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Digestate of Fecal Sludge Enhances the Tetracycline Removal in Soil Microbial Fuel Cells

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Abstract: The soil pollution of agricultural lands is increasingly being caused by the widely used antibiotic tetracycline (TC) in the animal husbandry industry. Soil microbial fuel cells (SMFCs) provide a promising strategy for the bioremediation of contaminated soil. However, our current understanding of the bioremediation of TC-contaminated soil by SMFC is still limited. Here, we investigated the influence of fecal sludge (FS) digestate on TC biodegradation efficiency and extracellular electron transfer in SMFCs. The addition of FS digestate was beneficial to electricity generation by SMFC, and thus enhanced the removal efficiency of TC in the SMFC. After 25 days, the SMFC with fecal sludge digestate showed a TC removal efficiency of 64.5%, compared to values of 25.2% and 21.4% observed for a SMFC and an open-circuit SMFC operating without the addition of fecal sludge digestate, respectively. Moreover, the addition of FS digestate was favorable for electricity generation by SMFCs, and the average current density and the maximum power density of the SMFC with fecal sludge digestate were 0.054 A/m^3 and 8.85 W/m^3, respectively. The enrichment of *Desulfuromonas* and *Pseudomonas* in the electrode biofilms might account for their high TC removal efficiency and electricity generation. The SMFC with fecal sludge digestate provides a promising approach for the simultaneous disposal of fecal sludge digestate and the bioremediation of antibiotics-contaminated-soil.

Keywords: fecal sludge digestate; antibiotics; tetracycline; soil microbial fuel cell; microbiome

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1. Introduction

Tetracycline (TC) is widely used in animal husbandry worldwide for its low cost and broad-spectrum antibacterial activity [1]. TC production and use rank second in the world and first in China, respectively, with 12,000 tons of TC produced annually (approximately 70% of which is used by veterinarians) [2]. TC is poorly absorbed in animals, and the overuse of TC in the animal husbandry industry may result in high TC residues (40–90%) in livestock manure and urea [3]. Significantly, TC inevitably accumulates in the soil through manure spreading, direct discharge from grazing livestock, and wastewater irrigation [3]. It has been reported that TC can restrain the growth and metabolic activity of soil microorganisms, thereby affecting the ecological function of soils [4]. Crops can absorb TC and accumulate through the food chain, which may pose serious risks to human health [5].

Furthermore, soil-borne antibiotics might contaminate groundwater via rainfall and irrigation. Thus, there is a need for further research on antibiotic-related groundwater pollution. Hence, understanding the degradation mechanism of TC in soil and finding cost-effective remediation methods for TC contamination are urgent practical issues. Microbial fuel cells (MFCs) represent a cost-effective method for removing heavy metals from aquatic environments and degrading organic pollutants in soil [6,7]. Moreover, MFCs can spontaneously convert chemical energy in organics into electricity, independent of precious metal catalysts, further reducing manufacturing costs [8]. It is generally accepted that MFC is an effective method for remediating TC contamination and reducing antibiotic resistance

genes in wastewater [7,9]. MFCs provide sufficient electron acceptors, which make up for the deficiency of traditional biological treatment technology, and have recently come to widespread public attention [10]. A previous study has suggested that MFC can degrade TC with a removal efficiency of 79.1%, which was more effective than the traditional anaerobic degradation with a removal efficiency of 14.9% [9]. The microbial metabolism combines electrochemical redox reactions in MFCs [11]. The electron transfer process between electroactive bacteria also enhances the metabolic dynamics of TC-degrading microorganisms [12]. Compared with conventional technology for soil bioremediation, SMFC has advantages in the remediation of TC-contaminated soil.

Unlike the aquatic environment, soil has a higher internal resistance, which astricts electron transport capacity and thus impedes TC degradation [7]. Fecal sludge (FS) is inevitably produced during human activities and commonly accumulates in sanitation facilities. FS contains many un-stabilized organics and pathogenic microorganisms [13,14]. After fermentation, FS is frequently used as a soil regulator for agricultural production, which can provide nutrients for soil microbes, enhance microbial activities, accelerate the decomposition of organic matter, promote crops' growth, and improve the quality of agricultural products [15]. It has been reported that the availability of carbon sources in MFC has an essential impact on antibiotics degradation and electricity generation [16]. A previous study showed that wetland MFC fed with glucose of 200 mg/L had a better removal effect on SMX and TC than that fed with other carbon sources [17]. Moreover, the performance of MFC can be intensified by using primary sludge digestate containing a high concentration of macromolecule organics [18]. There is less information concerning applying FS digestate in soil MFCs, which might be an attractive strategy for enhancing the transfer of matters and energy in soil and TC degradation.

Our previous study demonstrated that potassium ferrate (PF) is a promising pretreatment method for FS decomposition and release of organic matter. The PF-pretreated FS digestate contained abundant short chain fatty acids and iron ions, which might enhance microbial activity [14]. In this study, SMFCs were constructed for TC degradation, and the effect of the addition of PF-pretreated FS digestate on the microbiome response and performance of SMFCs was investigated.

