



Article

Electricity Production and Population Dynamics of Microbial Community in a Co-Culture of Iron Mine Soil Biofilm and *Shewanella oneidensis* MR-1 with Anode as Electron Acceptor

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Abstract

Microbial communities that develop within biofilms on electrodes are necessary for the proper functioning of the microbial electrochemical system. However, the mechanism through which an exogenous exoelectrogen influences the population dynamics and electrochemical performance of biofilms remains unclear. In this study, we explored the community structure dynamics and electrochemical characteristics of iron mine soil biofilm co-cultured with *Shewanella oneidensis* MR-1, with the anode as the electron acceptor, and compared the results with those of iron mine soil biofilms alone on the anode. *Shewanella oneidensis* MR-1 improved the electrochemical activity of microbial biofilms, resulting in a higher maximum power density of $195 \pm 8 \text{ mW/m}^2$ compared with that of iron mine soil ($175 \pm 7 \text{ mW/m}^2$) and *Shewanella* ($88 \pm 8 \text{ mW/m}^2$) biofilms individually. The co-cultured biofilms could perform near the highest power density for a longer duration than the iron mine soil biofilms could. High-throughput 16S rRNA gene sequencing of the biofilms on the anode indicated that the relative abundance of Pelobacteraceae in the co-culture system was significantly ($p = 0.02$) increased, while that of Rhodocyclaceae was significantly ($p = 0.008$) decreased, compared with that in iron mine soil biofilms. After continuing the experiment for two months, the presence of *Shewanella oneidensis* MR-1 changed the predominant bacteria of the microbial community in the biofilms, and the relative abundance of *Shewanella* was significantly ($p = 0.02$) decreased to a level similar to that in iron mine soil. These results demonstrate that *Shewanella oneidensis* MR-1 could improve the performance of iron mine soil biofilms in electrochemical systems by altering the composition of the functional microbial communities.

Keywords: iron mine soil biofilm; *Shewanella oneidensis* MR-1; co-culture; microbial fuel cell; population dynamics



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1. Introduction

Electrochemically active bacteria can transfer electrons from respiration directly to extracellular insoluble electrodes and minerals through the extracellular electron transfer (EET) process [1–3]. The first extracellular respiratory process, depending on EET, to be discovered was iron and manganese respiration in *Shewanella* and *Geobacter* species [4,5].

Multiheme c-type cytochromes involved in the dissimilatory reduction of solid metal oxides in *S. oneidensis* MR-1 and *G. sulfurreducens* utilized the EET process [6,7]. In *S. oneidensis* MR-1, the extracellular c-type cytochromes can directly reduce solid metal oxides [3,8–10]. Similarly, in *Geobacter*, the electrons are hypothesized to be transferred from outer membrane c-type cytochromes to an external acceptor via the type IV pili [11–14]. The *Shewanella* and *Geobacter* species are also regarded as model bacteria in bioelectrochemical systems [15].

However, pure cultures cannot produce as much electrical power in MFCs as mixed cultures do [16–18]. Multispecies exoelectrogenic biofilms on electrodes play a significant role in the function of bioelectrochemical systems [19]. The current generation of microbial electrochemical systems is highly dependent on the exoelectrogenic community structure. Thus, further insight into the microbial composition of biofilms on electrodes is crucial for improving the function of bioelectrochemical systems and for gaining a better understanding of extracellular electron transfer [20]. The diversity of microbial communities influences both power generation and environmental remediation [21–26]. The increase in power has been known to coincide with a significant change in the microbial community associated with the electrode [22,27]. Different anode potentials and power generation could result in additional differences in population dynamics [28–30]. On the contrary, it seems the operation mode does not affect the microbial community, as ascertained after conducting a microbial community analysis of the single-equivalent sediment MFC (SMFC) and the scaled-up SMFC [28]. Anyhow, an understanding of the microbial community of these systems is essential for perfecting the MFC technology [31,32].

Co-culture of nonexoelectrogen and exoelectrogen improves the electrochemical performance compared with that achieved using a pure culture [18]. Co-culturing of phototrophs and *Geobacter* has previously been shown to help in energy conversion from light to electricity in microbial solar cells [17]. Co-culture experiments with an electrode as electron acceptor have shown that microbial interaction can specifically convert target substrates into electricity [16,33]. *Shewanella oneidensis* MR-1 was supplied with a microbial fuel cell to enhance the degradation of a recalcitrant organic compound, o-xylene [34]. Similarly, cocultured systems of denitrifying anaerobic methane oxidation (DAMO) microbes and *Shewanella* MR-1 indicate that DAMO is involved in iron reduction with methane as a carbon source [35]. However, precisely how an exogenous electricigen influences the community structure of the exoelectrogenic biofilm and the performance of the microbial fuel cell remains to be elucidated.

In this study, we investigated the population dynamics within a co-culture of iron mine soil biofilm/*Shewanella* MR-1 compared with that of iron mine soil biofilm alone using Illumina HiSeq sequencing of the 16S rRNA gene. Moreover, we also considered the electrochemical performance of the co-culture system compared with that of the iron mine soil biofilm and *Shewanella* MR-1, when considered separately.

2. Materials and Methods

2.1. Sample Collection and Enrichment of Electroactive Biofilms from Iron Mine Soil Sample Were Collected from Meishan Iron Mine, China, 300 m Underground (Latitude: 31.53° N, Longitude: 118.43° E)

All samples were stored at −20 °C in the dark and directly transported to the laboratory by low-temperature delivery, where MFCs of iron mine soil were immediately constructed. Two-chambered electrochemical fuel cells were designed and fabricated using glass. The total working volume of one chamber was 100 mL. Carbon paper (3 cm × 3 cm) was used as an electrode after ultrasonic cleaning in acetone and deionized water, respectively. Nafion-117 (DuPont, Wilmington, DE, USA) was used as a proton exchange

membrane (PEM). The anode was immersed in the anolyte (DM medium) with an iron soil. The cathode was immersed in the catholyte 50 mmol/L $K_3[Fe(CN)_6]$ and dissolved in PBS buffer. A 1000-ohm resistor was connected between the anode and cathode. After running for a month at 25 °C, the carbon paper with biofilms was taken out in a super-clean bench (via UV sterilization) for the co-culture experiment.

2.2. Co-Culture Experiment and Electrochemical Analysis

Fifteen reactors were set up with the same method in Section 2.1, which were divided into three groups. Each group included five parallel MFCs. All the cathodes were carbon paper, and the catholyte was 50 mmol/L $K_3[Fe(CN)_6]$, dissolved in Phosphate Buffer Solution (0.05 mol/L, pH = 7.0). The first group was operated with pure culture in the anode chamber using *Shewanella* MR-1 in DM medium. The second group was operated with iron soil biofilms, using carbon papers as anodes, and the anolyte was DM medium. The third co-culture group was operated with iron soil biofilms, carbon paper as anodes, and inoculated with MR-1 in DM medium. All anode chambers of MFCs were operated in anaerobic conditions by filling with nitrogen before being sterilized. A Data Acquisition Card (USB5936, Beijing Art Technology Development Co., Ltd., Beijing, China) was used to collect the data on voltage every day. The current and the power data were calculated using Ohm's law. Cyclic voltammetry measurements of MFCs were performed during operations on a CHI600E (Chenhua, Shanghai, China) at a scan rate of 10 mV/s.

