

Article

Microbial Structure and Energy Generation in Microbial Fuel Cells Powered with Waste Anaerobic Digestate

Dawid Nosek * and Agnieszka Cydzik-Kwiatkowska

Department of Environmental Biotechnology, University of Warmia and Mazury in Olsztyn, Słoneczna 45 G, 10-709 Olsztyn, Poland; agnieszka.cydzik@uwm.edu.pl

* Correspondence: dawid.nosek@uwm.edu.pl; Tel.: +48-89-523-4144; Fax: +48-89-523-4131

Received: 19 July 2020; Accepted: 7 September 2020; Published: 10 September 2020



Abstract: Development of economical and environment-friendly Microbial Fuel Cells (MFCs) technology should be associated with waste management. However, current knowledge regarding microbiological bases of electricity production from complex waste substrates is insufficient. In the following study, microbial composition and electricity generation were investigated in MFCs powered with waste volatile fatty acids (VFAs) from anaerobic digestion of primary sludge. Two anode sizes were tested, resulting in organic loading rates (OLRs) of 69.12 and 36.21 mg chemical oxygen demand (COD)/(g MLSS·d) in MFC1 and MFC2, respectively. Time of MFC operation affected the microbial structure and the use of waste VFAs promoted microbial diversity. High abundance of *Deftia* sp. and *Methanobacterium* sp. characterized start-up period in MFCs. During stable operation, higher OLR in MFC1 favored growth of exoelectrogens from *Rhodopseudomonas* sp. (13.2%) resulting in a higher and more stable electricity production in comparison with MFC2. At a lower OLR in MFC2, the percentage of exoelectrogens in biomass decreased, while the abundance of genera *Leucobacter*, *Frigoribacterium* and *Phenylobacterium* increased. In turn, this efficiently decomposed complex organic substances, favoring high and stable COD removal (over 85%). Independent of the anode size, *Clostridium* sp. and exoelectrogens belonging to genera *Desulfobulbus* and *Acinetobacter* were abundant in MFCs powered with waste VFAs.

Keywords: MFC; *Rhodopseudomonas*; anode surface; waste VFA; NGS

1. Introduction

In view of the increasing demand for electricity, sustainable production of electricity from renewable sources by microorganisms is considered an alternative to the generation of energy based on fossil fuels. One of the most attractive solutions are Microbial Fuel Cells (MFCs), which use the metabolism of microorganisms to produce electricity by oxidizing organic substances on the anode and transferring the produced electrons to the cathode without using mediators [1,2].

Most studies on MFCs have been conducted using simple substrates such as glucose or acetate [3,4]. The use of pure acetic acid or its salt in the form of sodium acetate allows for the efficient production of electricity at a high coulombic efficiency [5,6]. Unfortunately, such solution is expensive. To develop economical MFC technology, electricity production should be associated with waste management. The use of complex waste substrates may, however, result in a lower efficiency of electricity generation because microorganisms need time to degrade the complex organic compounds, often present in form of suspensions. To date, waste substances such as molasses, wastewater or food waste have been used to power MFCs [7–9]. The use of wastewater streams rich in short chain fatty acids (volatile fatty acids (VFAs)), which are formed as a result of hydrolysis of organic compounds during acid fermentation,

is an environment-friendly solution. Freguia et al. [10] investigated the use of VFAs in two-chamber MFCs. After about 30 days of operation, they reached a constant current level of 21 ± 2 mA, and mainly acetate and propionate were taken as electron donors. The remaining VFAs, i.e., butyric, valerian and capron acids, were removed at a lower rate. For waste treatment, MFC competes directly with anaerobic digestion (AD), which is already widely practiced and also commercially implemented in large factories, albeit no electricity is directly produced. In comparison with conventional AD, the MFC technology holds specific advantages, such as its applicability for the treatment of substrates with low concentration at temperatures below 20 °C. This provides specific niche applications that do not compete with, but complement, the AD technology.

The conversion of biomass to electricity depends on the composition of the microbial community. Microorganisms in MFC may play different roles. The most important group from the perspective of electricity generation are exoelectrogens, which transfer electrons to the anode [11]. Electrochemically inactive microorganisms are also important because they degrade complex substrates to a simpler one that can be used by exoelectrogens [12,13]. Some microorganisms support defense properties of biofilm and protect other bacteria from unfavorable environmental conditions e.g., a presence of heavy metals [14]. It has been shown that in acetate-based biofilms *Deltaproteobacteria* transfer electrons directly to the anode, while *Clostridia* are responsible for the transformation of complex substrates [15,16]. In MFCs fed with acetate, abundant populations of *Synergistetes*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes* have been observed in anode biofilms [17]. The main groups of anode microorganisms in a two-chamber MFC with graphite electrodes powered with food waste were *Firmicutes*, *Bacteroidetes* and *Proteobacteria* represented by classes *Bacilli*, *Bacteroidia*, *Deltaproteobacteria* and *Gammaproteobacteria* [18]. The predominant exoelectrogen was *Geobacter* sp., that directly transported electrons to the anode using highly conductive type c cytochromes and pili.

The up-scaling of MFCs requires investigations on new materials and reactor configurations because the enlargement of MFCs increases internal electricity losses, which results in a relatively low output power. Peixoto et al. [19] indicated the suitability of compact, flat MFCs for wastewater treatment as a solution for autonomous service in decentralized wastewater treatment systems. A larger active anode surface can increase the MFC output power until the cathode reaction becomes limited, because the large surface provides more space for microorganisms [20]. The impact of anode size on electricity generation in MFCs was investigated by Lanas et al. [21] and Rossi et al. [22]. Lanas et al. [21] used carbon fiber brushes and sodium acetate as a substrate in single-chamber MFCs. In batch mode, the configuration with three larger brushes (25 mm diameter) produced 80% more power than reactors with eight smaller brushes. Rossi et al. [22] used graphite fiber brushes and primary clarifier effluent in single-chamber MFCs. Reducing the diameter of the anode brushes from 5.1 to 2.5 cm did not improve the anode performance, however, the electrode spacing and hydraulic flow were important for MFC performance. Information about the impact of anode size on microorganism and energy production in MFCs powered with complex waste substrates is, however, insufficient and suggests the novelty of the study.

The aim of this study was to examine to the effect of the anode size on the microbiological structure of the anode and the efficiency of energy generation in MFCs fed with waste organics. In the study, volatile fatty acids (VFAs) from AD of primary sludge were used to generate electricity in line with the assumptions of a circular economy.

