

Article



# **Contrasting Effects of Sediment Microbial Fuel Cells** (SMFCs) on the Degradation of Macrophyte Litter in Sediments from Different Areas of a Shallow Eutrophic Lake

# Na Song \*, Helong Jiang and Zaisheng Yan

State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China

\* Correspondence: nsong@niglas.ac.cn; Tel.: +86-25-8688-2223

Received: 16 July 2019; Accepted: 29 August 2019; Published: 6 September 2019



**Abstract:** Eutrophication is one of the major ecological problems of our era. It accelerates the growth of aquatic plant and algae, eventually leading to ecological deterioration. Based on a 700-day lab experiment, this paper investigated the contrasting effects of sediment microbial fuel cells (SMFCs) on the removal of macrophyte litter in a macrophyte-dominated area and an algae-dominated area from two bay areas of a shallow eutrophic lake. The results revealed that the removal efficiencies of total organic carbon increased by 14.4% in the macrophyte-dominated area and 7.8% in the algae-dominated area became more humified and had a higher electricity generation compared to the sediment samples from the algae-dominated area. Pyrosequencing analysis further determined that SMFC promoted more aromatic compound-degrading bacteria growth in sediments from the macrophyte-dominated area than from the algae-dominated area. Our study demonstrated that SMFC could enhance organic matter degradation, especially plant litter degradation, but this influence showed different from sediment sources. Thus, SMFC is capable of providing a useful strategy for delaying the terrestrialization of lakes areas suffering from eutrophication.

Keywords: macrophyte litter; sediment microbial fuel cell; degradation; lake management

# 1. Introduction

Lake eutrophication has become one of the most serious ecological problems of the 21<sup>st</sup> century [1]. In aquatic ecosystems, especially for shallow lakes, eutrophication is often caused by excessive nutrient loading (i.e., N and P) [2,3]. Eutrophication is often accompanied by undesirable overgrowth of autotrophs, such as cyanobacteria and algae, which form a greenish slime layer on the surface of lake water and restrict light penetration [4]. Eutrophication also triggers instability of the ecosystems, which can easily be invaded by aquatic plants. Eutrophication can create enough nutrients for aquatic plant growth. Therefore, macrophytes usually spread rapidly in these shallow water areas, which include wetlands, fens, and peat lakes [5,6]. In some lakes, restoration of macrophytes has been proposed as an ecological strategy to improve water quality and to rehabilitate the degraded lake ecosystem, which also causes rapid aquatic plant growth [7]. Once decayed, plant tissues become abundant and settle into sediments as litter.

Decomposition of excess litter may generate a series of negative influences on lake ecosystems. First, because the refractory lignocellulose is the major component of plants, decomposition of plant litter causes an increase in sediment layer, reducing lake area, damaging animal habitats, and accelerating lake swamping [8]. Second, the accumulation and decomposition of dead litter consume oxygen and

generate greenhouse gases such as hydrogen sulfide ( $H_2S$ ) and methane ( $CH_4$ ) [9]. Once that happens, many fish species and macroinvertebrates can suffocate and die, while species that live at the bottom of such lakes can die off completely [10].

In sediments, the removal of lignocellulose is mostly mediated by microbial extracellular enzymes [11]. In fact, the biodegradation rate of plant litter in sediments is low and is usually due to the lack of suitable and adequate electron acceptors, such as oxygen, ferric iron, nitrate, and sulfate. Therefore, a complementary technique to effectively remove and obtain energy from lignocellulose is needed. Recent studies have shown that the anode of sediment microbial fuel cell (SMFC) can act as an effective electron acceptor [12,13]. SMFC technology can remove organic matter from sediment and provide a low-cost and long-term sink for electrons [14].

For a typical microbial fuel cell (MFC), organic matter is converted into electricity via the action of bacteria in an anode [15]. SMFCs are the main type of MFCs. They place an anode into organic rich sediment and a cathode on the surface of lake water. Electrons are released during substrate oxidation; then, they are transferred to an anode via anode-respiring bacteria. The anode serves as the electron acceptor. Subsequently, electrons are transported across a load to the cathode and a current is generated when oxygen is reduced in the water column [16]. Our previous research determined that SMFC can speed up the removal of plant litter [17], but it was still unknown whether this removal was influenced by different sources of sediment.

The overall objectives of this study were to investigate the effects of SMFC on litter removal and how that removal affects the different properties of sediments. During the 700 days of *ex-situ* pilot-scale lab experiments, sediment samples were extracted from a macrophyte-dominated area and an algae-dominated area in two bays of the shallow eutrophic lake-Taihu lake. The alteration of the organic matter in each of the two sampling sites was characterized and compared. Meanwhile, the composition of microbial communities was identified using high-throughput pyrosequencing technology after 700 days of incubation.

