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Community Structure Analyses of Anodic Biofilms in a Bioelectrochemical System Combined with an Aerobic Reactor

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Abstract: Bioelectrochemical system (BES)-based reactors have a limited range of use, especially in aerobic conditions, because these systems usually produce current from exoelectrogenic bacteria that are strictly anaerobic. However, some mixed cultures of bacteria in aerobic reactors can form surface biofilms that may produce anaerobic conditions suitable for exoelectrogenic bacteria to thrive. In this study, we combined a BES with an aerobic trickling filter (TF) reactor for wastewater treatment and found that the BES-TF setup could produce electricity with a coulombic efficiency of up to 15% from artificial wastewater, even under aerobic conditions. The microbial communities within biofilms formed at the anodes of BES-TF reactors were investigated using high throughput 16S rRNA gene sequencing. Efficiency of reduction in chemical oxygen demand and total nitrogen content of wastewater using this system was >97%. Bacterial community analysis showed that exoelectrogenic bacteria belonging to the genera *Geobacter* and *Desulfuromonas* were dominant within the biofilm coating the anode, whereas aerobic bacteria from the family Rhodocyclaceae were abundant on the surface of the biofilm. Based on our observations, we suggest that BES-TF reactors with biofilms containing aerobic bacteria and anaerobic exoelectrogenic bacteria on the anodes can function in aerobic environments.

Keywords: bacterial community structure; biofilms; bioelectrochemical system (BES); trickling filter (TF); *Geobacter*; *Desulfuromonas*; wastewater treatment

1. Introduction

Bioelectrochemical systems (BESs), comprising microbial fuel cells (MFCs) that generate electricity [1] and microbial electrolysis cells that produce hydrogen [2], are promising candidate technologies for wastewater treatment, chemical synthesis, and environmental remediation [3–5]. Exoelectrogenic bacteria [6], which adhere to the surfaces of electrodes in BESs, produce electrons by degrading organic matter. The bacteria transfer these electrons to the anode via various pathways, including short-range electron transfer through c-type cytochrome, direct electron transfer between redox-active metabolites and the electrode, and long-range electron transfer via electrically conductive pili [7]. The electrons generated in BESs can then be used to produce electricity and other valuable

chemical by-products [8]. Since electron transfer by exoelectrogenic bacteria can only take place under anaerobic conditions, BES-based reactors must maintain an anaerobic environment in the anode chambers for successful electricity generation. This requirement for maintaining anaerobic conditions limits the application range of BESs. However, it has been observed that in aerobic reactors fed with wastewater, biofilms formed of mixed microbial cultures could create anaerobic conditions suitable for electricity generation. This has recently been reported in a study describing an aerobic open-type biosensor for monitoring biochemical oxygen demand in wastewater generated by livestock [9]. The biofilms on the biosensor anodes allowed stable electricity generation, even though these anodes were inserted into an intermittently aerated tank.

The biofilms on BES anodes often contain bacteria of the *Geobacter* spp., which are well-characterized exoelectrogenic bacteria [10]. The *Geobacter* spp. are Fe(III)-reducing bacteria, which couple iron reduction with oxidation of acetate to CO_2 under anaerobic conditions [11]. These bacteria have been shown to generate electricity through pathways involving electron transfer between redox-active metabolites and the anode, as well as through electrically conductive pili [12,13]. Apart from the *Geobacter* spp., there are many other exoelectrogenic bacterial species which directly use simple substrates such as acetate and amino acids as a source of fuel for electricity generation [14]. However, when complex substrates with high molecular weights, such as starch, proteins, and glucose are to be utilized in BESs, non-exoelectrogenic bacteria are required to decompose these molecules into the simpler ones that are easily utilized by exoelectrogenic bacteria. Since substrates like municipal wastewater contain a mixture of both simple and complex substrates, BESs that utilize wastewater for electricity generation also require a complement of non-exoelectrogenic bacteria for optimal function.

Apart from producing electricity, BESs are an ideal option for wastewater treatment, as they require very little external energy inputs and are cheaper than traditional processes. However, although BESs can effectively reduce concentrations of organic pollutants in wastewater, the system is not very efficient at reducing the soluble nitrogen content. This is because nitrogen-removal processes require both anaerobic and aerobic conditions, whereas BESs usually operate best under anaerobic conditions. The development of BESs that can remove soluble nitrogen content for efficient wastewater treatment has therefore been a major challenge [8].