2.3. SEM Imaging of Biofilms on Anode

After the MFCs in Section 2.2 were running for two months, small pieces of carbon paper were picked in a sterile environment. The morphology of the biofilms was observed under a scanning electron microscope (SEM, Zeiss Ultra Plus, Oberkochen, Germany).

2.4. 16S rRNA Gene High-Throughput Sequencing

Metagenomic DNA was extracted and purified from carbon cloth anodes twice. The first sampling time was conducted one week after the MFCs in Section 2.2 were running, and the samples were signed as SiS1-5 (iron soil biofilms) and CoS1-5 (co-cultured biofilms). The second sampling time was two months later, after the MFCs in Section 2.2 were running, and the samples were signed as SiL1-5 (iron soil biofilms) and CoL1-5 (co-cultured biofilms). Metagenomic DNA was extracted and purified from carbon paper anodes by using the SunShineBio™ Microbial Genomic DNA Extraction Kit according to the manufacturer's instructions. Using 20 ng of metagenomic DNA of the samples as template, PCR was carried out using primers 515F (5-GTGCCAGCMGCCGCGTAA-3) and 806R (5-GGACTACVSGGGTATCTAAT-3) with the following cycling conditions: 98 °C for 3 min; 25 cycles of 98 °C for 15 s, 50 °C for 30 s, 72 °C for 30 s; 72 °C for 5 min. The final fragment of the PCR was isolated and purified by 2% agarose gel electrophoresis. Sequencing was performed on the Illumina HiSeq 2500 platform according to standard protocols (by Shanghai Personal Biotechnology Co., Ltd., Shanghai, China).

2.5. Analysis of 16S rRNA Gene Sequencing Data

The sequence data of 16S rRNA genes were analyzed using general methods, as described by Liu et al., 2018 [36]. The software involved included Fast Length Adjustment of SHort reads (FL-ASH) software (V1.2.7, <http://ccb.jhu.edu/software/FLASH/> (accessed on 4 April 2019)) [37], Quantitative Insights Into Microbial Ecology pipeline software (Qiime version 1.9.0, <http://qiime.org/> (accessed on 4 April 2019)) [38,39], and the Mothur program (<http://www.mothur.org/> (accessed on 4 April 2019)) [40–42]. Statistical software (SPSS 17.0) was used to evaluate significant differences between iron mine soil biofilms and co-cultured biofilms. A level of $p < 0.05$ was considered to be statistically significant. The

principal coordinates analysis (PCoA) plots based on the relative abundance of OTUs were generated by UniFrac algorithms. Principal components analysis (PCA) plot based on the relative abundance of OTUs was generated by the ggplots2 package of R (Version 2.15.3). The raw Illumina sequencing data were deposited in the National Center for Biotechnology Information BioProject under accession no. PRJNA530844.

3. Results

3.1. Electrochemical Activity of MFCs of Co-Cultured System

In the cyclic voltammetry curves, the iron mine soil biofilm/MR-1 co-cultures on anodes of microbial fuel cells (MFCs) exhibited the highest redox peak (Figure 1). Peak current increased in the order of iron mine soil/MR-1 co-culture, iron soil, and *Shewanella* MR-1. The oxidation peak was significantly higher in the co-cultured MFC than in the other MFCs. The differences among the redox peaks indicate that co-culturing of iron mine soil and MR-1 could enhance the microbial extracellular electron transfer between biofilm and anode. The maximum power density of MFCs of co-culture was found to be $195 \pm 8 \text{ mW/m}^2$, which was higher than that of iron mine soil ($175 \pm 7 \text{ mW/m}^2$) and *Shewanella* ($88 \pm 8 \text{ mW/m}^2$), individually. The co-culture reactor could run three cycles in 27 days in accordance with that of *Shewanella*. In contrast, the iron mine soil reactor ran three cycles in about 18 days, which was, by far, the shortest of the three (Figure 2). The co-cultured biofilm could perform near the highest power density for about 5 days per cycle, while the iron mine soil biofilm could only perform in the highest power density for 1 or 2 days per cycle.

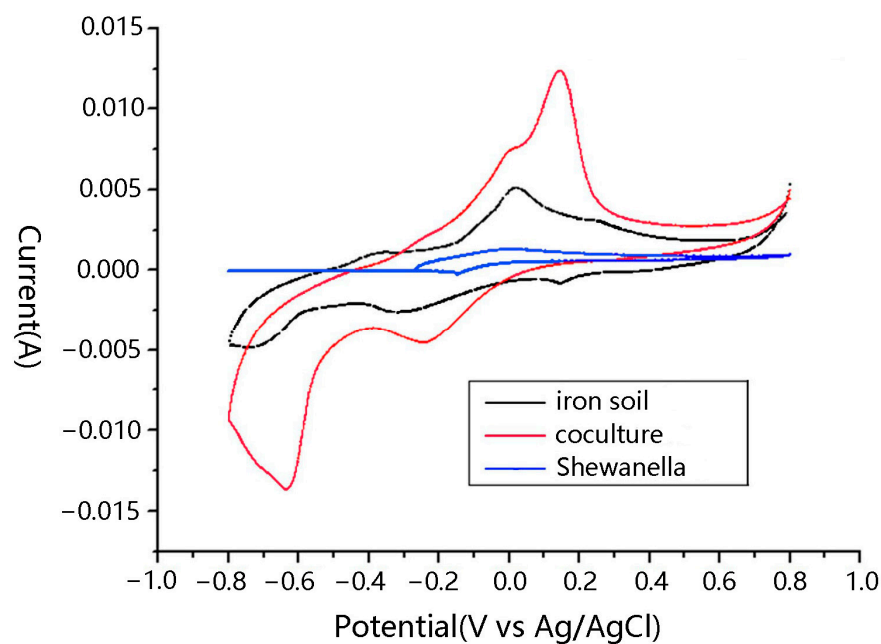


Figure 1. Cyclic voltammetry curves of microbial fuel cells with *Shewanella* MR-1, iron mine soil, and cocultured biofilms.

Upon filtering the raw tags of the samples, 51,027 to 284,267 high-quality effective tags of the 16S rRNA gene (Supplementary Table S1) were obtained for each sample, with an average length of 273 bp (Supplementary Table S2). Operational taxonomic unit (OTU) distribution of the iron mine soil and co-cultured biofilms is presented in Supplementary Table S3. The average OTUs of iron soil biofilms increased from 1355 to 1440 after running for two months. The average OTUs of co-cultured biofilms increased from 1515 to 1676 after running for two months. The average OTUs of co-cultured biofilm were less than those of the iron mine soil biofilm (Supplementary Table S3).

