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The Microbial Communities of Anaerobic Respiration and Fermentation Degrading Chitin Exist in the Anaerobic Sludge of Microbial Fuel Cell Anodes

Sheng-Hu Zhen [1], Yang-Yang Yu [2], Rong-Rong Xie [3], Wei Xu [1] and Shan-Wei Li [1,4,*]

[1] Institute of Environmental Health and Ecological Safety, School of Environmental and Safety Engineering, Jiangsu University, Zhenjiang 212013, China
[2] Information Materials and Intelligent Sensing Laboratory of Anhui Province, Anhui University, Hefei 230601, China
[3] Biofuels Institute, School of Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, China
[4] Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment, Suzhou University of Science and Technology, Suzhou 215009, China
* Correspondence: lsw3@ujs.edu.cn

Abstract: Chitin is one of the most abundant polymers in nature, with chitinous biomass often discarded as food waste and marine debris. To explore an effective way to degrade chitin, in this work, anaerobic sludge was inoculated at the anode of a two-chamber microbial fuel cell (MFC), and chitin was degraded via anaerobic respiration and fermentation. The results showed that the anaerobic sludge could degrade chitin under both the anaerobic respiration and fermentation modes, with similar degradation rates (7.10 ± 0.96 and 6.96 ± 0.23 C-mg/L·d^{-1}). The open-circuit voltage and output current density could roughly reflect the degradation of chitin in the MFC. The maximum current density generated through the anaerobic sludge degradation of chitin via anaerobic respiration was 160 mA/m^2, and the maximum power density was 26.29 mW/m^2. The microbial sequencing results revealed substantially different microbial community profiles, with electroactive bacteria (EAB) flora and fermentative bacteria (*Longilinea*) as the main microbial groups that degraded chitin via anaerobic respiration and fermentation, respectively. Therefore, anaerobic sludge may be a good choice for the treatment of refractory biomass due to its abundant electroactive and fermentative flora.

Keywords: anaerobic sludge; chitin; anaerobic respiration; fermentation; MFC

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1. Introduction

Advances in modern industry and technology have resulted in a constant demand for energy, and this enormous energy demand has been largely met by unsustainable natural fossil fuels. Extensive mining and refining operations of fossil fuels have resulted in further environmental pollution, shifting the focus of research to eco-friendly and sustainable resources [1]. Biomass has gained attention as a sustainable future energy source. Chitin is the second most abundant natural biopolymer in nature after cellulose, with a highly similar structure to cellulose, and is composed of N-acetyl-β-D-glucosamine (GlcNAc) linked by β-1,4-glycosidic bonds [2]. Crustaceans such as shrimp and crabs are the richest source of chitinous biomass, with approximately 10^{12}–10^{14} tons of chitinous biomass produced annually by the fish processing industry [3]. Chitinous biomass is mostly discarded as food waste and marine debris, making the effective degradation and use of chitin of great significance.

Aerobic and anaerobic degradation are the two common metabolic modes of chitin degradation. Boyer et al. compared chitin degradation using estuary aerobic and anaerobic sediments and found that metabolite acetate could be detected via the fermentation of chitin, while no measurable intermediates were detected via aerobic chitin degradation [4].

The aerobic respiration degradation of chitin was faster than fermentation degradation, but it could not recover energy or chemicals. Anaerobic respiration is another type of anaerobic metabolism besides fermentation. The MFC is a device of great concern; it is a biochemical-catalyzed system that uses microorganisms to generate bioelectricity by oxidizing biodegradable organic matter [5]. Microorganisms that are typically present in the anode chamber of fuel cells can act as biocatalysts, oxidizing organic substrates through anaerobic respiration to generate electrons (e^-) and protons (H^+). Electrons are transferred from the anode to the cathode through an external circuit, while protons diffuse into the cathode chamber through a proton exchange membrane, which separates the anode and cathode chambers. MFCs have been developed to generate electricity using different substrates, such as acetate, glucose, and starch [6,7]. However, these substrates have high biodegradation rates, are quickly exhausted, and are relatively expensive, making their use uneconomical. Therefore, chitin derived from shrimp and crab food waste is of great significance as a sustainable energy source. Using anaerobic respiration as the main mode of MFC systems to simultaneously degrade chitin and produce energy may be a better chitin treatment method.

As a biocatalyst for MFC, electrochemically active bacteria (EAB) can generate and transfer electrons exocellularly [8]. Using wastewater from a secondary clarifier as an inoculum to degrade chitin in a single-chamber MFC has been reported, but no analysis of EAB has been conducted [9]. The EAB pure cultures *Arenibacter palladensis*, *Aeromonas hydrophila*, and *Shewanella oneidensis* were reported to directly use chitin as a substrate to generate electricity in two-chamber MFCs [10–12]. Anaerobic sludge has the characteristics of a large biomass and a rich microbial diversity [13]. The bacterial genera contained in anaerobic sludge include fermentative bacteria such as *Hydrogenispora* [14], *Enterobacter*, *Bacillus*, and *Clostridium* [15], as well as common EAB such as *Geobacter* [16] and *Shewanella* [17] in MFC systems. Anaerobic sludge is widely used in water pollution control and is currently used to degrade refractory organic pollutants [18] and biomass, such as cellulose [19]. Therefore, anaerobic sludge may be suitable for degrading chitin and generating electricity in MFC systems, though no reports have been published on the degradation of chitin using MFC systems inoculated with anaerobic sludge.

This work investigates the anaerobic degradation of chitin by anaerobic sludge via anaerobic respiration and fermentation systems, and the degradation rates of the chitin were compared. The bioelectrical properties of the MFC system were obtained, including the open-circuit voltage, output current density, and polarization curve. In addition, the biofilm formed by the anaerobic sludge at the anode was analyzed to understand the evolution of the functional bacteria in the anaerobic respiration and fermentation systems.

2. Materials and Methods

2.1. Preparation of Suspended Chitin

First, 5 g of chitin flakes (Sangon Biotech, Shanghai, China) were shredded and mixed with 400 mL of HCl (37%) under stirring for 10 min. Then, the homogenous slurry was poured into 5 L of cold deionized water. After stirring, the solution was filtered with a cellulose membrane and the chitin precipitate was washed with deionized water until the pH reached 4. Subsequently, the precipitate was resuspended in deionized water and adjusted the pH to 7 using NaOH. The suspension was filtered again and the supernatant was discarded. Finally, the chitin precipitate was resuspended in 1 L of deionized water to obtain a final concentration of 2% (w/v, g/mL).

