

Article

# Effects of Modified Anodes on the Performance and Microbial Community of Microbial Fuel Cells Using Swine Wastewater

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**Abstract:** Microbial fuel cells (MFCs) have emerged as a sustainable technology for wastewater treatment that has potential to recycle bioelectricity from livestock wastewater. The performance of MFCs is influenced by the synergistic effect of anode material with nearby microorganisms. In this study, three identical double-chambered MFCs with different anode carbon clothes using swine wastewater are established. The optimization mechanism of MFC performance is analyzed by anode characteristics, cell performance, and microbial community, respectively. The results show that the surface structure and properties of the anode carbon cloth can be obviously improved by the acid–heat-modified treatment. The community structure of anodic biofilm, which varied with different modification methods, was mainly dominated by Proteobacteria, Firmicutes, and Bacteroidetes. These findings demonstrate efficient and simple methods for improving the performance of MFCs based on swine wastewater and may help to explore the influence mechanism of different modified anodes on the exoelectrogens.

**Keywords:** microbial fuel cells; swine wastewater; modified anodes; anode characteristics; microbial community

## 1. Introduction

With the development of intensive livestock farms, the production efficiency and economic benefits of the livestock farms were improved significantly, but the relative concentration of livestock wastewater cannot be handled properly in many areas, which means that the livestock-farm wastewater has become or will be becoming a source of pollution equivalent to industrial wastewater or domestic sewage [1–3]. Many traditional wastewater treatment techniques are available to remove high concentrations of organic compounds from livestock wastewater, such as the returning farmland mode, the natural mode, and the factory mode [4,5]. The returning farmland mode and natural mode are poorly adapted and have the risk of secondary pollution. Although the factory model can meet the wastewater discharge standards, it entails high operational costs [6]. It is important to develop cost-effective treatments for the promising livestock wastewater with its high level of organics.

Microbial fuel cells (MFCs) have emerged as a sustainable technology for wastewater treatment that have potential in recycle bioelectricity from livestock wastewater and have great application

prospects in livestock wastewater treatment, desalination, hydrogen production by electrolysis, and so on [7,8]. In a widely studied double-chambered MFC, some microorganisms suspended in the anode chamber degrade complex macromolecular organic compounds into small molecules, and exoelectrogens attached onto the anode oxidize simple organics and produce electrons and protons, simultaneously [9,10]. The generated electrons are transported to the anode surface in several ways and accepted by an electron acceptor in the cathode using an external circuit, while the protons are transferred to the cathode via the exchange membrane used to maintain the two chambers' (anode and cathode) relative independence [11,12]. The proton and electron arriving at the cathode have a three-phase reduction reaction with the electron acceptor in the cathode chamber, thus forming a complete current loop [13]. Applying MFCs to wastewater treatment can not only avoid many disadvantages of traditional techniques but also theoretically make the energy conversion efficiency of MFCs higher than that of biogas power generation without the limitation of the Carnot cycle, which accords with the purpose of social sustainable development [14–16]. However, the performance of MFCs is too poor to be applied in practice.

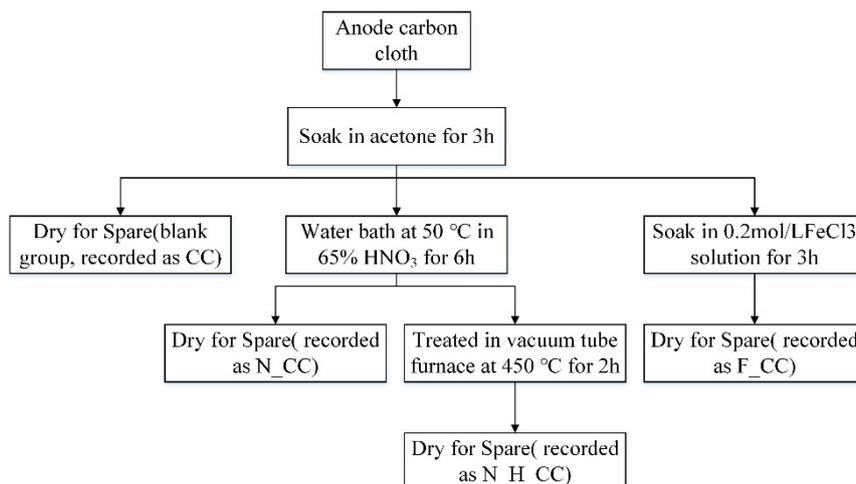
The performance of MFCs can be affected by the synergistic effect of the anode material with the surrounding microorganisms [17]. As the microorganism's attachment sites and electronic transit hub are in MFCs, the anode directly affects the electricity production and decontamination performance of the battery [18]. More and more researchers are trying to improve the anode materials reported or develop new anode materials and compare the effects of different anodes on the performance of MFCs for livestock wastewater treatment [19]. Although many studies have focused on the differences among modified anode materials characteristics and effects of various anodes on MFC performance, they did not analyze the effects of different anodes on the performance of MFCs using livestock wastewater as a substrate, especially swine wastewater, from the perspective of the biological community, and no scholars have determined the best modified anode of MFCs based on swine wastewater [20–22].

