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Real-time monitoring of sediment bulking through a multi-anode sediment microbial fuel cell as reliable biosensor

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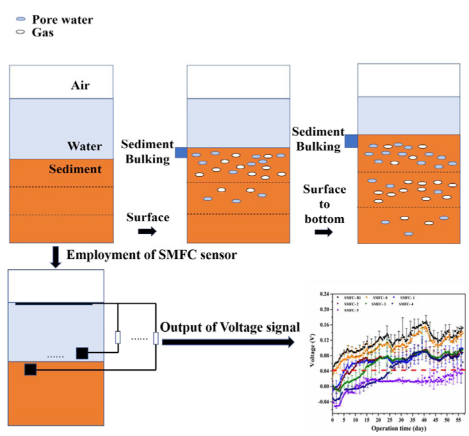
HIGHLIGHTS

- A novel sediment microbial fuel cell-based biosensor was developed.
- The SMFC sensor can monitor variation in sediment height of 1 cm.
- The rapid growth of sediment height is related to sediment bulking.
- Sediment bulking changes the sediment properties with accumulation of gases.
- The sensor could be used as an early-alert program in a shallow lake.

GRAPHICAL ABSTRACT



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ABSTRACT

Sediment bulking was closely related to the occurrence of black water agglomerate in anoxic aquatic sediments. Real-time monitoring of sediment bulking can be labor intensive and technically difficult, especially in dynamic environments where a record of variation in height over time is desired. In this study, a vertically distributed multi-anodes sediment microbial fuel cell (SMFC) as biosensor was developed for monitoring the changes in sediment height. According to the principle of sediment microbial fuel cell (SMFC), the voltage of SMFC would increase when the anode embedded into the sediment. The results showed that when the anode buried in the sediment, the biosensing system delivered voltage can increase to 40 mV, where the power density of SMFC exceeded 10 mW m^{-2} with overshoot of power density appeared. However, for the anodes above the water-sediment interface, the voltages and power densities kept at around 0. The redundancy analysis further indicated that the labile carbon pool-I of sediment was a key factor for sediment bulking, which led to drastic changes in sediment characteristics. The results from this study can provide a simple strategy for identifying sediment bulking in shallow lakes.

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1. Introduction

Sediments constitute an important compartment in aquatic environments. In lakes especially shallow lakes, the sediment is a primary site for organic matter (OM) mineralization due to high sediment area-to-water volume ratio as well as high area rate of organic matter mineralization (den Heyer and Kalff, 1998), and sediment processes dominate the overall metabolic activities (Pace and Prairie, 2005). Given the long-term drainage of industrial wastewater and municipal sewage as well as accumulation of autochthons OM, the surface layer of sediments would contain a significant amount of pollutants, such as organic matter, nitrogen, and phosphorus (Beg et al., 2001). With the resuspension and bioturbation, the rock weathering and soil erosion can carry pollutants into water again, and threaten the integrity of the aquatic ecosystem (Zhang et al., 2016).

Moreover, the accumulated organic matter in sediments can cause the depletion of oxygen and accelerate production of greenhouse gases such as methane (Flury et al., 2015). In recent years, black water agglomerate in aquatic ecosystems, which is also called "black spot" or "black spot algae", has occurred in many eutrophic lakes rich in organic matters with increased frequency (Zhang et al., 2010; Pucciarelli et al., 2008). For the black water agglomerate, studies have focused on the dissolved oxygen, oxidation-reduction potential (Eh), concentrations of odorous volatile organic sulfur compounds (Liu et al., 2015). On the occurrence of black water agglomerate, the reactive and labile organic matters in sediment were able to be easily and rapidly decomposed (He et al., 2013; Liu et al., 2018), resulting in accumulation of vast gases. Then the produced vast gases can lead to sediment bulking and foam up, which was directly related to the variation in sediment height, changed the sediment characteristics, released the hazardous substance into water and destroy the aquatic ecosystem (Zhou et al., 2015). Despite the harm of process of sediment bulking, there is still no research focus on monitoring the process of sediment bulking.

Recently, biosensors, which are integrated devices that combine biological materials with a transducer, have been developed for the quantitative or semi-quantitative monitoring of various analytes (Zhou et al., 2017). Electrical signals from microbial fuel cell (MFC) have frequently been applied as indicators for the monitoring of heavy metals and aromatic compounds due to their highly speed, sensitivity, and quantifiability (Chen et al., 2016; Kim et al., 2007; ElMekawy et al., 2018). The sediment microbial fuel cell (SMFC) is a type of MFC with an anode electrode embedded in anaerobic sediments and a cathode electrode suspended in the aerobic water column above the anode electrode (Reimers et al., 2001). Nowadays, this system is applicable as an electron supply device for biosensors for long term and *in situ* operation. The use of SMFCs as wireless sensors for the real-time detection of environmental and ecological conditions such as temperature, the concentration of chromium (VI) and the microbial metabolism have been reported (Zhang et al., 2011; Zhao et al., 2018; Wardman et al., 2014.). However, there are few reports on the development of SMFC as a biosensor for monitoring variation in height of sediment.