2.2. Experimental Operation

A two-compartment H-type MFC (working volume of 140 mL) was used in this work, with a carbon felt electrode (30 × 30 × 1 mm) used for both the anode and cathode. The anode and cathode chambers were separated by a cation exchange membrane (CMI-7000, Membranes International Inc., Ringwood, NJ, USA). This cation exchange membrane is a strong acid cation exchange membrane with a thickness of 0.45 ± 0.025 mm, allowing

positively charged cations to pass through. The cation exchange membrane was placed between the anode and cathode chambers and clamped with a clamp. The anode chamber was inoculated with 140 mL of anaerobic sludge (mixed liquor suspended solid—MLSS: 2500 mg/L) from the anaerobic tanks of the Jingkou Sewage Treatment Plant (Zhenjiang, Jiangsu, China), and the final concentration of chitin was 1.5 g/L. Meanwhile, ferricyanide solution (16.47 g of $K_3Fe(CN)_6$, 17.8 g of $Na_2HPO_4 \cdot 12H_2O$, and 3 g of KH_2PO_4 per liter) was used for the cathode. The MFCs were connected to a fixed external resistor (1000 Ω), and the output voltage was recorded via a data acquisition system (PISO-813, ICP DAS Co., Ltd., Taiwan, China) every 0.5 h. The experimental device of the MFC is shown in Figure 1a. After the output voltage reached the steady state, the MFCs were run in the open-circuit mode for 4 h, the polarization curves were obtained by varying the external resistance (300–50 kΩ), and the stable voltage was recorded.

To confirm the anaerobic fermentation degradation mode of chitin in the open-circuit MFC system, a control fermentation degradation experiment of chitin in anaerobic serum bottles was conducted. The fermentation control experiment was carried out in a 200 mL serum bottle, to which 140 mL of chitin and anaerobic sludge were added, with the same concentration as the MFC system. The experimental device of the serum bottle is shown in Figure 1b. Before starting the experiment, the anaerobic sludge was completely depleted of any remaining organic carbon (verified via chemical analysis) through an operation in the MFC under starvation conditions (without adding feed). The MFC anode and serum bottles were blown with nitrogen for 15 min and then sealed with a rubber stopper. All experiments were conducted in triplicates.

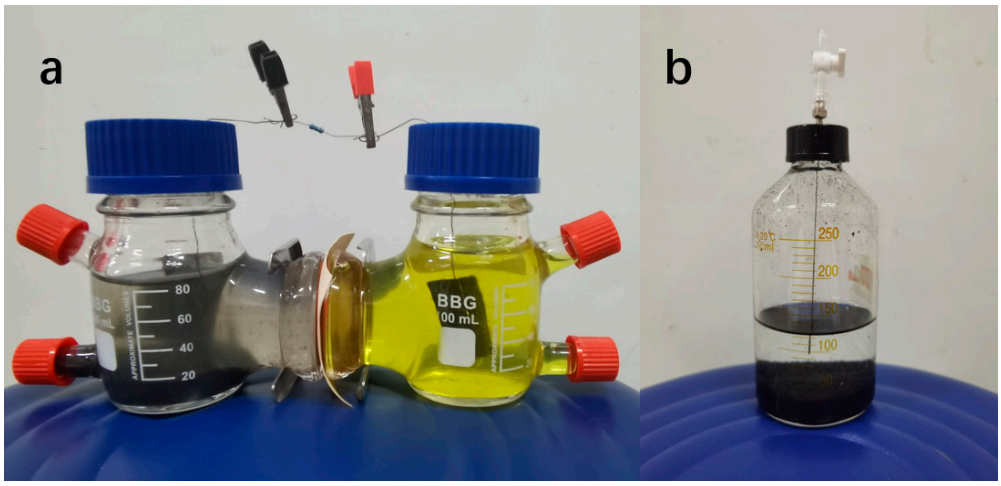

Figure 1. Photographs of experimental setup of two-compartment MFC (**a**) and serum bottle (**b**).

2.3. Chemical Analyses

Samples were periodically collected from the MFC and the serum bottle with sterilized syringes, which were filtered with 0.22 μm syringe filters. The TOC was measured using a TOC detector (TOC-L, Shimadzu, Kyoto, Japan). The NH_4^+ was analyzed using Nessler's reagent spectrophotometry method at a wavelength of 420 nm, the NO_2^- was determined via the N-1-naphthyl-1,2-diaminoethane dihydrochloride spectrophotometric method at a wavelength of 540 nm, and the NO_3^- was measured via the ultraviolet spectrophotometry method at wavelengths of 220 nm and 275 nm [20]. The concentrations of NH_4^+, NO_2^-, and NO_3^- were analyzed using a UV–visible spectrophotometer (L8, INESA, Shanghai, China).

2.4. Scanning Electron Microscope Observation

Upon the completion of the chitin degradation experiment, a small piece of the carbon felt electrode was harvested, fixed with 2.5% glutaraldehyde overnight, and dehydrated with gradient ethanol solutions (35%, 50%, 70%, 90%, and 100%) and hexamethyldisilane

solution (50% and 100%) [11], and finally deposited on a silica wafer. The samples were coated with gold using a sputter coating device and observed via thermal field emission scanning electron microscopy (SEM) (S-4800, Hitachi, Tokyo, Japan).

2.5. High-Throughput Sequencing

Sludge samples from the anode carbon felt surface were obtained after the completion of the experiments, and initial samples were obtained from the fresh inoculated sludge. The samples were sent to Sangon Biotech (Shanghai) Co., Ltd. for microbial community profiling. The V3 to V4 hypervariable regions of the bacterial 16S rRNA gene were amplified through a polymerase chain reaction (PCR) with primers 341F (5′-GTACTCCTACGGGAGGCAGCA-3′) and 805R (5′-GTGGACTACHVGGGTWTCTAAT-3′), using the Illumina MiSeq platform (BBI, China) for high-throughput sequencing. The raw sequencing data generated from this study have been deposited in the NCBI Sequence Read Archive database under the accession number SRP437213.

2.6. Data Analysis

The average degradation rate of chitin (v_{chitin}) was calculated according to the following equation:

$$v_{\text{chitin}} = \frac{C_{\text{TOC}}}{t}\left(\frac{\frac{\text{mg}}{\text{L}}}{\text{d}}\right),$$

where C_{TOC} represents the generated TOC concentration at steady-state time t, and t is the time when the TOC concentration reached the steady state. Because it was difficult to measure the actual amount of chitin during degradation, a complete conversion of chitin to organic carbon was assumed in the calculation [11].

3. Results and Discussion

3.1. Chitin Degradation in the Anaerobic Respiration and Fermentation Systems

Previous work reported that chitin degradation could theoretically yield ammonium [10], which could be transferred from the MFC anode to the cathode through the cation exchange membrane. In addition, the anaerobic sludge could convert ammonium to other nitrogen compounds [21]. Therefore, the nitrogen compound concentrations of the anode and cathode of the MFC, and the serum bottle samples were detected to reflect the degradation of chitin. In this work, ammonium was detected when chitin was degraded in the closed-circuit and open-circuit MFCs, and in the serum bottles. No nitrate or nitrite compounds were detected in the samples under all conditions. This is probably because ammonium was used as a sustainable proton shuttle in a CEM-equipped BES and was not consumed in the process [22]. The change in ammonium concentration during the chitin degradation process is shown in Figure 2. Under the closed- and open-circuit conditions, the ammonium concentration in the anode of the MFC initially increased and then decreased, while the ammonium concentration in the cathode continued to increase to a stable level (Figure 2a). This indicated that chitin degradation at the MFC anode to produce ammonium occurred simultaneously with the diffusion of ammonium from the anode to the cathode, and almost all the ammonium produced by the anode was transferred to the cathode. The changes in the sum of ammonium concentrations at the anode and cathode of the MFC, as well as the changes in ammonium concentration in the serum bottle, are shown in Figure 2b. The results indicated that the ammonium concentration produced due to the degradation of chitin in the closed-circuit and open-circuit MFCs and serum bottles showed a consistent increase and reached a steady-state concentration of approximately 45 mg/L after the ninth day. Since no other nitrogen-containing compounds other than ammonium were detected in the MFCs and serum bottles, the time at which the resultant ammonium concentration reached a steady state was the time at which the chitin was completely degraded.