2. Materials and Methods

2.1. FS Pretreatment and AD

FS was sampled from the dry pail latrine without running water in Harbin, and the top layer of FS was collected for subsequent analysis. The basic parameters of FS were described in our previous research [14]. The moisture content of the FS was 70% (v/v), the pH of the FS was 6.3, and the C/N ratio was 8.8. The soluble chemical oxygen demand (SCOD) of the FS was 7316 mg/L. The total chemical oxygen demand of the FS was 47,665 mg/L. The volatile solid was 36.5 g/L, and the total organic carbon was 28,742 mg/L. A total of 1500 mL of FS was added to a bottle and pretreated with potassium ferrate (0.5 g/g-VS) for 120 min. Digestate was made by a 10-day anaerobic digestion (AD) process of PF-treated FS in a 2-L fermenter. The AD process used activated sludge from Wenchang Wastewater Treatment Plant (Harbin, Heilongjiang Province, China) as inoculum mixing with the FS with a 1:10 (v/v) I/S ratio. The total suspended solid was 14.6 g/L, the pH of the sludge was 6.6. The SCOD of the sludge was 280 mg/L. Ultrahigh-purity N_2 (99.999%) was used for aeration in to ensure an anaerobic environment [19].

2.2. The Addition of FS Digestate on Simulated TC-Contaminated Soil

Soil was obtained from the second campus of Harbin Institute of Technology. TC was added into soil and mixed evenly with the soil to simulate TC-contaminated soil. The final simulated TC concentration in the soil was 0.13 mg/kg [20]. Some basic parameters of this simulated soil are shown in Table S1. A 60-mesh stainless steel screen was used to remove grass seeds and other herbs from the soil prior to use. In this process, the leaching cavity and MFC reactor used a rectangular polypropylene box measuring 28.5 cm in length,

20 cm in width, and 17 cm in height. A 100-mesh filter screen was set at the bottom of the reactor, and a 1.5 cm thick volcanic rock layer was laid on top of the mesh filter [21]. The screened soil particles were placed at the top of the volcanic rock layer. In particular, the soil was compacted prior to test, and the final volume of soil was 7.5 L. After that, 2.5 L of PF-pretreated FS digestate was sprayed into the soil at a rate of 1.8 mL/min using a sprinkler irrigation device with an automatic control valve [22]. The ratio of soil to FS was 3:1 (v/v, calculated by wet weight). A water tank was set at the bottom of the reactor to collect the leachate. The leaching process lasted for 24 h. The sketch map of the soil leaching process in this experiment is shown in Figure S1. The concentration of SCOD and TC during the leaching process was monitored every 4 h with sampling the leachate at the outlet of the reactor.

2.3. Soil Microbial Fuel Cell Construction

The electrodes were inserted into SMFC and placed in the center of the polypropylene box. The electrode adopted a hollow cylindrical sleeve structure. The outer anode was graphite felt, which was in direct contact with the soil. The inner cathode was the active carbon, and the middle layer was a glass fiber to prevent a short circuit [23]. The catalytic layer of the cathode was close to the glass fiber, and the diffusion layer was in contact with air. The bottom of the electrodes was sealed. The anode and cathode areas were both 112 cm^2. Both electrodes were connected to an external resistor of 500 Ω with a titanium wire. The voltage was monitored by a data acquisition system (2700, Keithley Instrument, OH) [24]. The leach solution in water tank A flowed through the electrode surface of the reactors. The flow rate of the leach solution was controlled at 1.2 mL/min by a microfluidic device. The leach solution was collected again in water tank B after it flowed through the electrode surface. The drenching solution was flowed back to tank A and then fed back to the electrode region at the same flow rate through the pump and microfluidic device [25]. The leach solution in water tank A and B were circulated every 24 h. Both water tanks were 3 L. The reactor ran for 25 days, and samples were taken from the water tank every 5 days.

There were three reactors set in this study, including SMFC without FS digestate addition (SMFC), SMFC with FS digestate (SMFC-FSD), and open-circuit SMFC without FS digestate addition (OC-SMFC). All reactors operated for 42 days at 25 ± 1°C.

2.4. MFC Performance Characterizations

The current density was calculated as previously described [26]. The power density was determined by changing external resistors from 5 Ω to 5000 Ω. The cyclic sweep voltammetry and linear sweep voltammetry were determined by an Autolab potentiostat (Metrohm, Swiss) with a scan rate of 0.1 mV/s. Membrane filters filtered the liquid samples with a pore size of 0.22 μm. SCOD was measured based on the stand method of the American Public Health Association (APHA) [27]. TC in the leach solution was concentrated by solid phase extraction (SPE) and determined by high-performance liquid chromatography (Agilent, CA, USA).

