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Overcoming the Bottlenecks of Cellulose Utilization in Microbial Fuel Cells via Bioaugmentation Strategy with Cellulose-Degrading Isolates

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Abstract

Although cellulosic biomass was among the most abundant substrates for producing renewable energy, its energy recovery in microbial fuel cells (**MFCs**) was often relatively low, owing to its low biodegradability. Here, the effect of bioaugmentation of three cellulose-degrading bacterial isolates (*Acinetobacter tandoii*, *Brevundimonas bullata*, and *Micrococcus endophyticus*) on MFCs efficiency was evaluated. Bioaugmented MFC with *Acinetobacter tandoii* strain showed the highest power densities (**373 mW m^{-3}**) compared with non-bioaugmented MFC (**240 mW m-^{3}**), associated with near-complete cellulose biodegradation. The results reveal that the bioaugmentation approach shaped the microbial community structure with the emergence of several phylotypes that were closely related to cellulose fermentation (*Firmicutes* and *Bacteroidetes*) and anode respiration (*Proteobacteria*), establishing a syntrophic partnership among cellulose-fermenting bacteria and electrochemically-active bacteria (EAB). In conclusion, our results confirm that the performance of mixed-culture MFCs fed with complex substrates (e.g., cellulose) could be improved by the bioaugmentation approach of fermenting isolates.

Keywords: Microbial Fuel Cell; Cellulose; Bioaugmentation; Microbial syntrophy; electrochemically active bacteria

Introduction

Concerns about climate change and environmental pollution caused by fossil fuel usage and rising energy costs necessitate further efforts toward finding robust and more sustainable ways to meet our Society's energy demand [1, 2]. For instance, the global carbon dioxide (CO_2) emissions reached an unprecedented level of 33 gigatonnes (Gt) in 2021, achieving its highest-ever level below the 2019 peak (i.e., 33.4 Gt) [3]. Cellulosic biomass, which includes waste products from agricultural and industrial operations, represents one of the most abundant feedstocks for renewable energy production due to its low cost and vast supply [4, 5]. Approximately 89 million dry tons of biomass feedstock are generated in Egypt annually with the possibility to replace up to 6 % of the entire country's petroleum consumption [6, 7]. Several approaches have been proposed to generate biofuel from cellulosic biomass, including ethanol, biodiesel, methane, hydrogen, and electricity. However, these approaches were restricted by the low biodegradability of cellulosic biomass [8–10]. In this context, microbial fuel cells (MFCs) have been introduced as advanced engineered platforms to convert chemical energy stored in various waste streams into electricity using electrochemically-active biofilm (EAB) [11, 12]. Compared to chemical and

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enzyme fuel cells, MFCs are more versatile for oxidizing a wide range of donor substrates, including simple compounds (e.g., acetate and glucose) and real wastewater (e.g., sewage, landfill leachate, and sewage sludge) [13–15].

Thus, MFCs offer new opportunities for achieving high wastewater treatment efficiency while providing access to sustainable, environmental-friendly renewable energy. However, the intrinsic electron losses during anode respiration often limit power generation, especially when the complex organic matter is used as the sole donor substrates [16, 17].