In this study, parallel-operated double-chambered MFCs equipped with untreated carbon cloth (CC), nitric acid-modified carbon cloth (N\_CC), acid-heat-modified carbon cloth (N\_H\_CC), and ferric chloride-modified carbon cloth (F\_CC) were constructed, using swine wastewater as a substrate, to compare the effects of different modification methods on the performance of MFCs. In particular, the effects of different modified anodes on the performance of MFCs based on swine wastewater were investigated from the view of microbial community structure, and the optimum method of CC anodic modification for MFCs with swine wastewater as a substrate was preliminarily determined. The experimental results have reference value for treating livestock wastewater, enhancing MFC performance and exploring the influence mechanism of different modified anodes on the production of microorganisms.

## 2. Materials and Methods

### 2.1. Electrode Pretreatment

First, all anode carbon cloths ( $\phi 16$  mm,  $0.21 \pm 0.01$  mm thick, HCP120, Shanghai Hesen Electric Co., Ltd., Shanghai, China) were soaked in acetone for 3 h and then rinsed with the ultrapure water to neutral and dried at  $60$  °C for spare, finally getting the CC. Then, one of the clean CCs was oxidized in 65% nitric acid at  $50$  °C for 6 h to obtain N\_CC, and the other was soaked in 0.2 mol/L ferric chloride solution for 4 h to obtain F\_CC. The N\_H\_CC was prepared by heating N\_CC at  $450$  °C for 2 h, as shown in Figure 1. All modified carbon cloths were stored separately at  $60$  °C prior to use for further experiments. All cathodes were the same carbon cloth as anodes containing  $0.35$  mg/cm<sup>2</sup> Pt catalyst [23].



**Figure 1.** Anode modification roadmap.

## 2.2. Construction and Operation

Four identical reactors were fabricated by using transparent plexiglass to study the effects of different modification methods on the performance in identical MFCs, while keeping all other factors identical. The MFC reactor was separated into anode (250 mL) and cathode (250 mL) chambers by the Nafion 117 proton exchange membrane (50 mm×50 mm, Du Pont China Holding Co., Ltd., Beijing, China). The anode chamber of the MFCs was inoculated with a prefermentation supernatant of swine wastewater, a medium containing 1.0 g/L sodium acetate and a 50 mM phosphate buffer solution (PBS) containing 10 mL/L trace minerals and 10 mL/L vitamins [24]. The cathode chambers were inoculated with 0.1 mol/L ferricyanide solution and a 50 mM PBS. When the outputted voltage of the MFC reactor declined to below 50 mV across an external loading resistance of 1000 Ω, a complete cycle was over. All reactors were operated in parallel-operated, fed-batch mode at 30 °C.

## 2.3. Analysis

The morphology of modified CCs were examined using a scanning electron microscope (SEM) (S-3400N, Hitachi, Ltd., Tokyo, Japan). The functional groups carried by modified electrodes were examined with Vertex 70 FTIR (Clus scientific instruments (Shanghai) Co., Ltd., Shanghai, China). The surface wettability was measured with contact angle meter (DSA100, KRUSS GmbH, Hamburg, Germany). The external resistance voltages were collected using a data acquisition board (USB-6343, National Instruments Co., Ltd., Austin, TX, USA) with on-line monitoring and recording every 10 min. When the voltage output became stable, data started to be recorded to obtain the polarization curve and power density curve by varying the resistance between the electrodes from 9999 Ω–50 Ω. The polarization slope method was used to calculate the internal resistance ( $R_{int}$ ) of MFC [25]. The power density of MFC were calculated according to the following equation [26]:

$$P_{An} = \frac{U^2}{R_{ex} \times S_{An}} \quad (1)$$

where  $P_{An}$  is power density (MW/m<sup>2</sup>);  $U$  is operating voltage (mV);  $R_{ex}$  is external circuit resistance (Ω);  $S_{An}$  is anode electrode surface area (m<sup>2</sup>).

After a week of stable operation of the MFCs, the sewage purified by MFCs was filtered with the drainage membrane (0.45 μm, Jingteng Experimental Equipment Co., Ltd., Tianjin, China), and we examined chemical oxygen demand (COD) concentrations with a water-quality analyzer (DR3900, HACH, Loveland, CO, USA).