2. Materials and Methods

2.1. Reactor Set-Up

The experiment was carried out in two identical dual-chamber reactors (H type) made of Plexiglas with cathode and anode chambers, each with a volume of 1L; the active volume of the anode chambers was 700 mL. A Nafion 117 (Alfa Aesar) proton exchange membrane (PEM) with a surface of 8.5 cm² was placed between anode and cathode chamber as shown in Figure S1. PEM was treated with 1M

HCl for 30 min and then rinsed with distilled water before use. The electrodes were made of carbon felt (CGT Carbon GmbH, Asbach, Germany) and, without pre-treatment, they were applied to a Zn-Fe wire. In MFC1, the anode was made of 20 carbon felt discs (each disc with a 30 cm² surface, total surface area was 600 cm²). In MFC2, the anode had a surface area of 1200 cm² (40 carbon felt discs). In both MFCs the cathode surface was 70 cm². The external circuit, connecting the anode and cathode, was made of copper wire connected with a 1-kΩ resistor. The catholyte consisted of 5 g NaCl and 50 mL of phosphate buffer in 1L of distilled water. The catholyte was adjusted to pH 7 with 1M NaOH and 1M HCl.

2.2. Medium and Operation

MFCs were inoculated with 50 mL of aerobic granular sludge from a full-scale wastewater treatment plant in Lubawa (Poland). The microbial characteristics of the inoculum are presented in Świątczak and Cydzik-Kwiatkowska [23]. The process was carried out using a VFAs mixture from AD of primary sludge in a wastewater treatment plant in Olsztyn (Poland) as an electron donor. Characteristics of the VFAs mixture were as follows: 2049 ± 505.5 mg COD/L; 1512.5 ± 164.3 mg BOD₅/L; 648 ± 123.2 mg VFA/L; 192.2 ± 32.1 mg TN/L; 45.4 ± 5.9 mg TP/L; 1750.0 ± 132.0 mg TSS/L. VFAs mixture was sieved through a 2-mm sieve and stored at 4 °C. The anolyte was replaced every 24 h and mixed with crude VFAs in such a ratio that the COD value was about 750 mg COD/L. The pH was adjusted to 7 with 1M NaOH and 1M HCl. The organic loading rate (OLR) in both MFCs was 750 mg COD/(L·d) corresponding to 69.12 and 36.21 mg COD/(g Mixed Liquor Suspended Solids (MLSS·d)) in MFC1 and MFC2, respectively. The MFCs were run for 90 days at room temperature, the OLRs were kept constant throughout the experiment.

2.3. Chemical Analyses

Chemical analyzes of the effluent from the anode chamber included COD concentration, VFA and pH. COD was determined by the cuvette test (Hach Lange, Wrocław, Poland), VFA by distillation according to APHA [24] and pH using TitroLine (Donserv, Warsaw, Poland). Biomass was determined by the weighting method in accordance with APHA [24]. Polarization and power curves were determined according to Watson and Logan [25] using a True-RMS multimeter changing the external resistance of the cell in the range of 30–120,000 Ω. For data collection, the 6600 Counts PC-LINK data acquisition unit was used to record voltage changes every minute. The current was calculated from external resistance using Ohm's law. All calculations were carried out in accordance with Logan et al. [1]. Coulombic efficiency (CE) was determined by using the Equation (1):

$$CE = \frac{M \int_0^{t_h} Idt}{F \cdot b \cdot v \cdot \Delta COD} \quad (1)$$

where M is molecular weight of oxygen, F is Faraday's constant, b is a number of electron exchanged per mole of oxygen, v is volume of liquid in the anode chamber and ΔCOD is change in COD concentration over time (t_h).

2.4. Microbial Analyses

During the operation of the reactors, samples of inoculum and biomass and supernatant from MFC1 and MFC2 powered with VFAs were collected to analyze microbial composition with next-generation sequencing (NGS). The biomass was collected at 6th, 42th, 61th, 72th, and 87th day of the experiment. The mixture of biomass and supernatant was centrifuged at 13,000 rpm for 10 min. The collected pellets were stored at −20 °C and at the end of the experiment DNA was isolated from all samples using FastDNA[®] SPIN[®] Kit For Soil (Q-BIO gene). The quantity and quality of DNA was assessed with the use of NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and agarose electrophoresis. The samples were then amplified using primers

515F/806R (GTGCCAGCMGCCGCGGTAA/GGACTACHVGGGTWTCTAAT) targeting the V4 region of the bacterial 16S rDNA gene and sequenced using MiSeq Illumina Platform in Research and Testing Laboratory (USA). Over 400 thousand full sequences were obtained (Table 1). The obtained sequences were analyzed bioinformatically as described in Świątczak and Cydzik-Kwiatkowska [23]. Because the samples were obtained in the same run and characterized by similar amounts of sequences, to avoid loss of data, the data were not normalized. For calculations and visualizations, the ampvis2 package [26] for R version 3.6.2 [27] in the RStudio environment, version 0.99 were used. Diversity of samples was assessed at a genus level using the Shannon index of diversity [28]. A value of $p \leq 0.05$ was defined as significant. The sequences have been deposited in the NCBI Sequence Read Archive (SRA) as the experiment entitled “MFC reactors Raw sequence reads” (Accession: PRJNA646600).

Table 1. Molecular indicators in Microbial Fuel Cell (MFC)1 and MFC2.

Day	MFC1			MFC2		
	Operational Taxonomic Units (OTUs)	Shannon	Reads	OTUs	Shannon	Reads
6	1019	3.963	38,439	1026	4.548	40,590
42	466	2.934	43,939	673	4.044	32,622
61	1026	4.869	57,866	1155	5.423	40,904
72	984	4.808	40,880	1027	5.219	37,040
87	905	4.907	41,054	823	4.906	41,304

The technological and molecular results were statistically analyzed in the Statistica 13.1 (StatSoft) using Student’s t-test. The results were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Electricity Generation

After the addition of a fresh substrate, cell voltage increased rapidly to 50–70 mV (Figure 1a) and 80–90 mV (Figure 1b) in MFC1 and MFC2, respectively, and then gradually decreased to about 20–30 mV.