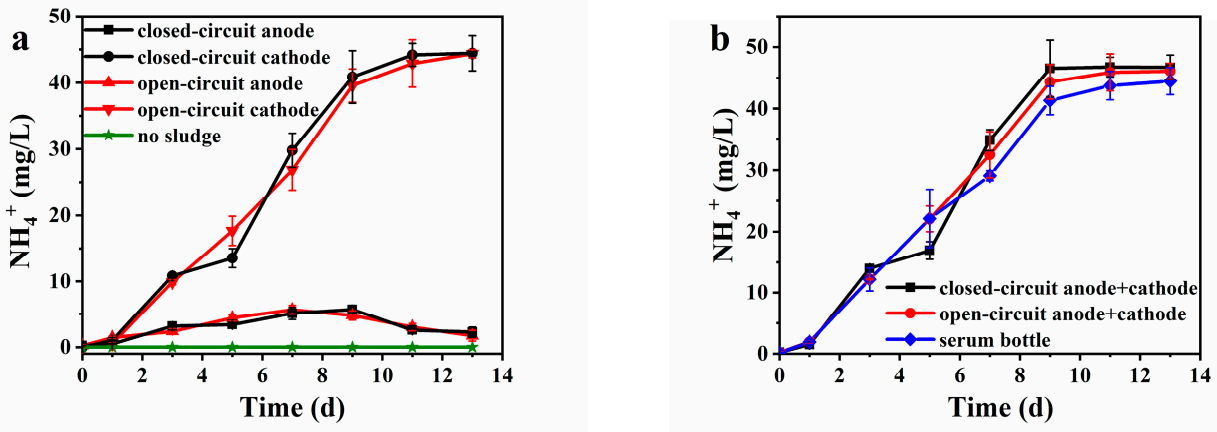

Figure 2. Ammonium concentration in closed-circuit anode, closed-circuit cathode, open-circuit anode, open-circuit cathode and sludge-free anode (**a**); total ammonium concentration in closed-circuit MFC, open-circuit MFC, and serum bottle (**b**).

The TOC represented the organic metabolites produced during chitin degradation. The changes in TOC concentration during the process of chitin degradation in the MFC and serum bottle experiments are shown in Figure 3. The change in TOC concentration with time was similar in the closed-circuit, open-circuit, and serum bottle conditions, with almost no metabolites produced under the sludge-free condition. The time of the steady-state TOC concentration was consistent with the time of the steady-state ammonium concentration (day 9), indicating that the concentration of produced organic metabolites was the largest when the chitin was completely degraded. As the chitin was difficult to effectively quantify, and the products were not easily determined, the TOC concentration increases could be used as a good method to reflect the degradation of chitin. Therefore, the TOC concentration generated at the steady-state time divided by this time was used to reflected the chitin degradation rate. This was similarly handled in a previous study [10]. Based on the similarity of the concentration changes in TOC or total ammonium (Figure 2b) in the closed-circuit and open-circuit MFCs and serum bottle culture conditions, we speculated that there was no difference in the efficiency of the anaerobic respiration and fermentation degradation of chitin. To accurately compare the chitin degradation rates under the three conditions, the rates were calculated, which are listed in Table 1. The degradation rates of chitin in the serum bottles as well as the open-circuit, closed-circuit, and sludge-free MFCs were 6.96 ± 0.32, 6.96 ± 0.23, 7.10 ± 0.96, and 0.05 ± 0.08 C-mg/L·d^{-1}, respectively, indicating similar degradation rates of chitin by the anaerobic sludge through fermentation and anaerobic respiration. Previous studies have shown that the EAB *Aeromonas hydrophila* and *Shewanella oneidensis* degraded chitin much faster in MFCs than in fermentation systems [10,11], whereas in this work, anaerobic sludge could degrade chitin equally effectively in anaerobic respiration and fermentation systems. This should be attributed to the microbial diversity of anaerobic sludge, which gives it great advantages in degrading refractory biomass.

Table 1. TOC generation concentration at steady-state time; degradation rates for chitin degradation by anaerobic sludge in both anaerobic respiration and fermentation modes.

Metabolic Mode	Experimental Group	Generated TOC Concentration (mg/L)	v_{chitin} (C-mg/L·d^{-1})
Anaerobic respiration	Closed-circuit MFC	63.93 ± 8.60	7.10 ± 0.96
Fermentation	Open-circuit MFC	61.19 ± 4.24	6.96 ± 0.23
	Serum bottle	62.60 ± 2.92	6.96 ± 0.32
Abiotic group	Sludge-free MFC	0.47± 0.72	0.05 ± 0.08

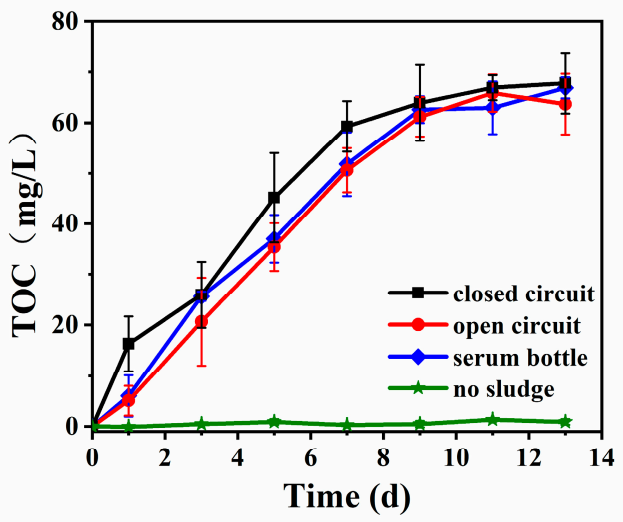

Figure 3. TOC concentration in closed-circuit MFC, open-circuit MFC, serum bottle, and sludge-free MFC.

3.2. Electrical Properties of Chitin Degradation in the MFC

The electrical properties of chitin degradation under the open- and closed-circuit conditions in the MFC are shown in Figure 4. The open-circuit voltage of the open-circuit MFC reached a maximum value of 784 mV, and then gradually decreased (Figure 4a). The output current density of the closed-circuit MFC supplemented with anaerobic sludge reached a maximum value of 160 mA/m^2 and then declined gradually, while the output current density of the uninoculated MFC was only approximately 4 mA/m^2 throughout the test (Figure 4b). This indicated that the anaerobic sludge could degrade chitin in both the fermentation and anaerobic respiration modes. When the output current of the closed-circuit MFC reached its maximum value, the polarization and power density curves were measured to determine the maximum current and power density (Figure 4c). The open-circuit voltage of the closed-circuit MFC was measured at 654.9 mV, while a maximum power density of 26.29 mW/m^2 was produced at a current density of 98.67 mA/m^2, with a resistance of 3 kΩ.