2.5. Microbiome Analysis Based on 16S rRNA Gene Sequencing

The top and bottom of the anode were cut and fragmented by sterile scissors from the two reactors. The anode biofilm samples named FSD-TOP and FSD-BOTTOM were from the SMFC-FSD reactor. Similarly, the biofilm samples named SMFC-TOP and SMFC-BOTTOM were from SMFC reactors, respectively. Cathode biofilms were scraped and collected from both reactors' cathode catalytic layer surfaces. The samples named FSD-C and SMFC-C came from SMFC-FSD and SMFC reactors, respectively. The soil samples in the SMFC-FSD and SMFC reactors were named FSD-S and SMFC-S. All of the samples mentioned above were obtained after 25 days of operation. The MP DNA Isolation Kit (Tiangen, China) was used in the DNA extraction process. The 16S rRNA gene with V4–V5 region was amplified with the bacterial universal primers 515F (5′-5GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) as previously described [28,29]. After library

construction, the 16S rRNA gene amplicons were sequenced using the Illumina HiSeq 2500 platform (Illumina Inc., Hayward, CA, USA) [30]. The quality control was conducted by SMRT Link (Version 6.0). The clean reads were analyzed using the Quantitative Insights Into Microbial Ecology software (QIIME; http://qiime2.org; ver. 2022.2.1). Effective reads were created after all of the chimeric sequences were removed. Operational taxonomic units (OTUs) were determined based on 97% similarity. A representative sequence of each OTU was selected for taxonomic determination. Alpha diversity analysis (Shannon and Simpson indices and species richness estimator) and principal coordinates analysis (PCoA) based on the Bray–Curtis distance were performed using the QIIME software (ver. 2022.2.1) [29].

2.6. Statistics Analysis

Statistical analysis was performed with the one-way analysis of variance (ANOVA). The significant differences were determined based on $p < 0.05$ or 0.01.

3. Results and Discussion

3.1. Effect of FS Digestate Addition on TC and Organics Leaching

The results of the leaching experiment showed that the leaching efficiency of TC was over 90% in all assays (Figure 1a). The TC concentration in the SMFC-FSD and SMFC showed a similar trend. TC concentration increased rapidly in the first 4 h, while the leaching rate slowed down in 4–12 h. TC concentration peaked at 12 h, and the maximum TC concentration at the bottom of the reactor of sampling point was each 86.4 and 82.6 μg/L in the SMFC-FSD and SMFC. After 12 h, the concentration of leached TC decreased with the decrease of TC concentration in the soil, and the TC concentration was 29.1 and 23.2 μg/L at the end of leaching in the SMFC-FSD and SMFC reactors, respectively. It is worth noting that the total concentrations of TC in the leach solutions of reactor SMFC-FSD and SMFC were 820.34 and 799.5 μg/L, respectively (Figure 1b). It could be inferred that the application of FS digestate might contribute to the leaching of TC, which were in agreement with the previous studies [31,32]. In general, the migration and transformation of contaminants mainly depend on their adsorption behavior in soil. The soil characteristics impact the adsorption capacity of contaminants, including pH value, soil organic carbon, and cation exchange capacity [20]. The adsorption behavior of TC is pH-dependent, and acid soil was more conducive to TC adsorption compared with alkaline soil [33]. Therefore, applying alkaline FS digestate and the alkaline pH of the soil might play an essential role in TC leaching (Table S1).

Except for TC, organic matters in FS digestate were inevitably leached through the soil. Less organic matters were leaching out in the SMFC reactor. Comparatively, the SCOD in the SMFC-FSD reactor grew from zero to 438.32 ± 46.3 mg/L measured at the bottom of the reactor at the sampling point within 12 h. The leached SCOD concentration decreased over 12 to 24 h (Figure 1a). The total volume of leach solution in the SMFC-FSD reactor was 1.6 L. The total concentration of SCOD was 1882 mg/L (Figure 1b). It is well known that FS digestate is rich in small organic acids. The considerable SCOD concentration in the SMFC-FSD might promote microbial activity and accelerate the degradation of TC in the subsequent MFC system [16].