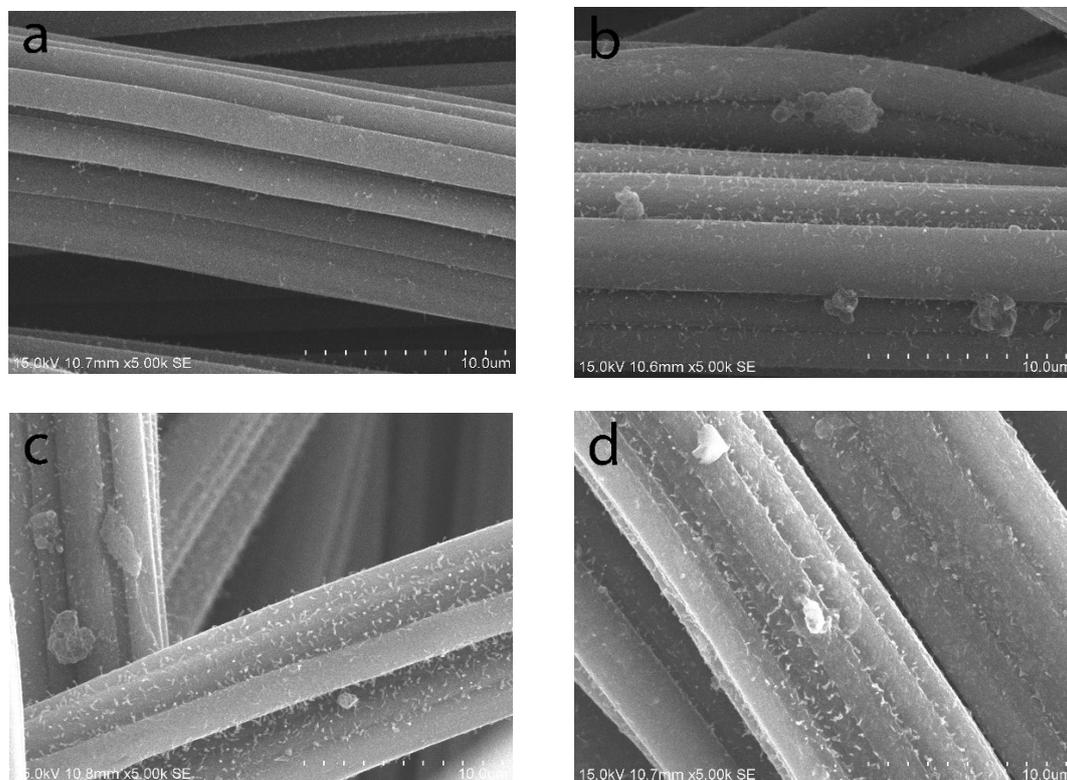
The DNA of MFC anode biofilm was picked up through a DNA isolation Kit (MoBio, Carlsbad, CA, USA). The V3-V4 regions of the 16S rDNA gene were amplified and purified by the 16S universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The high-throughput sequencing for polymerase chain reaction (PCR) amplification of barcoded 16S rDNA gene was carried out on the Illumina Miseq platform at Majorbio Co., Ltd. (Shanghai, China). Based on 97% similarity, operational taxonomic units (OTUs) clustering analysis of qualified sequences were developed by the Sequence Read Archive (SRA, <http://www.ncbi.nlm.nih.gov/Traces/sra>) and were obtained by cut off, which were used for the analysis of microbial community diversity and the microbial community in different aspects.

### 3. Results and Discussion

#### 3.1. Characterizations of Anodes

##### 3.1.1. SEM Analysis

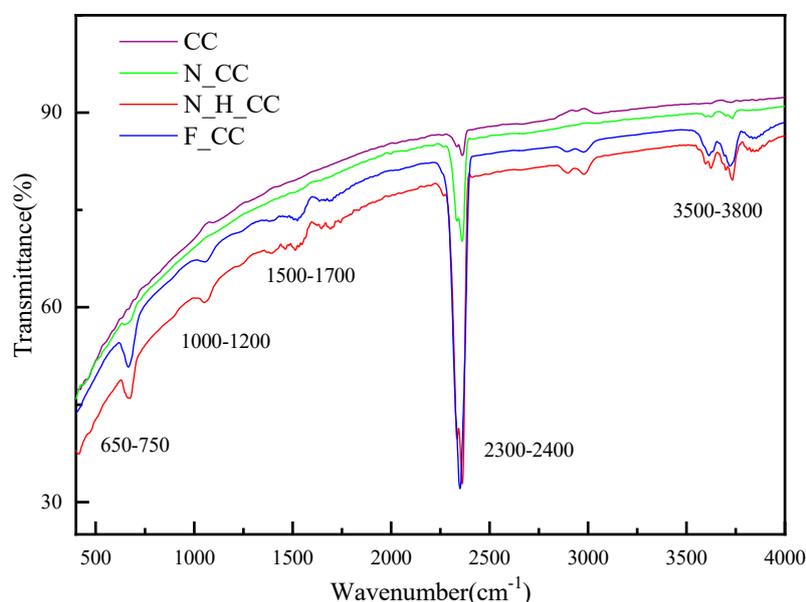
Different modification methods may affect the MFCs' performance by changing the anode characteristics. The SEM of carbon cloth anode treated by different methods is shown in Figure 2. SEM examination showed that the enlarged bulge structure makes the surface of the anode become rougher compared with the CC anode (Figure 2a), which may increase the specific surface area of the anode and be beneficial to the adhesion of microorganisms. The bulge structure of the N\_H\_CC (Figure 2c) anode was more uniform than N\_CC (Figure 2b) or F\_CC (Figure 2d), which may provide a better chemical reaction point for the anode and improve the battery performance. Particularly, the spatial distribution of carbon fibers on the F\_CC anode appeared more compact than the other ones rarely reported before, which may reduce the anode specific surface area and weaken the microbial adhesion on the anode surface [27].



**Figure 2.** SEM images of a carbon cloth anode treated with different methods: (a) CC, (b) N\_CC, (c) N\_H\_CC, and (d) F\_CC.