These losses were mainly due to either slow electron transfer kinetics, inhibition of fermentation, or a combination of both [14, 15, 18]. A literature survey yields a few studies that reported cellulose biodegradation in MFCs for electricity or hydrogen gas generation [19–22]. Even though different kinds of bacteria have been discovered to be electrochemically active, none of them exhibit a high capability to biodegrade cellulose and instead rely on cellulose hydrolysis and fermentation by-products as the sole donor substrates [23, 24]. For instance, Ren et al. [19] tested a defined co-culture of cellulose-degrading bacterium (*Clostridium cellulolyticum*) and electrochemically-active bacterium (*Geobacter sulfurreducens*) to synergistically convert cellulose into electricity in an MFC. They observed a maximum power density of 143 mW m^2 with up to 64 % cellulose utilization, whereas both pure cultures did not produce electricity from cellulose. In another study, Rismani-Yazdi et al. [20] used a consortium of rumen microorganisms as an anodic inoculum for generating electricity with cellulose as the sole donor substrate. Due to cellulose's slow hydrolysis and fermentation, they observed a maximum power density of 55 mW m^{-2}. Therefore, to overcome this bottleneck, a successful strategy for efficiently generating electricity from cellulose (and other complex organic matter, too) without external chemical catalysts requires building a synergistic microbial consortium of cellulose-fermenting microorganisms with EAB [19]. Bioaugmentation was considered a promising strategy for improving the overall efficiency of MFCs by enhancing the synergetic interaction between the pre-grown native species and robust indigenous isolates. A higher degradation rate of hardly-degraded substrates shortens the startup time and alleviates the toxicity effect of hazardous waste streams [25]. The hallmark of using bioaugmentation to improve the MFC's efficiency was to supplement MFCs with relevant organisms with specific metabolic capabilities to increase electron transfer and substrate utilization rate, which, in turn, improve the current generation [26]. Poor efficiency of MFCs was often associated with a lack of a certain trophic guild that harbors crucial metabolic pathways and cooperates synergistically to convert organic matter into electricity [27, 28]. Several studies demonstrate that bioaugmentation of MFCs with electrochemically-active bacteria resulted in a significant enhancement of electron transfer efficiency owing to the syntrophic partnership between augmented isolates and the native microflora [29]. However, few studies have documented the syntrophic cellulose-degrading consortia during bioaugmentation of MFCs, while the microbiological mechanism remains unclear. Our study aimed at exploring cellulose degradation efficiency in MFCs via bioaugmentation strategy. In this context, we evaluated the functional role of three fermenting bacterial isolates (*Brevundimonas bullata* (NBRC 13290), *Micrococcus endophyticus* (YIM 56238), and *Acinetobacter tandoii* (DSM 14970)) for improving the cellulose utilization rate and electricity generation in batch-fed MFCs. The selected isolates exhibited high metabolic capabilities to degrade polysaccharides, such as dextrin, cellulose, hemicellulose, and lignin [30, 31]. To enumerate the variation in cellulose utilization rate and electron transfer mechanism, we compared the augmented MFCs with the efficiency of non-augmented MFC as a control. The efficiency of MFCs was analyzed in terms of substrate utilization, power output, bioelectrochemical behavior and activity, and electron losses. Finally, the relative abundance and composition of the microbial community were characterized by high-throughput sequencing. To our knowledge, this was the first report that investigated these bacterial strains for improving cellulose utilization in MFCs via a bioaugmentation strategy, which would guide future research toward the scaling-up and commercialization of MFCs, particularly when complex organic compounds were used as the sole donor substrates.

Experimental

The cellulose-degrading bacterial strains (*Brevundimonas bullata* strain (NBRC 13290), *Micrococcus endophyticus* strain (YIM 56238), and *Acinetobacter tandoii* strain (DSM 14970)) used in this study were isolated in our laboratory from an MFC fed with sewage sludge for 18 months[32].

They were cultivated in nutrient broth media (yeast extract: 3 g/L, peptone: 5 g/L, beef extract: 3 g/L, and sodium chloride: 5 g/L; pH 7.0) under facultative anaerobic condition at 37 ºC for 24 h. The cultured isolates were harvested by centrifugation (10000 *g*, 5 min), washed three times, and then re-suspended in phosphate-buffered synthetic medium containing (per liter): 1 g microcrystalline cellulose, 2.5 g $NaHCO_3$, 0.2 NH_4Cl, 17.4 g K_2HPO_4, 13.6 KH_2PO_4, 0.33 g KCl, 0.3 NaCl, 0.15 $CaCl_2.2H_2O$, 3.15 g $MgCl_2$, 1 g yeast extract, and 10 mL trace minerals; pH = 7.0) (chemical oxygen demand concentration = 685 mg COD/L).

Construction, operation, and monitoring of microbial fuel cells

Air-cathode MFCs fabricated from a Plexiglass cylinder, with a working volume of 100 mL as described elsewhere [32, 33]. Three-dimensional carbon felts with a projected surface area of 18.50 cm^2 (Fuel cell store, TX, USA) were used as anodes. While non-catalyzed, wet-proofed carbon cloth electrodes with gas-diffusion layers (Fuel cell store, TX, USA) were used as cathodes. Both electrodes were positioned parallel to each other at a distance of ~ 5 cm. A titanium wire was used as a current collector. Before startup, MFCs were inoculated with 5 mL of anaerobic sludge collected from a local municipal wastewater treatment plant (Benha, Egypt). Then, MFCs fed with a cellulose-containing synthetic medium (COD concentration = 685 mg l^{-1}). The reactors were operated in a fed-batch mode at room temperature (25 ± 2 ℃), with the feeding medium replaced when the overall voltage decreased to ~ 30 mV. All MFCs were inoculated with bacterial strains at an optical density of 0.2 (OD_{600}). Out of the four MFCs, three MFCs were inoculated with the bacterial strains and denoted as MFC-BB, MFC-ME, and MFC-AT for MFCs bioaugmented with *Brevundimonas bullata*, *Micrococcus endophyticus*, and *Acinetobacter tandoii*, respectively. While a non-bioaugmented MFC was used as control (denoted as Non-B MFC).