One way to tackle this challenge is to integrate BESs with other conventional wastewater treatment processes [15,16]. So far, MFCs combined with an anaerobic fluidized bed membrane bioreactor [17] or aerated biological filters [18,19] have been reported. These hybrid reactors are two-stage processes, consist of two separate reactors, and are not integrated as a single reactor. In this study, we have integrated a BES with a biological trickling filter (TF) reactor as a single reactor to efficiently remove organic matter and soluble nitrogen compounds from wastewater by a one-step treatment. A TF reactor is an aerobic wastewater treatment system commonly used for the removal of organic matter and soluble nitrogen compounds using microbes [20]. In this method, wastewater is trickled through a growth bioreactor that contains an inert plastic or mineral medium supporting a microbial biofilm that degrades organic matter and soluble nitrogen compounds. As the biofilms are attached to a medium, the bacterial 'filters' have long retention times despite the very short hydraulic retention times of the system itself. In addition, due to the large gas–water interface area, this method requires no additional aeration, has low sludge production, is simple and easy to maintain, and therefore, cost-effective. We also analyzed the community structures of the biofilms formed at the anodes in BES-TF reactors to investigate the mechanism by which this system can support the current generation even under aerobic conditions.

2. Materials and Methods

2.1. Reactor Construction and Operation

A diagrammatic representation of the reactor we have developed is shown in Figure 1. The reactor was designed as a cuboid-shaped tank with a total volume of 16 L (dimensions: 20 cm length × 20 cm

width × 40 cm height) that can hold 4 L of liquid. The reactor has a carbon-fiber brush anode (length: 40 cm, and branch length: 16 cm) positioned at the middle of the setup (Figure 1). The entire carbon fiber was submerged in the wastewater of the reactor for the first two months to accumulate bacteria, following which, only half of the anode was left immersed in the wastewater to create an upper anode (without immersion) and a lower anode. A Pt-sheet counter electrode (8 mm × 8 mm × 0.15 mm) and Ag/AgCl reference electrode (International Chemistry, Chiba, Japan) were also inserted into the reactor. The electrodes were connected to a potentiostat (HA-151B; Hokuto Denko, Tokyo, Japan), and the anode potential was set to −0.2 V (vs. Ag/AgCl).

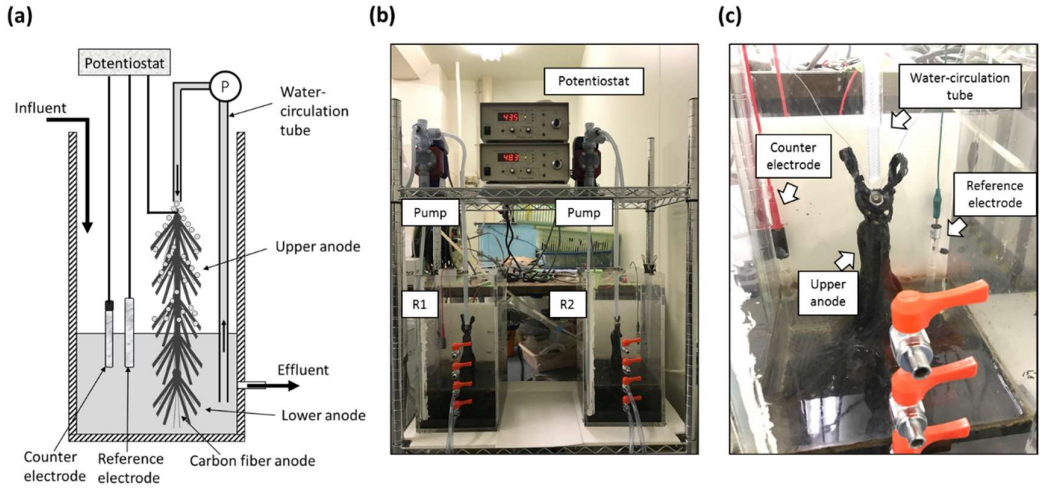

Figure 1. Diagrammatic representation (**a**) and pictures (**b**,**c**) of the bioelectrochemical system-trickling filter (BES-TF) reactor designed and tested in this study.

The composition of the artificial wastewater used in the reactor was as follows: 1.37 g/L CH_3COONa, 0.83 g/L beef extract, 0.27 g/L urea, 0.5 g/L $NaH_2PO_4{\cdot}2H_2O$, 0.097 g/L NaCl, 0.025 g/L $CaCl_2{\cdot}2H_2O$, 0.037 g/L KCl, 0.041 g/L $MgSO_4{\cdot}7H_2O$. The chemical oxygen demand (COD) loading rate was set to 0.5 g COD/L/day. Two reactors, reactor 1 (R1) and reactor 2 (R2), were set up and operated at 35 ± 2 °C in a sequential batch mode. In this mode, half of the reactor contents (2 L) were removed every two days and replaced with fresh artificial wastewater. The reactors were inoculated with 1 L each of activated sludge collected from an animal wastewater treatment plant at the institute of livestock and grassland science (Tsukuba, Japan). The wastewater in the reactors was pumped at a rate of 8 L/h to the top of the anode for circulation. The reactors were operated for a year, and thereafter, the anodic biofilm was sampled for the community structure analysis. The current produced was recorded every hour using a data logger (LR 5042, HIOKI, Nagano, Japan). To examine decreases in COD and total nitrogen (TN) levels, water samples at 3 h, 24 h, and 48 h after medium exchange were analyzed for each reactor. COD and TN levels were measured with a DBR 200 HACH apparatus and a TOC-V CPH analyzer (Shimadzu, Japan), respectively. Measures of the electric current generated and COD values were used to calculate the coulombic efficiencies of the reactors [21,22].