### 3.1.2. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

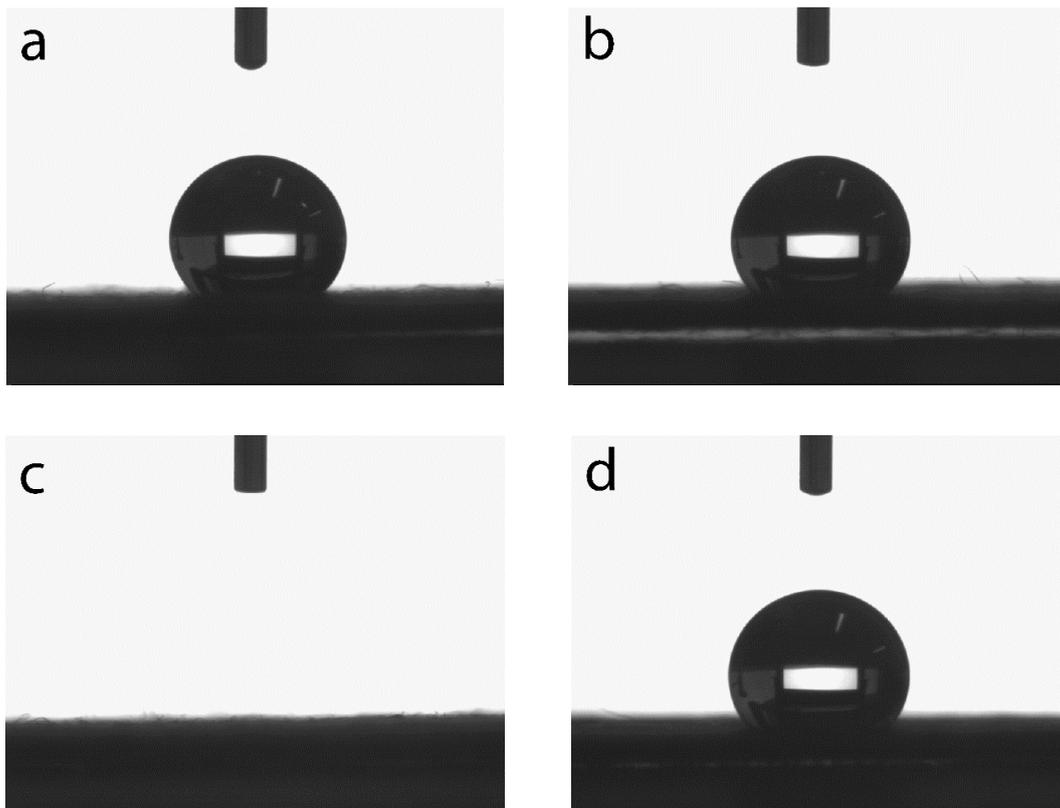
Modification treatment helped to increase the type of functional groups attached to the carbon cloth surface, which can be proved by the FT-IR spectra of the anode [28]. As shown in Figure 3, the infrared spectra of CC showed one peak at 2300–2400  $\text{cm}^{-1}$ , which was the asymmetric stretching vibration induced by a small amount of  $\text{CO}_2$  in the experiment. For N\_CC, peaks at 3500–3800  $\text{cm}^{-1}$  originate from the stretching vibration of O–H band and  $-\text{NH}_2$  band, which was caused by the infrared radiation of  $-\text{COOH}$ . The modified treatment resulted in more absorption peaks in the spectra of N\_H\_CC and F\_CC. The stretching vibration of C=O band at 1500–1700  $\text{cm}^{-1}$  and C–O band at 1000–1200  $\text{cm}^{-1}$  were observed, respectively, in the infrared spectra, which may be caused by the carboxyl group ( $-\text{COOH}$ ), while the absorption peak at 650–750  $\text{cm}^{-1}$  was the outward flexural vibration of carboxyl (O–H). The results indicated that the hydrophilic groups were attached to the surface of the modified CC, such as hydroxyl, carboxyl, and amino groups, greatly improving the hydrophilicity of anode, which indirectly promoted the electron transfer rate benefiting from more microorganisms attachment to the anode and improved the overall performance of the MFCs.



**Figure 3.** FT-IR spectra of the CC, N\_CC, N\_H\_CC and F\_CC.

### 3.1.3. Contact Angle Analysis

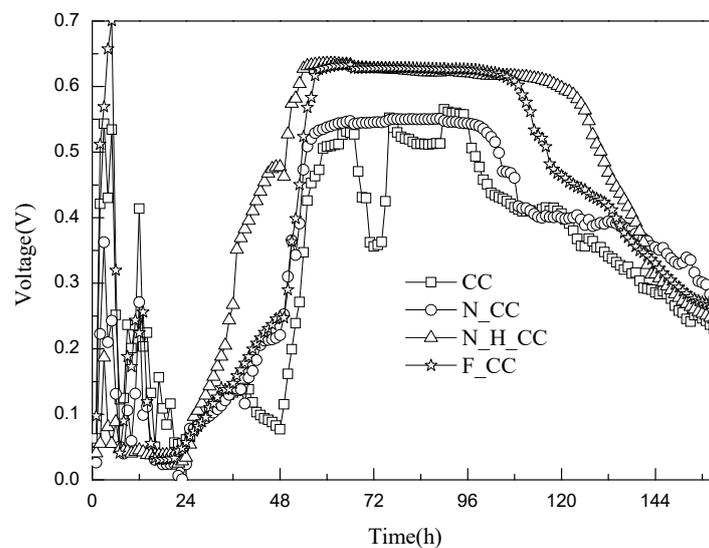
In order to further investigate the influence of different modification treatments on the characteristics of CC, especially the interaction between anolyte and anode, the wettability of different anodes was characterized by the contact angle [29]. As shown in Figure 4, the contact angle of CC ( $132.2^\circ$ ) was smaller than N\_CC ( $120.1^\circ$ ) and F\_CC ( $122.9^\circ$ ), indicating that the nitric acid and ferric chloride modification can enhance the wettability of carbon cloth relative to water, which was mainly due to the increased hydrophilic groups and bulge structure. The hydrophobicity of N\_H\_CC anode disappeared after modification, which may cause the partial change of the basic structure of carbon fiber and the decrease of the order of carbon fabric structure by modification, and the increased hydrophilic groups may be conducive to form hydrogen bonds with water. The results showed that the wettability of the anode was improved significantly after modification, which not only can enhance the compactness between the anode and the microorganism: by increasing the activity of microbial enzymes on the anode surface to advance the transfer between the microorganisms and anode, the potential of hydrophilic groups on a modified anode to act as electron-transfer carriers was also indirectly confirmed, and then the performance of the MFCs was improved.