Chemical analyses

COD was analyzed, in duplicate, according to the method described in the standard methods for examining water and wastewater (APHA, 2005)[34]. Short-chain fatty acids (SCFAs) were analyzed using a 1260 series high-performance liquid chromatography (HPLC) (Agilent Technologies, USA) equipped with Inert Sustain AQ-C18 HP column (GL-Sciences; 3 μm, 4.6 mm × 150 mm). Sulfuric acid at 1.0 mM concentration was used as the mobile phase at a 0.8 mL/min flow rate, and chromatographic peaks were detected using a photo-diode array (210 nm). The oven temperature was maintained at 30 °C.

Electrochemical analyses

The overall cell voltage was measured across a fixed external resistance of 10 kΩ using a data acquisition system (Lab jack U6-PRO, USA) every 5 min with application software (LJLogUD V1.20). The Coulombic efficiency (CE) was estimated by normalizing the electrons equivalent to the electric current produced by the COD removed during batch cycles as described in our previous study [35]. Polarization and power curves were performed using a single-cycle approach by recording the pseudo-steady-state voltage across different external resistors of 10 MΩ to 500 Ω in reducing order stepwise. We calculated current and power densities by normalizing current and power by liquid working volume, respectively. Cyclic voltammetry (CV) was carried out using an electrochemical workstation (Voltamaster 6 potentiostat (PST006)), in which the anode was used as the working electrode while the air cathode and an Ag/AgCl electrode were used as the auxiliary and reference electrodes, respectively. CV was recorded in the potential window of - 1000 to 1000 mV at a scan rate of 5 mV/s. Electrochemical impedance spectroscopy (EIS) was carried out using an electrochemical workstation (VSP, Bio-Logic, Clarix, France) over a frequency range of 100 kHz to 0.1 Hz at OCV with a perturbation signal of 10 mV.

DNA extraction and high-throughput sequencing

At the end of batch MFCs cycles, the entire biofilms biomass was harvested using a sterile pipet tip, re-suspended in a sterile centrifuge tube containing DNA-free PBS, and concentrated by centrifugation of the entire content at 10,000 *g* for 15 min. Finally, the biomass samples were stored at – 20 °C before DNA extraction. The total genomic DNA was extracted using the PureLink™ Microbiome DNA Purification kit (Invitrogen™, USA) following the manufacturer's instructions. The quality and quantity of the extracted DNA were determined using a nanodrop spectrophotometer (Thermo Scientific, USA) by monitoring absorbance at 260

and 280 nm. High-throughput microbial community analysis was performed using a MiSeq Illumina sequencer (Illumina Inc., USA) and the bar-coded primer combination (5′–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3′)/(5′–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC –3′) for amplifying V3/V4 regions of the bacterial 16S rRNA gene according to the manufacturer's guidelines. The raw sequence data were analyzed using QIIME 2 software [36]. After trimming off low-quality reads and chimeric sequences, taxonomic classification was performed at 97% sequence similarity, and taxonomy was assigned to operational taxonomic units (OTUs) by using the Ribosomal Database Project (RDP) classifier with a 50% confidence threshold [37]. The Alpha and beta diversity analyses were performed using python script in the QIIME software.

Results and Discussion

Electricity generation and substrate utilization with different anodic inocula

Before the bioaugmentation experiment startup, MFCs, which were inoculated with anaerobic sludge, were operated in batch-fed mode with acetate-containing media (20 mM) for 30 days. We observed a comparable open-circuit potential for all MFCs, implying a successful enrichment of the bacterial community (data not shown). Following the successful acclimation period, MFCs were bioaugmented with three bacterial isolates and their performance, in terms of electricity generation and cellulose degradation, compared to control MFC (Non-B MFC) (Figure 1a). Interestingly, MFC bioaugmented with *Acinetobacter tandoii* (MFC-AT) exhibited the highest electricity generation efficiency (maximum voltage = 0.56 V; 56 µA at 10 KΩ), which was ~ 1.8 - 2.1–fold higher than other tested MFCs (i.e., maximum voltage = 0.32 V and 32 µA for Non B-MFC; maximum voltage = 0.30 V and 30 µA for MFC-ME; and maximum voltage = 0.27 V and 27 µA for MFC-BB).