In this study, a multi-anodes SMFC system was developed for long term and real-time monitoring the changes in sediment height during the process of sediment bulking. A significant change in voltage signal of SMFC was recorded when the anode was imbedded into sediment. The relationship between voltage signal and sediment height change was built within 60 days. Meanwhile, the characteristics of sediment at different layers were analyzed with experiment. This study could provide clues for real-time monitoring of environmental risks in aquatic environments, also help improve our understanding about biogeochemical process within water-sediment surface (WSI).

2. Materials and methods

2.1. Setup and operation of vertically distributed multi-anode SMFC (VM-SMFC)

In this study, relatively undisturbed sediments and surface water were collected from Lake Xuanwu (Nanjing, China) in December 2017. The detail operations for samples were shown in Supporting Information (SI). Carbon felt with a thickness of 5 mm was used both as anode (6×6 cm^2) and cathode (12×6 cm^2). All electrodes were soaked in 1 M HCl for 24 h and washed thoroughly with deionized water prior to use. As described in Fig. 1, total 7 SMFCs was constructed with a cathode, and anodes were vertically employed in various heights. The horizontal and vertical distances between the two adjacent electrodes were 3 cm and 1 cm, respectively. The electrodes were connected to an external circuit using copper wires, the joints were sealed with conductive silver epoxy glue and insulating epoxy glue in succession. The circuit was connected through a resistance of 1000 Ω for SMFC. The VM-SMFC was installed in a container that had a total volume 36 L (dimensions: length, 46 cm; width, 34 cm; and height, 23 cm). The height of sediment inside the container was about 7.6 cm and had a volume of 11.9 L sediment at the initial stage. Then the lake water was used to fill the container, and water volume was about 11.6 L.

At the beginning of experiments, 7 anodes were vertically employed with different heights along the initial WSI. Two anodes were placed at the position of 1 cm below the initial WSI (SMFC-B1) and the positions of initial WSI (SMFC-0), the other five anodes were placed at the positions of 1 cm (SMFC-1), 2 cm (SMFC-2), 3 cm (SMFC-3), 4 cm (SMFC-4), and 5 cm (SMFC-5) above initial WSI, respectively. The shared cathode was fixed 15 cm above the bottom of container. The water level in the box was maintained 15 cm above the bottom of container through setting the position of effluent port. All experiments were conducted in duplication and operated at 25 °C for 60 days.

The heights of sediment were measured every day, and the core sediment samples were taken regularly (on days 5, 15, 25, 35, 45, and 55) during experimental operation and sliced into three layers: first layer (0–2 cm), second layer (2–4 cm), and third layer (4–6 cm). S0 was the original sediment sample, first layer (FL), second layer (SL), and the third layer (TL) taken on the above six different times were labelled by turn as FL-5 to FL-55, SL-5 to SL-55, and TL-5 to TL-55, respectively. Parts of sediment samples were stored in −80 °C refrigerator for chemical and microbial analysis.

2.2. Electrochemical measurement and calculation

Voltage measurements including whole-cell potential and cathode potential were collected with a Keithley model 2700 multimeter (Keithley Instruments 2700, USA) every 10 min. The measured voltage was then converted to a current according to Ohm's law: $V = IR$, and power according to $P = VI$. Electricity current and power density were both calculated based on anodic geometric surface areas (GA). For polarizations behavior test of SMFC, linear sweep voltammetry (LSV) were employed by recording the current at an applied voltage that was lowered in steps of 0.001 V from open-circuit voltage to 0 V with electrochemical workstation (CHI660D, Shanghai Chenhua Device company, China), during which anodes were considered as working electrodes and cathodes as counter electrodes. Internal resistance was calculated by the polarization slope method (Wang et al., 2014).

2.3. Chemical analysis

Loss on ignition (LOI) was determined gravimetrically by weighing the samples before and after combustion at 550 °C for 4 h. Water content of sediments was determined by drying at 60 °C until constant weight. Bulk density is the mass of dry solids in a given bulk volume of soil (Brady, 1984). Porosity of sediment was calculated according to

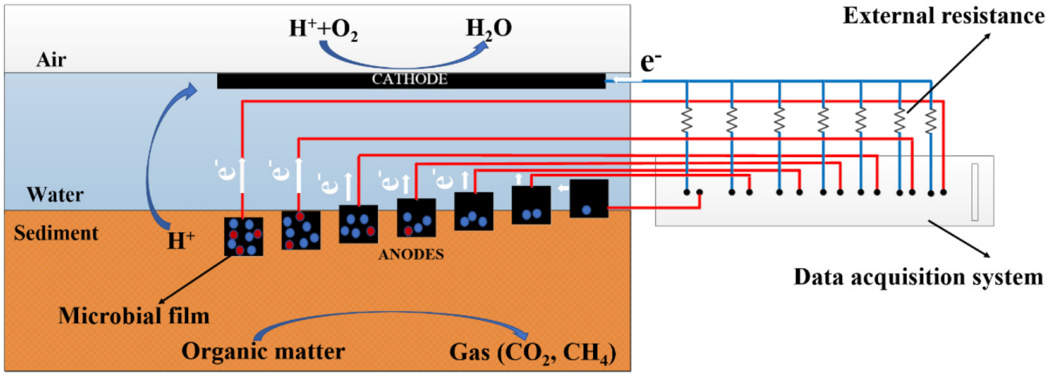

Fig. 1. Schematic and picture of vertically distributed multi-anodes SMFC.

the mass of sediment before and after combustion at 60 °C for 12 h (Urban et al., 1997). Total organic carbon (TOC) in sediments was measured with Walkley and Black's rapid titration method (Walkley and Black, 1934). The concentration of TP (total phosphorus) and TN (total nitrogen) were measured by a Shimadzu UV-2550 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using a high-pressure digestion method, a molybdenum blue method, and a Nessler's reagent method, respectively (Shen et al., 2014).