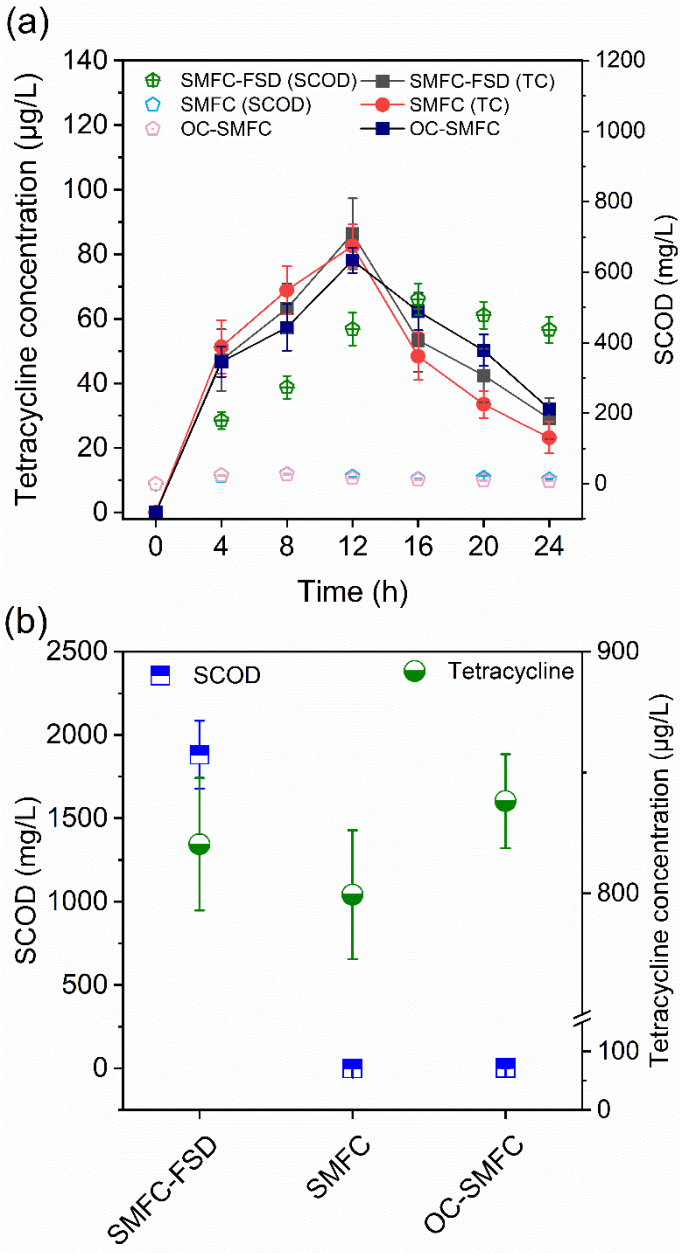

Figure 1. The concentration changes of soluble chemical oxygen demand (SCOD) and tetracycline concentration at the sampling point of the water tank during leaching process of SMFC-FSD were compared with those of SMFC (**a**). The final concentration of SCOD and tetracycline in the total leach solution of the two reactors after the leaching process (**b**).

3.2. Effect of FS Digestate Addition on TC Removal in SMFC

The removal efficiency of TC in SMFC-FSD, SMFC, and OC-SMFC increased in a 25-day reaction cycle (Figure 2a). Compared with the SMFC and OC-SMFC, SMFC-FSD showed better TC removal efficiency, indicating that the application of FS digestate was beneficial to TC degradation. After 25 days, the removal efficiency of TC in the SMFC-FSD was 64.5%, which was significantly higher than 25.2% in the SMFC and 21.4% in the OC-SMFC ($P < 0.05$). It should be noted that the highest TC removal rate of 34.4 μg/L/d was observed within 5–10 days in the SMFC-FSD. While the highest TC removal rate in the SMFC was detected within one to five days, which was 14.54 μg/L/d (Figure 2b). It could be asserted that the application of PF-pretreated FS digestate accelerated the removal rate of TC. In addition, SCOD removal efficiency exhibited a similar uptrend trend to TC. The SCOD results showed that compared with the SMFC and OC-SMFC, SMFC-FSD was more

efficient in organic matter consumption, manifesting a higher SCOD removal efficiency. This observation supported the optimal TC removal results observed in the SMFC-FSD.