The open-circuit voltage should be close to the cell's electromotive force when not considering potential loss, and the electromotive force of the cell is equal to the cathode potential minus the anode potential. The open-circuit voltage of the open-circuit MFC reached a maximum value after 2.1 days and then gradually decreased (Figure 4a). The increase in the initial open-circuit potential was due to the decrease in the anode potential, as a result of the anodic biofilm development [23]. With the continuous degradation of chitin, the ratio between oxidizing species and reducing species on the electrode surface increased, resulting in an increase in the anode's electrode potential and a decrease in the open-circuit voltage. The electrical potential between the microbial respiratory system and the electron acceptor generates the required current and voltage needed to generate electricity [24]. Similar to the open-circuit voltage variation trend of the open-circuit MFC, the output current density of the closed-circuit MFC reached a maximum value after 2.5 days, and then gradually declined (Figure 4b). These results indicated that the anode biofilm of the open-circuit and closed-circuit MFCs reached a steady state at 2.1–2.5 days, and the open-circuit voltage and output current densities gradually decreased with the consumption of chitin. In addition, the resultant ammonium concentration reached a maximum value in the open-circuit MFC at day 13, and the maximum value in the closed-circuit MFC was at day 11. Meanwhile, the open-circuit voltage in the open-circuit MFC dropped to the minimum value at day 13, and the output current density in the closed-circuit MFC dropped below the initial value after 11.1 days. It could be seen that both the open-circuit voltage and the output current density decreased to the minimum when the

chitin was completely degraded. In conclusion, the open-circuit voltage and output current density could roughly reflect the degradation of chitin in the MFC.

The closed-circuit MFC initially showed a marked increase in the output current density, which peaked within a short period (Figure 4b), and this was similar to the MFC using marine sediment as an inoculum to degrade chitin [25]. In addition, the resultant ammonium concentration (Figure 2b) and TOC (Figure 3) of the closed-circuit MFC were consistently elevated during this period. These results suggested that the microorganisms in the anaerobic sludge of the closed-circuit MFC could effectively degrade chitin and generate electricity, and the EAB must have been present in the anaerobic sludge to transfer electrons to the anode.

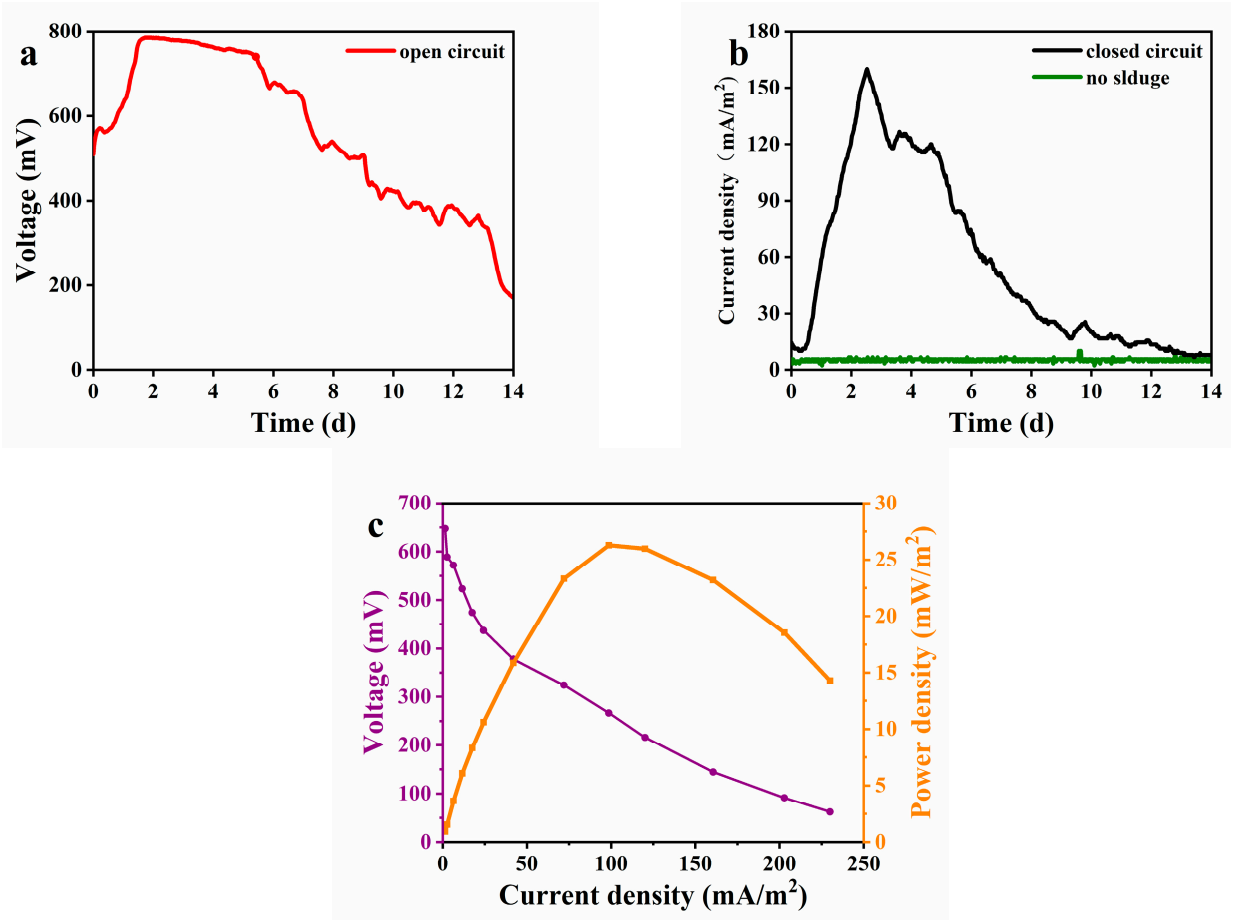

Figure 4. Open-circuit voltage curve of open-circuit MFC (**a**); output current density curve of closed-circuit MFC (**b**); polarization (purple) and power density (orange) curves of closed-circuit MFC (**c**).