Sediment organic matter (SOM) is a heterogeneous material including both labile and refractory organic matters. The labile and recalcitrant carbon pools in sediment organic matters were measured using the three-pool approach through a total organic carbon analyzer (TOC-Vcph, Shimadzu) (Rovira and Vallejo, 2007). The detail methods were described in SI.

2.4. DNA extraction, sequencing and microbial community analysis

After 60 days of experiments, sediment samples at different layer were taken for molecular analysis. Genomic DNA was extracted with a PowerSoil®DNA Isolation Kit (MO BIO Laboratories Inc., USA), according to the manufacturer's instructions. DNA concentrations were measured by Nanodrop 2000 instrument (NanoDrop Technologies, Wilmington, USA), and a highly pure genomic DNA (A260/A280 ≈ 1.8) was collected. Then 16S rRNA was partially amplified from the DNA extracts using a nearly universal bacterial primer set and processed by the MAGIGENE Biotechnology Company (Guangzhou, China) for high-through-put DNA sequencing with the Illumina MiSeq System (New England Biolabs, MA, USA). Detailed procedures of DNA extraction, PCR amplification, high-throughput sequencing, and sequence analysis are given in the

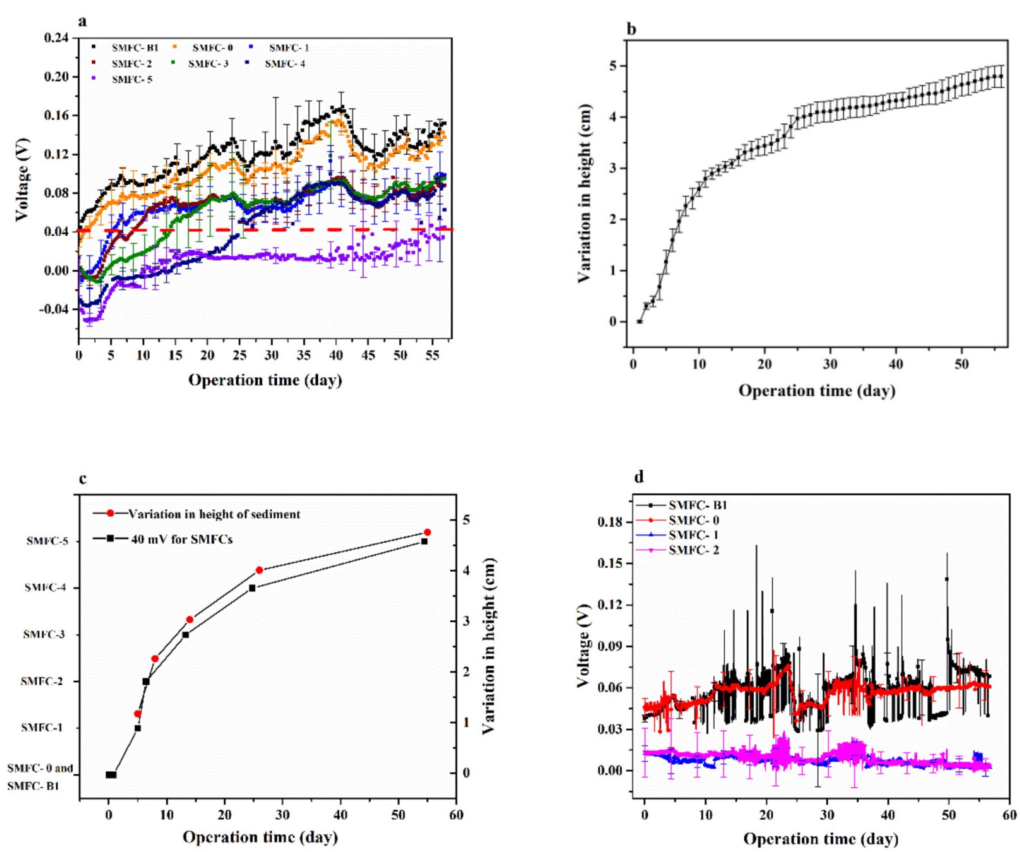

Fig. 2. Variations in voltage values of SMFCs (a) and sediment height (b) for VM-SMFC within 60 days, the red dashed line in (a) is 40 mV. Operation time for SMFCs' voltage reached to 40 mV with sediment height variation is 1 cm (c), and the voltage signals of VM-SMFC with non-bulking sediment (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SI. The nucleotide sequences were all deposited in the GenBank database (accession number: SRP 211918).