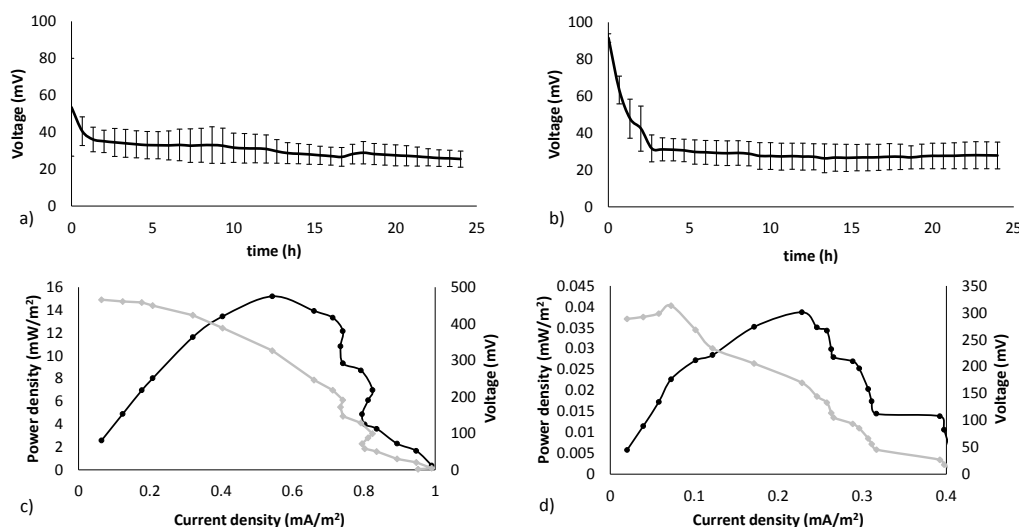


Figure 1. The averaged voltage in an MFC cycle ($n = 7$) in (a) MFC1 and (b) MFC2 and polarization (grey line) and power curves (black line) in (c) MFC1 and (d) MFC2; bars represent standard deviation; the arrows show the affiliation of the curve to the axis.

Although the anode in MFC2 was larger, it did not result in a better power generation, because the voltages were only initially higher than in MFC1, and after reaching a plateau they oscillated at a

similar level. The current density after reaching the plateau in MFC1 ($836 \pm 128 \mu\text{A}/\text{m}^2$) was about 2.6 times higher than in MFC2 ($316 \pm 101 \mu\text{A}/\text{m}^2$). The power density was $3.5 \text{ mW}/\text{m}^2$ in MFC1 and $1.03 \cdot 10^{-2} \text{ mW}/\text{m}^2$ in MFC2. In studies of Choi et al. [29], it was observed that for 100 h after feeding of MFC (1 k Ω resistance) with VFAs mixture, a stable, low voltage of 174 mV was generated, and the power density was $15.3 \text{ mW}/\text{m}^2$. The higher MFC power than in our study may have resulted from the use of a platinum coating on the cathode: such a coating accelerates the cathode reactions [30].

Power curves show that the power of the cells increased with the increase in external resistance. The highest power obtained in MFC1 was $15.2 \text{ mW}/\text{m}^2$ at a current density of $0.54 \text{ mA}/\text{m}^2$ (external resistance of 10 k Ω) (Figure 1c). Under normal conditions, the cell worked at a lower external resistance of 1 k Ω , which explains the lower power range ($1.3\text{--}3.5 \text{ mW}/\text{m}^2$). Data indicate that increasing the anode surface did not improve the cell power. In MFC2 at an external resistance of 6.2 k Ω , cell power reached a maximum of $0.038 \text{ mW}/\text{m}^2$ and a current density was $0.23 \text{ mA}/\text{m}^2$ (Figure 1d). Similar results to those observed in MFC2 were obtained by Revelo Romo et al. [31] in a two-chamber MFC with a biocathode and a salt bridge. The voltage and power density were 8.0 mV and $0.02806 \text{ mW}/\text{m}^2$, respectively. The authors observed voltage fluctuations that could have been associated with the activity of exoelectrogenic microorganisms, as well as those that are not able to transfer electrons to the anode, but degrade organic matter. In the present study, internal resistance of the cells was determined from the slope of the polarization curves in the linear region where the ohmic losses predominate. In MFC 1, the internal resistance was 10.72 k Ω , while in the MFC2 it was lower (9.12 k Ω), despite a higher ratio of anode to membrane surface. Such high resistances can be explained by the cell configuration: relatively small area of the membrane in relation to the reactor volume, which limited proton flow [22,32], and the long distance between the anode and the cathode.

Lanas et al. [21] investigated how the diameter of anode brush, an anode number and the distance between electrodes affected MFC performance. In batch mode, the configuration with three larger brushes provided 80% more power than reactors with eight smaller brushes. This was due to the greater negative and stable potential of the anode. After moving the smaller brushes closer to the cathode, the power in the batch mode increased significantly from 690 to 1030 mW/m². The distance between the anode and the cathode in our study was much larger (15 cm) than in the studies of Lanas et al. [21] (1.65 cm), which may explain the lower power of the cell. In the present study, PEM fouling was observed (Figure 2) due to the rapid growth of microorganisms fed with waste VFAs. Despite cleaning of the membrane, after several days of operation, a repeated overgrowing effect was observed. Such a phenomenon can reduce the efficiency of energy production in MFCs, due to reduced proton transport.

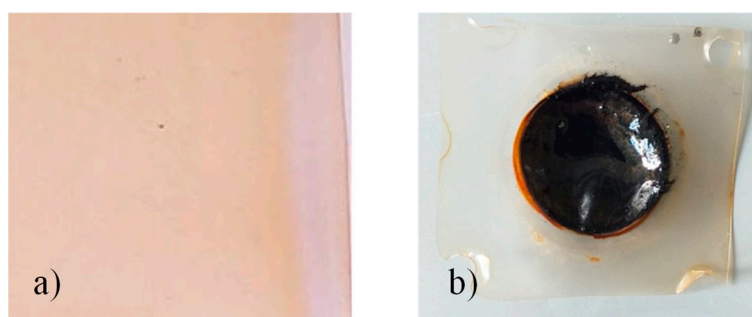


Figure 2. Nafion membrane at (a) day 0 and (b) after 30 days of operation.

Lower OLR in MFC2, despite lower energy production, contributed to more efficient and stable removal of organic compounds. In MFC1 (Figure 3a) the efficiency of COD removal ranged from 55 to 85% and averaged 65% at the end of the experiment. In MFC2, the COD removal efficiency increased from about 55% at the beginning of the experiment to 85% during the cell's stable operation and was significantly higher ($p = 0.03$, $F = 7.17$) than in MFC1. Higher COD removal in MFC2 with a

larger anode surface resulted from a higher biofilm mass (20.71 g/L) compared to MFC1 (10.85 g/L). The stable operation of the cell overlapped with the stabilization of the species composition of the microorganisms on the anode. The CE in both cases was low, indicating that only a limited fraction of organic matter was converted into electricity. In MFC2, a decrease in the CE value was noticeable during the experiment (Figure 3b). The highest CE was 0.024 and 0.028% in MFC1 and MFC2, respectively. Low CE could be caused by the consumption of electrons by microorganisms that do not transfer electrons to the electrode.