3.3. Microbial Morphology of the Anode-Attached Sludge

The SEM images of the initial carbon felt as well as the open-circuit and closed-circuit MFC anode carbon felt after the chitin degradation experiment are shown in Figure 5. The SEM images revealed the microbial morphology on the surface of the carbon felt electrode. Attached microorganisms were observed on the anode carbon felt of the open-circuit (Figure 5b) and closed-circuit (Figure 5c) MFC systems, compared to the initial carbon felt (Figure 5a). The carbon fiber surface of the initial carbon felt was smooth and the fibers were interwoven in three dimensions. In addition, the carbon fiber surface of the open-circuit MFC anode carbon felt was smooth, with most microorganisms adhering to the three-dimensional structure of the fibers in an agglomerated form. A large number of microorganisms were dispersed on the carbon fiber surface of the closed-circuit MFC anode carbon felt, with some fibers completely coated by microorganisms. The difference in microbial morphology on the surface of the closed-circuit and open-circuit MFC anode carbon felt could be related to the different metabolic modes of chitin degradation via

anaerobic respiration and fermentation. The magnification of the closed-circuit MFC anode carbon felt (Figure 5d) showed a large number of rod-like bacteria attached to the carbon fiber surface. A large number of microorganisms were attached to the surface of the closed-circuit MFC anode carbon fiber (Figure 5c,d) because the EAB needed to make contact with the carbon fiber for extracellular electron transfer to generate electricity [26].

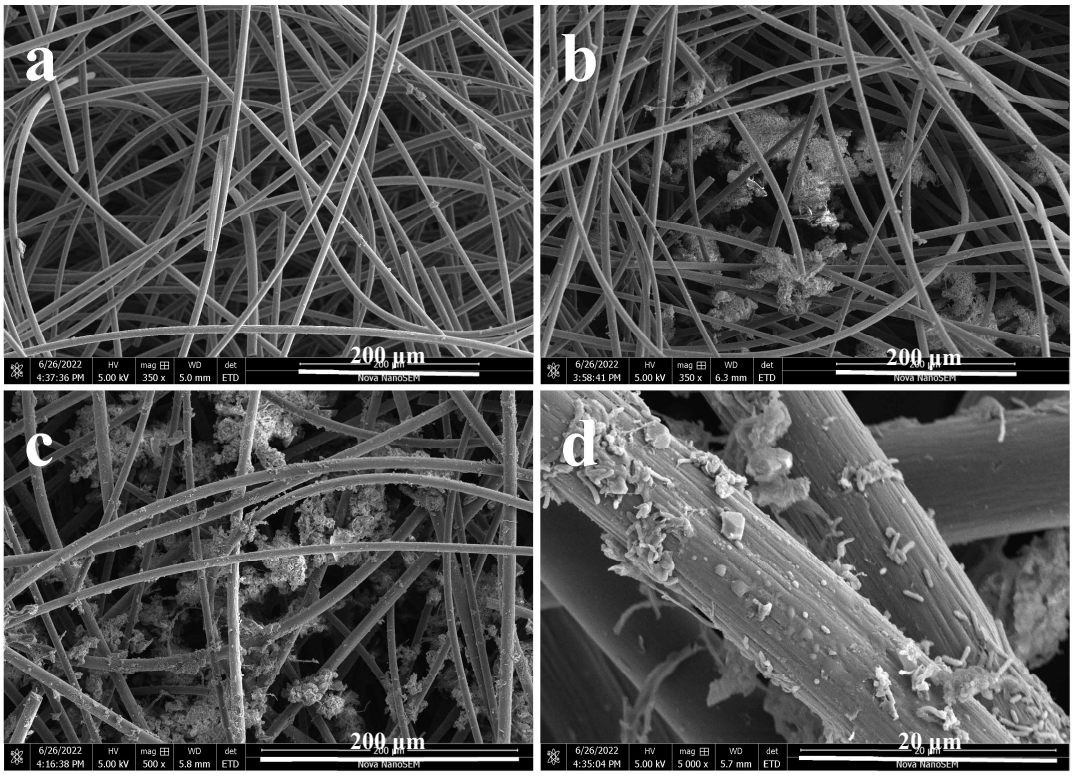

Figure 5. SEM images of the initial carbon felt electrode surface (**a**), the open-circuit anode carbon felt electrode surface (**b**), and the closed-circuit anode carbon felt electrode surface (**c**,**d**).

3.4. Microbial Community of Anode Sludge in the Anaerobic Respiration and Fermentation Systems

The microbial composition and abundance of the open-circuit and closed-circuit MFC anode-attached sludge are listed in Figure 6, to compare and explore the differences in microbial community between the chitin fermentation and anaerobic respiration degradation. The bacteria that accounted for more than 60% of the relative abundance in the three sludge samples belonged to three dominant groups, namely *Proteobacteria*, *Chloroflexi*, and *Bacteroidetes*. *Proteobacteria*, the main phylum of the closed-circuit MFC, increased significantly from 19.00% to 35.57%, while the open-circuit MFC showed no obvious increase (only 20.67%). *Chloroflexi*, the main phylum of the open-circuit MFC, increased significantly from 21.36% to 34.65%, while the closed-circuit MFC decreased to 10.08%. In addition, *Acidobacteria* increased from 2.04% to 4.34% in the closed-circuit MFC but decreased to 1.73% in the open-circuit MFC. Furthermore, *Nitrospirae* increased from 2.37% to 4.69% in the closed-circuit MFC, but decreased to 0.58% in the open-circuit MFC. *Spirochaetes* increased from 0.40% to 4.94% in the closed-circuit MFC, but decreased to 0.16% in the open-circuit MFC, while *Planctomycetes* decreased from 7.21% to 4.84% in the closed-circuit MFC, but increased to 8.37% in the open-circuit MFC.