Remarkably, bioaugmented MFCs showed a long-term performance for electricity production, which is an essential prerequisite to assessing the success of bioaugmentation in order to ensure better chances to transfer the desired characteristics to native microflora. A likely reason for high MEC-AT efficiency was that *Acinetobacter tandoii* relieved the bottleneck for efficient utilization of cellulose. *Acinetobacter tandoii* was known for its high capacity to oxidize hardly-biodegradable donor substrates [38], resulting in an efficient synergistic partnership with native anodic microflora and enhancing electricity generation. However, other bioaugmented strains exhibited less electricity generation, comparable to Non-B MFC (p-value > 0.05), implying high metabolic competition among anodic microflora.

In addition, we observed that the cellulose utilization and removal (expressed as mg COD $_{removed}$/L) varied as a function of bioaugmented strains (Figure 1b). Consistent with electricity generation profile, MFC-AT showed the highest COD removal efficiency (i.e., 93 ± 0.93 %) and cellulose utilization rate (i.e., 80 g COD/m^3.d) compared to 86 ± 3.9 % and 74 g COD/m^3.d; 84 ± 5.5 % and 72 g COD/m^3.d; and 81 ± 7.4 % and 69 g COD/m^3.d for MFC-BB, Non-B MFC, and MFC-ME, respectively. Consequently, we observed that ~ 36 % of removed cellulose was channeled to electricity generation for MFC-AT, achieving the highest CE followed by MFC-BB (23 ± 2.5 %), Non-B MFC (21 ± 0.06 %), and MFC-ME (19 ± 1.6 %). Our results reveal that the high cellulose utilization in bioaugmented MFCs most likely attributed to multiple metabolic responses manifested as a result of the enhanced syntrophic interactions among anodic consortia, which may not be possible with native consortia. In addition, higher MFC efficiency in terms of electricity production, CE, and cellulose removal, confirms that significant cellulose utilization was possible if hydrolysis and fermentation were substantially improved [18].

Figure 1c displays the fermentation efficiency (expressed as mg SCFA-COD/mg $COD_{removed}$) for the bioaugmented MFCs and Non-B MFC. Among all tested MFCs, MFC-AT exhibited the highest fermentation efficiency (0.37 ± 0.02 mg SCFA-COD/$COD_{removed}$) followed by Non-B MFC (0.25 ± 0.04 mg SCFA-COD/$COD_{removed}$), MFC-ME (0.2 ± 0.04 mg SCFA-COD/$COD_{removed}$, and MFC-BB (0.19 ± 0.01 mg SCFA-COD/$COD_{removed}$). Citrate was the most abundant SCFA with the highest accumulation rate during the fermentation of cellulose, followed by acetate, which was the second-largest, and valerate had a low concentration. These results suggest that *Acinetobacter tandoii* improves the fermentation efficiency of cellulose in MFCs. Given that acetate was one of the most preferred donor substrates for EAB [27, 39], its low accumulation in

bioaugmented MFCs, especially MFC-AT, implies high EAB activity.