**Figure 4.** Contact angle diagram of the CC (a), N\_CC (b), N\_H\_CC (c), and F\_CC (d).

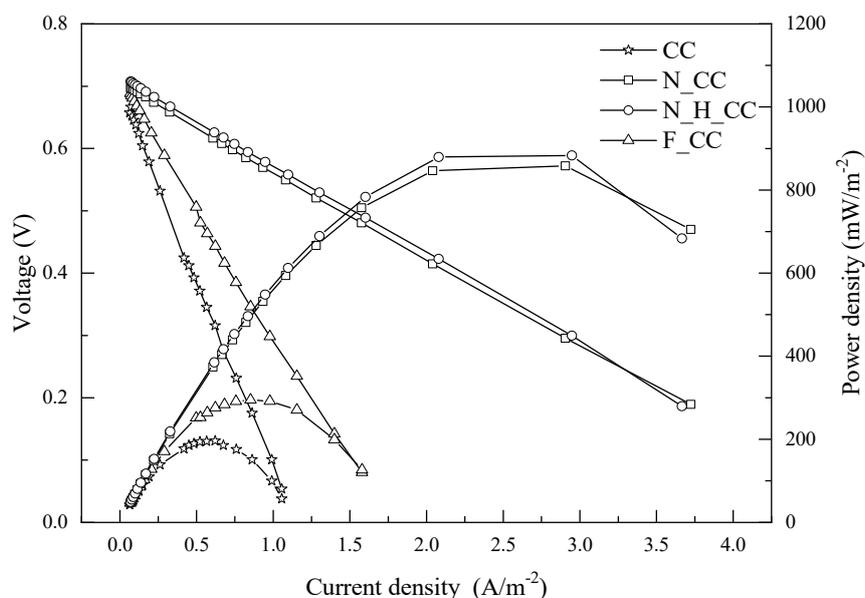
### 3.2. MFC Performance

Figure 5 shows the working voltage variation of four groups with different modified carbon cloth anodes. In the stable operation stage, the stable voltage of each group was 0.512 V (CC), 0.550 V (N\_CC), 0.634 V (N\_H\_CC), and 0.627 V (F\_CC). It showed that the N\_H\_CC group performed better, and the voltage of N\_H\_CC was the highest, which was 23.82% higher than that of the CC. After the anode was modified, MFCs showed stronger power-generation performance, including shorter start-up period, longer stable operation stage, and higher stable voltage.



**Figure 5.** Working voltage of MFCs.

The power densities and internal resistances of MFCs varied with the modification and could be obtained from the polarization curves [30]. Figure 6 showed that the maximum power densities for N\_CC (858.48 mW/m<sup>2</sup>) and N\_H\_CC (883.62 mW/m<sup>2</sup>) were similar, followed by F\_CC (295.02 mW/m<sup>2</sup>) and CC (196.68 mW/m<sup>2</sup>). The R<sub>int</sub> was the external resistance value when the highest power density was reached. The minimum R<sub>int</sub> for the N\_H\_CC reactor was 136 Ω, which was 0.27%, 34%, and 95.77% of those for the CC, F\_CC, and N\_CC. The COD removal rates were 58.3%, 71.2%, 80.1%, and 61.9% for MFCs with CC, N\_CC, N\_H\_CC, and F\_CC, respectively, which was consistent with the power density trend. These results suggested that the improvement of the anode wettability and the increase of specific surface area promoted the electron transfer between the electrode and the microorganisms, which reduced the resistance of the MFCs and improved the ability to produce electricity or purify sewage, and it may be the main reason for the optimal performance of N\_H\_CC anode. Compared with N\_CC, the average voltage of F\_CC was high, but the power density was low, which may be because the introduction of more hydrophilic groups by the strong oxidation of Fe<sup>3+</sup> reduced the internal resistance. However, agglomerated bundles of carbon fibers reduced the specific area of the F\_CC anode and the adhesion of microorganisms, which resulted in the power density of the F\_CC anode being lower than that of the N\_CC anode and indirectly showed that the structure of the carbon anode and the adhesion of microorganisms have more influence on the performance of MFCs than the functional group or electron transfer on the electrode surface.