2.2. Microbial Community Analysis

The bacterial community structures of the biofilms adhering to the anodes of the two reactors were analyzed by amplifying the 16S rRNA genes of the microbial assemblage using the Illumina (San Diego, CA, USA) high throughput sequencing system. The wastewater in the BES-TF reactor was first centrifuged at 6000 g for 10 min, following which, the precipitate obtained was washed in distilled water. Bacteria obtained in this precipitate were considered to be samples of the planktonic cell (PC) fraction. The upper and lower anodes (Figure 1) were scraped using disinfected spoons, and the biofilm samples obtained were considered as the outer biofilm from the upper (Outer-Up) and lower

(Outer-Low) anodes. Subsequently, the anodes were thoroughly washed with distilled water until the sludge visibly adhered to the surface was removed. The part of the biofilm adhering tightly to the anodes were now considered as the inner layers of the upper (Inner-Up) and lower (Inner-Low) anodes. Genomic DNA from these samples were extracted using the DNeasy PowerMax Soil Kit (Qiagen, Hilden, Germany). A two-step polymerase chain reaction (PCR) program focused on amplifying the 16S rRNA gene, as according to Illumina's dual indexing method (Illumina Inc., San Diego, CA, USA), was used to obtain gene sequences for analysis. The first PCR amplification was conducted using the universal forward and reverse primers Uni515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and Uni806R (5′-GGACTACHVGGGTWTCTAAT-3′), respectively [23]. The conditions for the first PCR program are as follows: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min; these steps were followed for 23 cycles. The amplicons were then purified using the Agincourt AMpure XP (Beckman Coulter, Brea, CA, USA) system, following which, the purified DNA fragments were amplified in a second PCR according to the manufacturer's manual (Illumina Inc., San Diego, CA, USA), to obtain PCR products incorporated with sequencing adapters. The amplicons were again purified using the AMpure XP system, pooled, and sequenced using the Illumina MiSeq platform utilizing V2 Nano chemistry. Raw sequence data from the MiSeq platform were quality-trimmed using Trimmomatic software [24]. High-quality reads were joined by FLASH software [25]. The primers were removed using Cutadapt software [26]. Dereplication, discarding of singletons, and clustering into operational taxonomic units (OTUs) at a 97% similarity level were conducted using the UPARSE Pipeline [27]. The OTUs were assigned to different taxa using the GreenGenes reference database [28], and aligned with PyNAST [29]. Non-metric multidimensional scaling (NMDS) analyses based on the Bray–Curtis distance of rarefied abundance data were used to summarize the variations in community compositions. Diversity indices and NMDS analyses were conducted using the R package Vegan [30]. The sequence data for all samples were deposited in the DNA data bank of Japan (DDBJ) under the accession numbers DRA008656–008665.

3. Results and Discussion

3.1. Decomposition of Organic Matter and Soluble Nitrogen Compounds

Two BES-TF reactors, labelled R1 and R2, were operated in parallel to serve as replicates for our experiments. The reactors were inoculated with seed sludge from activated sludge used for animal waste treatment, since animal waste contains many useful bacteria including methanogens, hydrogen-producing bacteria [31], and exoelectrogenic bacteria [32]. Although neither reactor was able to maintain anaerobic conditions, both reactors produced current (Figure 2a,b), and the time courses of electric current generation were similar between R1 and R2. The intensity of electric current produced in both reactors increased sharply in the first 2 h after medium exchange, was stable at a maximum intensity of 165–189 mA for 6 h, and then decreased to 20–30 mA. After 24 h, the electric current generated in both reactors was very low. The pH of the liquid contents of the reactors remained stable (8.0–8.5) over 48 h. The COD value at 0 h was ~1000 mg/L, but dropped to <5% at 24 h after medium exchange (Figure 2c,d). The TN levels at 0 h were ~132 mg/L, which gradually decreased to 3.4–4.2 mg/L after 48 h (Figure 2c,d). Table 1 shows the decomposition efficiencies of COD and TN in R1 and R2. COD and TN decomposition efficiencies at 48 h ranged between 96.2%–99.7% and 97.2%–97.5%, respectively. Coulombic efficiencies at 24 h ranged between 14.1%–16.8% (Table 1), suggesting that on average, ~15.4% of the COD was removed by exoelectrogenic bacteria. These results indicate that the BES-TF reactors can generate electric currents even in the absence of a strictly anaerobic environment, and that the system can successfully remove both organic matter and soluble nitrogen compounds from wastewater.