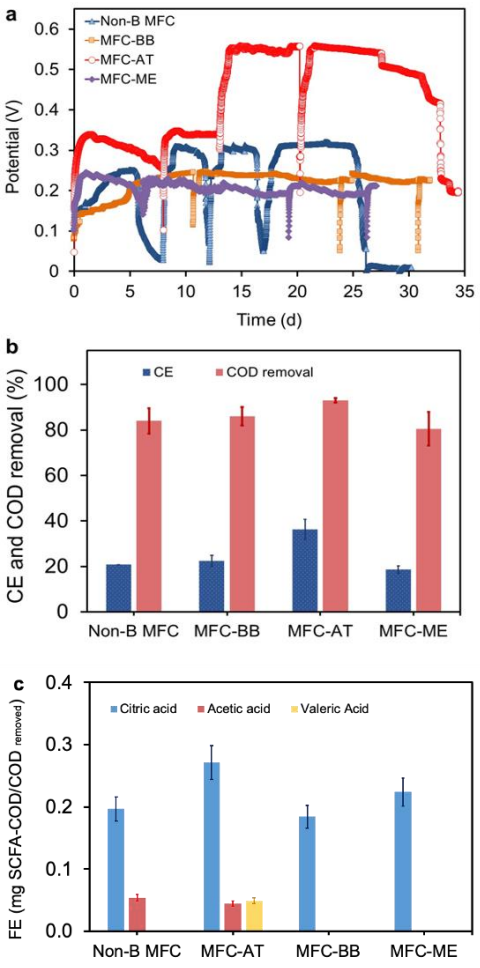

Figure 1. (a) Steady-state potential evolution versus time. (b) COD removal and Coulombic efficiencies. (c) Fermentation efficiency of control and bioaugmented MFCs. Error bars represent the standard deviation of three replicates.

Bioelectrochemical analysis

Power and polarization curves were performed to evaluate the overall performance of MFCs after long-term operation. Poor enrichment of anodic biofilm often causes high internal resistance due to sluggish electron transfer into the anode surface in MFCs. Figure 2a shows a significant voltage drop in the low current region for Non-B MFC and MFC-ME compared to MFC-AT and MFC-BB, indicating more activation losses and sluggish electron transfer rate. Among the 5 tested cathode catalysts, MFC-AT exhibited a superior activity with a maximum power density of (PD_{max}) of ~ 373 mW m^{-3}, which was ~ 1.55-fold higher than that of Non-B MFC (240 mW m^{-3}) and ~ 2.52-fold higher than that of MFC-ME (148 mW.m^{-3}) (Figure 2b). The improved power generation with *Acinetobacter tandoii* was probably attributed to the high electrochemical activity of anodic biofilm in MFC-AT. On the other hand, MFC-ME showed much lower power output due to the poor fermentation efficiency of cellulose, resulting in a low accumulation of readily available fermentation by-products, and hence much low EAB activity [27]. The anode acts as an electron acceptor, and its nature and structure might alter the nature of the EAB community. In the interfacial region between the electrode and the solution, electrode responses normally occur where charge distribution differs from bulk phases. The optimal external resistance based on the polarization curves was 1000 Ω for bioaugmented reactors and 5000 Ω for Non-B MFC. Given that we used MFCs with the same configuration (e.g., anode material and size, donor substrate, and cell volume), the differences in the overall performance of MFCs were mainly attributed to the metabolic capability of the anodic microbial community to degrade cellulose, producing simple fermentation by-products, which can be efficiently utilized by EAB biofilm [37].

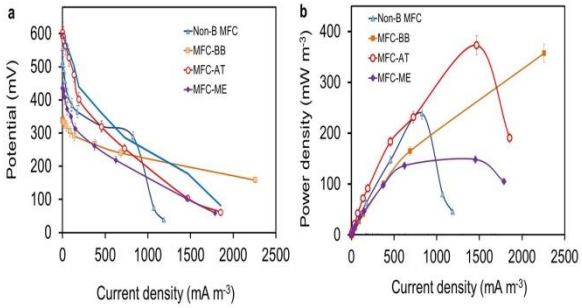

Figure 2. (a) Polarization curve. (b) Power curve of control and bioaugmented MFCs after 18 weeks of operation.

We performed CV analysis for MFCs in a potential window of − 1000 to 1000 mV at a scan rate of 5 mV s^{-1} (Figure 3a). For all bioaugmented MFCs, we observed two distinguished oxidation peaks at ~ − 0.04 V (vs. Ag/AgCl) and 0.28 V (vs. Ag/AgCl), and one reduction peak at ~ − 0.34 V (vs. Ag/AgCl). Although a couple of the distinct redox oxidation peaks with the same peak shape were observed for all bioaugmented MFCs, they were of significantly different peak heights and peak positions. Among all bioaugmented MFCs, MFC-AT showed the highest redox catalytic currents, followed by MFC-BB and MFC-ME, implying

more electrochemical activity and robustness of anodic biofilm. On the other hand, Non-B MFC exhibited a different pattern for having only an oxidation and reduction peak at – 0.04 and – 0.43 V, respectively. Redox-peak height differences were related to the number of redox-active components present in the biofilms, showing the catalytic activity to convert substrates into electricity. The redox peak positions were linked to various types of redox-active components, which may reflect a change in the community composition of the anodic biofilm. The voltammetric profiles indicated a higher charge for bioanode of MFC-AT (254 mC) followed by MFC-BB (138 mC), MFC-ME (187 mC), and Non-B MFC (68 mC), which showed higher availability of electrons on the anode surface of bioaugmented MFCs compared to Non-B MFC. This might be attributed to enhanced electron transfer and consequently higher EAB activity.