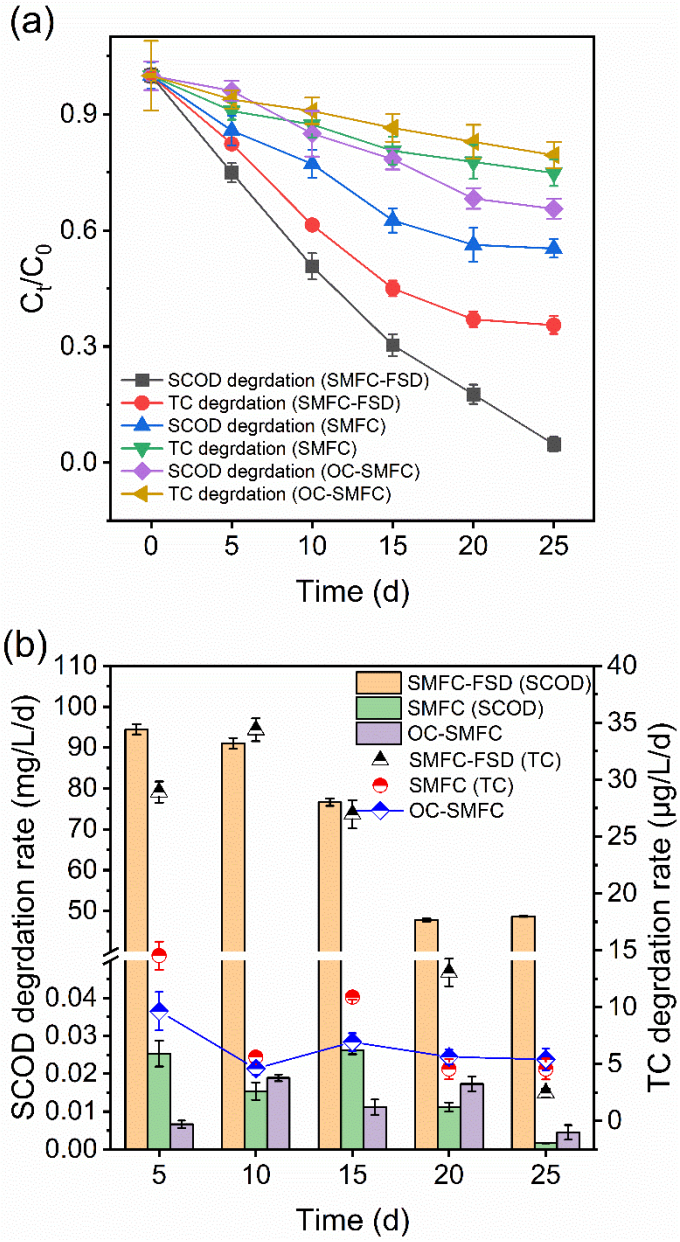

Figure 2. Degradation performance (**a**) of SCOD and TC in the drenching solution of SMFC, SMFC-FSD and OC-SMFC with a five-day interval. The degradation rate (**b**) of SCOD and TC in different SMFC reactors: 5. 10, 15, 20, and 25 represent the average degradation rate in the 0–5th day, 5–10th day, 10–15th day, 15–20th day and 20–25th day, respectively.

TC is a refractory organic pollutant which has a complex molecular structure and ecological toxicity. Microbes commonly degrade TC via co-metabolism and electrochemical reduction. Specifically, microbes can use electrons to disintegrate TC into micromolecule organics. TC can be further degraded by electroactive bacteria (EAB) at the MFC anode [34,35]. As research suggests, organicism is a critical factor affecting MFC performance as a result of their availability to anaerobes. This availability is especially for refractory pollutants biodegradation such as antibiotics by co-metabolism processes [17,36]. Similar results were observed in a previous study, in which the addition of sodium acetate enhanced oxytetracycline removal from MFC systems [37]. Sufficient carbon sources can satisfy the growth and

metabolic requirements of microorganisms and stimulate more microorganisms to involve in TC degradation, thereby enhancing the TC removal efficiency of SMFC-FSD [37].

3.3. Electricity Generation by SMFC

The maximum output voltage was achieved at 250 mV as the SMFC-FSD reactor entered a stable operation stage (Figure 3a). The power density curve results showed that no power overshoot was observed in the stable operation stage of SMFC-FSD, indicating that the status of the anode biofilm tended to be stable (Figure 3b). The average current density and the maximum power density of the SMFC-FSD anode were 0.054 A/m^3 and 8.85 W/m^3, respectively, which were considerably higher than those of the SMFC anode (Figure 3b,c). The above experimental results revealed that the addition of PF-pretreated FS digestate reinforced the electricity generation by SMFC, which was consistent with the higher TC and SCOD removal efficiencies observed in the FS-SMFC (Figures 2 and 3a,b). Furthermore, the cyclic voltammetry curve showed a redox peak of SMFC-FSD electronic generation ability of the reactors.