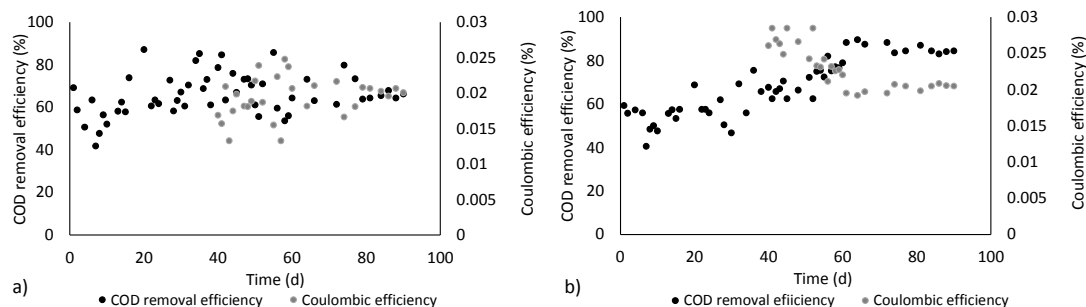


Figure 3. Chemical oxygen demand (COD) removal and coulombic efficiency in (a) MFC1 and (b) MFC2.

To conclude, while using VFAs to generate energy in MFCs, the cell should be designed to minimize the internal resistance and a rapid biofilm fouling. In the present study, more electricity was generated in MFC with a smaller anode, i.e., in MFC operated at the higher OLR. A large anode surface, although beneficial for the development of microorganisms and a more effective and stable decomposition of the complex substrate, was not conducive to energy generation. Larger anode surfaces can cause a large dispersion of electrons, some of which are consumed in other metabolic processes, which increases electron losses.

3.2. Microbial Community

Analysis of rarefaction curves indicated that in all samples the sequencing depth was sufficient (Figure S2). The results of NGS indicated that the number of operational taxonomic units (OTUs) did not differ significantly between MFC1 and MFC2 (Table 1). The largest difference in the number of OTUs was recorded on day 42, indicating a sharp decrease in the diversity of microorganisms in both MFCs. From this point, the Shannon index value increased in both MFCs reaching around 4.9 at the end of the study.

Principal Component Analysis (PCA) showed that the time of operation significantly affected the composition of microbial community in anode biofilm in MFCs (Figure 4). The most abundant groups of microorganisms throughout the entire experiment were *Rhodopseudomonas*, *Acidovorax*, *Acinetobacter*, *Clostridium*, *Methanobacterium* and *Leucobacter* (Figure 5). For both MFCs at the end of the study there was a large number of sequences that were not yet classified in databases. A high percentage of unclassified microorganisms indicates that the potential of microorganisms important for electricity generation in MFC powered with waste VFAs is still to be discovered.

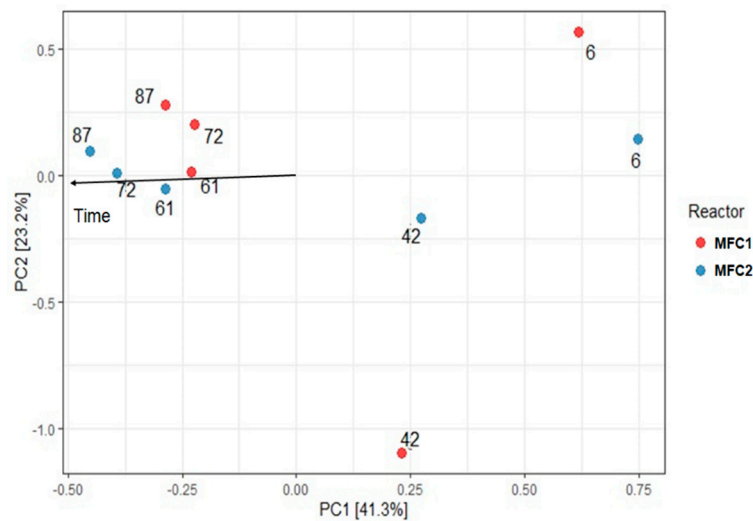


Figure 4. Results of PCA; numbers next to points indicate days of sampling during the MFC operation.

Unclassified	19.5	8	23	22.8	28.1	21.3	16.8	26.6	20.6	17
Rhodopseudomonas	28.9	2.9	10.4	17	13.2	15.8	19.3	2	2.6	2
Acidovorax	18.3	17	5.3	1.7	1.6	5.2	12.2	7.9	5.1	3.3
Acinetobacter	1	18.9	11	7.3	3.7	0.1	3.1	5.6	7	2.6
Clostridium	1.6	1.4	5.5	4.9	5.9	5.2	5.3	5.7	5.5	6.4
Deftia	0.7	27.8	0.1	0	0	9.2	5.9	0.2	0.1	0.1
Methanobacterium	2.1	0.3	2.5	3.9	3.5	2.4	12.7	4.6	3.3	4.6
Leucobacter	0	1.7	3.7	4.5	3.9	0.1	0.8	3.8	7.2	11.5
Candidatus Competibacter	6.1	0	0.1	0.1	0	10.5	0	0	0	0
Phenylobacterium	0.1	0.3	1.4	1.6	2.5	0.1	0.8	1.4	3.5	4.8
No Hit	0.9	0.4	1.8	1.5	1.6	1	0.2	2.5	2.9	3.3
Pseudomonas	0.4	1.5	3.9	3.1	0.3	1.7	1.7	1.2	2.2	0.1
Frigonibacterium	0	0.6	1	1.4	1	0	0.5	1.5	3.4	5.2
Desulfobulbus	0.1	0	1.7	1.8	3.6	0	0.1	1.3	2	2.8
Stenotrophomonas	0	2.9	1.1	0.7	0.5	0.2	2.8	0.1	0.9	0.8
Legionella	0.2	0.1	2	1.2	0.9	0.2	0.3	0.4	1.2	2.7
Anaerovorax	0.5	0.2	0.8	1.1	1.3	0.7	1.4	1.2	1	0.8
Fusibacter	1	0.5	0.3	0.5	0.4	2.1	1	0.9	0.7	0.4
Rhizobium	0.4	0.2	0.7	0.7	1.4	2.1	0.1	0.2	0.4	1.4
Aminobacterium	0.3	0.2	1.5	1.5	1.6	0	0.2	0.4	0.9	0.8
Methanobrevibacter	0.3	0	0.6	0.6	0.4	0.1	0.3	2	1.5	0.8
Bacteroides	0.5	0.3	1.1	0.7	0.4	0.7	1.6	0.9	0.3	0.1
Thaueria	0.3	0.3	0.1	0.1	0.3	0.2	0.1	4	0.8	0.1
Brevundimonas	0	0.7	0.2	0.2	0.2	0.3	3.1	0.2	0.5	0.8
Hydrogenophaga	1.2	0	0.6	0.4	0.2	0.6	0.1	1.6	1.1	0.2
Trichococcus	0.9	0	0.3	0.3	0.4	1.1	0.2	0.4	0.9	1.1
Fluviicola	0	5.5	0	0	0	0.2	0	0	0	0
Bifidobacterium	0.1	0	0.4	0.6	0.4	0.1	0	1.3	1.2	1.1
Lactobacillus	0.1	0	0.1	0.6	1.4	0.1	0	0.1	0.7	1.8
Pseudonocardia	1.5	0	0	0.1	0	2.5	0.1	0	0	0
	6d	42d	61d	72d	87d	6d	42d	61d	72d	87d

Figure 5. Heatmap presenting the percentage of sequences belonging to particular taxa in MFC1 and MFC2; in the bottom row, days of MFC operation are given.