Figure 3a shows the Nyquist curves and electrochemical impedance fitting results for all tested bioanodes. We observed that all MFCs exhibited a single characteristic impedance semi-circle with different R_{ct} values, which follows the following order: ME-MFC (16.64 Ω) > Non-B MFC (13.25 Ω) > AT-MFC (7.78 Ω) > BB-MFC (5.15 Ω). Lower values of R_{ct} for MFC-AT and MFC-BB were mainly attributed to their ability to facilitate the electron transfer to the anode surface, reducing the voltage losses of anodic reactions and increasing the current generation.

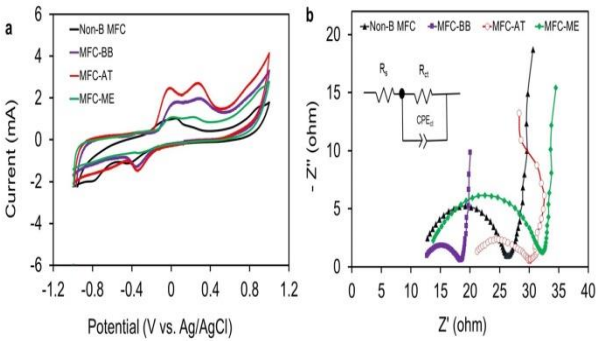

Figure 3. (a) Cyclic voltammogram. (b) Nyquist plots of different bioanodes (inset picture shows the equivalent circuit used for data fitting).

Table 1: The charge transfer resistance (R_{ct}) and solution resistance (R_s) values of MFCs

	Solution resistance (R_s)	Charge transfer resistance (R_{ct})
MFC-AT	22.43	7.775
MFC-BB	13.31	5.154
MFC-ME	14.91	16.64
Non-B MFC	13.44	13.25

Microbial community analysis

Figure 4a shows the bacterial sequence results of the V3/V4 region in the bacterial 16S rRNA gene in the anodic biofilm samples at the family level. The majority of bacterial sequences were belonged to four phyla: *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Planctomycetota*, which was in agreement with previous research. Members of *Bacteroidetes* and *Firmicutes* were well-known to ferment polysaccharides, whereas members of *Proteobacteria* phyla can perform anode respiration [27, 40]. On the other hand, *Planctomycetes*-dominant biofilm was commonly found in the MFCs bioanodes fed with fermentable substrates [41]. Although members of *Proteobacteria* phyla were known to perform anode respiration, the *Planctomycetes*-dominant biofilm might be a feature of the anodic EAB microbial community fed with complex polysaccharides (e.g., cellulose). The high relative abundance of *Proteobacteria* phylum in the biofilm samples of MFC-AT and MFC-BB was consistent with our previous results, confirming that bioaugmentation gave the anodic EAB community an ecological advantage, which improves electron recovery from cellulose.

Figure 4b shows the results of the Principal Coordinate analysis, in which principal components (PC) 1 and 2 described 61.7 % and 17.0 % of the bacterial sequence variations, respectively. We observed that the overall MFC efficiency, in terms of cellulose utilization and electricity production, correlated well with the PC2 vector. Within the *Proteobacteria* phylum, we observed an increase in the relative abundance of the *Rhodocyclaceae* family in MFC-AT and MFC-BB compared to Non-B MFC, which was known for performing anode respiration and electricity generation in MFCs [42], which followed by *Lentimicrobiaceae*, *Rubinisphaeraceae*, and *Chthoniobacteraceae*. This increase in the relative abundance was associated with a decrease in the relative abundance of several families, including *Synergistaceae*, *Rikenellaceae*, *Dysgonomonadaceae*, and *Victivallaceae*.

More importantly, the relative abundance of several phyla (i.e., *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*) increased in MFC-AT and MFC-BB, which was correlated to an increase in electricity generation and cellulose utilization rate. The genera within these phyla were often detected in MFCs owing to their capability to ferment complex donor substrates and respire electrons to anode surface,

which has a crucial influence on electricity generation in MFCs fed with complex substrates (e.g., cellulose). Our findings suggest that the high efficiency of MFC-AT and MFC-BB in comparison to other MFCs was due to a synergistic interaction between the bioaugmented strains (*Acinetobacter tandoii and Brevundimonas bullata*) and native microbial consortia, which increases the electron transfer rate and cellulose utilization. This high efficiency of MFCs was associated with an increase in the relative abundance of the *Proteobacteria* subgroup with *Geobacter* as the predominant genus (Figure 5).

