Development of bioelectrochemical systems using various biogas fermenter effluents as inocula and municipal waste liquor as adapting substrate

Péter Bakonyi[1], László Koók[1], Enikő Keller[1], Katalin Bélafi-Bakó[1,*], Tamás Rózsenberszki[1], Ganesh Dattatraya Saratale[2], Dinh Duc Nguyen[3], J. Rajesh Banu[4], Nándor Nemestóthy[1]

[1] Research Institute on Bioengineering, Membrane Technology and Energetics, University of Pannonia, Egyetem ut 10, 8200 Veszprém, Hungary

[2] Department of Food Science and Biotechnology, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea

[3] Department of Environmental Energy Engineering, Kyonggi University, Suwon 16227, Republic of Korea

[4] Department of Civil Engineering, Regional centre of Anna University, Tirunelveli, India

[*]Corresponding Author: Katalin Bélafi-Bakó

Tel: +36 88 624726

E-mail: bako@almos.uni-pannon.hu

Abstract

The purpose of this research was to improve microbial fuel cell (MFC) performance – treating landfill-derived waste liquor – by applying effluents of various biogas fermenters as inocula. It turned out that the differences of initial microbial community profiles notably influenced the efficiency of MFCs. In fact, the adaptation time (during 3 weeks of operation) has varied significantly, depending on the source of inoculum and accordingly, the obtainable cumulative energy yields were also greatly affected (65% enhancement in case of municipal wastewater sludge inoculum compared to sugar factory waste sludge inoculum). Hence, it could be concluded that the capacity of MFCs to utilize the complex feedstock was heavily dependent on biological factors such as the origin/history of inoculum, the microbial composition as well as proper acclimation period. Therefore, these parameters should be of primary concerns for adequate process design to efficiently generate electricity with microbial fuel cells.

Keywords: microbial fuel cell; inoculum role; municipal waste treatment; energy recovery; microbial community analysis

1. Introduction

Microbial fuel cells (MFC) are emerging applications in the field of bioelectrochemical systems (BES), which is attributed to the offered potential of achieving energy recovery from the environmental-friendly remediation of organic waste materials (Dahiya et al., 2018). Nonetheless, to realize adequate efficiency, BES such as MFCs should undergo a careful design to be concerned with a number of non-biological and biological and factors affecting their performance (Kumar et al., 2017; Santoro et al., 2017). Among the former ones, the properties of materials and constructing elements i.e. electrodes, membranes and their arrangement (often referred as architecture) can be of importance (Rahimnejad et al., 2015; Sleutels et al., 2017; Wei et al., 2011). In the latter group of variables, actual MFC behavior is substantially determined by the characteristics of active biocatalysts, called exoelectrogenic, anode-respiring bacteria (Kumar et al., 2015). These microbes release electrons from substrate conversion, which, to be able to harvest electricity, have to be successfully conveyed to the anode as terminal electron acceptor under anoxic conditions.

From practical point of view, the MFC power output and obtainable treatment efficiency of pollutants are two important parameters and are heavily dependent on the underlying community of electroactive-microbes. Hence, an enriched and better adapted population of these bacteria can be a key to improve the process and help its cost-effective expansion to larger-scales. These electroactive-bacteria are found in a wide range of seed sources such as wastewater, soil, marine sediment, compost, etc. (Chabert et al., 2015; Miceli et al., 2012)

For a process taking into account practicality, mixed communities ought to be used as inoculum because of reasons such as their metabolic flexibility and better robustness to withstand fluctuations in operating circumstances (i.e.

process disturbances) relative to pure isolates matching more the demand of fundamental studies (Hasany et al., 2016; Jung and Regan, 2007). However, in case of versatile bacterial consortia applied for MFC inoculation, considerable variations of efficiency can be expected. This may be ascribed to particular differences in the history of the inoculum (i.e. features of its origin) and its population diversity. Consequently, the proper enrichment and adaptation of microbial communities to given operating circumstances can be a requirement to establish a sufficient BES (Kim et al., 2005; Liu et al., 2011; Park et al., 2017) and furthermore, the utilization of feedstock (based on its type and complexity) could be notably influenced by the above-said inoculum traits (Park et al., 2017).