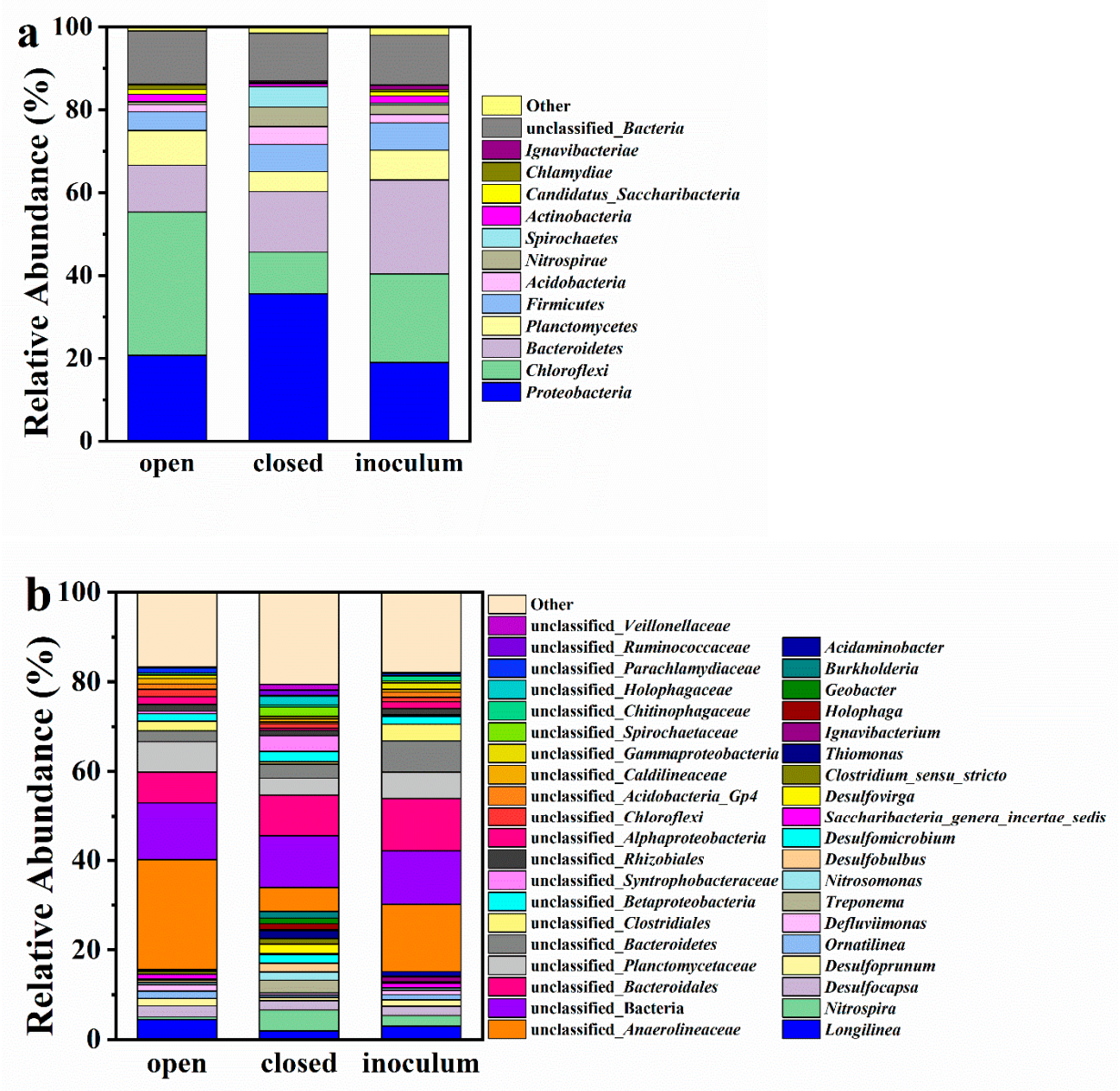

Figure 6. Relative abundance of microbial community in open-circuit MFC and closed-circuit MFC anode sludge and inoculated sludge at phylum level (**a**) and genus level (**b**).

At the phylum level, in the closed-circuit MFC, the abundance of *Proteobacteria*, *Acidobacteria*, *Nitrospirae*, and *Spirochaetes* increased, while the abundance of *Chloroflexi* and *Planctomycetes* decreased. In the open-circuit MFC, the abundance of *Chloroflexi* and *Planctomycetes* increased, while the abundance of *Nitrospirae* decreased. These results indicate that *Proteobacteria*, *Acidobacteria*, *Nitrospirae*, and *Spirochaetes* were the predominant anaerobic respiration phyla in the anaerobic sludge, while *Chloroflexi* and *Planctomycetes* were the main fermentation phyla. *Proteobacteria* played a crucial role in the electrochemical activity, and most of the species from *Proteobacteria* were recognized as EAB [27]. *Acidobacteria* are physiologically diverse and could use different substrates in the MFC and produce electricity [28]. *Nitrospirae* is a group of Gram-negative bacteria, with the genus *Nitrospira* affiliated with the phylum *Nitrospirae* considered the most widely distributed nitrifying bacteria [29]. *Spirochaetes* have been shown to play an important role in electron transfer [30]. This indicates that a variety of EAB were enriched at the closed-circuit MFC anode. *Chloroflexi* are abundant in sediment and involved in carbon cycling in the subsurface [31]. *Planctomycetes* have been isolated from sewage treatment sludge, using N-acetyl-glucosamine as the sole

carbon and nitrogen source [32], which was the hydrolysis product of chitin [10]. This suggests that *Chloroflexi* and *Planctomycetes* were the common phyla involved in the organic carbon and chitin degradation. These results indicate that the closed-circuit MFC mainly enriched bacteria related to electrogenesis, while the open-circuit MFC mainly enriched organic carbon (such as chitin)-degrading bacteria.

At the genus level, *Nitrospira*, *Treponema*, *Desulfovirga*, *Desulfomicrobium*, *Desulfobulbus*, *Nitrosomonas*, *Thiomonas*, *Burkholderia*, and *Geobacter* all increased in the closed-circuit MFC. Meanwhile, only *Longilinea* increased in the open-circuit MFC. It can be clearly seen in the relative abundance heatmap (Figure 7) that *Treponema* and *Nitrospira*, with more increased abundance in the closed-circuit MFC, were considered the main anaerobic respiration genera, while *Longilinea* was the main fermentation genus with increased abundance in the open-circuit MFC. *Nitrospira* contributed to high nitrogen removal and bioelectricity production [33]. *Treponema* were the EAB and involved in the denitrification process [34]. *Desulfovirga*, *Desulfomicrobium* and *Desulfobulbus* were sulfate-reducing bacteria and EAB; *Desulfovirga* and *Desulfobulbus* were enriched using $NaHCO_3$ and acetate as carbon sources, while *Desulfomicrobium* were enriched using ethanol as the carbon source [35]. *Nitrosomonas* were the nitrogen transformation functional bacteria in the microbial fuel cell system [36]. *Thiomona*, *Burkholderia*, and *Geobacter* were EAB [37–39]. These findings indicated that bacteria associated with electrogenesis were the domain genera in MFCs. *Longilinea* were the anaerobes in the anaerobic sludge, which could undergo anaerobic (fermentative) growth on carbohydrates and/or peptides (amino acids) [40]. These indicated that under different chitin degradation modes (anaerobic respiration and fermentation), the corresponding metabolic bacteria in the anaerobic sludge became the main functional bacteria.

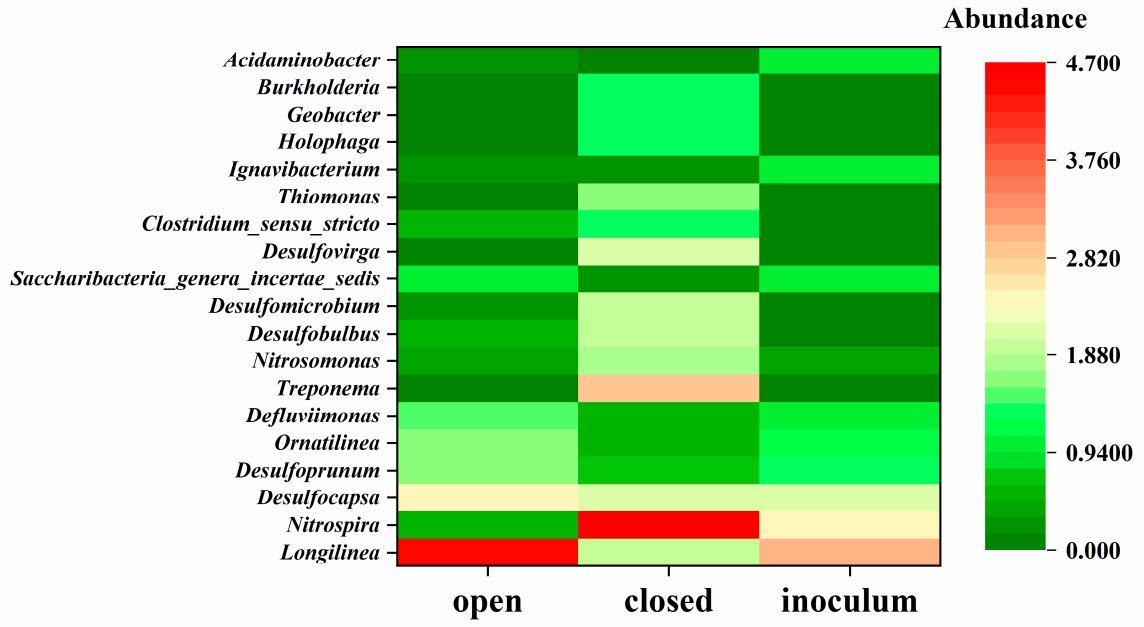

Figure 7. Relative abundance heatmap of dominant genera.