2.5. Statistical analysis

The means and standard deviations were calculated using Origin 8.5. The principal component analysis (PCA) and redundancy analysis (RDA) were analyzed by the CANOCO for windows 4.5.

3. Results and discussion

3.1. Changes in VM-SMFC voltages and sediment height

Voltages from VM-SMFC during 60 days of experiments were shown in Fig. 2a. It was found that different voltage signals were produced from the VM-SMFC on basis of the sediment height. When the anodes were embedded in the sediment, produced voltages would exceed 40 mV (the dashed red line), however, the voltages from SMFCs even with part of anode above the WSI, still kept around 0 mV. This phenomenon was attributed to the anode at position where oxygen exists, would cause a decline of the anode performance (Lowy and Tender, 2008). Previous studies indicated that the electron produced from the biofilm can be utilized by the oxygen directly with anode above the WSI (Song and Jiang, 2011). In the meantime, the study found that the gases produced by microbes can cause the occurrence of sediment bulking (Zhou et al., 2015). As shown in Fig. 2b, during 60 days, sediment bulking occurred with 4.8 cm increase in sediment height. Through comparing the height change with the voltage output of VM-SMFC, the results showed that the changes of sediment height can be depicted by the voltage change of VM-SMFC (Fig. 2c). When the anode of SMFC was completely embedded into the sediment, the produced voltage signal would reach around 40 mV. For examples, the variation in sediment height was 1.2 cm on day 5, the anode of SMFC-1 was embedded into sediments with the voltage increasing to ~40 mV. For the other SMFCs with anodes above the initial WSI, the voltage of SMFCs arrived at around 40 mV on days 8, 15, 25 and 56 in sequence with ~1 cm increase in the sediment height. And the voltage of SMFCs with anode above the WSI, almost kept at 0 mV. Furthermore, the control experiment was conducted by placing VM-SMFC in sediment with no obvious variation in sediment height for 60 days. As showed in Fig. 2d, the voltage of SMFCs can reach around 40 mV immediately only when anodes were placed in sediment, however, the voltage of SMFCs with anodes above the WSI, almost kept at 0 mV during 60 days. Hence, the VM-SMFC could monitor the height change of sediment through detecting voltage change.

3.2. Electrochemical properties and internal resistance of the VM-SMFC

The power density and open circuit voltage of SMFCs were performed to investigate the relation with variation in sediment height. Here, electrochemical properties of seven SMFCs were measured on days 5, 15, 25, 35, 45 and 55 respectively. It was found that the maximum power density of SMFC would exceed 10 mW m^{-2} when the anode was fully amended into sediments. As shown in Fig. 3, the maximum power density of SMFC-0 was 17.59 mW m^{-2} on day 5. On day 15, the maximum power densities of SMFC-0 and SMFC-2 were 29.38 and 12.69 mW m^{-2} with 3.1 cm increase in sediment height, but the maximum power densities of SMFC-4 was 0.35 mW m^{-2}. When an increase in the sediment height reached 4.0 cm on day 25, the maximum power densities of SMFC-0, SMFC-2, SMFC-4 were all beyond 10 mW m^{-2}. These results indicated that the maximum power density of SMFC would increase to ~10 mW m^{-2} after anodes were completely embedded into the sediments. The other SMFCs were shown in Fig. S1. Similarly, the open circuit voltage of SMFCs increased when anodes were below the WSI (Fig. S2). Accordingly, the results showed that the power density of VM-SMFC can reflect the variation in sediment height.

