(b) that the microorganisms are mainly bacilli, the bacteria length is about 1 μm, the cell surface is smooth and flat, and there is no cell structure extending outward. During the voltage decay period of oily sludge bio-electrical (Figure 6(c)), particles are 0.5–0.8 μm, and the microorganisms are mostly spherical. It can be seen that the morphological characteristics of the microbial flora at different periods of MFC are quite different. This must be related to the pollution control and power generation performance of the system in different periods.

3.3.2. Effect of power generation period on microbial diversity index of oily sludge bio-electrical system

In order to further analyze the effect of different power generation periods on the microbial flora of sludge bio-electrical system, the Illumina high-throughput sequencing technology was used to analyze the microbial community structure on different power generation periods of oily sludge. As can be seen from Figure 7, compared with the Chao1 index and ACE index (22,327.10, 50,398.64) of the voltage rise period, the voltage stabilization period (15,617.76, 31,064.92) all decreased, while the voltage decay period (25,595.76, 62,013.62) all increased. This indicates that the microbial diversity during the period of voltage stabilization decreases, while the microbial diversity during the period of voltage decay increases. Studies have shown that this is because MFC promotes the enrichment of electricity-producing microorganisms in the system, which

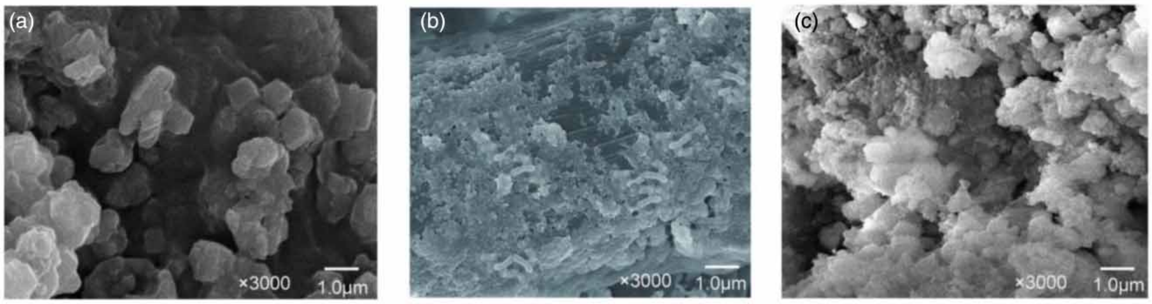


Figure 6 | Different period SEM of oily sludge bio-electrical system: (a) voltage rise period, (b) voltage stabilization period (c) voltage decay period.

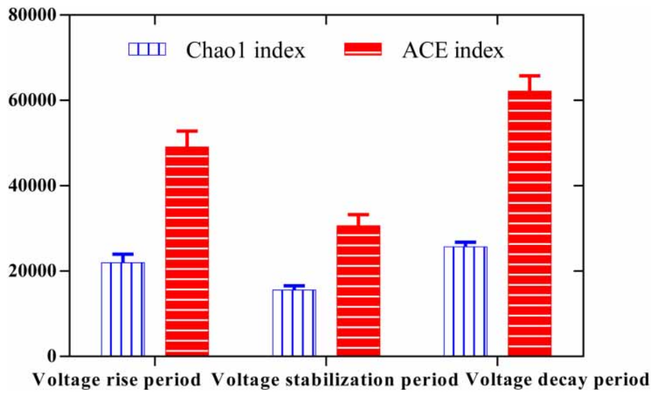


Figure 7 | Different period SEM of oily sludge bio-electrical system.

promotes the system to generate electricity and the microbial abundance is low (Liu *et al.* 2012). As the electricity production decreased, the bacterial diversity in the system increased again.

3.3.3. Effect of power generation period on microbial composition of oily sludge bio-electrical system

Figure 8(a) shows the composition and relative abundance at the phylum level of the flora in different power generation periods. As can be seen from Figure 8, at the phylum level, and the dominant flora were Proteobacteria, Firmicutes, Chloroflexi and Bacteroidetes. The sum of the four types of bacteria accounted for more than 85% of the community, occupying an absolute advantage.

But the community composition of different power generation periods in the oily sludge bio-electrical system is very different. Among them, Proteobacteria (more than 45%) have the largest number of bacteria in the stable period of electricity production. Studies have shown that Proteobacteria contains many electricity-producing bacteria, which do not only secrete redox mediators but also provide electron transfer mediators to other microorganisms or direct electron transfer to the anode (Figure 9), while increasing the power generation capacity of the system. Firmicutes have the largest number of bacteria in the other two power generation stages. Studies have shown that Firmicutes can carry out extracellular electron transfer as shown in Figure 9, and are an important part of the MFC microbial community (Finch *et al.* 2011). Chloroflexi is suitable for survival under strict anaerobic conditions at around 37 °C and the optimum pH for growth is 7.0. Therefore, it widely exists in MFC (Jiang *et al.* 2016b). At the genus level (Figure 8(b)), compared with the relative abundance of the period of voltage rise and voltage decay, the bacteria with the greatest difference in the stable period of electricity production is *Pseudomonas*. Studies have shown that *Pseudomonas* is an electricity-producing bacteria, which secretes redox mediators and also provides