4. Conclusions

In this work, two modes of anaerobic respiration and fermentation were used to degrade chitin, and the functional microbial groups, mainly the EAB and fermenting bacteria, were enriched in the anaerobic sludge, respectively. Chitin degraded at the MFC anode to produce ammonium, which diffused from the anode to the cathode at the same time, and eventually almost all the ammonium produced at the anode was transferred to the cathode. Chitin was completely degraded in both modes of anaerobic respiration and fermentation using anaerobic sludge as the inoculum, which was confirmed through TOC and ammonium concentration detection. There was no significant difference in

the degradation rate of chitin by the anaerobic sludge in the anaerobic respiration and fermentation modes (7.10 ± 0.96, and 6.96 ± 0.23 C-mg/L·d^{-1}). Due to the anaerobic properties of the MFC anodes, although MFC anodes can serve as extracellular insoluble electron acceptors, not all bacteria can directly utilize anodes as electron acceptors, which made the fermentation process in the anode environments highly competitive. The open-circuit voltage and output current density could roughly reflect the degradation of chitin in the open-circuit and closed-circuit MFCs. Chitin degradation via anaerobic respiration in MFCs may be an attractive energy recovery approach, and waste biomass can be used as a source of organic carbon and nitrogen for wastewater treatment and energy recovery. Anaerobic sludge could effectively degrade chitin under different metabolic modes due to the abundance of electroactive and fermentation bacteria, providing a good choice for the treatment of refractory biomass.

Author Contributions: S.-W.L. conceived and designed the research. S.-H.Z. conducted the experiments. R.-R.X. and W.X. contributed the analytical tools. S.-H.Z. and S.-W.L. analyzed the data. S.-H.Z., S.-W.L., and Y.-Y.Y. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Boulaiche, W.; Hamdi, B.; Trari, M. Removal of heavy metals by chitin: Equilibrium, kinetic and thermodynamic studies. *Appl. Water Sci.* **2019**, *9*, 39. [CrossRef]
2. Sherin, P.; Nathalie, L.; Deepu, G.; Hanna, J.M.; Ange, N.; Sabu, T. Chitin and chitosan based composites for energy and environmental applications: A review. *Waste Biomass Valori.* **2020**, *12*, 4777–4804.
3. Lv, J.; Lv, X.; Ma, M.; Oh, D.-H.; Jiang, Z.; Fu, X. Chitin and chitin-based biomaterials: A review of advances in processing and food applications. *Carbohyd. Polym.* **2023**, *299*, 120142. [CrossRef] [PubMed]
4. Boyer, J.N. Aerobic and anaerobic degradation and mineralization of 14C-chitin by water column and sediment inocula of the York River estuary, Virginia. *Appl. Environ. Microb.* **1994**, *60*, 174–179. [CrossRef] [PubMed]
5. Logan, B.E. Biologically extracting energy from wastewater biohydrogen production and microbial fuel cells. *Environ. Sci. Technol.* **2004**, *38*, 159–167.
6. Zhang, E.R.; Xu, W.; Diao, G.W.; Shuang, C.D. Electricity generation from acetate and glucose by sedimentary bacterium attached to electrode in microbial-anode fuel cells. *J. Power Sources* **2006**, *161*, 820–825. [CrossRef]
7. Herrero-Hernandez, E.; Smith, T.J.; Akid, R. Electricity generation from wastewaters with starch as carbon source using a mediatorless microbial fuel cell. *Biosens. Bioelectron.* **2013**, *39*, 194–198. [CrossRef]
8. Logan, B.E. Exoelectrogenic bacteria that power microbial fuel cells. *Nat. Rev. Microbiol.* **2009**, *7*, 375–381. [CrossRef]
9. Rezaei, F.; Richard, T.L.; Logan, B.E. Analysis of chitin particle size on maximum power generation, power longevity, and coulombic efficiency in solid-substrate microbial fuel cells. *J. Power Sources* **2009**, *192*, 304–309. [CrossRef]
10. Li, S.W.; Zeng, R.J.; Sheng, G.P. An excellent anaerobic respiration mode for chitin degradation by *Shewanella oneidensis* MR-1 in microbial fuel cells. *Biochem. Eng. J.* **2017**, *118*, 20–24. [CrossRef]
11. Li, S.W.; He, H.; Zeng, R.J.; Sheng, G.P. Chitin degradation and electricity generation by *Aeromonas hydrophila* in microbial fuel cells. *Chemosphere* **2017**, *168*, 293–299. [CrossRef] [PubMed]
12. Gurav, R.; Bhatia, S.K.; Moon, Y.M.; Choi, T.R.; Jung, H.R.; Yang, S.Y.; Song, H.S.; Jeon, J.M.; Yoon, J.J.; Kim, Y.G.; et al. One-pot exploitation of chitin biomass for simultaneous production of electricity, n-acetylglucosamine and polyhydroxyalkanoates in microbial fuel cell using novel marine bacterium *Arenibacter palladensis* YHY2. *J. Clean. Prod.* **2019**, *209*, 324–332. [CrossRef]