## 2. Material and Methods

## 2.1. Sediment, Lake Water and Macrophyte Sampling

In this study, samples were collected from two sampling sites of Taihu Lake ( $31^{\circ}10'$  N,  $120^{\circ}24'$  E) as shown in Figure S1. The first site, referred to as Taihu 1 (TH1) with a latitude and longitudinal location of  $31^{\circ}10'$  N,  $120^{\circ}25'$ E was Dongtaihu Bay, a eutrophic bay with an abundance of submerged and emergent aquatic plants; The second site TH2 ( $31^{\circ}30'$  N,  $120^{\circ}11'$ E) was Meiliang Bay, a eutrophic bay in the northern part of Taihu lake. Submerged macrophytes were planted in this area to restore the local ecology [18]. Surface sediment (at 0–10 cm depth) was collected using a grab bucket. Samples of surface water (at 0–20 cm depth) were also sampled using a Niskin bottle. Samples were transported to the laboratory as soon as possible and then stored in refrigerator at 4 °C. Sediments were first sieved through a 2 mm mesh screen; then, they were mixed and homogenized for subsequent experiments. Lake water was filtered through a 45  $\mu$ m membrane filter to remove large detritus and zooplankton. *Potamogeton malaianus*, as one of the dominating macrophytes in Taihu Lake, were also sampled.

#### 2.2. Experimental Design

Eight plexiglass columns (12 cm wide  $\times$  35 cm tall), each with an approximate 4 L volume capacity, were used as reaction columns (RCs) to carry out the experiment. The columns were divided into two groups (Table S1 in supporting information): Group I: RC 1 to RC 4, which were set up using sediments from the TH1 site; Group II: RC 5 to RC 8, using sediments from the TH2 site. Each group included two SMFCs treatments and two non-SMFC controls. Schematic diagram and photo of the experiments were shown in Figure S2. All parts of *P. malaianus* were mixed together and dried below 60 °C. They were then crushed and sieved through a 100 mesh screen. Crushed *P. malaianus* material (48 g) and wet sediments (3000 g) were put into each bioreactor, and then were mechanically homogenized and mixed.

After adding the sediment/plant mixture, the remaining volume of the bioreactor was filled with overlying lake water, which continuously flowed using the peristaltic pump at 10 L min<sup>-1</sup> from the lower basin into the upper reactors. All the columns were covered with foil to avoid light and were kept at room temperature.

Graphite felts (5 mm in thickness, Nengkang Carbon, Shanghai, China) were used to make both the anode and cathode. At first, graphite felt were activated by soaking in 1 mol HCl solution for 24 h. Then they were rinsed several times using deionized water to obtain the neutral pH. The anode with 22 cm long and 20 cm wide was fixed on stents. The whole structure was buried 8 cm below the surface of the sediment. A circular cathode with a 9.5 cm diameter was suspended above overlying water. The distance between the cathode and anode was about 7 cm. All electrodes were connected by wires. The external resistor between the cathode and anode was 100  $\Omega$ . The sediments incubated under each treatment were collected every three months. Graphite felt is adopted as a typical electrode material because of its wide operating potential, high corrosion resistance, good electrical conductivity, large porosity and low cost [19]. Because of the special properties of the graphite felt, both the anodes and cathodes were not replaced during the whole experiment.

#### 2.3. Chemical Analyses

The total organic carbon (TOC) in sediments were measured according to the standard method [20]. Fulvic acid (FA) and humic acid (HA) were extracted based on their different solubility in alkaline solutions and acid solutions [17]. TOC contents of FA and HA were quantified using elemental analyzer (CE-440, PerkinElmer, MA, USA).

Cell voltage (mV) during the 700 days of experiments were monitored using a data collector (Model 2700, Keithley Instruments, OH, USA). Polarization curves were tested using the linear sweep voltammetry method with an electrochemical work station (CHI660D, China). Cathodes were considered as counter electrodes and anodes as working electrodes [21]. Power (P) curves that describe the power density as the function of the current density were obtained. The current was calculated according to Ohm's law: U = IR, where U is voltage and R is external resistance (100  $\Omega$ ) and power was calculated according to P = IU. Current density and power density were also calculated by normalizing I and P by the anode surface area (0.044 m<sup>2</sup>).