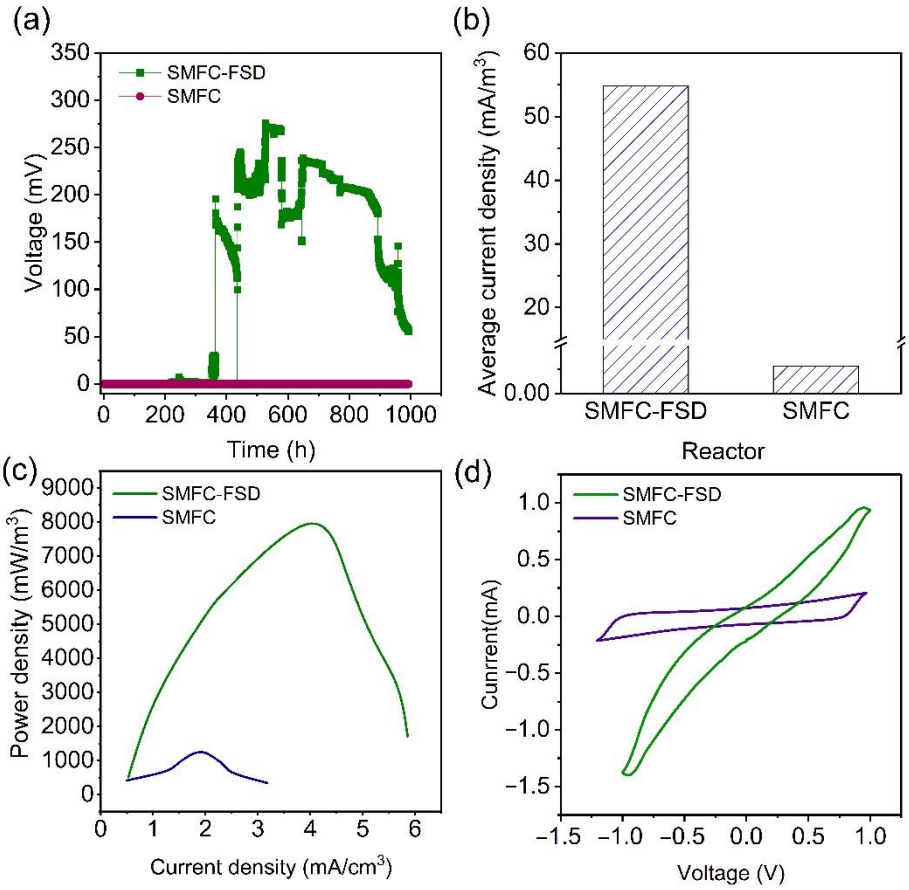

Figure 3. Voltage curve (**a**); average current density (**b**); power density (**c**); and cyclic voltammetry curve (**d**) of the SMFC-FSD and SMFC reactors.

3.4. Anodic Biofilm Microbiome

The Chao1 index revealed that the species richness of microbiome of SMFC-FSD anode biofilm decreased compared to the FSD-S (Table S2). Furthermore, the higher Simpson and lower Shannon indices (vs. FSD-S) observed in the SMFC-FSD anode suggested that the biofilm of SMFC-FSD anode decreased the community diversity. It is worth noting that the species richness and diversity were higher at the anode bottom of the SMFC-FSD than at the anode top, manifesting as a higher Chao1 and Shannon indices and lower Simpson index in the FSD-BOTTOM (vs. FSD-TOP). Likewise, the anode biofilms of SMFC

decreased the species richness and diversity compared to the SMFC-S. At the same time, the biofilm of SMFC-BOTTOM exhibited higher species diversity and lower species richness than those in the SMFC-TOP. Venn's results indicated that 706 OTUs were expressed in all samples and that 1093 and 1969 OTUs were shared in anode biofilms of the SMFC and SMFC-FSD, respectively (Figure S2a). A rarefaction curve is often used to assess whether a fair comparison of richness between microbial communities measured with unequal sequencing depths is possible [38]. Based on the rarefaction curves, one might conclude that the samples sufficiently covered the original communities they were taken from (Figure S3). PCoA demonstrated that the samples of two anode biofilm samples in SMFC-FSD have high similarity. In contrast, the bacterial communities of the anode biofilm in SMFC-FSD were distinct from that of FSD-S, and the bacterial communities of the anode biofilms of SMFC and SMFC-S were also different (Figure 4).

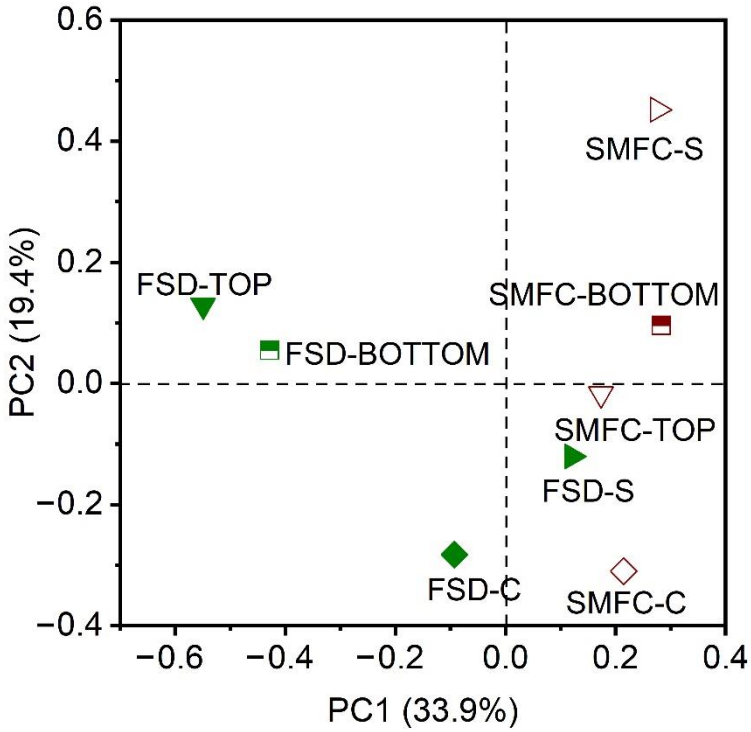

Figure 4. Principal coordinate analysis (PCoA) of different samples based on Bray–Curtis distance.