On day 42, in MFC1 high percentages of *Acidovorax* sp., *Acinetobacter* sp., *Deftia* sp. and *Fluviicola* sp. were observed. In contrast, in MFC2, next to *Acidovorax* sp., in the biofilm *Rhodopseudomonas* sp., *Methanobacterium* sp. and *Brevundimonas* sp. predominated (Figure 5). The high share of *Methanobacterium* sp. reaching 12.7% in MFC2 was unfavorable because methanogenesis may consume up to 26% of all electrons generated in the cell [33]. The hydrogen generated in the cell can also be used as an electron donor to produce methane in the oxidation of carbon dioxide to methane and water [34], lowering electricity generation. The results of molecular studies indicating a higher share of *Methanobacterium* sp. in MFC2 compared to MFC1 at the end of the study are in agreement with technological data indicating a lower cell power due to higher electron losses. In MFC1, the abundance of *Deftia* sp. reached 27.8% on day 42 and then this genus nearly disappeared in both MFCs (Figure 5). The presence of *Deftia* sp. is desirable because it is a potential exoelectrogen [35] capable of efficient generation of electricity in MFCs fed with organics such as sodium pyruvate [36]. Jangir

et al. [37] observed that after inoculation of MFC with *Deftia* sp., the anode current immediately rose above the typical background of 50 nA obtained for a sterile medium to about 400 nA after 11 h of testing. Percentage of *Acidovorax* sp. at day 42 was 17.0 and 12.2% in MFC1 and MFC2, respectively. This denitrifying bacterium is able to grow using fatty acids [38] and prefers lower operating temperatures of MFC [39]. Its high share in the initial stages of the experiment resulted from the fact that for inoculation of MFCs, denitrifying aerobic granular sludge was used. The higher decrease in the share of *Acidovorax* sp. in MFC1 indicated that this genus preferred lower OLR in MFC.

Rhodopseudomonas sp. predominated in biomass in MFC1, except from day 42, reaching 13.2% at the end of the study. In contrast, in MFC2 the share of *Rhodopseudomonas* sp. gradually decreased from 19.3% on day 42 to around 2% at the end of the study. *Rhodopseudomonas* sp. can produce electricity using a wide range of substrates at higher power densities than mixed cultures in the same device [40] and can also generate hydrogen. Generation of hydrogen by this genus is well documented. According to previous studies, hydrogen production by *Rhodopseudomonas* sp. from acetate and butyrate was 318.9 and 468.3 mL H₂/g COD, respectively [41] while wild strain *Rhodopseudomonas palustris* WP3-5 produced up to 1053.6 mL H₂/g acetate [42]. *Rhodopseudomonas* sp. share in biomass in MFC operated with acetate and glucose can reach over 60% [43]. *Acinetobacter* sp. that use hydrogen as an electron donor [44,45] were abundant in both MFCs and at the end of the experiment, their percentage was 3.7 and 2.6% in MFC1 and MFC2, respectively. *Acinetobacter* sp. is involved not only in the transfer of electrons to the anode, but also in a reduction of oxygen on the biocathode [9,46]. The share of *Acinetobacter* sp. in biomass on biocathode can reach as much as 57.81% [47]. In the presented study, the coexistence in the anode biofilm of hydrogen-consuming exoelectrogens belonging to *Acinetobacter* sp. with hydrogen-producing exoelectrogens of *Rhodopseudomonas* sp. could have resulted in a high current density.

In the present study, in MFCs powered by waste VFAs, different bacterial groups able to metabolize a very broad range of organic substrates were identified. *Clostridium* sp. was present in both reactors. Its participation in biomass in MFC1 increased from 1.4 to 1.6% at the beginning of the study to 5.9% at the end of the study. In MFC2, operated at the lower OLR, its share reached 6.4% at the end of the experiment. *Clostridium* sp. participate in decomposition of both simple and complex organic compounds, such as cellulose [48], producing butyrate, acetate, lactate, capronate (hexanoate), butanol, acetone, acetoin, and ethanol [49]. *Desulfobulbus* sp. constituted 3.6 and 2.8% of biomass in MFC1 and MFC2 at the end of the study, respectively. *Desulfobulbus* sp. can metabolize organic acids such as propionate, lactate and pyruvate [50], however, electron transfer to the anode resulting from metabolism of these acids is relatively inefficient; only about 25% of the available electrons are transferred to the electrode surface [51]. This inefficient electron transfer by *Desulfobulbus* sp. may have negatively affected electricity generation in MFCs in our study. Microorganisms from the genera *Bifidobacterium*, *Lactobacillus* and *Aminobacterium* constituted a small percentage of biomass at the beginning of the experiment, however, their number increased at the end of the experiment. The abundance of *Lactobacillus* sp. that ferments lactose to VFAs [52,53] was similar in both MFCs. The percentage of *Aminobacterium* sp., capable of protein degradation to monomeric by-products [54,55] was nearly two times higher in MFC1 than in MFC2; this indicated that these microorganisms preferred higher OLRs. The increase in *Aminobacterium* sp. abundance can be explained by the composition of the anolyte—proteins comprise about 30% of volatile suspended solids in a primary sludge [56].