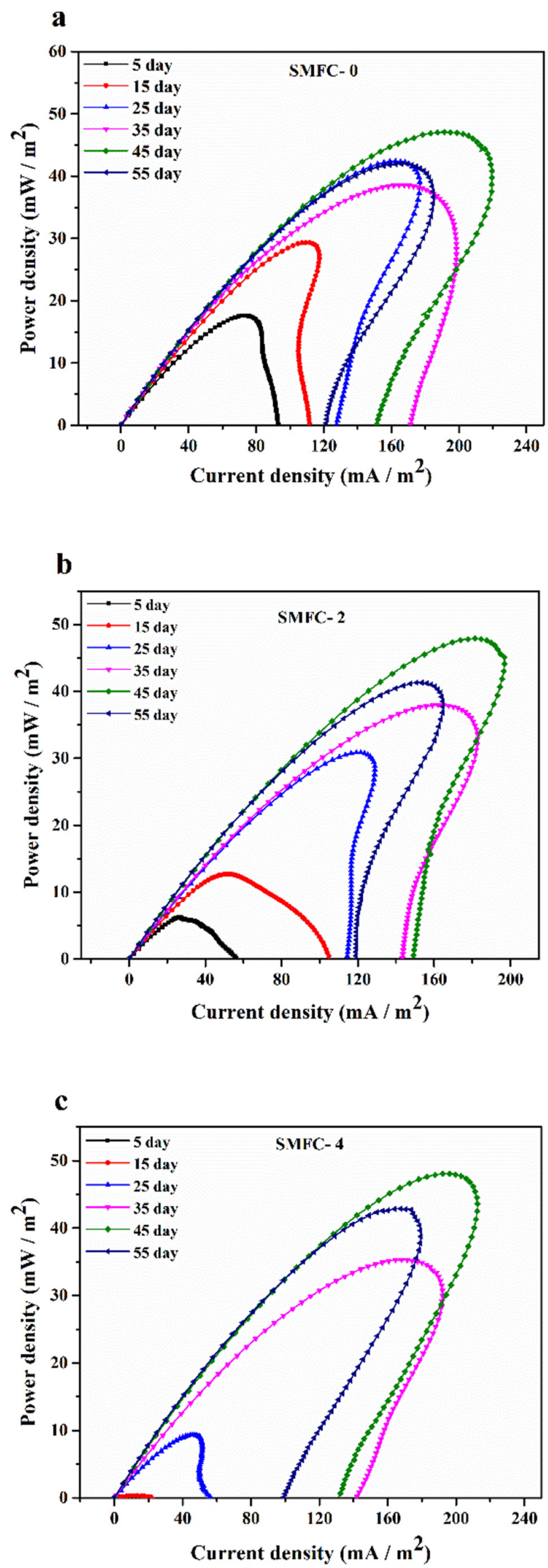

Fig. 3. Power densities as function of current densities obtained from SMFC-0, SMFC-2, SMFC-4 (a–c).

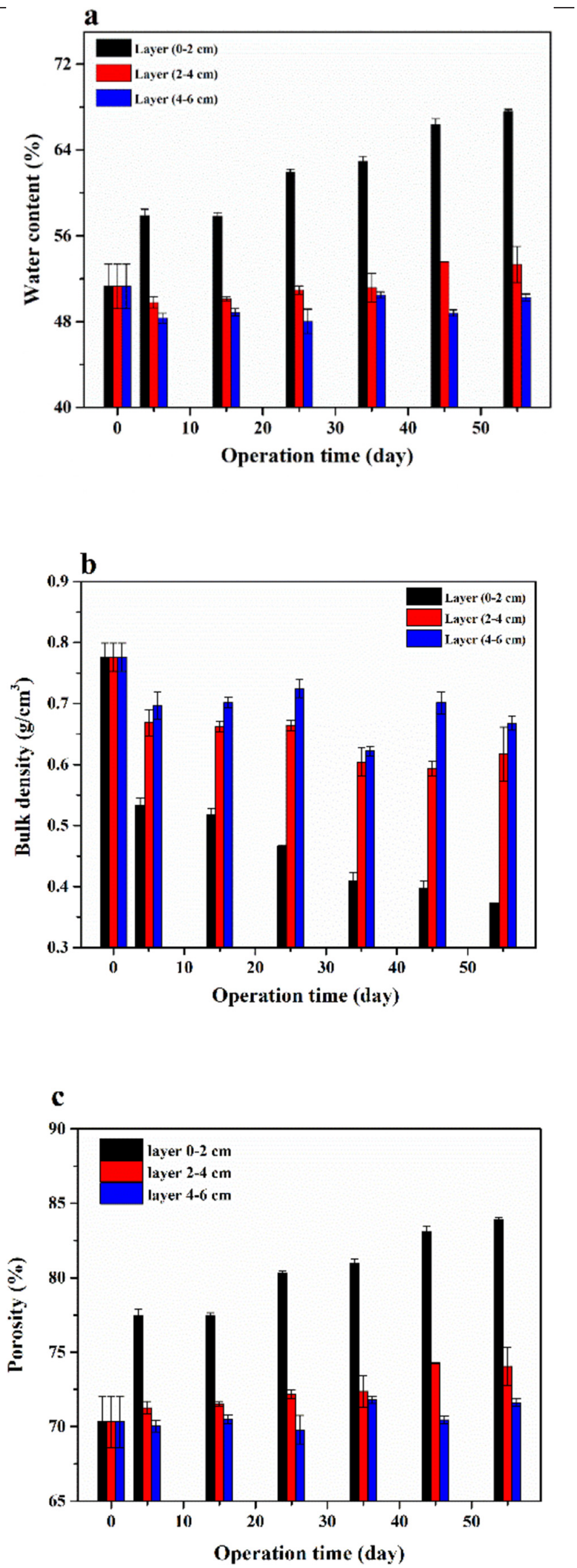

Fig. 4. Changes in water content (a), bulk density (b), and porosity (c) of sediments at different layers during experimental operation.