**Figure 6.** Power density and polarization curves of MFCs operated with CC, N\_CC, N\_H\_CC, and F\_CC.

### 3.3. Microbial Community Diversity

The Illumina sequencing analysis was adopted to generate a total of 139,591 raw sequences and obtained a total of 26,270, 31,232, 33,164, 34,536 high-quality reads from the anode biofilms of MFCs operated with CC, N\_CC, N\_H\_CC, F\_CC, respectively. A total of 509 (CC), 552 (N\_CC), 425 (N\_H\_CC), 412 (F\_CC) operational taxonomic units (OTUs) were determined at 97% nucleotide identify. The species richness and diversity of environmental communities were analyzed by calculating indexes.

As shown in Table 1, the observed Chao and Shannon estimators showed that the biofilm of N\_CC had the highest richness and diversity, followed by CC, F\_CC and N\_H\_CC. The Heip of F\_CC was the highest among the four samples, implying that species evenness of F\_CC was higher than CC, N\_CC, and N\_H\_CC. The results showed that the space structure or hydrophilic groups attached to the carbon cloth after nitric acid modification can improve the microbial community diversity in the anode chamber, but it does not help much in the performance of MFCs. It is possible that more nonelectric

microorganisms are attached to the anode, resulting in a decrease in the production of MFC with N\_CC, and we should not neglect the fact that many dominant bacteria were degraded in the process of organic-matter consumption, which also proved that microbial community diversity of an MFC anode based on swine wastewater was not positively correlated with MFC performance. The F\_CC and N\_H\_CC anode showed a more obvious selective effect on microorganisms, which caused a lower microbial community diversity than that of CC. The microbial community evenness of N\_H\_CC was lowest, indicating that the selection on the microbial community by the N\_H\_CC anode may develop better exoelectrogens or related cooperative communities to improve the power generation and decontamination performance of MFCs using swine wastewater. The values of coverage tend to be 1, indicating that the sequencing data in the microbial diversity analysis was sufficient to reflect species diversity in the samples.

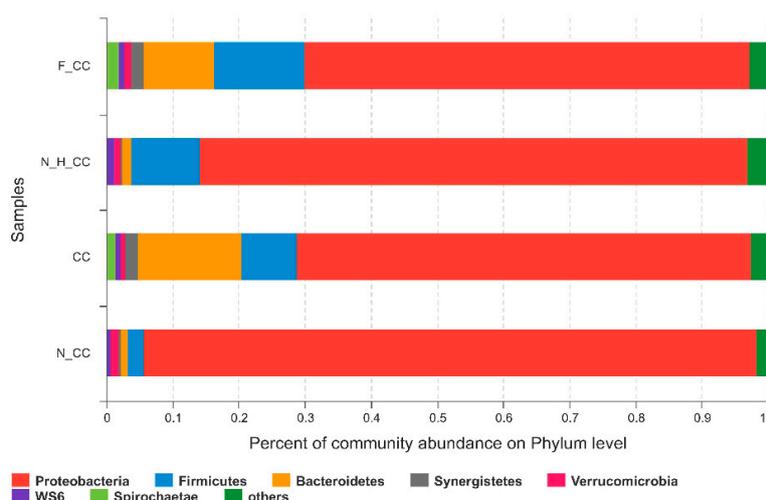
**Table 1.** Similarity based OTUs and species richness estimates.

Sample	Reads	OTU	Chao	Heip	Shannon	Coverage
CC	26,270	509	543 (527, 574)	0.1852	4.56 (4.54, 4.58)	0.9977
N_CC	31,232	552	565 (557, 585)	0.1189	4.83 (4.81, 4.85)	0.9989
N_H_CC	33,164	425	475 (452, 517)	0.0856	3.63 (3.61, 3.65)	0.9980
F_CC	34,536	412	491 (458, 550)	0.2251	3.91 (3.9, 3.93)	0.9975

### 3.4. Microbial Community Structure

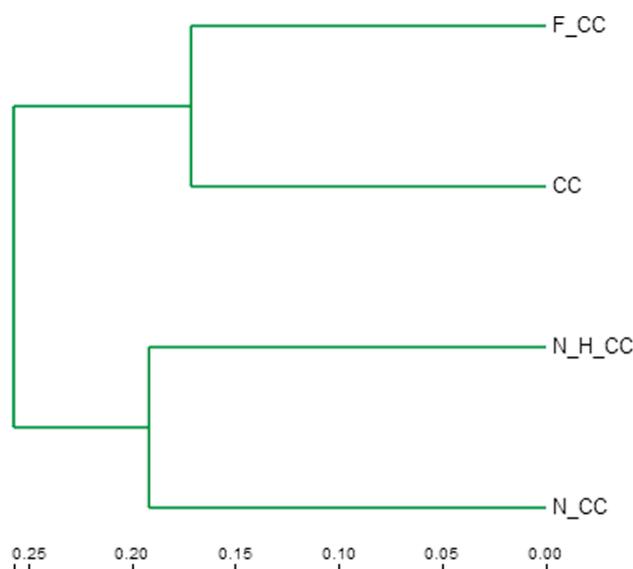
The predominant phyla in the anode biofilms of all MFCs belonged to *Proteobacteria* (78.71%), *Firmicutes* (8.52%), *Bacteroidetes* (7.44%), *Synergistetes* (1.04%), *Verrucomicrobia* (0.95%), *WS6* (0.86%), *Spirochaetes* (0.8%), and *Saccharibacteria* (0.47%) in decreasing order. The highest relative abundance of *Proteobacteria* among the four samples were 68.7% (CC), 92.65% (N\_CC), 82.83% (N\_H\_CC), and 67.17% (F\_CC). *Bacteroidetes* can better adapt to the micro-ecological environment changes and help to improve the performance of MFCs. *Synergistetes* can degrade amino acids and pyruvate in an anaerobic environment and may play a role in degrading intermediate products of swine wastewater [31]. *Verrucomicrobia*, *WS6*, and *Spirochaetes* were commonly found in feces or soil but at low abundance in the reported anodic biofilm [32].