The lower OLR in MFC2 favored the growth of *Phenylobacterium* sp., *Frigoribacterium* sp., *Leucobacter* sp., and *Bifidobacterium* sp. (Figure 5), that metabolize very diverse substrates, including hardly degradable ones. *Phenylobacterium* sp. use e.g., chloridazone, antipyrin, pyrimidone for growth and simple compounds such as sugars, alcohols, amino acids or carboxylic acids are not used by them [57]. *Frigoribacterium* sp. are mainly involved in transformations of sugars such as N-acetyl-D-glucosamine, L-arabinose, p-arbutin, D-fructose or D-cellobiose, while with organic acids they use only citrate and fumarate [58]. Abundance of *Leucobacter* sp. increased over time; at the end of the study, *Leucobacter* sp. constituted 3.9 and 11.5% of biomass in MFC1 in MFC2, respectively. Generally, *Leucobacter* sp.

show poor substrate utilization with a limited ability to assimilate many carbohydrates and carbon sources [59]. The abundance of these aerobic bacteria in MFC is, however, desirable. In MFCs powered with sodium acetate, glucose and ethanol in which air was introduced to the anode compartment during a start-up period, the presence of aerobic *Leucobacter* sp. in the anode biofilm increased electricity generation [60].

4. Conclusions

The use of waste VFAs from AD in MFCs favors energy recovery in wastewater treatment plants and is in line with assumptions of a circular economy. To optimize the operation of MFCs powered with waste substrates, knowledge of the effect of MFC operation and microbiological composition on energy production should be broadened. In this study, it was shown that the higher OLR in MFC1 favored growth of exoelectrogens from *Rhodospseudomonas* sp. (13.2%) resulting in a higher and more stable electricity production in comparison with MFC2. At a lower OLR in MFC2, the abundance of genera *Leucobacter*, *Frigoribacterium* and *Phenylobacterium* increased which resulted in the efficient decomposition of organics favoring high and stable COD removal. Independent of the anode size, *Clostridium* sp. and exoelectrogens belonging to genera *Desulfobulbus* and *Acinetobacter* were abundant in MFCs powered with waste VFAs. Future work will focus on a possibility of increasing electricity generation by increasing the surface area of PEM.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1996-1073/13/18/4712/s1>, Figure S1: Design of a dual-chamber MFC reactor, Figure S2: Rarefaction curves obtained for biomass samples collected from MFC1 and MFC2 during the experiment (in Sample_ID the name of MFC and the day of sampling are given).

Author Contributions: Conceptualization, A.C.-K. and D.N.; methodology, A.C.-K.; formal analysis, D.N., A.C.-K.; investigation, D.N.; resources, A.C.-K.; data curation, D.N.; writing—original draft preparation, D.N.; writing—review and editing, A.C.-K.; visualization, A.C.-K. and D.N.; supervision, A.C.-K.; project administration, A.C.-K.; funding acquisition, A.C.-K. All authors have read and agreed to the published version of the manuscript.

Funding: Project was financially supported by Minister of Science and Higher Education in the range of the program entitled “Regional Initiative of Excellence” for the years 2019–2022, project No. 010/RID/2018/19, amount of funding PLN 12,000,000.

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

References

1. Logan, B.E.; Hamelers, B.; Rozendal, R.; Schröder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40*, 5181–5192. [[CrossRef](#)] [[PubMed](#)]
2. Kumar, R.; Singh, L.; Zularisam, A.W.; Hai, F.I. Microbial fuel cell is emerging as a versatile technology: A review on its possible applications, challenges and strategies to improve the performances. *Int. J. Energy Res.* **2018**, *42*, 369–394. [[CrossRef](#)]
3. Tan, Y.C.; Kharkwal, S.; Chew, K.K.W.; Alwi, R.; Mak, S.F.W.; Ng, H.Y. Enhancing the robustness of microbial fuel cell sensor for continuous copper(II) detection against organic strength fluctuations by acetate and glucose addition. *Bioresour. Technol.* **2018**, *259*, 357–364. [[CrossRef](#)]
4. Christwardana, M.; Frattini, D.; Accardo, G.; Yoon, S.P.; Kwon, Y. Optimization of glucose concentration and glucose/yeast ratio in yeast microbial fuel cell using response surface methodology approach. *J. Power Sources* **2018**, *402*, 402–412. [[CrossRef](#)]
5. Mateo, S.; Cañizares, P.; Rodrigo, M.A.; Fernandez-Morales, F.J. Driving force behind electrochemical performance of microbial fuel cells fed with different substrates. *Chemosphere* **2018**, *207*, 313–319. [[CrossRef](#)] [[PubMed](#)]
6. Foudhaili, T.; Rakotonimaro, T.V.; Neculita, C.M.; Coudert, L.; Lefebvre, O. Comparative efficiency of microbial fuel cells and electrocoagulation for the treatment of iron-rich acid mine drainage. *J. Environ. Chem. Eng.* **2019**, *7*, 103149. [[CrossRef](#)]