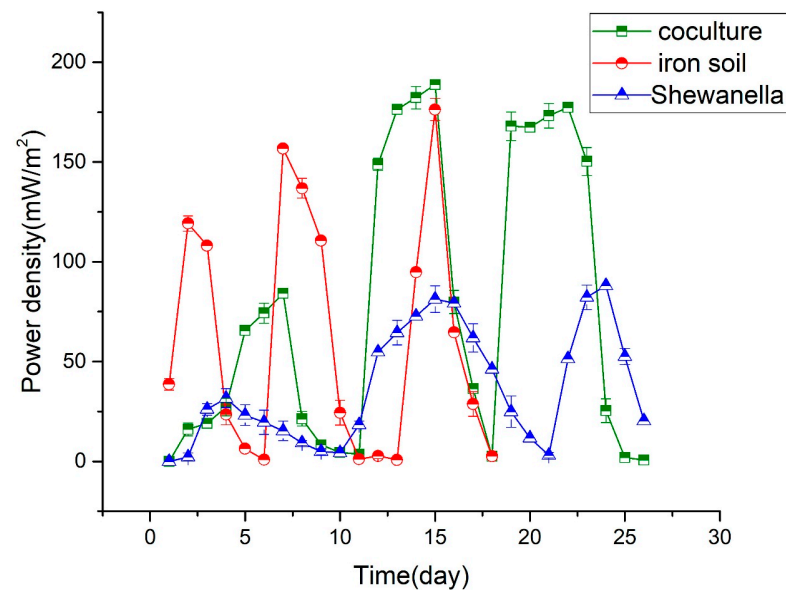


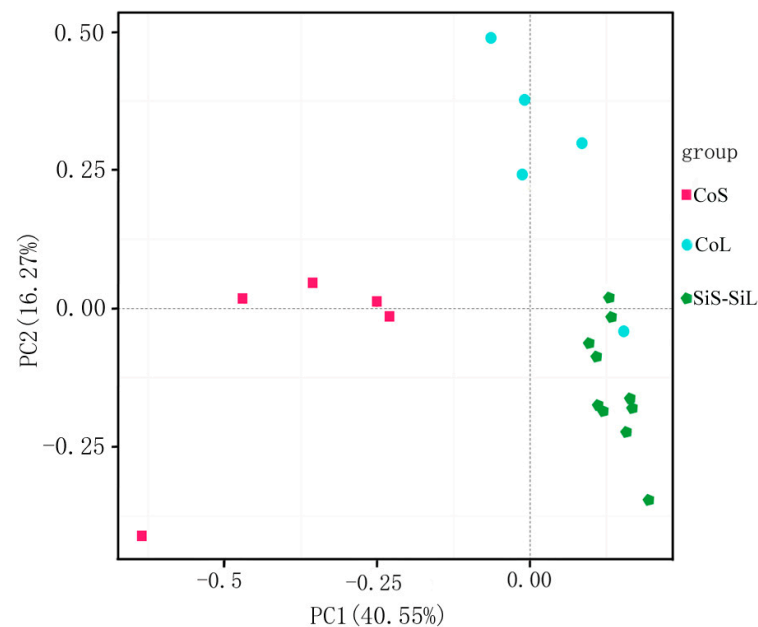
Figure 2. Power density curves of *Shewanella* MR-1, iron mine soil, and co-cultured biofilms.

3.2. Anode-Associated Bacterial Community Structure of the Iron Soil Biofilm and the Co-Cultured Biofilm

Table 1 shows the alpha diversity indices of the cultures, including the Simpson, Shannon, Chao1, and ACE indices. The Simpson and Shannon indices evaluate the abundance and uniformity of microbial communities [43]. After running for two months, the diversity of the bacterial community had a relatively higher density than that of the biofilm at the early stage of culturing. The Shannon indices of co-cultured biofilm were 4.89 ± 0.39 at the early stage and 5.21 ± 0.19 after running for two months. Similarly, the Shannon indices of iron soil biofilm were 6.01 ± 0.15 in the early stage and 6.55 ± 0.12 after running for two months. The diversity of the bacterial community of co-cultured biofilm had a relatively lower diversity than that of the iron soil biofilm, at both the early stage and the post-two-month stage. Furthermore, uniformity measured in the bacterial community indicated the same trend as diversity via the Simpson indices. The iron soil biofilm showed the highest uniformity among all the biofilms (see Simpson indices in Table 1). The bacterial communities of co-cultured biofilm had a relatively lower diversity (Simpson indices of 0.86–0.96) than those of the iron soil biofilms (Shannon indices of 6.01 ± 0.15 and 6.55 ± 0.12) (Table 1). Furthermore, the value of Chao1 and ACE could be used to estimate the richness of the microbial community structure [44]. The Chao1 and ACE indices of the co-cultured biofilm were lower than those of the iron soil biofilm, either in the early stage or in the two months later stage (Table 1). Thus, the diversity and uniformity had no direct relationship with electricity production in our experiment. Three clusters were indicated by a principal coordinate analysis (PCoA) according to unweighted UniFrac distance based on the relative abundance of OTUs (Figure 3). This proved a separation between the iron soil biofilm and the co-cultured biofilm, whereas there was no separation between different stages of the iron soil biofilm. The co-cultured biofilm proved to be two separate clusters. The bacterial community of the co-cultured biofilms changed substantially after running for two months. However, Co-cultivation promoted an increase in the total abundance of microbial communities, resulting in an increase in electricity production (see Figures 2 and 6).

Table 1. Richness indices of microbial community composition in the reactors.

	Chao1	ACE	Simpson	Shannon
CoS	1698.16 ± 102.67	1658.61 ± 106.83	0.87 ± 0.04	4.89 ± 0.39
CoL	1812.95 ± 68.18	1781.50 ± 59.69	0.91 ± 0.01	5.21 ± 0.19
SiS	1879.36 ± 136.45	1859.55 ± 135.01	0.95 ± 0.00	6.01 ± 0.15
SiL	2073.69 ± 51.77	2033.65 ± 74.18	0.96 ± 0.00	6.55 ± 0.12

**Figure 3.** Principal coordinate analysis (PCoA) based on operational taxonomic units of iron mine soil biofilms and co-cultured biofilms in microbial fuel cells.