## 2.4. Microbial Diversity Analysis with High-Throughput Pyrosequencing

After 700 days of incubation, sediment and electrode samples under different treatments were sampled for microbial diversity analysis. The DNA of bulk sediment samples was extracted directly without any pretreatment. However, the electrodes required treatment before DNA extraction. At first, sterile water was used to rinse electrodes, so as to remove large adherent sediment on each electrode surface. Then small pieces of the anode and cathode were randomly obtained using sterile scissors for subsequent DNA extraction. Genomic DNA for 454 pyrosequencing tests was extracted with a DNA Isolation Kit (MOBIO Laboratories, CA, USA) according to the manufacturer's instructions. The extracted DNA was detected with a Nanodrop 2000 instrument (NanoDrop Technologies, Wilmington, USA) and a highly pure genomic DNA (260/280 nm  $\approx$  1.8) was used for pyrosequencing [22]. Then a PCR-based pyrosequencing technology was used to study the diversity of the bacterial communities. Bacterial 16S rRNA gene fragments were amplified with the two primers 341F (5'-CCTACGGGAGGCAGCAG-3') and 1073R (5'-CGAGCTGACGACARCCATG-3'). Bacterial 16S rRNA amplification was performed in a PCR system (ABI GeneAmp®9700), and the detailed steps were shown as follows: an initial denaturation step at 95 °C for 2 min, then 25 cycles of 30 s at 95 °C, 30 s at 72 °C and a final extension at 72 °C for 5 min.

The sequence data were analyzed using QIIME 1.9.0 [23]. OTUs were generated through UCLUST based on a 97% similarity level, and singletons were removed during the process. UCHIME was used to remove chimeras of 16S and ITS data [24]. The greengenes database (http://greengenes.lbl.gov) was

used to assign the taxonomic identity of phylotype of bacteria [25]. The assignment method is BLAST using assign\_taxonomy.py script with default parameters in QIIME.

#### 2.5. Calculation and Statistical Analysis

The degradation of plant litter was characterized according to TOC removal efficiencies. The TOC removal efficiencies in sediments during the whole experiments were analysed as expressed by Equation (1):

$$\text{FOC removal efficiencies (\%)} = (m_t - m_0)/m_0 * 100 \tag{1}$$

where  $m_0$  was the TOC content in original sediments when added plant litter,  $m_t$  was the TOC content in sediments under all treatments at time t.

The statistical significance of differences under different treatments was analyzed by a one-way ANOVA using the SPSS 19 software. The levels p < 0.05 is considered significant difference.

#### 3. Results and Discussion

# 3.1. Chemical Properties of the Original Sediments and Lake Water and Electrochemical Performance from SMFCs

Chemical properties of the original sediments from sites TH1 and TH2 were analyzed and are shown in Table S2: The TP amounts were 763.61 and 625.50 mg kg<sup>-1</sup> dry sediments; the TN amounts were 1250.28 and 1191.50 mg kg<sup>-1</sup> dry sediments; the cellulose amounts were 269.32 and 168.0 mg kg<sup>-1</sup> dry sediments; the TOC amounts were 15.70 and 9.70 mg kg<sup>-1</sup> dry sediments. The properties of the water samples from sites TH1 and TH2 were also measured (Table S2): pH were 8.0 and 7.8, respectively with a conductivity of 0.50 and 0.60 mS cm<sup>-1</sup>, and a dissolved oxygen (DO) content of 11.79 and 12.47 mg L<sup>-1</sup>. More nutrients were found in the macrophyte-dominated area.

The cell voltages of SMFCs from the two sampling sites during the whole experimental period of 700 days showed the same changing tendencies (Figure 1). The voltage output in SMFCs showed a changing fluctuation throughout the experimental period. On the whole, the voltage from SMFCs increased quickly during the former 35 days. This was the startup stage of SMFC and an electrochemically active biofilm on the anode surfaces was formed during this period [26,27]. After 80 days of operation, SMFCs for sediments from TH1 and TH2 reached the highest voltage of 123 mV and 145 mV respectively. The phenomenon indicated microbial population enrichment in this stage, which oxidized some easily biodegradable organic matters in sediments [28]. As the contents of easily biodegradable organic matters decreased, the voltages dropped drastically between Day 80 and Day 125. Then they maintained a relatively stable value between Day 125 and Day 282, and began dropping between Day 282 and Day 370. Voltages increased again on Day 370, followed by a gradual decrease to a minimum of 40 mV and 20 mV from TH1 and TH2 sediments, respectively on Day 700. The voltage drop might be due to the microorganisms in the sediment utilizing the stable, recalcitrant organic matter when the labile carbon pools were depleted. Although the overall trend of voltage change from the two sampling sites was the same, the maximum voltages were different. The voltage in sediments from site TH1 were lower in the early stage of the experiments but became higher after about 400 days of incubation compared to the voltages from site TH2. Current and power generation during the whole experiment were also obtained according to voltage output and the fixed external resistance (100  $\Omega$ ) (Figure S3). The maximum current and power were 1.4 mA and 210 mW from site TH1.