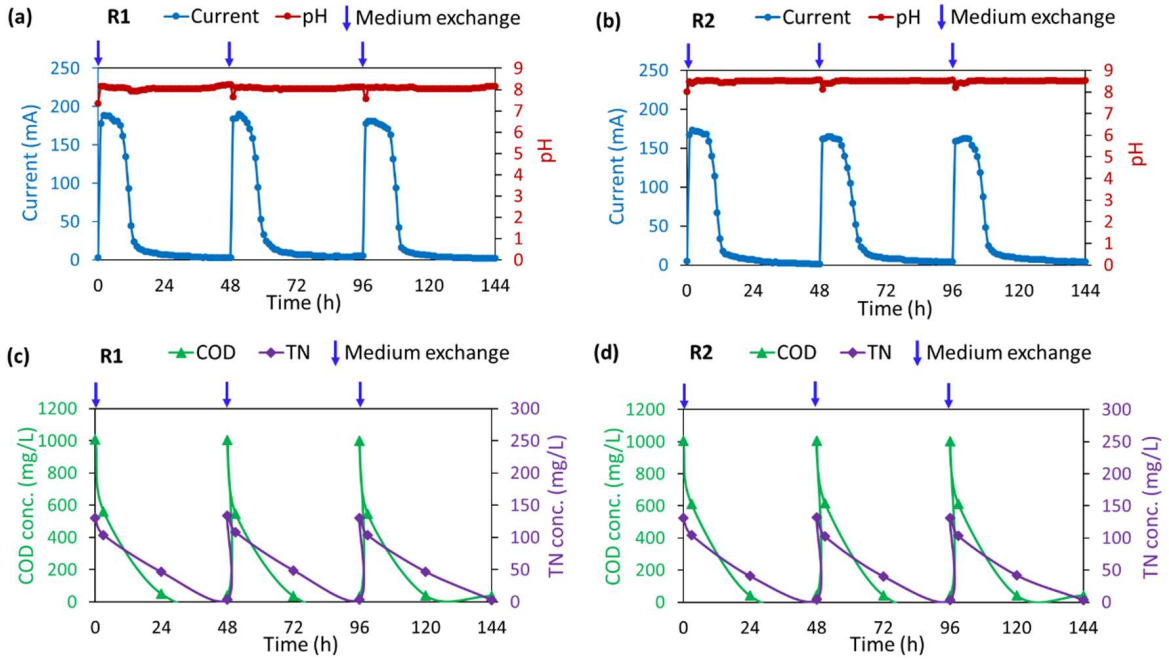

Figure 2. Representative profiles of current generation in the bioelectrochemical system-trickling filter (BES-TF) reactors (**a**) R1, and (**b**) R2. Variations in the levels of chemical oxygen demand (COD) and total nitrogen (TN) values in (**c**) R1, and (**d**) R2. Arrows indicate the time points at which medium exchange had occurred.

Table 1. The coulombic efficiencies and decomposition efficiencies of chemical oxygen demand (COD) and total nitrogen (TN) in the two bioelectrochemical system-trickling filter (BES-TF) reactors, R1 and R2.

Performance Indicators	R1	R2
Coulombic efficiency (%) at 24 h	16.8 ± 0.7	14.1 ± 0.4
COD decomposition efficiency (%) at 48 h	99.7 ± 0.03	96.2 ± 0.6
TN decomposition efficiency (%) at 48 h	97.5 ± 0.1	97.2 ± 0.4

3.2. Analyses of Alpha and Beta Diversities

A total of 143,265 sequencing reads were produced, which were grouped into a total of 266 OTUs. Table 2 shows the numbers of OTUs, along with the values of alpha diversity index, Shannon's diversity index, and Simpson's diversity index in various samples from the BES-TF reactors. The outer anode samples showed higher diversity values than the inner anode samples ($p < 0.05$). All rarefaction curves for each sample appeared to reach a plateau (Figure 3a). The NMDS plot indicates that the different communities could be grouped clearly by sampling-site identity (Figure 3b). The structures of microbial communities sampled from the same sites in R1 and R2 are reasonably similar, since the two reactors were operated under the same conditions.

Table 2. Numbers of reads and operational taxonomic units (OTUs), along with values of alpha diversity index, Shannon's diversity index, and Simpson's diversity index for the microbial communities of different samples from the anodes of the bioelectrochemical system-trickling filter (BES-TF) reactors R1 and R2. PC: planktonic cells, Outer-Low: Outer biofilm of the lower part of the anode (submerged in wastewater), Outer-Up: Outer biofilm of the upper part of the anode (not submerged in the wastewater), Inner-Low: Inner biofilm of the lower part of the anode (submerged in wastewater), Inner-Up: Inner biofilm of the upper part of the anode (not submerged in the wastewater).