3.3. *Shewanella* MR-1 Altered the Bacterial Structure of Iron Mine Soil Biofilms

Figure 4 shows the morphology of *Shewanella* MR-1 (Figure 4a), iron mine soil (Figure 4b), and co-cultured biofilms (Figure 4c). In these biofilms, *Gammaproteobacteria*, *Deltaproteobacteria*, *Clostridia*, *Alphaproteobacteria*, *Bacteroidia*, and *Betaproteobacteria* were the most predominant bacterial classes (Figure 5). Thus, the predominant class in all biofilms was Proteobacteria (Figure 5). Furthermore, the genus and family level structure of the microbial community was distinctively different between the iron soil biofilm and the co-cultured biofilm (Figure 6). In iron soil biofilms, *Proteiniclasticum* decreased significantly to 1.52 ± 0.52 ($p = 0.04$) after two months. *Rhodocyclaceae* was the most abundant bacterium, followed by *Desulfuromonas*, *Pseudomonas*, and *Thermomonas*. In the co-cultured biofilms, *Shewanella* decreased significantly to 1.47 ± 0.78 ($p = 0.02$) after two months. *Pseudomonas* (18.28 ± 4.85) was the most abundant bacterium in these biofilms, followed by *Pelobacteraceae*, *Desulfuromonas*, and *Comamonadaceae*. *Desulfuromonas* decreased from 11.8 ± 2.10 to 1.10 ± 0.45 after the addition of *Shewanella* MR-1 to the iron soil biofilm. After two months, the *Desulfuromonas* population recovered to 10.28 ± 4.58 ; however, *Shewanella* decreased significantly from 26.27 ± 2.83 to 1.47 ± 0.78 . This result implied that *Desulfuromonas* and *Shewanella* may have a mutual exclusion effect within a certain concentration range. *Pelobacteraceae* was significantly higher in the co-cultured biofilm (15.98 ± 3.71 and 16.9 ± 4.69 , at the early stage and post two months, respectively) than in the iron soil biofilm (2.43 ± 0.68 and 1.95 ± 0.32 , respectively). *Rhodocyclaceae* was significantly lower in the co-cultured biofilms (0.14 ± 0.05 and 0.90 ± 0.54 , respectively) than in the iron soil biofilm (9.58 ± 3.23 and 8.92 ± 2.63). This result implied that *Rhodocyclaceae* tended to exert a mutual exclusion effect on *Shewanella* MR-1 and *Pelobacteraceae*, while

Pelobacteraceae could synergize with *Shewanella* MR-1. *Shewanella* (0.50 ± 0.06 and 2.50 ± 0.42) and *Geobacter* (3.68 ± 1.55 and 1.66 ± 0.59) were, however, not the most abundant genera in the iron soil biofilm.

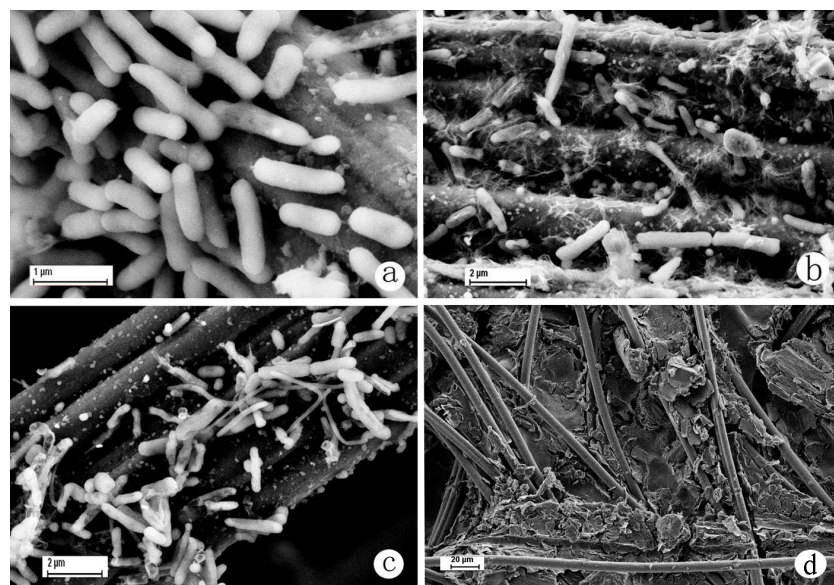


Figure 4. Scanning electron micrographs of biofilms of *Shewanella* MR-1, iron mine soil, and co-cultured biofilms on anode (a) *Shewanella* MR-1, (b) iron mine soil, (c) co-cultured of *Shewanella* MR-1/iron mine soil, (d) and empty carbon paper.

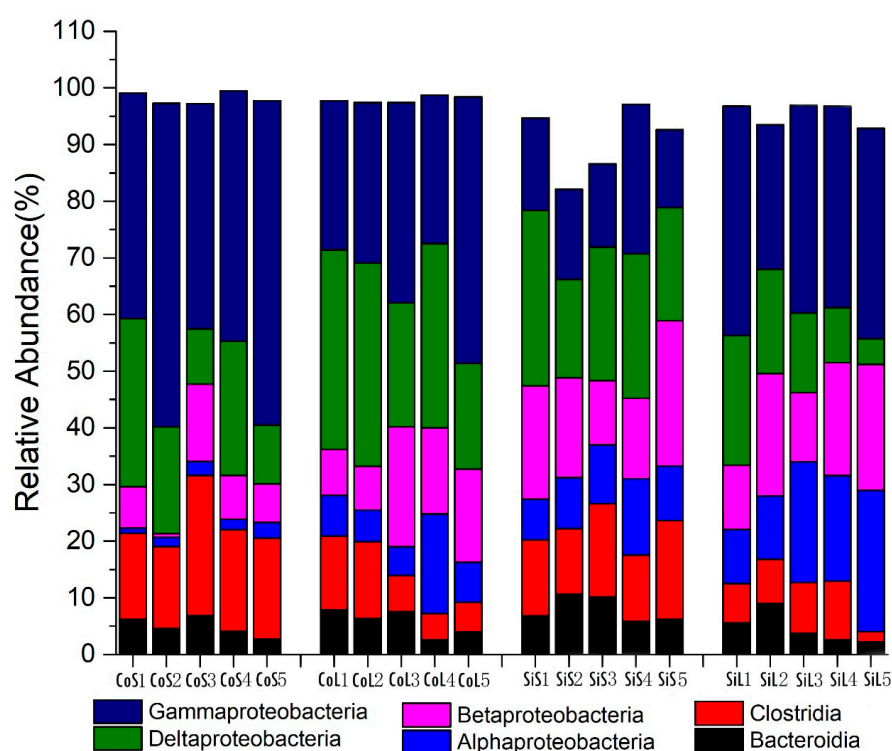


Figure 5. Relative abundance of 16S rRNA sequences of the iron mine soil biofilms and co-cultured biofilms in microbial fuel cells at the class level.