Figure 2 shows the polarization curves for SMFC produced with sediments from sites TH1 and TH2 on Day 450. Notably, the SMFC from site TH1 had a significantly higher maximum power density (5.34 mW m<sup>-2</sup>) compared to that from site TH2 (1.82 mW m<sup>-2</sup>). Previous researches determined that the bioelectrochemical system of an SMFC was influenced by several operational characteristics, such as differently pretreated carbon felts, electrodes' resistance, microorganisms' growth and development, and electrode surface inhomogeneity [29,30]. The same electrodes were designed in each reaction

column, so the same electrodes' resistances were obtained (5.5  $\Omega$ ), which led to the speculation that the different electrical parameters from sites TH1 and TH2 might be mainly due to the microbial community composition. Polarization curves showed that the maximun power densities were produced using an 820  $\Omega$  and a 2000  $\Omega$  resistor, respectively, for sediments from the sites TH1 and TH2. *P. malaianus* is a complex mixture of lignin, hemicellulose, and cellulose, which makes it much more difficult to break down or biodegrade than pure fibrous material.



**Figure 1.** Voltage generation along with time from sediment microbial fuel cells (SMFCs) inoculated with TH1 and TH2 sedimentary samples. Voltages were represented as mean voltages and standard deviations (n = 2).



**Figure 2.** Polarization and power curves for SMFCs produced with TH1 and TH2 sediments on Day 450. Error bars were represented as mean values (n = 2).

Cell voltages in this experiment (the maximum value was about 120 mV) were higher and power densities (5.4 mW m<sup>-2</sup>) were lower than those from other sediments under the same load resistance (100  $\Omega$ ) (about 20 mV and 25 mW m<sup>-2</sup>) (Table S3) [31]. The higher voltages in our experiments might be due to the higher content of organic matter in the sediments, but the larger anode area resulted in a smaller power density compared to other studies. Cell voltages were also obviously lower than those from the two-chambered SMFC which fed on chitin and cellulose [32] (Table S3). The most likely reason was due to the higher load resistance (1000  $\Omega$ ) in other experiments and lower internal resistances obtained compared to our results. It should be noticed that the SMFC using plant litter generated

current for a longer time (700 days in this study) than that using cellulose. The results determined that the slower degradation of lignocelluloses in SMFC could be used as a long term energy source which does not require extensive maintenance or frequent loading.

### 3.2. Changing of TOC Removal Efficiencies during SMFC Operation

TOC is a measurement of the organic richness of sedimentary rocks. Therefore, TOC was applied to characterize the utilization of lignocellulose in sediments. TOC removal efficiencies in sediments from the sites TH1 and TH2 with and without SMFC employment were determined as shown in Figure 3. It was found that, during the whole experimental period, removal efficiencies of TOC in sediments with the employment of SMFCs were higher than those without SMFCs. For site TH1, the removal efficiency of TOC in sediments with an SMFC increased by 14.4% compared to the sediment under the control treatment. Similarly, the TOC removal efficiency for site TH2 and its SMFC sediments increased 7.8% compared to that site's control treatment, but the values were significantly lower compared to site TH1 (one-way ANOVA, p < 0.05). The different removal efficiencies of TOC in different types of sediments were coupled with anaerobic microbial oxidation processes. Most likely, the sediments from site TH1 provided a critical growth factor, which was essential to the success of the anode-respiring bacteria. These results demonstrated unequivocally that the application of SMFC technology could greatly enhance lignocellulose biodegradation in different types of sediments.



**Figure 3.** Removal efficiencies of total organic carbon (TOC) in TH1 and TH2 sediments by control and SMFCs during the 700 days of incubation.

## 3.3. Alternation of Sediment Humic Substances in SMFCs

Humus is the supramolecular association and relatively stable and recalcitrant fraction of organic matter [33]. HA and FA are the major components of the humic substances, and they have different solubility in water under different pH values [14]. Contents of HA and FA in sediments under different treatments on Day 700 are shown in Figure 4. The original values of HA were 2.32 and 1.13 mg-C g<sup>-1</sup> from site TH1 and TH2, respectively. It was found that the HA content in sediments with SMFCs increased (10.43 mg-C g<sup>-1</sup> from site TH1 and 4.93 mg-C g<sup>-1</sup> from site TH2); moreover, the HA content was substantially higher than the HA content in the control sediments (6.13 mg-C g<sup>-1</sup> from site TH1 and 2.92 mg-C g<sup>-1</sup> from site TH2) (one-way ANOVA, p < 0.05). FA content showed a similar tendency (Figure 4B), which markedly increased from 4.0 and 3.75 mg-C g<sup>-1</sup> respectively from sites TH1 and TH2 of the control sediment to 8.0 and 6.4 mg-C g<sup>-1</sup> respectively from site TH1 and TH2 with SMFCs (one-way ANOVA, p < 0.05). However, both of the HA and FA contents from site TH1 were higher than those from site TH2 at the end of the experiment. From this, when compared with the control sediments, without SMFC, it is clear that SMFCs stimulated the humification of organic matter in sediment,

especially for the macrophyte-dominated area. The phenomenon was also found elsewhere [34], which researched the degradation of the polycyclic aromatic hydrocarbons in freshwater sediments.



**Figure 4.** Organic carbon contents of humic acid (HA) (**A**) and FA (**B**) in TH1 and TH2 sediments after 700 days of incubation.