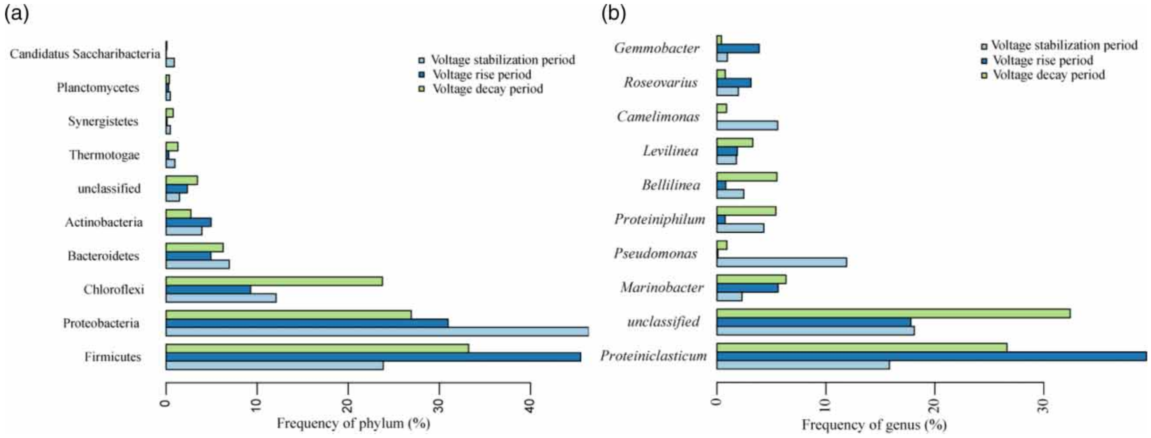


Figure 8 | Microbial species composition and relative abundance at different level. (a) Phylum level, (b) genus level.

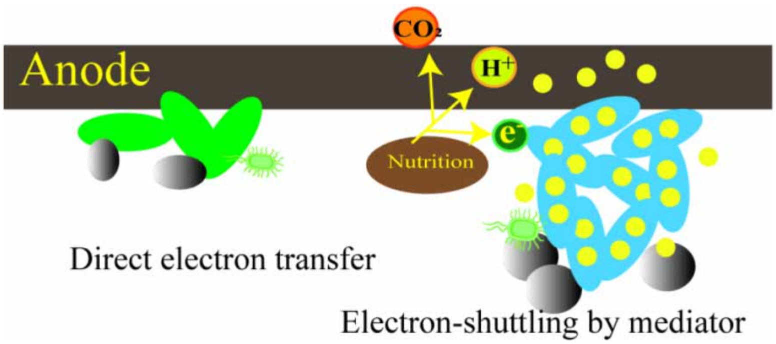


Figure 9 | Schematic diagram of the electron transfer mode of electrogenic bacteria.

electron transfer mediators to other microorganisms. It further shows that there are different electricity-generating bacteria groups in the system of different electricity-generating periods. Different types of power generation flora determine the power generation and oil degradation performance of the oily sludge bio-electrical system.

4. CONCLUSION

Oily sludge MFCs can effectively degrade crude oil and convert it into electricity. The power generation, oil degradation performance and bacterial community morphology, diversity, bacterial composition and relative abundance of oily sludge MFC during different power generation periods were systematically compared. The research results are as follows:

(1) Comparing the power generation and oily sludge degradation performance of MFC in different power generation periods, it is found that the stable power generation period is the best. The results of this study showed that the crude oil removal rate during the voltage stabilization period (43.73%) was greater than the voltage rise period (15.61%) and voltage decay period (32.75%). The voltage, EMF, maximum power density and the crude oil removal rate of the voltage stabilization period were 280 mV, 134.93 mW m^3, 493.1 mV and 43.73%, respectively.

(2) Compared with the Chao1 index and ACE index (22,327.10, 50,398.64) of the voltage rise period, the voltage stabilization period (15,617.76, 31,064.92) all decreased, while the voltage decay period (25,595.76, 62,013.62) all increased because there would be more electricity-generating bacteria in the system during the stable power generation period. The microorganisms in the system during the stable power generation period were mainly bacilli, with a length of about 1 μm, and the microorganisms are mostly Proteobacteria (more than 45%).

It can be seen that the bio-electrical system cannot only utilize oily sludge as a resource during different periods but can also gather different electricity-generating bacteria and avoid oil sludge separation and output electric energy, which has a good industrialization prospect.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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