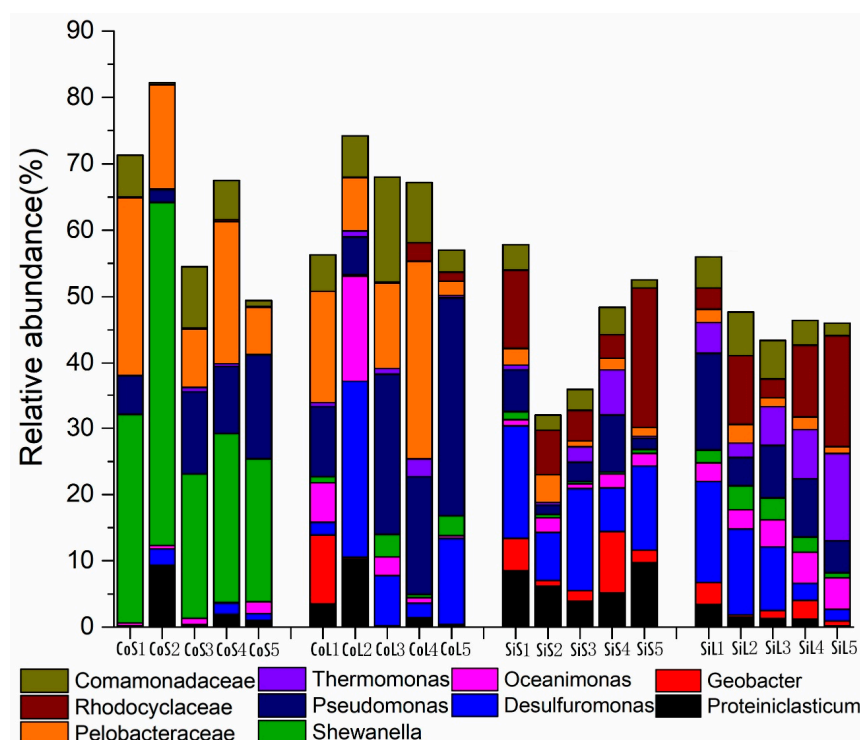


Figure 6. Relative abundance of 16S rRNA sequences of the iron mine soil biofilms and co-cultured biofilms in microbial fuel cells at the family and genus levels.

The proportion of the top ten genera and families was higher in the co-cultured biofilms (61.79 ± 8.76 and 67.37 ± 6.50) than in the iron mine soil biofilms (45.53 ± 3.68 and 48.17 ± 2.98). Furthermore, the highest proportion (67.37 ± 6.50) was found in mature co-cultured biofilms, indicating that the proportion of predominant functional bacteria could influence the electricity generation in MFCs.

4. Discussion

Mixed-culture biofilm essentially produces more electricity than the pure culture catalyst [18]. In a mixed-culture biofilm community, individual species appear to play specific roles in maintaining synergistic communities [15]. In our results, *Shewanella* considerably improved the performance of anode-associated exoelectrogenic biofilms from iron mine soil. Not only was the power density improved, but it also extended the electricity production time (Figure 1). The structure of the functional microbial community in the iron mine soil biofilm was obviously altered following the addition of *Shewanella* MR-1 (Figure 2). Many factors might influence the structure of microbial communities on electrodes, including temperature, electrode modification, pH, inoculum, and ferric ion concentration [44–52]. Our results indicated that an exogenous exoelectrogen could also be the main factor that alters the microbial community composition on the anode. In Figure 6, after running for a period of time with the addition of *Shewanella*, the microbial community structure in the iron soil biofilm changed; however, the abundance of *Shewanella* returned to the original abundance of the soil microbial community.

Our soil sample came from an iron mine, and the microbes in it were consequently related to iron metabolism. In the iron mine soil biofilm collected from the anode, several bacterial families such as *Rhodocyclaceae*, *Pelobacteraceae*, *Desulfuromonadaceae*, and genera known to reduce Fe^{3+} were found [53,54]. These bacteria were all detected and showed the highest abundance near the $\text{Fe(III)}/\text{Fe(II)}$ redox boundary [53]. Dissimilatory iron-reducing bacteria could produce electricity on an electrode through extracellular electron

transfer. The typical exoelectrogens were metal-reducing bacteria such as *Geobacter* spp. and *Shewanella* spp. [4,5].

The predominant bacteria in our biofilm included Gammaproteobacteria, Deltaproteobacteria, Clostridia, Alphaproteobacteria, Bacteroidia, and Betaproteobacteria. Proteobacteria, such as Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria, were the main exoelectrogens associated with previously conducted research [15,55]. Even though several exoelectrogens proved to be useful in the MFC systems, Proteobacteria possessed the largest numbers amongst all electrochemically active bacteria [15]. In fact, Proteobacteria and Firmicutes have previously been reported to be electrochemically active, as well as to play a significant role in the EET process [56].

At the class level, there was no definite difference in community structure between the co-cultured biofilms and the iron soil biofilms. However, at the genus level, the functional exoelectrogenic communities changed significantly (Table 2). Our results demonstrate that the microbial proportions were altered considerably after the MFC had run steadily for two months (Figure 2). Furthermore, *Shewanella* MR-1 possibly exerts a mutual exclusion effect on Rhodocyclaceae and Desulfuromonas, while MR-1 could synergize with Pelobacteraceae. *Geobacter* was detected in the biofilm, but was not significant in either content or ratio (Table 2). *Geobacter* has previously been found to be the main exoelectrogen in the microbial communities on electrodes in some MFC systems [36,57–59]. The interaction between *G. sulfurreducens* and *S. oneidensis* has been reported to be likely in the presence of a hydrogen cycle, with *S. oneidensis* as a producer and *G. sulfurreducens* as a consumer [53]. *G. sulfurreducens* cells were observed in the planktonic phase only in the presence of *S. oneidensis* [53]. In our experiments, adding *S. oneidensis* did not seem to influence *Geobacter*, most probably because the latter was not the main contributor in our iron mine soil biofilms. Although the proportion of *Geobacter* did decrease after adding *S. oneidensis*, the difference was not statistically significant (Table 1). The ratio of the predominant bacteria in co-cultured biofilm was higher than that in the iron soil biofilm (Table 1), which may be implicated in the resultant improvement of the performance of MFCs. Meanwhile, the UniFrac distance based on the relative abundance of OTUs showed separation between co-culture biofilms and iron soil biofilm, which also indicated altered microbial community structure. Our results provide evidence that a single exoelectrogen is capable of modifying the exoelectrogenic microbial community. An exogenous bacterial species was discovered as one of the main factors influencing the exoelectrogenic microbial communities. Additionally, the potential interaction among the related bacterial families was revealed, which could provide further clues for designing co-culture experiments.

Table 2. Functional microbes in the biofilms on anodes.