## 3.4. Microbial Communities Diversity in Sediments and on the Surfaces of Electrodes

The degradation of lignocellulosic biomass in sediment needs the synergistic effect of different microbial populations, including the hydrolytic, celluloytic, hemicellulolytic, and syntrophic hydrogen-producing bacteria [35]. In this study, a 16S rRNA gene pyrosequencing approach was used to analyze the bacterial community in sediments and on the surfaces of electrodes after 700 days of incubation. A total of 75,557 bacterial sequences and 7348 OTUs were obtained from the 10 samples (Table 1). Both of the two original sediments had the highest OTU values, whereas only 351 and 254 OTUs were identified on the Site TH1 and Site TH2 cathode samples. Coverage covered 88.5–92.1% of the bacterial communities (Table 1). This means that most of the libraries have been established as a matter of record. Shannon diversity indicates that bacteria diversity decreased in sediments with SMFC compared to the control without SMFC. However, higher diversities and more sequences were found in sediments from site TH1 under all treatments.

Figure 5A,B show the relative bacterial community abundance in sediments under different treatments on the phylum level. Proteobacteria (14.5–45.3% from site TH1 and 18.7–75.9% from site TH2), and Bacteroidetes (11.6–38.9% from site TH1, 10.0–32.2% from site TH2) were the most abundant

phyla in all samples. Proteobacteria and Bacteroidetes were the dominated organic matter degradation microorganisms and most frequently implicated in facilitating cellulose degradation in sediment [36]. The abundance of Proteobacteria was relatively more pronounced on the cathode surface in site TH1 and on both electrodes in site TH2. The phylum of Bacteroidetes could degrade waste lignocelluloses and enhance biogas production under the mesophilic condition [37] and the abundance was higher in sediments with added SMFCs, especially for the samples from the macrophyte-dominated area.



**Figure 5.** Relative abundances percentages of bacterial phyla (**A**,**B**) communities in original sediments, bulk sediments and electrode biofilm from the two sampling sites at the end of the experiments. The minor designation represents the sum of bacteria in a sample with a relative abundance < 3%.

Samples		Number of Sequences <sup>a</sup>	OTUs <sup>b</sup>	Shannon <sup>c</sup>	Coverage <sup>d</sup>
Site TH1	original	12,503	1329	7.94	92.1%
	anode	5671	567	5.63	90.2%
	cathode	3257	351	4.36	88.5%
	control	10,276	1026	7.68	91.3%
	SMFC	9876	756	6.65	90.9%
Site TH2	original	11,165	1021	7.78	91.5%
	anode	3524	452	5.21	89.3%
	cathode	2367	254	4.02	89.1%
	control	9056	956	7.25	88.6%
	SMFC	7862	636	6.01	90.2%

**Table 1.** Observed bacterial richness and diversity estimated based on 97% OTUs clusters for sediments under different treatments.

<sup>a</sup> Number of sequences indicates high quality sequences that passed quality trimming, denoising, and chumeria checking; <sup>b</sup> OTUs, calculated with QIIME at the 3% cutoff level; <sup>c</sup> Shannon, shannon diversity index, indicates both species abundance and evenness; <sup>d</sup> Coverage, Good's coverage.

Besides phylum taxa, the analyses were based on the genus level and were, therefore, more conducive to further verification of the function of the bacterial community. Relative percentages of the top 30 genera in both sites are listed in Figure 6. The sediments from both site TH1 and site TH2, especially with SMFCs in site TH1, Treponema was the dominant genera and belonged to the family of Spirochaetaceae, which could utilize the more recalcitrant carbon pool [38]. Spirochaetaceae has been known to contain digestive enzymes which can decompose carbohydrates of the food and algal cell walls [39]. The second dominated genera of site TH1 and TH2, especially on the surface of electrodes was *Clostridium* which belongs to Firmicutes. Firmicutes mainly include a large number of species that produces butanol, acetone, lactic acid, and ethanol through fermentation of a variety of organic matter [40]. In fact, *Clostridium* was also known to utilize a broad range of carbohydrates to ferment, such as monosaccharides, disaccharides, starches, organic acids, and other substrates [41]. The genera of GOUTA19 and LCP-6 were abundant in all samples, especially in the sediments with SMFCs and electrodes, but their functions are not known. It is also worth noting the obvious enrichment of Geobacter (belonging to Delta-proteobacteria) in the SMFC sediments, obviously on the anode surface in site TH2. Microorganisms in the cathodic biofilm consortium have been found to contribute to the overall SMFC performance [42]. Chitinophagaceae and Cytophagaceae were found on the surface of cathode both from site TH1 and TH2, and they are the more abundant of the two microbes found on cathodes from site TH1. Some bacteria often included in the Cytophagales are alternatively regarded as apochlorotic cyanobacteria, and the Chitinophagaceae species are capable of degrading chitin and cellulose.