Samples	No. of Reads	No. of OTUs	Shannon Index	Simpson Index
PC-R1	16449	189	3.342	0.943
PC-R2	18835	190	3.278	0.934
Outer-Low-R1	10280	171	3.428	0.944
Outer-Low-R2	17104	201	3.548	0.948
Outer-Up-R1	8754	157	3.215	0.928
Outer-Up-R2	18199	183	2.978	0.881
Inner-Up-R1	7424	151	2.695	0.834
Inner-Up-R2	17343	203	2.512	0.771
Inner-Low-R1	8515	134	3.007	0.9
Inner-Low-R2	20362	168	2.725	0.856

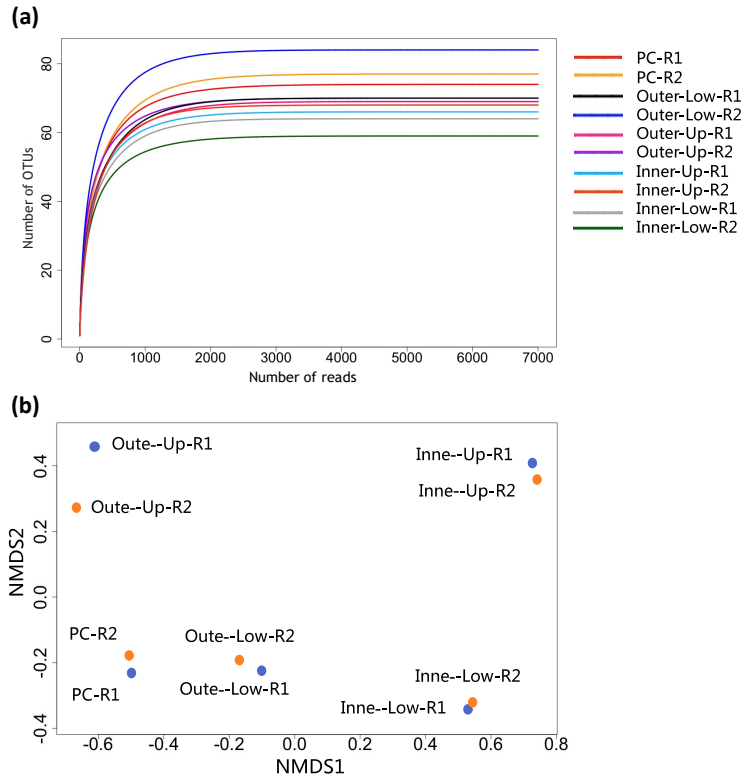

Figure 3. (**a**) Rarefaction curves and (**b**) Non-metric multidimensional scaling (NMDS) plot showing the relationships between the microbial communities of different biofilm samples obtained from the anodes of the bioelectrochemical system-trickling filter (BES-TF) reactors R1 and R2. PC: planktonic cells, Outer-Low: Outer biofilm of the lower part of the anode (submerged in wastewater), Outer-Up: Outer biofilm of the upper part of the anode (not submerged in the wastewater), Inner-Low: Inner biofilm of the lower part of the anode (submerged in wastewater), Inner-Up: Inner biofilm of the upper part of the anode (not submerged in the wastewater).

3.3. Structures of the Microbial Community in the Anodic Biofilms of BES-TF Reactors at the Phylum and Genus Levels

Figure 4 shows the distributions of the microbial communities of different samples of the anodic biofilms from within the reactors. Three representative phyla—Proteobacteria, Bacteroidetes, and Firmicutes—which are frequently observed in BESs [33,34], were dominant in all communities. Proteobacteria, which includes exoelectrogenic bacteria such as *Geobacter*, were most commonly detected in all communities. The abundance of Proteobacteria was highest (72.4%–73.9%) in the Inner-Up samples. The abundance of this phylum was also high in the biofilms of the lower anodes, and lowest in the PC samples (28.3%– 33.5%). Phylum Bacteroidetes was dominant (22.6%–29.7%) in the PC, Outer-Up, and Outer-Low samples, while its abundance was low (2.8%–10.1%) in the communities of the Inner-Up and Inner-Low samples. Phylum Firmicutes was abundant (7.9%–20.0%) in the communities of the PC, Outer-Low, and Inner-Low samples, and low in the communities of the Outer-Up and Inner-Up samples (2.4%–4.6%). On the other hand, phylum Chloroflexi, which includes nitrifying bacteria, was detected in all communities and was especially abundant (5.9%–12.6%) in the Outer-Low, Inner-Low, and Inner-Up samples. Phylum Planctomycetes, which includes aerobic heterotrophic bacteria and anaerobic ammonium oxidation (anammox) bacteria [33], was also detected in all communities. Although there are no known exoelectrogenic members in this phylum, a Planctomycetes-dominant biofilm has been reported to have developed on the anode of a BES fed with livestock wastewater [9].