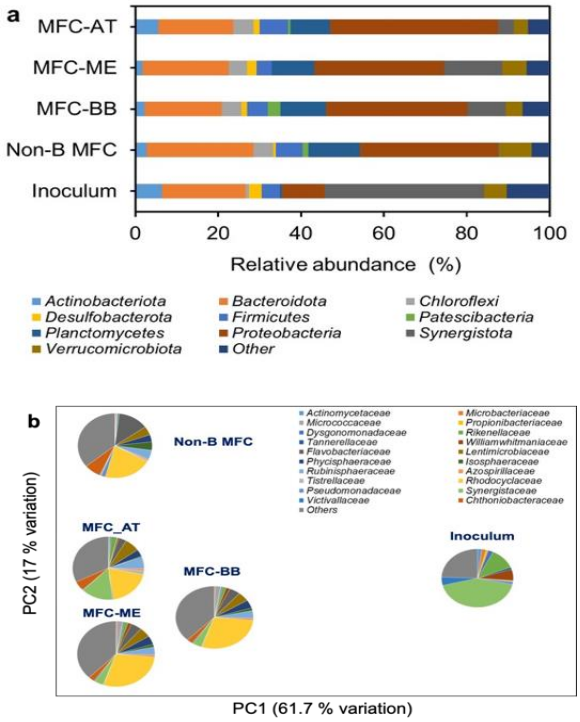

Figure 4. Bacterial community distribution at the phylum level (Phyla that have < 1% of total sequences are grouped as "others"). (b) Principal Coordinate analysis displays that the relative abundance of family-level phylotypes on the Principal Coordinates

On the other hand, MFCs bioaugmented with *Micrococcus endophyticus* exhibited much lower efficiency, in terms of electricity production, cellulose utilization, and CE. A likely reason for this low efficiency was the poor capability of *Micrococcus endophyticus* to ferment cellulose and produce simple substrates that could be efficiently consumed by EAB [43].

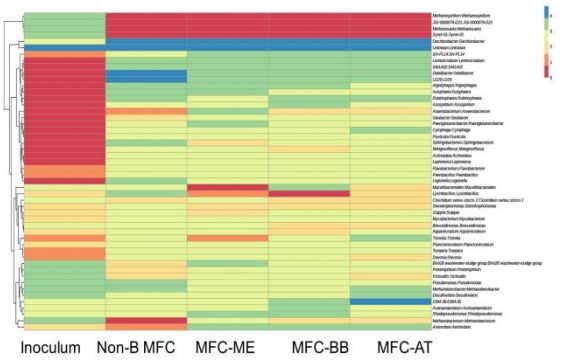

Figure 5. Phylogenetically clustered heatmap showing the relative abundance of the major genera in anodic microbial communities

Bacterial diversity within samples (alpha diversity) was estimated using richness and three diversity indices, Simpson diversity index, Shannon diversity index, and Chao-1 richness. The sequencing indices of the community diversity analysis were presented in Table 2. Our results indicate that the inoculum had the highest richness and diversity, followed Non-B MFC and MFC-ME, while MFC-AT and MFC-BB had the lowest diversity. These results might be attributed to the selection of microbial communities that can generate electricity from cellulose (Figure 6).

Table 2: Richness and diversity indices for bacterial sequences

	Observed	**chao 1**	**ACE**	**Shannon**	**Simpson**
Inoculum	4831	5403	5401	7.58	0.99899
MFC-BB	3163	3376	3376	7.45	0.99911
MFC-ME	3345	3576	3560	7.31	0.99868
MFC-AT	2743	2852	2847	7.32	0.99900
Non B-MFC	3413	3699	3712	7.31	0.99889