**Figure 6.** Relative abundance percentages of bacterial genus communities in original sediments, bulk sediments and electrode biofilm from the two sampling sites at the end of the experiments.

For the past few years, the terrestrialization of shallow freshwater lakes has become increasingly serious because of the deposition of abundant of organic matter. The accumulation of decayed macrophyte litter as the slowly decomposition rate has a direct influence on accelerating the swamping process. From this study, sediments formed different sources confirming that the enhanced litter degradation under sediments with SMFCs can slow down the lake swamping process. So, SMFC has promising potential implications in terrestialized lakes for multiple functions.

# 4. Conclusions

This study demonstrated that the technology of SMFC can speed up the removal of the macrophyte litter in sediments from a large shallow lake. Electricity was also produced during the 700 days of operation without frequent loading or extensive maintenance, which realized energy recycling. However, the application effect was significantly influenced by the sediment sources. The main reason was due to the differences in the microbial community structure of sediments. In this study, we found that SMFCs using the sediment from the macrophyte-dominated area promoted more bacterial abundance of *Treponema*, *Clostridium*, *GOUTA*19 and *LCP*-6, which could degrade the more recalcitrant substances. Moreover, it was found that sediments from the macrophyte-dominated area became more humified and had a higher electricity generation compared to that from algae-dominated area. Considering enhanced organic matter biodegradation, SMFCs could be used as a new promising remediation technology for the grass type lakes caused by eutrophication and rehabilitation.

Further works should also be explored, such as the influence of environmental conditions, which contain the changing of temperature, pH, electron sinks, and the appropriate electrical load.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3417/9/18/3703/s1, Figure S1: Location and photos of sampling sites in Lake Taihu, China, Figure S2: Schematic diagram (A) and photo (B) of the experiments, Figure S3: Current (A) and power (B) generation along with time from SMFCs inoculated with TH1 and TH2 sedimentary samples. Current and power were represented as mean voltages and standard deviations (n = 2), Table S1: Characteristic of the experimental set-up, Table S2: Characteristics of the basic physicochemical properties of sediments and water in the experimental sites, Table S3: Performance of solid organic matter-fed SMFCs to date.

**Author Contributions:** N.S., preparation, creation and/or presentation of the published work, specifically writing the initial draft; H.J., ideas; formulation or evolution of overarching research goals and aims; Z.Y., review and editing.

Funding: This research received no external funding.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (41501528, 51879256, 51679228, 51839011, 51861125201), and the Special Foundation on Water Pollution Control and Treatment of China (2017ZX07204005).

Conflicts of Interest: The authors declare no conflict of interest.