13. Liu, Y.; Xu, H.-L.; Yang, S.-F.; Tay, J.-H. Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor. *Water Res.* **2003**, *37*, 661–673. [CrossRef] [PubMed]
14. Liu, Y.; Qiao, J.-T.; Yuan, X.-Z.; Guo, R.-B.; Qiu, Y.-L. *Hydrogenispora ethanolica* gen. nov., sp. nov., an anaerobic carbohydrate-fermenting bacterium from anaerobic sludge. *Int. J. Syst. Evol. Micr* **2014**, *64 Pt 5*, 1756–1762. [CrossRef]
15. Kotay, S.M.; Das, D. Microbial hydrogen production with *Bacillus coagulans* IIT-BT S1 isolated from anaerobic sewage sludge. *Bioresource Technol.* **2007**, *98*, 1183–1190. [CrossRef] [PubMed]
16. Holmes, D.E.; Bond, D.R.; O'Neil, R.A.; Reimers, C.E.; Tender, L.R.; Lovley, D.R. Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microb. Ecol.* **2004**, *48*, 178–190. [CrossRef]
17. Logan, B.E.; Murano, C.; Scott, K.; Gray, N.D.; Head, I.M. Electricity generation from cysteine in a microbial fuel cell. *Water Res.* **2005**, *39*, 942–952. [CrossRef]
18. Veeresh, G.S.; Kumar, P.; Mehrotra, I. Treatment of phenol and cresols in upflow anaerobic sludge blanket (UASB) process: A review. *Water Res.* **2005**, *39*, 154–170. [CrossRef]
19. Lu, Y.; Lai, Q.; Zhang, C.; Zhao, H.; Ma, K.; Zhao, X.; Chen, H.; Liu, D.; Xing, X.H. Characteristics of hydrogen and methane production from cornstalks by an augmented two- or three-stage anaerobic fermentation process. *Bioresour. Technol.* **2009**, *100*, 2889–2895. [CrossRef]
20. Gao, Q.; Li, S.W.; Xie, Y.J.; Zheng, M.X.; Wei, J.; Luo, Z.J.; Zhou, X.T.; Liu, Z.G.; Li, Y.; Wu, Z.R. Rapid cultivation of anammox sludge based on Ca-alginate cell beads. *Water Sci. Technol.* **2022**, *85*, 2899–2911. [CrossRef]
21. Lv, Y.; Wang, L.; Wang, X.; Yang, Y.; Wang, Z.; Li, J. Macroscale and microscale analysis of anammox in anaerobic rotating biological contactor. *J. Environ. Sci.* **2011**, *23*, 1679–1683. [CrossRef]
22. Cord-Ruwisch, R.; Law, Y.; Cheng, K.Y. Ammonium as a sustainable proton shuttle in bioelectrochemical systems. *Bioresour. Technol.* **2011**, *102*, 9691–9696. [CrossRef] [PubMed]
23. Kamel, M.S.; Abd-Alla, M.H.; Abdul-Raouf, U.M. Characterization of anodic biofilm bacterial communities and performance evaluation of a mediator-free microbial fuel cell. *Environ. Eng. Res.* **2020**, *25*, 862–870. [CrossRef]
24. Logan, B.E.; Hamelers, B.; Rozendal, R.; Schröder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40*, 5181–5192. [CrossRef] [PubMed]
25. 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]
26. Ramasamy, R.P.; Ren, Z.; Mench, M.M.; Regan, J.M. Impact of initial biofilm growth on the anode impedance of microbial fuel cells. *Biotechnol. Bioeng.* **2008**, *101*, 101–108. [CrossRef]
27. Srivastava, P.; Abbassi, R.; Yadav, A.K.; Garaniya, V.; Lewis, T.; Zhao, Y.; Aminabhavi, T. Interrelation between sulphur and conductive materials and its impact on ammonium and organic pollutants removal in electroactive wetlands. *J. Hazard. Mater.* **2021**, *419*, 126417. [CrossRef]
28. Ramanaiah, S.V.; Cordas, C.M.; Matias, S.C.; Reddy, M.V.; Leitão, J.H.; Fonseca, L.P. Bioelectricity generation using long-term operated biocathode: RFLP based microbial diversity analysis. *Biotechnol. Rep.* **2021**, *32*, e00693. [CrossRef]
29. Zhao, Y.Z.; Wang, H.Y.; Dong, W.Y.; Chang, Y.; Yan, G.K.; Chu, Z.S.; Ling, Y.; Wang, Z.M.; Fan, T.F.; Li, C.Y. Nitrogen removal and microbial community for the treatment of rural domestic sewage with low C/N ratio by A/O biofilter with *Arundo donax* as carbon source and filter media. *J. Water Process Eng.* **2020**, *37*, 101509. [CrossRef]
30. Zhang, J.; Chen, R.; Du, C.; Dong, S.; Sun, J. Effects of continuous sulfamonomethoxine shock on the power generation performance and microbial community structure of MFCs under seasonal temperature variation. *Biochem. Eng. J.* **2021**, *167*, 107909. [CrossRef]
31. Hug, L.A.; Castelle, C.J.; Wrighton, K.C.; Thomas, B.C.; Sharon, I.; Frischkorn, K.R.; Williams, K.H.; Tringe, S.G.; Banfield, J.F. Community genomic analyses constrain the distribution of metabolic traits across the *Chloroflexi* phylum and indicate roles in sediment carbon cycling. *Microbiome* **2013**, *1*, 22. [CrossRef] [PubMed]
32. Fuerst, J.A. The *planctomycetes* emerging models for microbial ecology, evolution and cell biology. *Microbiology* **1995**, *141*, 1493–1506. [CrossRef] [PubMed]
33. Tao, M.; Jing, Z.; Tao, Z.; Luo, H.; Zuo, S.; Li, Y.-Y. Efficient nitrogen removal in microbial fuel cell—Constructed wetland with corncobs addition for secondary effluent treatment. *J. Clean. Prod.* **2022**, *332*, 130108. [CrossRef]
34. Li, J.; Li, Y.; Chen, P.; Sathishkumar, K.; Lu, Y.; Naraginti, S.; Wu, Y.; Wu, H. Biological mediated synthesis of reduced graphene oxide (rGO) as a potential electron shuttle for facilitated biological denitrification: Insight into the electron transfer process. *J. Environ. Chem. Eng.* **2022**, *10*, 108225. [CrossRef]
35. Pan, J.-J.; Tan, L.-Y.; Fan, Q.-Q.; Cao, X.-Y.; Huang, J.; Gu, Y.-K.; Chen, T.-M. Effect of different carbon sources on sulfate reduction and microbial community structure in bioelectrochemical systems. *Environ. Sci. Pollut. R.* **2023**, *30*, 18312–18324. [CrossRef]
36. Zhu, X.; Shen, C.F.; Huang, J.X.; Wang, L.M.; Pang, Q.Q.; Peng, F.Q.; Hou, J.; Ni, L.X.; He, F.; Xu, B. The effect of sulfamethoxazole on nitrogen removal and electricity generation in a tidal flow constructed wetland coupled with a microbial fuel cell system: Microbial response. *Chem. Eng. J.* **2022**, *431*, 134070. [CrossRef]
37. Yu, Y.; Ali, J.; Yang, Y.; Kuang, P.; Zhang, W.; Lu, Y.; Li, Y. Synchronous Cr(VI) remediation and energy production using microbial fuel cell from a subsurface environment: A review. *Energies* **2022**, *15*, 1989. [CrossRef]
38. Franco, A.; Elbahnasy, M.; Rosenbaum, M.A. Screening of natural phenazine producers for electroactivity in bioelectrochemical systems. *Microb. Biotechnol.* **2023**, *16*, 579–594. [CrossRef]

39. Reguera, G.; Nevin, K.P.; Nicoll, J.S.; Covalla, S.F.; Woodard, T.L.; Lovley, D.R. Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Appl. Environ. Microbiol.* **2006**, *72*, 7345–7348. [CrossRef]
40. Yamada, T.; Sekiguchi, Y. Cultivation of uncultured *Chloroflexi* subphyla: Significance and ecophysiology of formerly uncultured *Chloroflexi* 'subphylum i' with natural and biotechnological relevance. *Microbes Environ.* **2009**, *24*, 205–216. [CrossRef]