Name of Bacteria	Level	CoS	CoL	SiS	SiL
<i>Shewanella</i>	Genus	26.27 ± 2.83 a	1.47 ± 0.78 b	0.50 ± 0.06 b	2.50 ± 0.42 b
<i>Thermomonas</i>	Genus	0.26 ± 0.13 b	1.10 ± 0.41 b	2.14 ± 1.27 b	6.66 ± 1.84 a
<i>Geobacter</i>	Genus	0.02 ± 0.02 a	2.14 ± 2.07 a	3.68 ± 1.55 a	1.66 ± 0.59 a
<i>Desulfuromonas</i>	Genus	1.10 ± 0.45 b	10.28 ± 4.58 ab	11.8 ± 2.10 a	8.46 ± 2.71 ab
<i>Oceanimonas</i>	Genus	0.74 ± 0.29 a	5.18 ± 2.86 a	1.56 ± 0.32 a	3.84 ± 0.42 a
<i>Pseudomonas</i>	Genus	9.28 ± 2.43 b	18.28 ± 4.85 a	4.22 ± 1.42 b	8.12 ± 1.88 b
Pelobacteraceae	Family	15.98 ± 3.71 a	16.9 ± 4.69 a	2.43 ± 0.68 b	1.95 ± 0.32 b
Rhodocyclaceae	Family	0.14 ± 0.05 b	0.90 ± 0.54 b	9.58 ± 3.23 a	8.92 ± 2.63 a
Proteiniclasticum	Family	2.48 ± 1.73 ab	3.14 ± 1.86 ab	6.70 ± 1.06 a	1.52 ± 0.52 b
Comamonadaceae	Family	4.52 ± 1.70 ab	7.98 ± 2.16 a	2.92 ± 0.53 b	4.54 ± 0.82 ab
Total (percent)		61.79 ± 8.76	67.37 ± 6.50	45.53 ± 3.68	48.17 ± 2.98

Note: Different letters across treatments mean a significant difference at the $p < 0.05$ level.

5. Conclusions

This study demonstrated that co-cultured biofilm on the anode between *Shewanella* and iron soil biofilm improved the performance of MFC. A maximum power density increased from $175 \pm 7 \text{ mW/m}^2$ to $195 \pm 8 \text{ mW/m}^2$, and the highest power density plateau extended to 5 days from 2 days. The oxidation peak was significantly higher in the co-cultured MFC than in the other MFCs. *Shewanella* MR-1 altered the microbial communities of the iron mine soil biofilms. Rhodocyclaceae was the most abundant bacterium in the iron soil biofilms, followed by Desulfuromonas, Pseudomonas, and Thermomonas. Contrastingly, Pseudomonas was the most abundant bacterium in the co-cultured biofilms, followed by Pelobacteraceae, Desulfuromonas, and Comamonadaceae. The bacterial community of the co-cultured biofilm had a relatively lower diversity than the iron soil biofilm, at both early and mature stages of culturing. The proportion of the predominant functional bacteria population in the biofilm microbial community may, thus, influence electricity generation in the MFCs. The molecular origin of the observed relationship is currently unclear and needs further study.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms13102383/s1>; Supplementary Table S1: effective tags of all the sequencing samples; Supplementary Table S2: sequencing length of all the samples; Supplementary Table S3: OUT distribution of all the samples.

Author Contributions: Conceptualization, H.C.; methodology, H.C., J.B., and M.F.; software, H.C.; formal analysis, H.C. and M.F.; resources, M.F.; data curation, J.B.; writing—original draft preparation, H.C.; writing—review and editing, H.C.; supervision, H.C.; funding acquisition, H.C. All authors have read and agreed to the published version of the manuscript.

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

References

1. Neal, A.L.; Rosso, K.M.; Geesey, G.G.; Gorby, Y.A.; Little, B.J. Surface structure effects on direct reduction of iron oxides by *Shewanella oneidensis*. *Geochim. Cosmochim. Acta* **2003**, *67*, 4489–4503. [\[CrossRef\]](#)
2. Logan, B.E. Exoelectrogenic bacteria that power microbial fuel cells. *Nat. Rev. Microbiol.* **2009**, *7*, 375–381. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Shi, L.; Dong, H.; Reguera, G.; Beyenal, H.; Lu, A.; Liu, J.; Yu, H.-Q.; Fredrickson, J.K. Extracellular electron transfer mechanisms between microorganisms and minerals. *Nat. Rev. Microbiol.* **2016**, *14*, 651–662. [\[CrossRef\]](#)
4. Lovley, D.R.; Phillips, E.J. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* **1988**, *54*, 1472–1480. [\[CrossRef\]](#)
5. Myers, C.R.; Nealson, K.H. Bacterial manganese reduction and growth with manganese oxide as the sole electron-acceptor. *Science* **1988**, *240*, 1319–1321. [\[CrossRef\]](#)
6. Portela, P.C.; Silva, M.A.; Almeida, A.; Damas, G.F.; Salgueiro, C.A. Tweaking the redox properties of PpcA from *Geobacter metallireducens* with protein engineering. *Biochem. J.* **2024**, *481*, 2017–2036. [\[CrossRef\]](#)
7. Jiang, Z.; Liu, M.; Chen, Y.; Qian, X.; Dai, Q.; Yao, J.; Chen, J. Extracellular electron transfer in bioelectrochemical system and its role in denitrification: Mechanisms, enhancement strategies, and application prospects. *Bioresour. Technol.* **2025**, *437*, 133188. [\[CrossRef\]](#)
8. Xu, S.; Barrozo, A.; Tender, L.M.; Krylov, A.I.; El-Naggar, M.Y. Multiheme Cytochrome Mediated Redox Conduction through *Shewanella oneidensis* MR-1 Cells. *J. Am. Chem. Soc.* **2018**, *140*, 10085–10089. [\[CrossRef\]](#)