7. Xin, X.; Ma, Y.; Liu, Y. Electric energy production from food waste: Microbial fuel cells versus anaerobic digestion. *Bioresour. Technol.* **2018**, *255*, 281–287. [[CrossRef](#)]
8. Hassan, S.H.A.; el Nasser, A.; Zohri, A.; Kassim, R.M.F. Electricity generation from sugarcane molasses using microbial fuel cell technologies. *Energy* **2019**, *178*, 538–543. [[CrossRef](#)]
9. Pepè Sciarria, T.; Arioli, S.; Gargari, G.; Mora, D.; Adani, F. Monitoring microbial communities' dynamics during the start-up of microbial fuel cells by high-throughput screening techniques. *Biotechnol. Rep.* **2019**, *21*, e00310. [[CrossRef](#)]
10. Freguia, S.; Teh, E.H.; Boon, N.; Leung, K.M.; Keller, J.; Rabaey, K. Microbial fuel cells operating on mixed fatty acids. *Bioresour. Technol.* **2010**, *101*, 1233–1238. [[CrossRef](#)]
11. Chen, C.-Y.; Cheng, C.-Y.; Chen, C.-K.; Hsieh, M.-C.; Lin, S.-T.; Ho, K.-Y.; Li, J.-W.; Lin, C.-P.; Chung, Y.-C. Hexavalent chromium removal and bioelectricity generation by *Ochrobactrum* sp. YC211 under different oxygen conditions. *J. Environ. Sci. Health Part A* **2016**, *51*, 502–508. [[CrossRef](#)] [[PubMed](#)]
12. Chae, K.J.; Choi, M.J.; Lee, J.W.; Kim, K.Y.; Kim, I.S. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. *Bioresour. Technol.* **2009**, *100*, 3518–3525. [[CrossRef](#)] [[PubMed](#)]
13. Freguia, S.; Rabaey, K.; Yuan, Z.; Keller, J. Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. *Environ. Sci. Technol.* **2008**, *42*, 7937–7943. [[CrossRef](#)] [[PubMed](#)]
14. Costerton, J.W. Control of all Biofilm Strategies and Behaviours. In *The Biofilm Primer*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 85–105. [[CrossRef](#)]
15. Jiang, Y.B.; Zhong, W.H.; Han, C.; Deng, H. Characterization of electricity generated by soil in microbial fuel cells and the isolation of soil source exoelectrogenic bacteria. *Front. Microbiol.* **2016**, *7*. [[CrossRef](#)] [[PubMed](#)]
16. Kondaveeti, S.; Lee, S.H.; Park, H.D.; Min, B. Specific enrichment of different *Geobacter* sp. in anode biofilm by varying interspatial distance of electrodes in air-cathode microbial fuel cell (MFC). *Electrochim. Acta* **2020**, *331*, 135388. [[CrossRef](#)]
17. Almatouq, A.; Babatunde, A.O.; Khajah, M.; Webster, G.; Alfodari, M. Microbial community structure of anode electrodes in microbial fuel cells and microbial electrolysis cells. *J. Water Process Eng.* **2020**, *34*, 101140. [[CrossRef](#)]
18. Asefi, B.; Li, S.L.; Moreno, H.A.; Sanchez-Torres, V.; Hu, A.; Li, J.; Yu, C.P. Characterization of electricity production and microbial community of food waste-fed microbial fuel cells. *Process Saf. Environ. Prot.* **2019**, *125*, 83–91. [[CrossRef](#)]
19. Peixoto, L.; Rodrigues, A.L.; Martins, G.; Nicolau, A.; Brito, A.B.; Silva, M. Flat microbial fuel cell in small and deconcentrated applications: Performance and optimization. *Environ. Technol.* **2013**, *34*, 1947–1956. [[CrossRef](#)]
20. Chen, S.; Patil, S.A.; Brown, R.K.; Schröder, U. Strategies for optimizing the power output of microbial fuel cells: Transitioning from fundamental studies to practical implementation. *Appl. Energy* **2019**, *233–234*, 15–28. [[CrossRef](#)]
21. Lanas, V.; Ahn, Y.; Logan, B.E. Effects of carbon brush anode size and loading on microbial fuel cell performance in batch and continuous mode. *J. Power Sources* **2014**, *247*, 228–234. [[CrossRef](#)]
22. Rossi, R.; Evans, P.J.; Logan, B.E. Impact of flow recirculation and anode dimensions on performance of a large scale microbial fuel cell. *J. Power Sources* **2019**, *412*, 294–300. [[CrossRef](#)]
23. Świątczak, P.; Cydzik-Kwiatkowska, A. Performance and microbial characteristics of biomass in a full-scale aerobic granular sludge wastewater treatment plant. *Environ. Sci. Pollut. Res.* **2018**, *25*, 1655–1669. [[CrossRef](#)] [[PubMed](#)]
24. APHA. *Standard Methods for the Standard Methods for the Examination of Water and Wastewater*, 18th ed.; American Public Health Association (APHA), American Water Works Association (AWWA), Water Pollution Control Federation (WPCF): Washington, DC, USA, 1992.
25. Watson, V.J.; Logan, B.E. Analysis of polarization methods for elimination of power overshoot in microbial fuel cells. *Electrochem. Commun.* **2011**, *13*, 54–56. [[CrossRef](#)]
26. R Development Core Team. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
27. RStudio Team *RStudio: Integrated Development for R*, Computer Software v0.98.1074; RStudio, Inc.: Boston, MA, USA, 2015.

28. Hill, M.O. Diversity and evenness: A unifying notation and its consequences. *Ecology* **1973**, *54*, 427–432. [[CrossRef](#)]
29. Choi, J.-d.-r.; Chang, H.N.; Han, J.-I. Performance of microbial fuel cell with volatile fatty acids from food wastes. *Biotechnol. Lett.* **2011**, *33*, 705–714. [[CrossRef](#)]
30. Tardy, G.M.; Lóránt, B.; Lóka, M.; Nagy, B.; László, K. Enhancing substrate utilization and power production of a microbial fuel cell with nitrogen-doped carbon aerogel as cathode catalyst. *Biotechnol. Lett.* **2017**, *39*, 993–999. [[CrossRef](#)]
31. Revelo Romo, D.M.; Hurtado Gutiérrez, N.H.; Ruiz Pazos, J.O.; Pabón Figueroa, L.V.; Ordóñez Ordóñez, L.A. Bacterial diversity in the Cr(VI) reducing biocathode of a Microbial Fuel Cell with salt bridge. *Rev. Argent. Microbiol.* **2019**, *51*, 110–118. [[CrossRef](#)]
32. Oh, S.E.; Logan, B.E. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Appl. Microbiol. Biotechnol.* **2006**, *70*, 162–169. [[CrossRef](#)]
33. Tandukar, M.; Huber, S.J.; Onodera, T.; Pavlostathis, S.G. Biological chromium(VI) reduction in the cathode of a microbial fuel cell. *Environ. Sci. Technol.* **2009**, *43*, 8159–8165. [[CrossRef](#)]
34. Boone, D.R.; Genus, I. Methanobacterium. In *Bergey's Manual of Systematic Bacteriology*; George, M.G., Ed.; Springer: New York, NY, USA, 2001; pp. 215–218.
35. Chen, C.Y.; Chen, T.Y.; Chung, Y.C. A comparison of bioelectricity in microbial fuel cells with aerobic and anaerobic anodes. *Environ. Technol.* **2014**, *35*, 286–293. [[CrossRef](#)]
36. Leiva-Aravena, E.; Leiva, E.; Zamorano, V.; Rojas, C.; Regan, J.M.; Vargas, I.T. Organotrophic acid-tolerant microorganisms enriched from an acid mine drainage affected environment as inoculum for microbial fuel cells. *Sci. Total Environ.* **2019**, *678*, 639–646. [[CrossRef](#)] [[PubMed](#)]
37. Jangir, Y.; French, S.; Momper, L.M.; Moser, D.P.; Amend, J.P.; El-Naggar, M.Y. Isolation and characterization of electrochemically active subsurface *Delftia* and *Azonexus* species. *Front. Microbiol.* **2016**, *7*. [[CrossRef](#)] [[PubMed](#)]
38. Heylen, K.; Lebbe, L.; de Vos, P. *Acidovorax caeni* sp. nov., a denitrifying species with genetically diverse isolates from activated sludge. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 73–77. [[CrossRef](#)] [[PubMed](#)]
39. Mei, X.; Xing, D.; Yang, Y.; Liu, Q.; Zhou, H.; Guo, C.; Ren, N. Adaptation of microbial community of the anode biofilm in microbial fuel cells to temperature. *Bioelectrochemistry* **2017**, *117*, 29–33. [[CrossRef](#)] [[PubMed](#)]
40. Xing, D.; Zuo, Y.; Cheng, S.; Regan, J.M.; Logan, B.E. Electricity generation by *Rhodospseudomonas palustris* DX-1. *Environ. Sci. Technol.* **2008**, *42*, 4146–4151. [[CrossRef](#)] [[PubMed](#)]
41. Wu, X.; Wang, X.; Yang, H.; Guo, L. A comparison of hydrogen production among three photosynthetic bacterial strains. *Int. J. Hydrog.* **2010**, *35*, 7194–7199. [[CrossRef](#)]
42. Yang, C.F.; Lee, C.M. Enhancement of photohydrogen production using phbC deficient mutant *Rhodospseudomonas palustris* strain M23. *Bioresour. Technol.* **2011**, *102*, 5418–5424. [[CrossRef](#)]
43. Zheng, W.; Cai, T.; Huang, M.; Chen, D. Comparison of electrochemical performances and microbial community structures of two photosynthetic microbial fuel cells. *J. Biosci. Bioeng.* **2017**, *124*, 551–558. [[CrossRef](#)]
44. Gross, R.; Simon, J. The *hydE* gene is essential for the formation of *Wolinella succinogenes* NiFe-hydrogenase. *FEMS Microbiol. Lett.* **2003**, *227*, 197–202. [[CrossRef](#)]
45. Iino, T.; Mori, K.; Uchino, Y.; Nakagawa, T.; Harayama, S.; Suzuki, K.I. *Ignavibacterium album* gen. nov., sp. nov., a moderately thermophilic anaerobic bacterium isolated from microbial mats at a terrestrial hot spring and proposal of *Ignavibacteria classis* nov., for a novel lineage at the periphery of green sulfur bacteria. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 1376–1382. [[CrossRef](#)] [[PubMed](#)]
46. Liu, X.W.; Li, W.W.; Yu, H.Q. Cathodic catalysts in bioelectrochemical systems for energy recovery from wastewater. *Chem. Soc. Rev.* **2014**, *43*, 7718–7745. [[CrossRef](#)]
47. Cao, X.; Liang, P.; Song, X.S.; Wang, Y.H.; Qiu, Y.; Huang, X. Trickling filter in a biocathode microbial fuel cell for efficient wastewater treatment and energy production. *Sci. China Technol. Sci.* **2019**, *62*, 1703–1709. [[CrossRef](#)]
48. Rismani-Yazdi, H.; Christy, A.D.; Dehority, B.A.; Morrison, M.; Yu, Z.; Tuovinen, O.H. Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. *Biotechnol. Bioeng.* **2007**, *97*, 1398–1407. [[CrossRef](#)] [[PubMed](#)]