# References

- 1. McCrackin, M.L.; Jones, H.P.; Jones, P.C.; Moreno-Mateos, D. Recovery of lakes and coastal marine ecosystems from eutrophication: A global meta-analysis. *Limnol. Oceanogr.* **2017**, *62*, 507–518. [CrossRef]
- 2. Conley, D.J.; Paerl, H.W.; Howarth, R.W.; Boesch, D.F.; Seitzinger, S.P.; Havens, K.E.; Lancelot, C.; Likens, G.E. Controlling eutrophication: Nitrogen and phosphorus. *Science* **2009**, *323*, 1014–1015. [CrossRef] [PubMed]
- 3. Lewis, W.M.; Wurtsbaugh, W.A.; Paerl, H.W. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environ. Sci. Technol.* **2011**, *45*, 10300–10305. [CrossRef] [PubMed]
- 4. Paerl, H.W.; Hall, N.S.; Calandrino, E.S. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Sci. Total Environ.* **2011**, *409*, 1739–1745. [CrossRef] [PubMed]
- 5. Chimney, M.J.; Pietro, K.C. Decomposition of macrophyte litter in a subtropical constructed wetland in south Florida (USA). *Ecol. Eng.* **2006**, *27*, 301–321. [CrossRef]
- 6. Geurts, J.J.M.; Smolders, A.J.P.; Banach, A.M.; de Graaf, J.P.M.V.; Roelofs, J.G.M.; Lamers, L.P.M. The interaction between decomposition, net N and P mineralization and their mobilization to the surface water in fens. *Water Res.* **2010**, *44*, 3487–3495. [CrossRef] [PubMed]
- 7. Qiu, D.R.; Wu, Z.B.; Liu, B.Y.; Deng, J.Q.; Fu, G.P.; He, F. The restoration of aquatic macrophytes for improving water quality in a hypertrophic shallow lake in Hubei Province, China. *Ecol. Eng.* **2001**, *18*, 147–156. [CrossRef]
- Li, X.; Cui, B.S.; Yang, Q.C.; Lan, Y.; Wang, T.T.; Han, Z. Effects of plant species on macrophyte decomposition under three nutrient conditions in a eutrophic shallow lake, North China. *Ecol. Model.* 2013, 252, 121–128. [CrossRef]
- 9. Carmichael, M.J.; Helton, A.M.; White, J.C.; Smith, W.K. Standing dead trees are a conduit for the atmospheric flux of CH<sub>4</sub> and CO<sub>2</sub> from wetlands. *Wetlands* **2018**, *38*, 133–143. [CrossRef]
- 10. Nyenje, P.M.; Foppen, J.W.; Uhlenbrook, S.; Kulabako, R.; Muwanga, A. Eutrophication and nutrient release in urban areas of sub-Saharan Africa—A review. *Sci. Total Environ.* **2010**, *408*, 447–455. [CrossRef]
- 11. Sygmund, C.; Kracher, D.; Scheiblbrandner, S.; Zahma, K.; Felice, A.K.G.; Harreither, W.; Kittl, R.; Ludwig, R. Characterization of the two neurospora crassa cellobiose dehydrogenases and their connection to oxidative cellulose degradation. *Appl. Environ. Microbiol.* **2012**, *78*, 6161–6171. [CrossRef] [PubMed]
- 12. Yan, Z.S.; He, Y.H.; Cai, H.Y.; Nostrand, J.D.V.; He, Z.L.; Zhou, J.Z.; Krumholz, L.R.; Jiang, H.L. Interconnection of key microbial functional genes for enhanced Benzo[a] pyrene biodegradation in sediments by microbial electrochemistry. *Environ. Sci Technol.* **2017**, *51*, 8519–8529. [CrossRef] [PubMed]
- 13. Drendel, G.; Mathews, E.R.; Semenec, L.; Franks, A.E. Microbial fuel cells, related technologies, and their applications. *Appl. Sci.* **2018**, *8*, 2384. [CrossRef]
- 14. Hong, S.W.; Kim, H.S.; Chung, T.H. Alteration of sediment organic matter in sediment microbial fuel cells. *Environ. Pollut.* **2010**, *158*, 185–191. [CrossRef] [PubMed]
- 15. Gregoire, K.P.; Becker, J.G. Design and characterization of a microbial fuel cell for the conversion of a lignocellulosic crop residue to electricity. *Bioresour. Technol.* **2012**, *119*, 208–215. [CrossRef] [PubMed]
- 16. Sajana, T.K.; Ghangrekar, M.M.; Mitra, A. Effect of presence of cellulose in the freshwater sediment on the performance of sediment microbial fuel cell. *Bioresour. Technol.* **2014**, *155*, 84–90. [CrossRef] [PubMed]
- Song, N.; Jiang, H.L.; Cai, H.Y.; Yan, Z.S.; Zhou, Y.L. Beyond enhancement of macrophyte litter decomposition in sediments from a terrestrializated shallow lake through bioanode employment. *Chem. Eng. J.* 2015, 279, 433–441. [CrossRef]