9. Okamoto, A.; Hashimoto, K.; Nakamura, R. Long-range electron conduction of *Shewanella* biofilms mediated by outer membrane C-type cytochromes. *Bioelectrochemistry* **2012**, *85*, 61–65. [\[CrossRef\]](#)
10. Okamoto, A.; Tokunou, Y.; Saito, J. Cation-limited kinetic model for microbial extracellular electron transport via an outer membrane cytochrome C complex. *Biophys. Physicobiology* **2016**, *13*, 71–76. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Reguera, G.; McCarthy, K.D.; Mehta, T.; Nicoll, J.S.; Tuominen, M.T.; Lovley, D.R. Extracellular electron transfer via microbial nanowires. *Nature* **2005**, *435*, 1098–1101. [\[CrossRef\]](#)
12. Clark, M.M.; Reguera, G. Biology and biotechnology of microbial pilus nanowires. *J. Ind. Microbiol. Biotechnol.* **2020**, *47*, 897–907. [\[CrossRef\]](#)
13. Alves, M.N.; Fernandes, A.P.; Salgueiro, C.A.; Paquete, C.M. Unraveling the electron transfer processes of a nanowire protein from *Geobacter sulfurreducens*. *Biochim. Biophys. Acta* **2016**, *1857*, 7–13. [\[CrossRef\]](#)
14. Malvankar, N.S.; Vargas, M.; Nevin, K.; Tremblay, P.-L.; Evans-Lutterodt, K.; Nykypanchuk, D.; Martz, E.; Tuominen, M.T.; Lovley, D.R. Structural basis for metallic-like conductivity in microbial nanowires. *MBio* **2015**, *6*, e00084. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Li, M.; Zhou, M.; Tian, X.; Tan, C.; McDaniel, C.T.; Hassett, D.J.; Gu, T. Microbial fuel cell (MFC) power performance improvement through enhanced microbial electrogenicity. *Biotechnol. Adv.* **2018**, *36*, 1316–1327. [\[CrossRef\]](#)
16. Rosenbaum, M.A.; Bar, H.Y.; Beg, Q.K.; Segrè, D.; Booth, J.; Cotta, M.A.; Angenent, L.T. *Shewanella oneidensis* in a lactate-fed pure-culture and a glucose-fed co-culture with *Lactococcus lactis* with an electrode as electron acceptor. *Bioresour. Technol.* **2011**, *102*, 2623–2628. [\[CrossRef\]](#)
17. Nishio, K.; Hashimoto, K.; Watanabe, K. Light/electricity conversion by defined cocultures of *Chlamydomonas* and *Geobacter*. *J. Biosci. Bioeng.* **2013**, *115*, 412–417. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Qu, Y.; Feng, Y.; Wang, X.; Logan, B.E. Use of a coculture to enable current production by *Geobacter sulfurreducens*. *Appl. Environ. Microb.* **2012**, *78*, 3484–3487. [\[CrossRef\]](#)
19. Prokhorova, A.; Sturm-Richter, K.; Doetsch, A.; Gescher, J. Resilience, dynamics, and interactions within a model multispecies exoelectrogenic-biofilm community. *Appl. Environ. Microbiol.* **2017**, *83*, e03033-16. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Wrighton, K.C.; Thrash, J.C.; Melnyk, R.A.; Bigi, J.P.; Byrne-Bailey, K.G.; Remis, J.P.; Schichnes, D.; Auer, M.; Chang, C.J.; Coates, J.D. Evidence for direct electron transfer by a gram-positive bacterium isolated from a microbial fuel cell. *Appl. Environ. Microbiol.* **2011**, *77*, 7633–7639. [\[CrossRef\]](#)
21. Xing, W.; Li, D.; Li, J.; Hu, Q.; Deng, S. Nitrate removal and microbial analysis by combined micro-electrolysis and autotrophic denitrification. *Bioresour. Technol.* **2016**, *211*, 240–247. [\[CrossRef\]](#)
22. Brandel, J.; Humbert, N.; Elhabiri, M.; Schalk, I.J.; Mislin, G.L.; Albrecht-Gary, A.M. Pyochelin, a siderophore of *Pseudomonas aeruginosa*: Physicochemical characterization of the iron(III), copper(II) and zinc(II) complexes. *Dalton Trans.* **2012**, *41*, 2820–2834. [\[CrossRef\]](#)
23. Ou-douaou, A.; Akhtach, S.; Oulakhir, A.; Benhida, R.; Danouche, M. Advances in microbial biosurfactants for mineral flotation: Towards sustainable phosphate ore beneficiation. *J. Water Process Eng.* **2025**, *76*, 108267. [\[CrossRef\]](#)
24. Handley, K.M.; Wrighton, K.C.; Miller, C.S.; Wilkins, M.J.; Kantor, R.S.; Thomas, B.C.; Williams, K.H.; Gilbert, J.A.; Long, P.E.; Banfield, J.F. Disturbed subsurface microbial communities follow equivalent trajectories despite different structural starting points. *Environ. Microbiol.* **2015**, *17*, 622–636. [\[CrossRef\]](#)
25. Handley, K.M.; Wrighton, K.C.; Piceno, Y.M.; Andersen, G.L.; DeSantis, T.Z.; Williams, K.H.; Wilkins, M.J.; Lucie, N.A.; Peacock, A.; Bargar, J.; et al. High-density PhyloChip profiling of stimulated aquifer microbial communities reveals a complex response to acetate amendment. *FEMS Microbiol. Ecol.* **2012**, *81*, 188–204. [\[CrossRef\]](#)
26. Aftab, Q.; Wang, X.; Lu, J.; Tariq, M.; Liu, Y. Advancing soil microbial fuel cells: Exploring bioelectrogenesis mechanisms for integration into environmental bioremediation. *Renew. Sustain. Energy Rev.* **2025**, *214*, 115495. [\[CrossRef\]](#)
27. Nielsen, M.E.; Reimers, C.E.; White, H.K.; Sharma, S.; Girguis, P.R. Sustainable energy from deep ocean cold seeps. *Energy Environ. Sci.* **2008**, *1*, 584–593. [\[CrossRef\]](#)
28. Yang, Y.; Ren, G.; Peng, Z.; Wang, X. Bacterial diversity analysis of coal mine drainage enriched by different energy sources. *Energy Fuels* **2013**, *27*, 5552–5558. [\[CrossRef\]](#)
29. Jiang, H.L.; He, Q.; He, Z.; Hemme, C.L.; Wu, L.; Zhou, J. Continuous Cellulosic Bioethanol Fermentation by Cyclic Fed-Batch Cocultivation. *Appl. Environ. Microbiol.* **2013**, *79*, 1580–1589. [\[CrossRef\]](#)
30. Yan, X.; Peng, P.; Li, X.; Miao, Y.; Chen, L.; Zhao, F. Bridging energy and microbes: How the anodic potential of microbial electrolysis cell improves anaerobic digestion. *J. Power Sources* **2024**, *623*, 235489. [\[CrossRef\]](#)
31. Sasamoto, H.; Hama, K.; Seo, T. Characterization of redox conditions in the excavation disturbed zone of a drift in the Kamaishi Mine, Japan. *Eng. Geol.* **2012**, *151*, 100–111. [\[CrossRef\]](#)
32. Shimizu, M.; Zhou, J.; Schröder, C.; Obst, M.; Kappler, A.; Borch, T. Dissimilatory Reduction and Transformation of Ferrihydrite-Humic Acid Coprecipitates. *Environ. Sci. Technol.* **2013**, *47*, 13375–13384. [\[CrossRef\]](#)
33. Ren, Z.; Ward, T.E.; Regan, J.M. Electricity Production from Cellulose in a Microbial Fuel Cell Using a Defined Binary Culture. *Environ. Sci. Technol.* **2007**, *41*, 4781–4786. [\[CrossRef\]](#) [\[PubMed\]](#)