The dominant phylum in all samples was affiliated with Proteobacteria (Figure 5a). The relative abundance of Proteobacteria at the biofilm of anode bottom was lower than that of at the anode top, which was 70.0%, 53.5%, 66.5%, and 53.9% in the FSD-TOP, FSD-BOTTOM, SMFC-TOP, and SMFC-BOTTOM, respectively. Compared with SMFC, SMFC-FSD contributed to the enrichment of *Desulfuromonas*, the most dominant population on the biofilm of SMFC-FSD, with its relative abundance of 48.2% and 20.2% at the top and bottom of anode in the SMFC-FSD (Figure 5b). *Desulfuromonas* is an anaerobic sulfur-reducing bacterium that can oxidize acetate, ethanol, and propanol to CO_2 with elemental sulfur as an electron acceptor [39]. More importantly, *Desulfuromonas* is also an electroactive bacterium that can extracellularly transfer electrons to the electrodes of the MFC [40]. The enrichment of *Desulfuromonas* might account for the enhanced electricity generation performance found in the SMFC-FSD (Figure 3b, c). The predominant population in the SMFC were *Geobacter* and *Thauera*. Compared with the SMFC-BOTTOM, *Geobacter* was most dominant in the SMFC-TOP, and the relative abundance of *Geobacter* was 11.7% and 1.6% in the SMFC-TOP and SMFC-BOTTOM, respectively. *Geobacter* is a common EAB, playing a crucial role in electricity generation by MFCs [41]. The enrichment of *Desulfuromonas* and *Geobacter* on the biofilm in the two reactors demonstrated that EAB were enriched in

anode biofilm during operation. In contrast, the low abundance of *Geobacter* relative to *Desulfuromonas* might be behind the lower electricity generation performance of SMFC than SMFC-FSD.

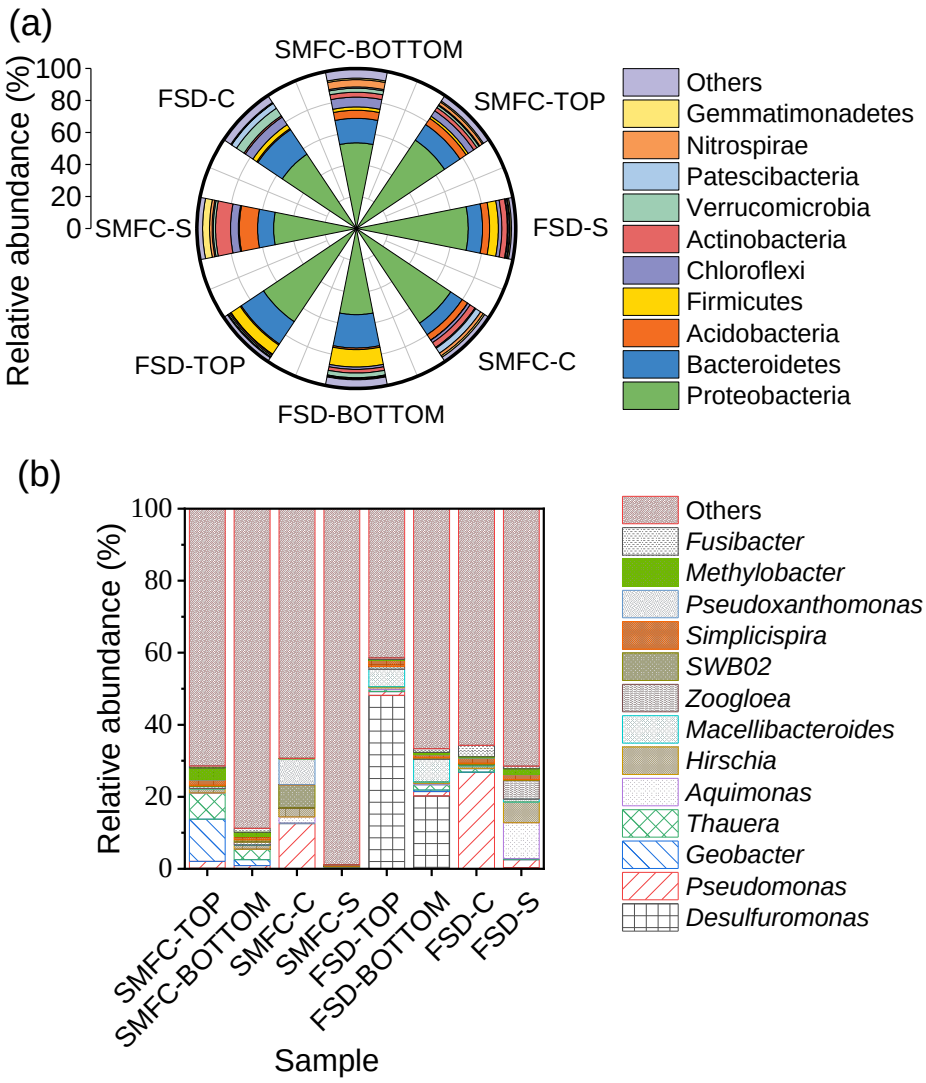

Figure 5. Taxonomic classification of dominant phyla (**a**) and genera (**b**) based on 16S rRNA sequences of anode biofilms, cathode biofilms, and soil samples from different reactors. The "others" represents the dominant genera with a relative abundance of < 1%.