The relative abundance of the dominant populations showed that a response of microbial community to modification methods change had occurred, and the composition and relative abundance of microbial community at the phylum level were basically the same, but there were still differences, as shown in Figure 7. The relative abundance of dominant bacteria *Proteobacteria* was low in the microbial communities of CC or F\_CC, which may be one of the main reasons leading to the poor performance of MFCs with CC or F\_CC. The relative abundance of *Proteobacteria* in the N\_CC anode was the highest, while its *Firmicutes* and *Bacteroidetes* relative abundance were the lowest, which affected the performance of MFCs to a certain extent and explained that the performance of N\_CC was better than that of CC or F\_CC. The optimal distribution of three dominant bacteria groups (*Proteobacteria*, *Firmicutes*, and *Bacteroidetes*) in the community structure of N\_H\_CC accounted for the optimum performance of MFCs with N\_H\_CC. The inexistence or low abundance of *Synergistetes* and *Spirochaetes* in the MFCs with N\_CC and N\_H\_CC illustrated that they were of little benefit to the performance improvement of MFCs based on swine wastewater. *Saccharibacteria* existed only in N\_H\_CC and may effectively promote the fermentation or oxidation of swine wastewater.



**Figure 7.** Relative ratio of the dominant bacterial at phylum level in CC, N\_CC, N\_H\_CC, and F\_CC.

Different samples of the hierarchical clustering tree on genus level is shown in Figure 8. The community composition of CC and F\_CC was closer and so were N\_CC and N\_H\_CC, which indicated that N\_CC and N\_H\_CC had a more obvious selective effect on microorganisms than CC and F\_CC.



**Figure 8.** Hierarchical clustering tree on genus level for CC, N\_CC, N\_H\_CC, and F\_CC.

As shown in Table 2, the relative abundance >1% species mainly distributed in *Betaproteobacteria*, *Gammaproteobacteria*, and *Epsilonproteobacteria* at the anode community. The dominant genus of relative abundance >5% species in CC mainly included *Methylomonas* (10.52%), *Arcobacter* (9.15%), and *Stenotrophomonas* (7.94%) in decreasing order, concentrated in *Gammaproteobacteria* and *Epsilonproteobacteria*. The dominant bacteria in F\_CC were *Thauera* (6.17%), *Thermomonas* (6.73%), *Comamonas* (5.35%), and the dominant genus in N\_H\_CC were *Adevenella* (27.63%), *Hydrogenophaga* (11.62%), and *Comamonas* (6.91%). The number of dominant bacteria in N\_CC was the largest, including *Comamonas* (11.96%), *Adevenella* (11.04%), *Hydrogenophaga* (10.34%), *Thermomonas* (8.36%) and *Azoarcus* (6.70%). The dominant bacteria on the surface of the anode changed significantly after modification in which the relative abundance >5% species mainly distributed in *Betaproteobacteria* and *Gammaproteobacteria*, and the content of dominant bacteria in N\_CC and N\_H\_CC became less than

before. The relative abundance <1% species exceeding 1/3 of the total sequence in CC and F\_CC, which seriously limits the electricity production of microbial communities.