- Song, N.; Yan, Z.S.; Cai, H.Y.; Jiang, H.L. Effect of temperature on submerged macrophyte litter decomposition within sediments from a large shallow and subtropical freshwater lake. *Hydrobiologia* 2013, 714, 131–144. [CrossRef]
- Jiang, H.R.; Shyy, W.; Wu, M.C.; Wei, L.; Zhao, T.S. Highly active, bi-functional and metal-free B4C-nanoparticle-modified graphite felt electrodes for vanadium redox flow batteries. *J. Power Sources* 2017, 365, 34–42. [CrossRef]
- 20. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
- Song, N.; Jiang, H.L. Effects of initial sediment properties on start-up times for sediment microbial fuel cells. *Int. J. Hydrogen Energy* 2018, 43, 10082–10093. [CrossRef]
- 22. Thakuria, D.; Schmidt, O.; Mac Siurtain, M.; Egan, D.; Doohan, F.M. Importance of DNA quality in comparative soil microbial community structure analyses. *Soil Biol. Biochem.* **2008**, *40*, 1390–1403. [CrossRef]
- Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 2010, 7, 335–336. [CrossRef] [PubMed]
- 24. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [CrossRef] [PubMed]
- Kõljalg, U.; Nilsson, R.H.; Abarenkov, K.; Tedersoo, L.; Taylor, A.F.S.; Bahram, M.; Bates, S.T.; Bruns, T.D.; Callaghan, T.M.; Douglas, B.; et al. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* 2013, 22, 5271–5277. [CrossRef] [PubMed]
- Zhou, Y.L.; Yang, Y.; Chen, M.; Zhao, Z.W.; Jiang, H.L. To improve the performance of sediment microbial fuel cell through amending colloidal iron oxyhydroxide into freshwater sediments. *Bioresour. Technol.* 2014, 159, 232–239. [CrossRef] [PubMed]
- Song, N.; Yan, Z.S.; Xu, H.C.; Yao, Z.B.; Wang, C.H.; Chen, M.; Zhao, Z.W.; Peng, Z.L.; Wang, C.L.; Jiang, H.L. Development of a sediment microbial fuel cell-based biosensor for sinultaneous online monitoring of dissolved oxygen. *Sci. Total Environ.* 2019, 673, 272–280. [CrossRef]
- 28. Hong, S.; Chang, I.; Choi, Y.; Kim, B.; Chung, T. Responses from freshwater sediment during electricity generation using microbial fuel cells. *Bioproc. Biosyst. Eng.* **2009**, *32*, 389–395. [CrossRef] [PubMed]
- 29. Babanova, S.; Hubenova, Y.; Mitov, M.; Mandjukov, P. Uncertainties of yeast-based biofuel cell operational characteristics. *Fuel Cells* **2011**, *11*, 824–837. [CrossRef]
- Christwardana, M.; Frattini, D.; Accardo, G.; Yoon, S.P.; Kwon, Y. Early-stage performance evaluation of flowing microbial fuel cells using chemically treated carbon felt and yeast biocatalyst. *Appl. Energy* 2018, 222, 369–382. [CrossRef]
- Mitov, M.; Bardarov, I.; Mandjukov, P.; Hubenova, Y. Chemometrical assessment of the electrical parameters obtained by long-term operating freshwater sediment microbial fuel cells. *Bioelectrochemistry* 2015, 106, 105–114. [CrossRef] [PubMed]
- Rezaei, F.; Richard, T.L.; Brennan, R.A.; Logan, B.E. Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems. *Environ. Sci. Technol.* 2007, 41, 4053–4058. [CrossRef] [PubMed]
- Valenzuela, E.I.; Avendaño, K.A.; Balagurusamy, N.; Arriaga, S.; Nieto-Delgado, C.; Thalasso, F.; Cervantes, F.J. Electron shuttling mediated by humic substances fuels anaerobic methane oxidation and carbon burial in wetland sediments. *Sci. Total Environ.* 2019, 650, 2674–2684. [CrossRef] [PubMed]
- 34. Yan, Z.S.; Song, N.; Cai, H.Y.; Tay, J.H.; Jiang, H.L. Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide. *J. Hazard. Mater.* **2012**, *199*, 217–225. [CrossRef] [PubMed]
- 35. Song, N.; He, Y.H.; Jiang, H.L. Inferior adaptation of bay sediments in a eutrophic shallow lake to winter season for organic matter decomposition. *Environ. Pollut.* **2016**, *219*, 794–803. [CrossRef] [PubMed]
- Yan, Z.S.; Jiang, H.L.; Cai, H.Y.; Zhou, Y.L.; Krumholz, L.R. Complex interactions between the macrophyte acorus calamus and microbial fuel cells during Pyrene and Benzo[a]Pyrene Degradation in sediments. *Sci. Rep.* 2015, *5*, 10709. [CrossRef] [PubMed]
- Yan, L.; Gao, Y.M.; Wang, Y.J.; Liu, Q.; Sun, Z.Y.; Fu, B.R.; Wen, X.; Cui, Z.J.; Wang, W.D. Diversity of a mesophilic lignocellulolytic microbial consortium which is useful for enhancement of biogas production. *Bioresour. Technol.* 2012, 111, 49–54. [CrossRef]

- 38. Lucey, K.S.; Leadbetter, J.R. Catechol 2,3-dioxygenase and other meta-cleavage catabolic pathway genes in the 'anaerobic' termite gut spirochete Treponema primitia. *Mol. Ecol.* **2014**, *23*, 1531–1543. [CrossRef]
- Tulupova, Y.R.; Parfenova, V.V.; Sitnikova, T.Y.; Sorokovnikova, E.G.; Khanaev, I.B. First report on bacteria of the family Spirochaetaceae from digestive tract of endemic gastropods from Lake Baikal. *Microbiology* 2012, *81*, 460–467. [CrossRef]
- 40. Gu, Y.; Ding, Y.; Ren, C.; Sun, Z.; Rodionov, D.A.; Zhang, W.W.; Yang, S.; Yang, C.; Jiang, W.H. Reconstruction of xylose utilization pathway and regulons in Firmicutes. *BMC Genom.* **2010**, *11*, 255. [CrossRef]
- Dos Passos, V.F.; Marcilio, R.; Aquino-Neto, S.; Santana, F.B.; Dias, A.C.F.; Andreote, F.D.; de Andrade, A.R.; Reginatto, V. Hydrogen and electrical energy co-generation by a cooperative fermentation system comprising Clostridium and microbial fuel cell inoculated with port drainage sediment. *Bioresour. Technol.* 2019, 277, 94–103. [CrossRef] [PubMed]
- 42. Bardarov, I.; Mitov, M.; Ivanova, D.; Hubenova, Y. Light-dependent processes on the cathode enhance the electrical outputs of sediment microbial fuel cells. *Bioelectrochemistry* **2018**, *122*, 1–10. [CrossRef] [PubMed]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).