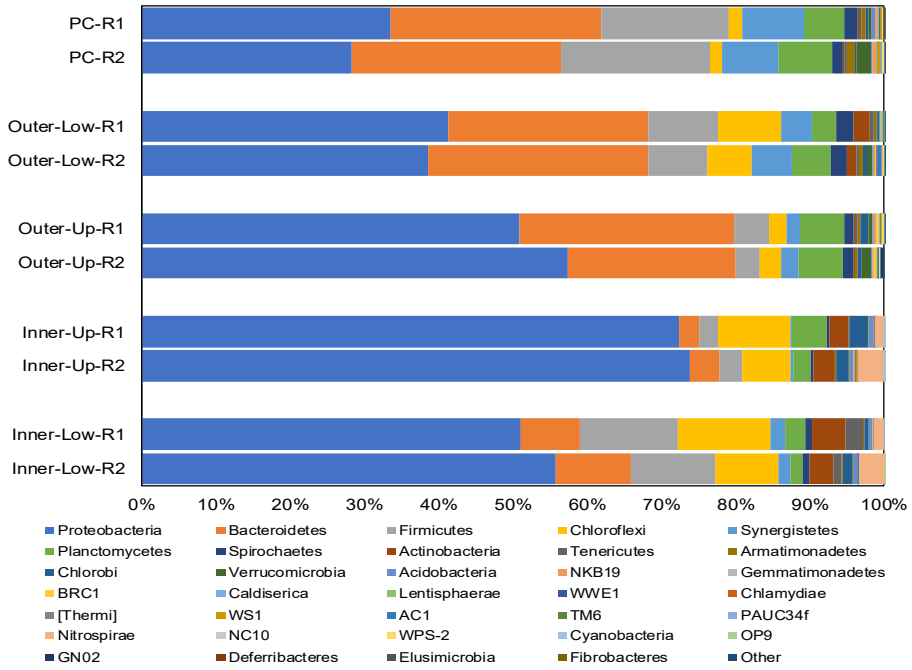

Figure 4. Phylogenetic makeup of the microbial community from different samples of the anodic biofilms from the bioelectrochemical system-trickling filter (BES-TF) reactors R1 and R2. PC: planktonic cells, Outer-Low: Outer biofilm of the lower part of the anode (submerged in wastewater), Outer-Up: Outer biofilm of the upper part of the anode (not submerged in the wastewater), Inner-Low: Inner biofilm of the lower part of the anode (submerged in wastewater), Inner-Up: Inner biofilm of the upper part of the anode (not submerged in the wastewater).

Figure 5 shows the genus-level analysis of the microbial communities on the anodes of R1 and R2. Two representative exoelectrogenic genera, *Geobacter* and *Desulfuromonas*, occurred at high frequencies (16.7%–30.3%) in the communities of the Inner-Low samples. The two genera were also abundant (4.6%–24.4%) in the Inner-Up communities, which were not in contact with the wastewater in the reactor. Low frequencies of these two genera were also found in the Outer-Low communities but

were negligible in the remaining communities (Figure 6). Both the genera belong to the same order, Desulfuromonadales, which belongs to the phylum Proteobacteria. *Geobacter* spp., which are strictly anaerobic [11], are exoelectrogenic bacteria with Fe(III) reducing activity. *Desulfuromonas* spp. are also strict anaerobes that couple sulfur and Fe(III) reductions with acetate oxidation [35–38]. *Desulfuromonas* spp. have also been detected in other BES reactors [39,40], and some species in this genus, such as *Desulfuromonas acetexigens* and *Desulfuromonas acetoxidans*, can produce electricity using acetate [41,42]. In this study, the OTUs affiliated with *Desulfuromonas* showed 99% identity in DNA sequence to *D. acetexigens* (Figure 7).