3.5. Cathodic Biofilm Microbiome

Similar to SMFC-FSD and SMFC anodes biofilm, SMFC-FSD and SMFC cathodes decreased the species richness of the microbiome compared to the FSD-S and SMFC-S, respectively, reflected in the reduction of the Chao1 index (Table S2). Compared with the FSD-S, the lower Simpson value in the FSD-C showed that the cathode biofilm increased the species' evenness, which agreed with the results observed in the SMFC-C. 774 OTUs are shared in the SMFC-C and FSD-C (Figure S2b). PCoA results indicated that the bacterial communities of SMFC-C and FSD-C were quite distinct from their corresponding soil samples (Figure 4).

Proteobacteria and Bacteroidetes were predominant phyla on the cathode biofilm in FSD-C, and the relative abundance was 55.8% and 18.5%, respectively (Figure 5a). In addition, the relative abundance of Proteobacteria was 71.14% in the SMFC-C, which remarkably increased compared with the SMFC-S. *Pseudomonas* was an overwhelming superiority on the cathode biofilm in the FSD-C and took 26.7% of the total relative abundance (Figure 5b).

It is reported that *Pseudomonas* is an electroactive microorganism that participates in the bio-degradation of TC and enhances extracellular electron transfer [3,42,43]. Accordingly, the enrichment of *Pseudomonas* in the cathode of the SMFC-FSD contributed to the biodegradation of TC and bioelectricity generation. As expected, this was consistent with the above results (Figures 2a and 3a). The dominant genera on the cathode biofilm in the SMFC-C comprised *Pseudomonas*, *Pseudoxanthomonas*, and SWB02, with a relative abundance of 12.6%, 7.1%, and 6.5%. This result revealed that in addition to *Pseudomonas*, *Pseudoxanthomonas* and SWB02 could also tolerate TC stress. *Pseudoxanthomonas* was reported to be related to the denitrification process, and the enrichment of *Pseudoxanthomonas* might favor nitrate removal [44]. It is worth noting that the lower relative abundance of *Pseudomonas* on the cathode biofilm in the SMFC-C might partly explain why the SMFC-FSD reactor had better TC removal and power generation performance than the SMFC reactor.

The high concentration of soluble organics in the pretreated-FS digestate provide sufficient volatile fatty acids for the growth of electroactive bacteria. The electron transfer generated by electroactive bacteria including *Desulfuromonas* and *Geobacter* could enhance the extracellular electron transport of other microorganisms [45]. Moreover, the oxygen as an electron acceptor on the cathode enhance the growth of *Pseudomonas*, *Pseudoxanthomonas* and SWB02 [46]. As a result, the cathode biofilm has the ability to degrade the TC. It is worth noting that the lower relative abundance of *Pseudomonas* in the SMFC-C might partly explain why the SMFC-FSD reactor had better TC removal and power generation performance than SMFC reactor.

3.6. Economic Analysis

The whole system mainly contained electrode system, automatic control system and water tank. The anode of the SMFC is the carbon fiber which costs 0.14 USD/cm^2, and the cathode was the active carbon which costed 0.37 USD/cm^2. The water tank was made up of polymethyl methacrylate which the costed 0.04 USD/mL/reactor (include processing fee). The biological TC treatment method with anaerobic digestion by using active sludge usually took 0.03 USD/mL. Although the total cost of MFC-FSD was higher than that of the anaerobic digestion, the TC degradation rate was higher than the anaerobic digestion.

4. Conclusions

In this study, PF-pretreated FS digestate was used to enhance TC removal of SMFC. It was noteworthy that adequate carbon sources in FS digestate could meet the growth and metabolic needs of EAB and TC biodegradation-related microorganisms, thus further improving TC removal efficiency. Moreover, the enrichment of *Pseudomonas* in the cathode biofilm of SMFC-FSD accounted for the enhancement of TC removal. The increase in the relative abundance of electroactive *Desulfuromonas* and *Pseudomonas* in the anode and cathode biofilms might account for the intensified electricity generation by the SMFC-FSD. In consequence, the addition of FS digestate for the enhancement of TC removal in the SMFC was an attractive strategy for the disposal of FS digestate as well. For further study, the actual effect of tetracycline degradation on SMFC placing in the tetracycline-contaminated soil should be determined based on this research. The authors believe that the pretreated septic tank supernatant could be the digestate which brought the soluble organic matters and tetracycline to the electrodes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14172752/s1. Figure S1: Sketch map of the soil leaching process in this experiment; Figure S2: Venn map based on the shared and unique OTUs of anode (a) and cathode (b) of the SMFC-FSD and SMFC. Figure S3: Rarefaction curves of all the samples in SMFC-FSD and SMFC reactors; Table S1: Basic information of the simulated tetracycline-contaminated soil; Table S2: Alpha diversity of microbial communities of anode biofilm, cathode biofilm, and soil samples based on 16S rRNA gene sequencing.

Author Contributions: H.C.: Investigation, Data curation, Writing-original draft, Writing-review & editing. J.W.: Investigation, Data curation. K.F.: Data curation, Formal analysis. D.X.: Data curation, Formal analysis, Writing-original draft, Writing-review & editing, Project administration, Funding acquisition, Supervision. All authors have read and agreed to the published version of the manuscript.

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