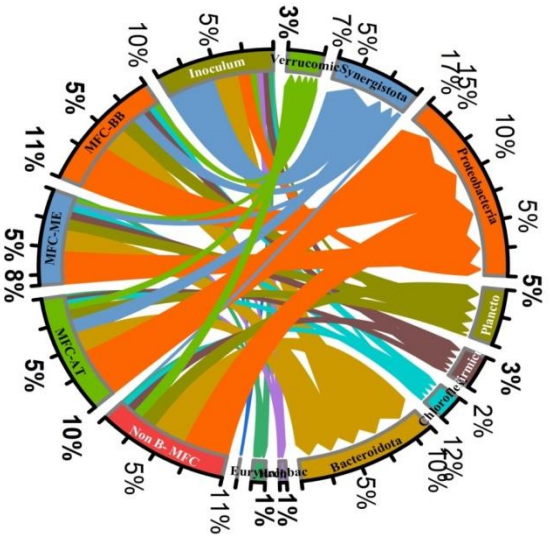

Figure 6. Chord diagram representing the microbial communities of different anodic biofilms

Implications and engineering significance

The possibility of converting cellulosic biomass into renewable energy has recently become a research hotspot due to its low cost and vast supply. However, traditional MFCs exhibited minimal capability to produce electricity from cellulosic biomass due to the low biodegradability of the cellulose [18]. In our study, we used the bioaugmentation strategy to enhance the synergistic partnership among the functional mixed-culture anodic consortia in MFCs. In our experimental setup, cellulose is hydrolyzed and fermented into glucose, which is subsequently metabolized into SCFAs (mainly acetate, citrate, and valerate) through Embden–Meyerhof–Parnas (EMP) glycolytic pathway as illustrated in Figure (7). Relieving the cellulose hydrolysis and fermentation bottlenecks resulted in efficient consumption of simple SCFAs by the EAB community. Consequently, the existence of bioaugmented strains remarkably altered the anodic microbial community structure. Our results reveal that it was possible to generate electricity from complex donor substrates if the fermentation bottleneck was significantly relieved by establishing a syntrophic partnership between fermenting bacteria and EAB. The bioaugmented systems developed in this study pave the path for efficiently converting hardly-biodegradable cellulosic biomass into electricity using a cellulose-degrading strains-based MFC. Our findings may potentially give a generalizable strategy for reducing cellulose content in several polluted systems in both engineering and natural environments with similar environmental conditions.

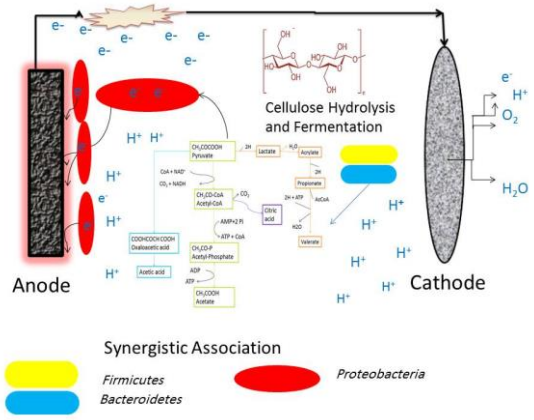

Figure 7. Schematic diagram for the synergistic interaction between Firmicutes , Bacteroidetes and Proteobacteria in bioaugmented microbial fuel cell.

Conclusion

In this study, bioaugmented systems were built up by supplementing cellulose-degrading isolates (i.e., *Acinetobacter tandoii*, *Brevundimonas bullata*, and *Micrococcus endophyticus*) into mixed-culture MFCs fed with cellulose as the sole donor substrate. In terms of electricity generation, cellulose utilization, and microbial community structure, their efficiency was evaluated and compared with a non-bioaugmented MFC (Non-B MFC). Bioaugmenated MFCs with *Acinetobacter tandoii* and *Brevundimonas bullata* exhibited high maximum power densities of 373 and 357 mW m^{-3}, respectively, associated with near-complete removal of cellulose. Compared with Non-B MFC, the bioaugmentation approach seems to relieve the cellulose and fermentation bottleneck, evidenced by high fermentation efficiency and accumulation of SCFAs. Upon supplementing cellulose-degrading isolates, the microbial community structure was shifted towards more predominant phyla that have the capability to ferment complex substrates and respire recovered electrons to the anode surface (i.e., *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*). In conclusion, these findings reveal that the bioaugmentation approach could enhance fermentation efficiency and anode respiration by establishing syntrophic association among anodic consortia in MFCs fed with hardly-biodegradable complex substrates. In addition, our results would help guide future efforts of developing more sustainable and economically flexible MFCs.

Conflicts of interest

The authors declare no competing interests

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