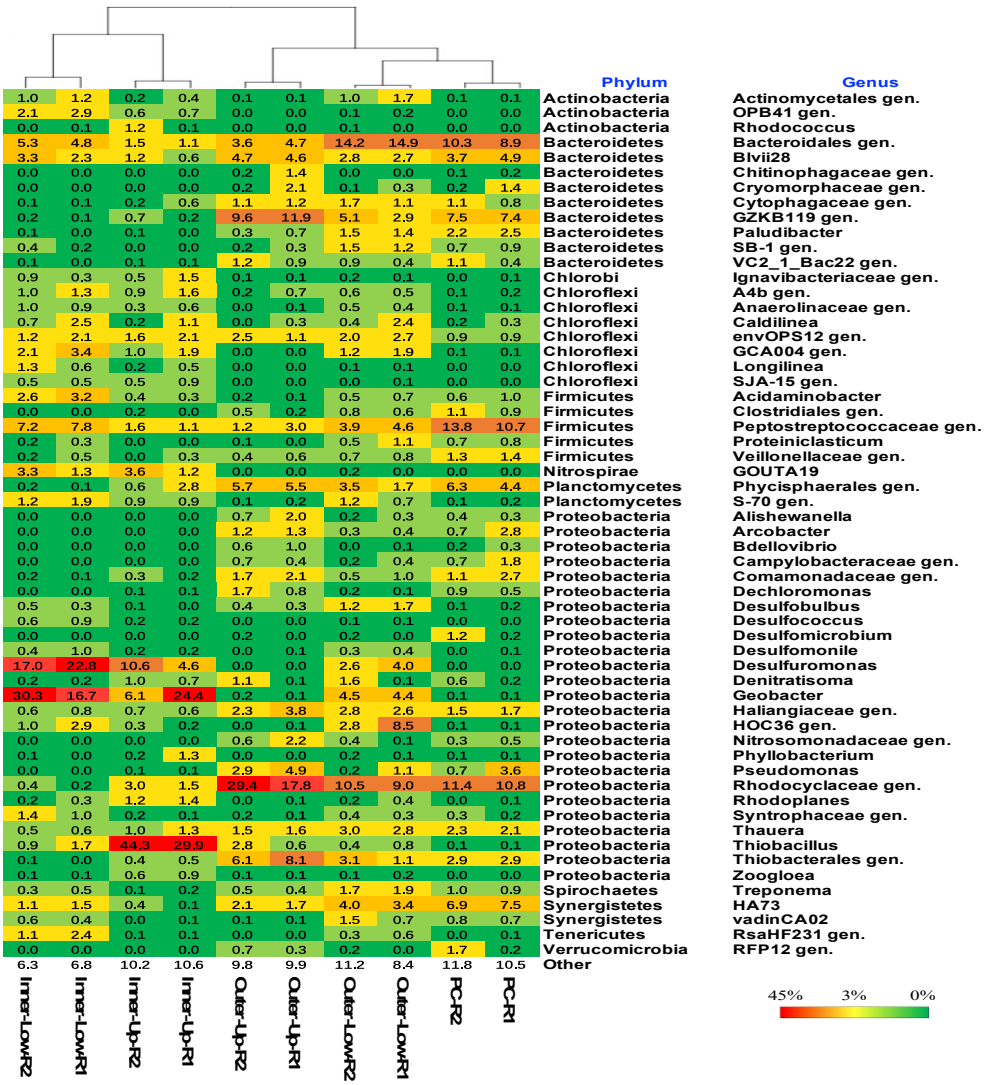

Figure 5. Phylogenetically clustered heat map of the representative genera (with abundances >0.9%) found in the anodic biofilm communities in the bioelectrochemical system-trickling filter (BES-TF) reactors R1 and R2. PC: planktonic cells, Outer-Low: Outer biofilm of the lower part of the anode (submerged in wastewater), Outer-Up: Outer biofilm of the upper part of the anode (not submerged in the wastewater), Inner-Low: Inner biofilm of the lower part of the anode (submerged in wastewater), Inner-Up: Inner biofilm of the upper part of the anode (not submerged in the wastewater).

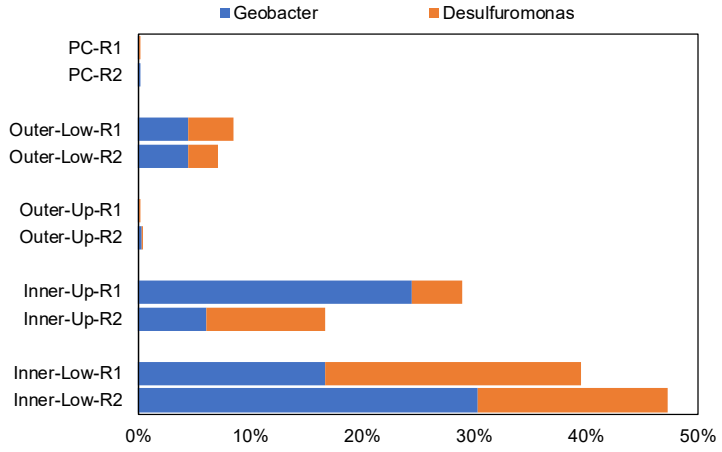

Figure 6. Relative abundances of exoelectrogenic bacteria (at the genus level) in the bioelectrochemical system-trickling filter (BES-TF) reactors R1 and R2. PC: planktonic cells, Outer-Low: Outer biofilm of the lower part of the anode (submerged in wastewater), Outer-Up: Outer biofilm of the upper part of the anode (not submerged in the wastewater), Inner-Low: Inner biofilm of the lower part of the anode (submerged in wastewater), Inner-Up: Inner biofilm of the upper part of the anode (not submerged in the wastewater).

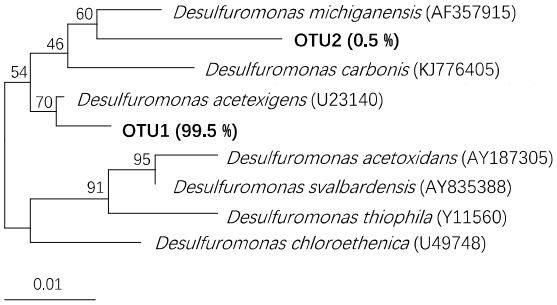

Figure 7. Phylogenetic tree of similarity between OTUs obtained in this study and different *Desulfuromonas* species. The tree was calculated using the neighbor-joining method. The percentages indicate the number of reads assigned to the OTUs for that assigned to the genera *Desulfuromonas* in the communities. The number of bootstrap replications was 1000. The bar indicates the difference of the genome sequences.