**Table 2.** Differences in relative abundance of the bacterial genera with CC, N\_CC, N\_H\_CC, and F\_CC.

Phylum or Class	Genus	Abundance (%)			
		CC	N_CC	N_H_CC	F_CC
Alphaproteobacteria	<i>Sphingopyxis</i>	1.82	2.16	0.4	0.81
	<i>Unclassified-f-Sphingomonadaceae</i>	0.37	2.18	3.6	0.9
	<i>Novosphingobium</i>	0.89	1.63	2.34	0.98
	<i>Unclassified-f-Caulobacteraceae</i>	0.99	1.94	0.24	0.68
	<i>Phenylobacterium</i>	0.24	1.68	0.28	0.87
	<i>Sphingobium</i>	0.09	1.60	0.74	0.63
	<i>Brevundimonas</i>	0.95	1.14	0.58	0.74
Betaproteobacteria	<i>Comamonas</i>	4.89	11.96	6.91	5.35
	<i>Aquabacterium</i>	3.44	1.26	1.75	1.42
	<i>Adevenella</i>	2.73	11.04	27.63	6.26
	<i>Hydrogenophaga</i>	2.59	10.34	11.62	4.2
	<i>Castellaniella</i>	2.05	1.69	0.28	2.56
	<i>Methylophilus</i>	1.09	0	0	0.13
	<i>Unclassified-f-Comamonadaceae</i>	1.46	2.27	1.22	1.58
	<i>Thauera</i>	0.95	7.76	3.98	6.17
	<i>Azoarcus</i>	0.6	6.70	2.96	2.09
	<i>Azovibrio</i>	0.29	0.24	1.41	0.12
	<i>Norank-f-Alcaligenaceae</i>	0.17	3.34	1.08	0.97
	<i>Simplicispira</i>	0.18	0.23	1.06	0.24
	<i>Unclassified-f-Alcaligenaceae</i>	0.02	1.39	0	0.23
Gammaproteobacteria	<i>Methylomonas</i>	10.52	1.51	0	1.87
	<i>Stenotrophomonas</i>	7.94	4.24	3.52	4.76
	<i>Thermomonas</i>	4.57	8.36	1.78	6.73
	<i>Acinetobacter</i>	1.17	0.26	0	0.3
	<i>Pseudomonas</i>	0.5	0.22	0.16	1.69
Deltaproteobacteria	<i>Desulfomicrobium</i>	2.01	0.48	3.38	3.42
	<i>Desulfobulbus</i>	0.44	1.10	1.9	0.63
Epsilonproteobacteria	<i>Arcobacter</i>	9.15	0.64	0.53	4.3
Bacteroidetes	<i>vadinBC27-wastewater-sludge-group</i>	2.31	0	0.44	2.13
	<i>Norank-c-Bacteroidetes-vadinHA17</i>	1.5	0.12	0.1	1.75
	<i>Flavobacterium</i>	1.44	0.12	0	0.99
Firmicutes	<i>Clostridium-sensu-stricto-1</i>	1.14	0.48	3.76	2.45
	<i>Erysipelothrix</i>	0.5	0.27	0.75	1.15
	<i>Terrisporobacter</i>	0.55	0.39	1.9	1.1
Synergistetes	<i>Norank-f-Synergistaceae</i>	1.56	0.18	0.23	1.56
P-WS6	<i>Norank-p-WS6</i>	0.92	0.56	1.16	0.83
others		27.98	10.31	12.06	27.41

The four samples had the same dominated species at genus level, such as *Comamonas*, *Hydrogenophaga*, *Stenotrophomonas*, and *Thermomonas*. *Comamonas* and *Thermomonas* have been reported to be beneficial for improving MFC performance, and similar results have been obtained in this study, which were more densely distributed in MFCs with N\_CC and N\_H\_CC positively correlated with cell performance. *Hydrogenophaga* and *Stenotrophomonas* were efficient bacteria reported for sewage treatment, and their enrichment was beneficial to the stability of microbial communities [33].

A small amount of *Methylomonas* was beneficial to the production of electricity cooperated with exoelectrogens, which was the only highly abundant genus of CC, but too much of it may consume too many organics and limit the rest of exoelectrogens [34]. After modification, the abundance of

*Methylomonas* on the anode surface decreased obviously, which indicated that the selection by modified anode on the biological community was beneficial for improving the performance of MFCs. The highest relative abundance species in N\_H\_CC was *Advenella* (27.6%), which was widely distributed in swine wastewater and not proven to be effective for MFC. *Advenella* was demonstrated to enhance the degradation of substrate by sludge system, which may be one of the main reasons for the best decontamination capacity of N\_H\_CC. *Azovibrio* and *Simplicispira* has been shown to be better adapted to low temperature environments both of which belonged to N\_H\_CC peculiarly. Ferric chloride modification enabled *Thauera* to become the dominant genus in the F\_CC, which, due to the strong ability of *Thauera* to reduce  $Fe^{3+}$ , effectively promoted electron transfer to improve the performance of the MFCs.

From the analysis above, the effects of different modifications on microbial community structure on the carbon cloth anode was obvious. Although the electricity generation by many dominant genera in swine wastewater remains to be further confirmed, there is no doubt that the performance of MFC with N\_H\_CC using swine wastewater was the best among the four samples in this study.

#### 4. Conclusions

Three different methods were used to modify the anode carbon cloth to explore the influence of anode modification on the power generation and decontamination performance of MFCs. From the perspective of the microbial community, the mechanism of different modification methods on the performance of MFCs based on swine wastewater was explored. The results showed that anode modification clearly influenced electricity generation and microbial community structure of MFCs. The space structure and properties of the carbon cloth anode can be improved obviously by acid thermal modification with which MFCs can produce the maximum power density ( $883.62 \text{ mW/m}^2$ ) and COD removal rate (80.1%). Compared with the untreated carbon cloth, the power density was increased by 350%. The microbial community structure and function analysis revealed that the selection by modified anode on the biological community was beneficial to improve the performance of MFCs. The dominant bacteria in MFCs were mainly distributed in *Proteobacteria* (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*), *Firmicutes*, and *Bacteroidetes*. Acid-heat modification was the best method for improving the MFC anode carbon cloth based on swine wastewater. Swine wastewater treated by MFCs can effectively solve the problem of environmental pollution and provide reference and future usage for improving the performance of MFCs using livestock wastewater. It also provides a new way for the development of renewable energy in practice and further research.

#### 5. Patents

The authors of this paper have carried out research on the microbial fuel cell for many years. Four China invention patents related to this paper have been granted, the patent numbers are CN106784952B, CN106876760B, CN106848361B, and CN108075162B. In addition, four China invention patents have been published: the patent numbers are CN110713899A, CN110669644A, CN109081424A and CN109081450A.

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