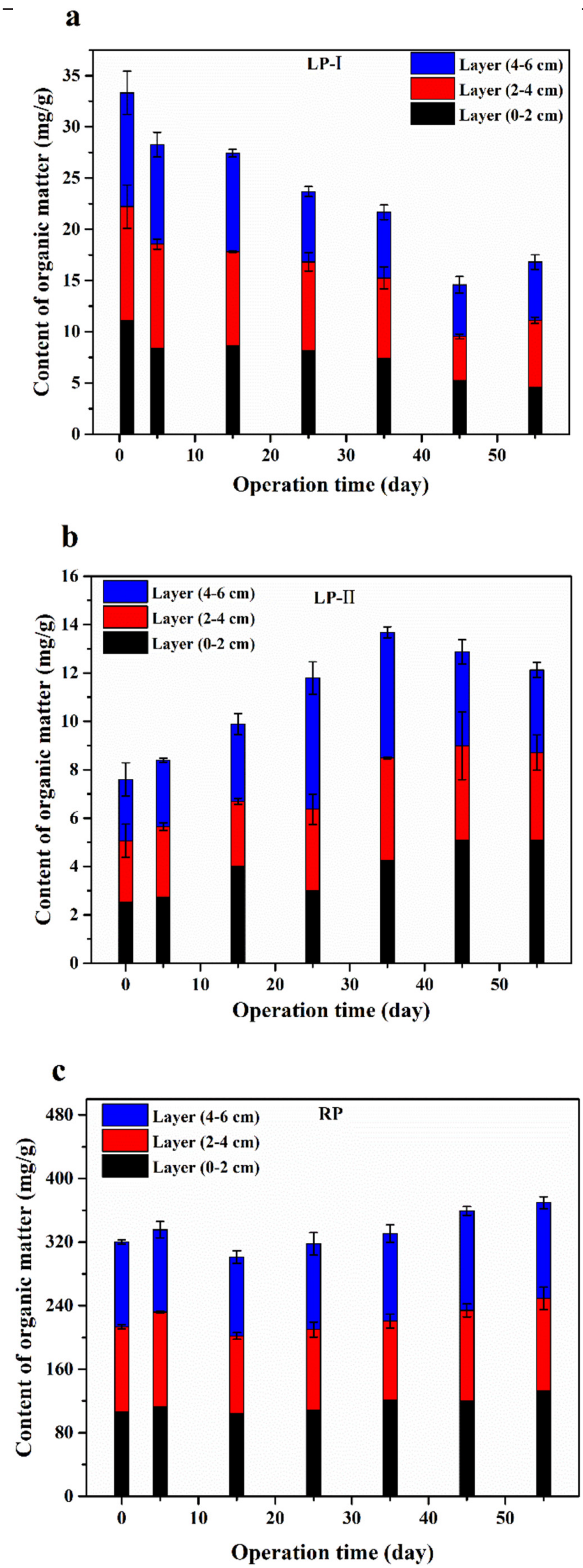

Fig. 5. Organic carbon contents of LP-I (a), LP-II (b) and RP (c) in sediments at different layers (0–2, 2–4 and 4–6 cm) during experimental operation.

Additionally, doubling back of the power density curve at higher current densities appeared in electrochemical experiments. As reported previously (Hong et al., 2011), the power overshoot of power density was observed when anode biofilm acclimated to higher but not lower resistances. The internal resistances of SMFCs decreased below 500 Ω when the anodes were covered with sediments, which were lower than the external resistance (1000 Ω) (Fig. S3). When the anode was above the WSI, the circuit of SFMC was in an open-circle state with enormous internal resistance. After the anode was amended into sediments, the circuit of SMFC was in a closed state with a decrease in internal resistance. The results suggested that the power overshoot of power density appeared with the anode below the WSI.

3.3. Changes of sediment characteristics within experiments

3.3.1. The water content, bulk density and porosity

Within experiments, the sediments at different layers had obvious changes in morphology and properties. As shown in Fig. 4a, the water content of sediments increased with time and decreased with depth. For example, the water content at FL increased from 51.3 to 67.6% during 60 days, and water content at SL and TL were 53.3% and 50.2% on day 55, respectively. The bulk density of sediments at FL decreased from 0.78 to 0.37 g cm^{-3}. Opposite to the water content, the bulk density increased with depth (Fig. 4b). Meanwhile, the porosity of sediment at layers increased from 70.33% to 83.91% (FL), to 74.05% (SL), and only to 71.62% (TL) (Fig. 4c). The results were expected as the pore waters were forced upwards and out of the bottom sediments (Jepsen et al., 2000).

3.3.2. Carbon pools of sediment

The labile (LP-I and LP-II) and recalcitrant (RP) pools of carbon in sediments with experiments were shown in Fig. 5. During experiments, the amount of LP-I decreased rapidly. As shown in the Fig. 5a, the contents of LP-I at FL, SL and TL decreased from 11.11 mg g^{-1} to 4.58, 6.54 and 5.68 mg g^{-1} respectively; the amounts of RP of FL, SL and TL increased from 106.65 mg g^{-1} to 132.53, 116.63 and 120.60 mg g^{-1} respectively (Fig. 5b, c). It was previously revealed that RP contains not only lignin but humic substances, and degradation of lignin-like compounds can improve the content of humic-like substances (Song et al., 2015). During organic matter decomposition in sediments, some carbon was observed to be transformed into humus, which is widely regarded as carbon sink because it is either physically protected inside aggregates or chemically too complex to be used by most organisms (Prescott, 2010; Boddy et al., 2007). So, the amount of LP-I in sediment was strongly related to the process of sediment bulking.

3.4. Microbial communities in sediments

3.4.1. The role of microorganisms in sediments

Microbial communities in sediments were analyzed, and a total of 8836 bacterial 16S RNA gene sequences and 9419 OTUs were obtained from 19 samples.