49. Tracy, B.P.; Jones, S.W.; Fast, A.G.; Indurthi, D.C.; Papoutsakis, E.T. Clostridia: The importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications. *Curr. Opin. Biotechnol.* **2012**, *23*, 364–381. [[CrossRef](#)] [[PubMed](#)]
50. El Houari, A.; Ranchou-Peyruse, M.; Ranchou-Peyruse, A.; Dakdaki, A.; Guignard, M.; Idouhammou, L.; Bennisse, R.; Bouterfass, R.; Guyoneaud, R.; Qatibi, A.I. *Desulfobulbus oligotrophicus* sp. Nov., a sulfate-reducing and propionate-oxidizing bacterium isolated from a municipal anaerobic sewage sludge digester. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 275–281. [[CrossRef](#)]
51. Holmes, D.E.; Bond, D.R.; Lovley, D.R. Electron Transfer by *Desulfobulbus propionicus* to Fe(III) and Graphite Electrodes. *Appl. Environ. Microbiol.* **2004**, *70*, 1234–1237. [[CrossRef](#)]
52. Vilas Boas, J.; Oliveira, V.B.; Marcon, L.R.C.; Pinto, D.P.; Simões, M.; Pinto, A.M.F.R. Effect of operating and design parameters on the performance of a microbial fuel cell with *Lactobacillus pentosus*. *Biochem. Eng. J.* **2015**, *104*, 34–40. [[CrossRef](#)]
53. Wenzel, J.; Fuentes, L.; Cabezas, A.; Etchebehere, C. Microbial fuel cell coupled to biohydrogen reactor: A feasible technology to increase energy yield from cheese whey. *Bioprocess Biosyst. Eng.* **2017**, *40*, 807–819. [[CrossRef](#)]
54. Zhao, Z.; Zhang, Y.; Ma, W.; Sun, J.; Sun, S.; Quan, X. Enriching functional microbes with electrode to accelerate the decomposition of complex substrates during anaerobic digestion of municipal sludge. *Biochem. Eng. J.* **2016**, *111*, 1–9. [[CrossRef](#)]
55. Kumar, S.S.; Malyan, S.K.; Basu, S.; Bishnoi, N.R. Syntrophic association and performance of *Clostridium*, *Desulfovibrio*, *Aeromonas* and *Tetrathlobacter* as anodic biocatalysts for bioelectricity generation in dual chamber microbial fuel cell. *Environ. Sci. Pollut. Res.* **2017**, *24*, 16019–16030. [[CrossRef](#)]
56. Jimenez, J.; Vedrenne, F.; Denis, C.; Mottet, A.; Déléris, S.; Steyer, J.P.; Cacho Rivero, J.A. A statistical comparison of protein and carbohydrate characterisation methodology applied on sewage sludge samples. *Water Res.* **2013**, *47*, 1751–1762. [[CrossRef](#)] [[PubMed](#)]
57. Lingens, F.; Blecher, R.; Blecher, H. *Phenylobacterium immobile* gen. nov., sp. nov., a gram-negative bacterium that degrades the herbicide chloridazon. *Int. J. Syst. Bacteriol.* **1985**, *35*, 26–39. [[CrossRef](#)]
58. Kämpfer, P.; Nurmiäho Lassila, E.L.; Ulrych, U.; Busse, H.J.; Weiss, N.; Mikkola, R.; Salkinoja-Salonen, M. Frigoribacterium. In *Bergey's Manual of Systematics of Archaea and Bacteria*; John Wiley & Sons, Ltd.: Chichester, UK, 2000; pp. 1–8.
59. Takeuchi, M.; Weiss, N.; Schumann, P.; Yokota, A. *Leucobacter komagatae* gen. nov., sp. nov., a new aerobic gram-positive, nonsporulating rod with 2,4-diaminobutyric acid in the cell wall. *Int. J. Syst. Bacteriol.* **1996**, *46*, 967–971. [[CrossRef](#)]
60. Quan, X.; Quan, Y.; Tao, K.; Jiang, X. Comparative investigation on microbial community and electricity generation in aerobic and anaerobic enriched MFCs. *Bioresour. Technol.* **2013**, *128*, 259–265. [[CrossRef](#)] [[PubMed](#)]