34. You, J.; Deng, Y.; Chen, H.; Ye, J.; Zhang, S.; Zhao, J. Enhancement of gaseous o-xylene degradation in a microbial fuel cell by adding *Shewanella oneidensis* MR-1. *Chemosphere* **2020**, *252*, 126571. [\[CrossRef\]](#)
35. Fu, L.; Li, S.W.; Ding, Z.W.; Ding, J.; Lu, Y.Z.; Zeng, R.J. Iron reduction in the DAMO/*Shewanella oneidensis* MR-1 coculture system and the fate of Fe(II). *Water Res.* **2016**, *88*, 808–815. [\[CrossRef\]](#)
36. Liu, Q.; Yang, Y.; Mei, X.; Liu, B.; Chen, C.; Xing, D. Response of the microbial community structure of biofilms to ferric iron in microbial fuel cells. *Sci. Total Environ.* **2018**, *631*, 695–701. [\[CrossRef\]](#)
37. Magoc, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Bokulich, N.A.; Subramanian, S.; Faith, J.J.; Gevers, D.; Gordon, J.I.; Knight, R.; Mills, D.A. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. Methods* **2013**, *10*, 57–59. [\[CrossRef\]](#)
40. Pitta, D.W.; Pinchak, W.E.; Dowd, S.E.; Osterstock, J.; Gontcharova, V.; Youn, E.; Dorton, K.; Yoon, I.; Min, B.R.; Fulford, J.D.; et al. Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. *Microb. Ecol.* **2010**, *59*, 511–522. [\[CrossRef\]](#)
41. Shannon, C.E. The mathematical theory of communication. 1963. *MD Comput.* **1997**, *14*, 306–317.
42. Mahaffee, W.F.; Kloepper, J.W. Temporal Changes in the Bacterial Communities of Soil, Rhizosphere, and Endorhiza Associated with Field-Grown Cucumber (*Cucumis sativus* L.). *Microb. Ecol.* **1997**, *34*, 210–223. [\[CrossRef\]](#)
43. Lu, L.; Xing, D.; Ren, Z.J. Microbial community structure accompanied with electricity production in a constructed wetland plant microbial fuel cell. *Bioresour. Technol.* **2015**, *195*, 115–121. [\[CrossRef\]](#)
44. Ebrahimi, S.; Gabus, S.; Rohrbach-Brandt, E.; Hosseini, M.; Rossi, P.; Maillard, J.; Holliger, C. Performance and microbial community composition dynamics of aerobic granular sludge from sequencing batch bubble column reactors operated at 20 °C, 30 °C, and 35 °C. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 1555–1568. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Wu, X.; Xiong, X.; Owens, G.; Brunetti, G.; Zhou, J.; Yong, X.; Xie, X.; Zhang, L.; Wei, P.; Jia, H. Anode modification by biogenic gold nanoparticles for the improved performance of microbial fuel cells and microbial community shift. *Bioresour. Technol.* **2018**, *270*, 11–19. [\[CrossRef\]](#)
46. Sulonen, M.L.; Kokko, M.E.; Lakaniemi, A.M.; Puhakka, J.A. Electricity generation from tetrathionate in microbial fuel cells by acidophiles. *J. Hazard. Mater.* **2015**, *284*, 182–189. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Mei, X.; Guo, C.; Liu, B.; Tang, Y.; Xing, D. Shaping of bacterial community structure in microbial fuel cells by different inocula. *RSC Adv.* **2015**, *5*, 78136–78141. [\[CrossRef\]](#)
48. Bardarov, I.; Mitov, M.; Ivanova, D.; Hubenova, Y. Light-dependent processes on the cathode enhance the electrical outputs of sediment microbial fuel cells. *Bioelectrochemistry* **2018**, *122*, 1–10. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Liu, Z.; He, Y.; Shen, R.; Zhu, Z.; Xing, X.; Li, B. Performance and microbial community of carbon nanotube fixed-bed microbial fuel cell continuously fed with hydrothermal liquefied cornstalk biomass. *Bioresour. Technol.* **2015**, *185*, 294–301. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Lu, L.; Xing, D.; Ren, N. Bioreactor performance and quantitative analysis of methanogenic and bacterial community dynamics in microbial electrolysis cells during large temperature fluctuations. *Environ. Sci. Technol.* **2012**, *46*, 6874–6881. [\[CrossRef\]](#)
51. White, H.K.; Reimers, C.E.; Cordes, E.E.; Dilly, G.F.; Girguis, P.R. Quantitative population dynamics of microbial communities in plankton-fed microbial fuel cells. *ISME J.* **2009**, *3*, 635–646. [\[CrossRef\]](#)
52. Yan, X.; Bu, J.; Chen, X.; Zhu, M. Comparative genomic analysis reveals electron transfer pathways of *Thermoanaerobacterium thermosaccharolyticum*: Insights into thermophilic electroactive bacteria. *Sci. Total Environ.* **2023**, *905*, 167294. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Reyes, C.; Dellwig, O.; Dähnke, K.; Gehre, M.; Noriega-Ortega, B.E.; Böttcher, M.E.; Meister, P.; Friedrich, M.W. Bacterial communities potentially involved in iron-cycling in Baltic Sea and North Sea sediments revealed by pyrosequencing. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw054. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Yu, R.; Gan, P.; Mackay, A.A.; Zhang, S.; Smets, B.F. Presence, distribution, and diversity of iron-oxidizing bacteria at a landfill leachate-impacted groundwater surfacewater interface. *FEMS Microbiol. Ecol.* **2010**, *71*, 260–271. [\[CrossRef\]](#)
55. Kersters, K.; De Vos, P.; Gillis, M.; Swings, J.; Vandamme, P.; Stackebrandt, E. Introduction to the proteobacteria. *Prokaryotes* **2006**, *5*, 3–37.
56. Schilirò, T.; Tommasi, T.; Armato, C.; Hidalgo, D.; Traversi, D.; Bocchini, S.; Gilli, G.; Pirri, C.F. The study of electrochemically active planktonic microbes in microbial fuel cells in relation to different carbon-based anode materials. *Energy* **2016**, *106*, 277–284. [\[CrossRef\]](#)
57. Chignell, J.F.; De Long, S.K.; Reardon, K.F. Meta-proteomic analysis of protein expression distinctive to electricity-generating biofilm communities in air-cathode microbial fuel cells. *Biotechnol. Biofuels* **2018**, *11*, 121. [\[CrossRef\]](#)

-
58. Ishii, S.; Suzuki, S.; Yamanaka, Y.; Wu, A.; Nealson, K.H.; Bretschger, O. Population dynamics of electrogenic microbial communities in microbial fuel cells started with three different inoculum sources. *Bioelectrochemistry* **2017**, *117*, 74–82. [[CrossRef](#)]
 59. Elreedy, A.; Harrer, D.; Ali, R.; Hille-Reichel, A.; Gescher, J. Efficacious enrichment of butyrate-oxidizing exoelectrogens upgrades energy recovery in relevant bioelectrochemical systems. *Environ. Technol. Innov.* **2024**, *36*, 103871. [[CrossRef](#)]

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