At phylum levels (Fig. 6a), the most abundant sequences of the sediment samples within 60 days belonged to *Proteobacteria* (24.8–47.1%), *Chloroflexi* (10.0–19.3%), *Bacteroidetes* (7.3–18.6%), and *Verrucomicrobia* (6.6–13.1%), *Firmicutes* (2.0–9.7%), *Actinobacteria* (1.8–9.0%), *Planctomycetes* (1.5–3.9%). Interestingly, among those phyla, the abundances of *Planctomycetes* and *Actinobacteria* decreased, and *Bacteroidete* increased with sediment depth within 60 days. It was found that the *Planctomycetes* and *Actinobacteria* were able to catalyze the oxidation reaction of recalcitrant and xenobiotic substances including humic substances and toxic chemicals (Li et al., 2014). Meanwhile, the previous studies indicated that the *Bacteroidete* was very diverse and their closest relatives were sequences from anaerobic digestors in sediment (Briée et al., 2007). Members of this phylum are typical degraders for hydrolyzing complex organic molecules (cellulolytic or chitinolytic) or fermenting under strict anaerobic conditions (Madigan et al., 2002).

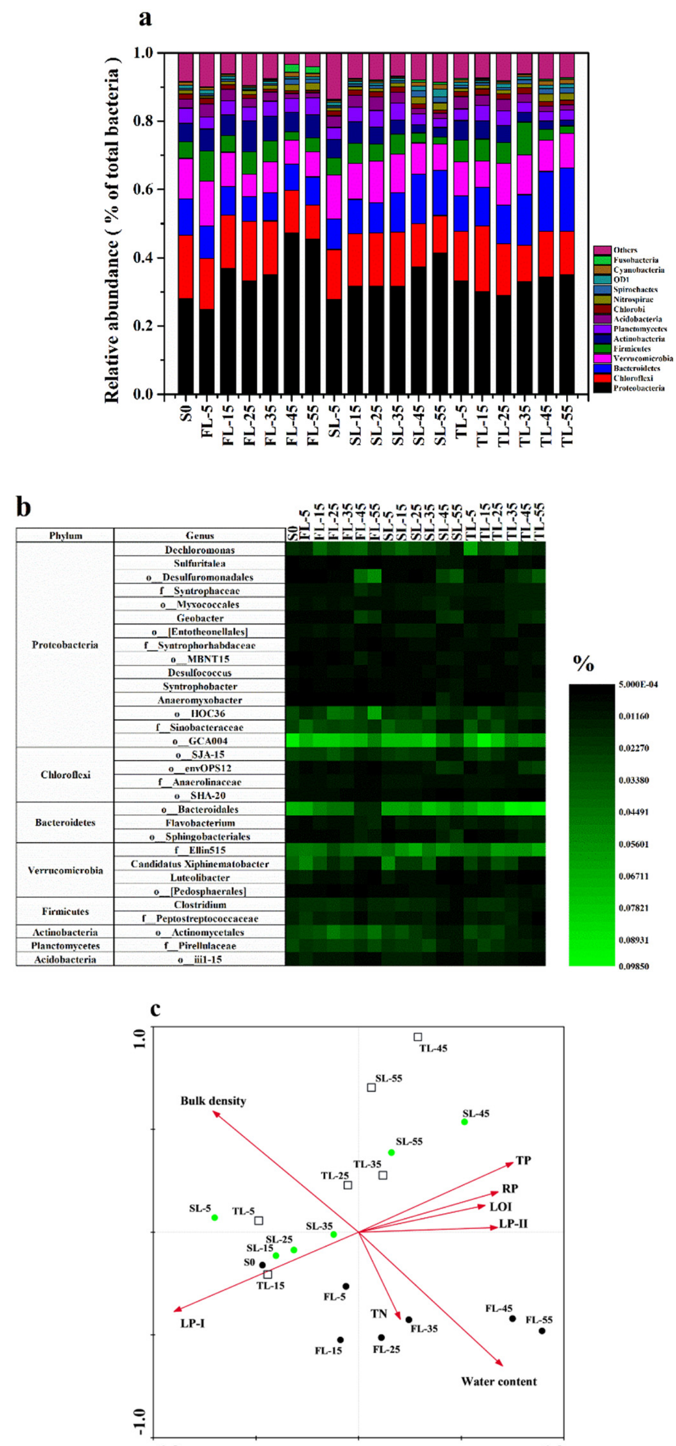

Fig. 6. Relative abundance of bacterial phylogenetic groups at the phylum level of different sediments samples (a), the heat map at the genus level (b), and the bioplot of redundancy analysis (c) of the bacterial community of sediment samples at different layers (0–2, 2–4 and 4–6 cm).

The genus (g.) level characterization further illustrated variation in the bacterial community, though some were only classified at the phylum (p.), class (c.), order (o.), or family (f.). The heatmap (Fig. 6b) showed the obvious difference in abundance of bacterial genera among the 19 samples within 60 days of experiments. Through comparing sediment samples taken from FL with those from TL, it was found that sediment bulking led to an obvious decrease in abundances of o. *Bacteroidales*. The *Bacteroidetes* are well known degraders of polysaccharide by fermentation process (Mann et al., 2013). The decrease in abundances of *Bacteroidales* with time may involve the reduction in liable carbon pool in sediments. And the increase in depth suggested that the fermentation process take place from the surface layer to bottom layer. Through comparing the samples at same layers within 60 days, it was also found that *Dechloromonas* appeared in the layer 0–2 cm. The genus *Dechloromonas* contained many heterotrophic and facultative anaerobic chlorate-, perchlorate- or nitrate respiring bacteria (Haller et al., 2011).