Both *Geobacter* and *Desulfuromonas* genera were detected at high frequencies, not only from the inner biofilms of the lower anodes (immersed in the wastewater), but also, interestingly, from the inner biofilms of the upper anodes (which was not immersed in wastewater), indicating that the electric current in this system was produced at both the upper and lower anodes.

Facultative aerobes and aerobic bacteria, which consume oxygen, were abundantly detected in the Outer-Up and Outer-Low samples. These belong predominantly to family Rhodocyclaceae (9.0%–29.4%), which includes facultative bacteria that degrade organic matter using oxygen, nitrates, and other electron acceptors [43,44]. In addition, Rhodocyclaceae also contains bacteria such as *Ferribacterium* [45] that can generate electricity through iron reduction; this family of bacteria has also been found to occur widely in MFCs [46,47]. We also detected aerobic bacteria belonging to the genus *Pseudomonas* in the Outer-Up and Outer-Low samples (0.2–4.9%). In all, these results indicate that facultative aerobes and aerobic bacteria in the outer biofilm on the anodes of the BES-TF system remove oxygen to establish an anaerobic environment within the biofilm. This in turn, provides suitable conditions for the growth of exoelectrogenic bacteria and the production of current.

Our genus-level analyses also detected bacteria capable of performing nitrification and denitrification. An unclassified genus in the family *Nitrosomonadaceae* was found in the Outer-Up samples (0.6%–2.2%); the bacteria in this family are nitrifying bacteria that can oxidize ammonia to

nitrite. Our analysis also uncovered that the denitrifying bacterium, *Thiobacillus*, which can reduce nitrate or nitrite to N_2 gas [48,49], was abundant in the Inner-Up samples (29.9%–44.3%). In addition, unclassified bacterial genus GOUTA19, belonging to the phylum *Nitrospirae*, were detected at higher frequencies in the inner samples (1.2–3.6%) than in the other samples (0–0.2%). GOUTA19 is related to the sulfate-reducing anaerobic thermophile, *Thermodesulfovibrio* [50], and sulfate was present in the wastewater used. Thus, the unclassified genus might reduce sulfate in the anodic biofilm.

In conclusion, our analysis of the microbial community in the BES-TF system has uncovered that exoelectrogenic, aerobic, nitrifying, and denitrifying bacteria can coexist in biofilms adhering to the anode in BES-TF reactors.

3.4. Application of BES Technology to Aerobic Bioreactors

The development of BES-based reactors that can function in aerobic conditions can greatly extend the application range and utility of BESs. So far, BES-based reactors are mostly utilized in anaerobic environments, which are essential for the growth of exoelectrogenic bacteria as well as electricity production. Maintaining anaerobic conditions requires a closed configuration which often makes the manufacture and operation of BES-based reactors expensive and complicated.

In this study, we have developed and demonstrated the working of a BES-TF reactor that can be used to produce current, even under aerobic conditions. The dominant exoelectrogenic bacteria in the biofilms coating the anodes of the BES-TF reactors were from the genera *Geobacter* and *Desulfuromonas*. We have found that the presence of other aerobic, nitrifying, and denitrifying bacteria in the anodic biofilm creates anaerobic conditions for exoelectrogenic bacteria to thrive in; the presence of all these bacteria also ensures high COD and TN decomposition rates. This combination of BES with an aerobic wastewater treatment system would make the wastewater treatment process more consuming-power saving, cost-effective, and easy to manage.

4. Conclusions

In this study, we combined a BES with an aerobic TF system to create a BES-TF reactor that can be used to treat wastewater, as well as generate electrical current even in aerobic environments. The BES-TF reactor we have designed can reduce the COD and TN of wastewater by >97%. This reactor produces current through the activities of exoelectrogenic bacteria which occupy an inner layer in the biofilms that form on the anodes; since the outer layers of the biofilms have aerobic bacteria which remove oxygen, anaerobic environments are ideal for exoelectrogenic bacteria in which the inner layers of the biofilms are established. The coulombic efficiencies of our BES-TF reactors were ~15%. Analyses of the microbial community structures in the anodic biofilms showed that the strictly anaerobic and exoelectrogenic genera, *Geobacter* and *Desulfuromonas*, were mostly found in the inner layers of the biofilm, whereas aerobic bacteria from the family *Rhodocyclaceae* were abundant in the outer biofilm. Nitrifying and denitrifying bacteria were also detected in these biofilm communities. In conclusion, this combination of BESs with aerobic reactors can make the wastewater treatment process more consuming-power saving, cost-effective, and easier to manage. Our results suggest that anodic biofilms can help modify BES-based systems to also function effectively in aerobic environments.

Author Contributions: Conceptualization, H.Y.; methodology, Q.L. and T.Y.; validation, Q.L. and R.Y.-I.; formal data analysis, Q.L. and N.M.; experimental analysis, Q.L. and T.Y.; writing—original draft preparation, Q.L.; writing—review and editing, Q.L., R.Y.-I., and H.Y.

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