3.4.2. Relationship between microbial community and environmental variables

Redundancy analysis (RDA) was performed to identify correlations among changes in the microbial community and environmental variables. The relationship between the microbial community and eight parameters (LP-I, LP-II, RP, water content, bulk density, total nitrogen, total phosphorus, LOI) in the RDA ordination plot was shown in Fig. 6c. The samples during initial 15 days had close relationship with content of LP-I. As microbes can use LP-I rapidly with accumulation of a large amount gas (Boddy et al., 2007), LP-II and RP were difficultly utilized by microorganisms. Therefore, LP-I was a key factor leading to the process of sediment bulking. It seemed that the surface-bulking sediment samples (FL) were related to the water content of sediment. This result was ascribed to the production of gases, then the pore water in sediment would increase (Maeck et al., 2014). So, it seemed that the content of LP-I was a key factor for the sediment bulking, resulting in the increase of sediment water content.

From principal component analysis (PCA), it was found that bacterial communities changed from surface to the bottom. As shown in Fig. S4, 19 different communities were grouped into three distinct clusters: (a) complete bulking sediment samples (FL-45 and FL-55); (b) partial bulking sediment samples (TL-35, SL-45, TL-45, SL-55, TL-55); (c) sediment samples during initial 30 days. Fig. S4 also showed the internal relation existed in three distinct clusters. Firstly, the black circles, representing samples at surface layer (FL), gradually shifted from the cluster (c) to cluster (a) during the process of sediment bulking; secondly, the green circles were samples at SL, and gradually moved from cluster (c) to cluster (b) within 60 days; finally, the squares denoting samples at TL, gradually changed from cluster (c) to cluster (b) within 60 days. This result suggested that sediment bulking occurred from the surface part to the bottom part.

3.5. Mechanism of sediment bulking and prospect for the VM-SMFC

Lake sediments are important in the global carbon cycle as they can act as both a sink and a source depending on relative carbon mineralization and sedimentation rates. When considering biodegradation of sediment organic matters in lake sediments, the type of organic matters is a key factor in degradation process (Song et al., 2013). With rapid decomposition of labile organic matters in sediments, microorganisms could produce a large amount of gases (Johnson et al., 2002), which resulting in occurrence of sediment bulking (Zhou et al., 2015). Fig. 5 showed clearly that the amount of LP-I was a key factor for sediment bulking. It can be reflected in the variation rate of sediment height. The variation in sediment height was around 3 cm during the initial 15 days, but around 2 cm for the remaining 45 days (Fig. 2b). The rapid rate of variation in sediment height was believed to have a significant effect on sediment physical properties, erosion rates and aquatic environment (Jepsen et al., 1997; Lick and Joe, 2001). In this study, the water content and porosity of sediments decreased with depth, while the bulk density increased with depth in 60 days. Interestingly, when looking at different layers, it was found that water content of sediments at SL and TL had little decrease during initial 35 days, which was expected as pore waters are forced upward and out of the sediments from the weight of the overlying solids (Jepsen et al., 2000). Though water content of sediment at SL and TL had a little decrease, the bulk density of sediments in SL and FL did not increase but decreased with time (Fig. 4b). This is because the forcing of water out of the sediments is approximately balanced by gases generated from biodegradation. Once sediment gas storage capacity is exceeded, gas exits the sediment by bubbling (Liu et al., 2016).

In this study, the VM-SMFC biosensor exhibited many improvements and compared favorably to conventional manual measurement methods for variation in sediment height. First, the VM-SMFC biosensor can monitor the variation in sediment height at 1 cm scale range by employing only one set of VD-SMFC system with multi-anode single cathode configuration, not multiple-unit MFC systems. Second, this VD-SMFC biosensor can realize long term and real-time monitoring the variation in sediment height. Traditional monitoring methods have relied on manual measurement, approaches which are often time-consuming and inaccurate. From the above, the VM-SMFC system has the potential to be integrated well with biosensing and real-time monitoring for the lake system. In the meantime, it will be credible to combine the monitoring of overlying water with sediment for forecasting the sudden pollution of aqueous environment. The further research is planned as we improve the online real time VM-SMFC biosensor. The integrated system can also be validated to illustrate the general application of this biosensor in lakes after investigating effects of other factors, such as the sediment deposition, nutrient levels of sediment, and the water depth.

4. Conclusion

In this study, the SMFC-based biosensor was developed for long term and real-time monitoring of the variation in sediment height during sediment bulking. The amount of LP-I of sediment was demonstrated as a key factor for sediment bulking. During the operation of VM-SMFC, once the anodes were completely amended into sediments, the changes in voltage and power density of biosensor would relate to the variation in sediment height. Thus, the VM-SMFC sensor can realize real-time monitoring of variation in sediment height and have potential application as an early-warning system to help identify sediment bulking.

Declaration of Competing Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134009.

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