To ensure appropriate start-up of BESs and promote electro-active biofilm formation on the electrode surface, several strategies can be carried out, for example the application of a given fixed anode potential or the addition of an alternative electron acceptor (Liu et al., 2011). However, more commonly, the acclimation can be properly improved by feeding various adapting substrates (among which acetate is the widely-used, or by using pre-enriched effluent of an electrochemical reactor as inocula (Kumar et al., 2017).

Actually, as stated by Ieropoulos et al. (2010), a robust community of microorganisms is a solid requirement for MFC involved in wastewater management, which seems coincide with the findings of Mathuriya (2013), observing the enhancement of MFC performance by adapted (vs. non-adapted) inoculum selection for harnessing electricity from tannery wastewater. In this aspect, it should be achieved as a result of dynamic, competition mechanism between electro-active and non-electro-active bacteria that the former ones grow faster, more in numbers and dominate the consortium (Liu et al., 2017b; Xiang et al., 2017). Hence, screening of seed sources and appropriate choice for a specific substrate might be a beneficial strategy and can be worthy for research.

So far, previous articles applying bioelectrochemical systems have dealt with the degradation of municipal waste streams, in particular a liquid fraction acquired from municipal solid waste by mechanical pressing, referred as liquid pressed waste (LPW). For instance, Rózsenberszki et al. (2015), Koók et al. (2016) and Zhen et al. (2016) tested this substrate in single-stage anaerobic degradation processes involving MFC and microbial electrohydrogenesis cells (MEC). Later on, cascade systems with MFCs attached have been investigated as well (Rózsenberszki et al., 2017). From these research works, it has turned out that several factors i.e. the type of system as well as the operating parameter settings could play a significant role to attain enhanced performance. However, the effect that inoculum properties can have on actual, LPW-fed MFC performance has not been systematically studied so far.

Therefore, the primary objective of this paper is to elaborate the effect of sludge inocula (having different history/background) on the start-up and acclimation of MFCs fed with LPW as substrate. The MFCs were started-up with seed sources of two distinguishable origins:

- In one case, the effluent of anaerobic digester built to a municipal waste water treatment plant was used

- In the other case, the effluent of biogas plant processing sugar manufacturing waste was applied.

The systems were evaluated for more than three weeks with various loads of LPW based on cell voltages and energy yields and moreover,

- The development of bioelectrochemical system was assessed by undertaking microbial community analysis to follow population shifts taking place in the MFCs with time. This is useful approach to get a better understanding of the process and establish correlations between MFC power output, obtainable

treatment efficiency of pollutants and community structure dynamics (Liu et al., 2017a; Zhi et al., 2014).

These points make this work distinguishable from those we have performed in previous studies and in our opinion, the present investigation can have a novel contribution in the sequence of existing literature studies.

2. Materials and Methods

2.1. Inoculum (seed) sources and substrate for MFCs

In this work, two different sludges were used as seed source to inoculate MFCs. The first one, referred as MWW-S, had been collected from an anaerobic digester treating the secondary sludge of municipal waste water treatment plant located in a Hungarian countryside city and had the following initial characteristics: pH: 7.8; COD content: 13 g L^{-1}. The second one, denoted by SFW-S, had been taken from the biogas fermenter of Hungarian sugar factory utilizing the processed, solid residue i.e. beet pulp, which is a typical by-product of this manufacturing technology. SFW-S was characterized as follows: pH: 7.8; COD content: 12 g L^{-1}.

An obvious difference occurs in the history of MWW-S and SFW-S, which is the nature of feedstock. In the former case, the sludge (before collection) was continuously processing a diverse mixture of components present in the municipal wastewater. In the latter case, however, the mixed community was routinely fed with a monosubstrate-like organic matter (beet pulp) over a long time. Hence, it was presumed that MWW-S could have a faster/greater adaptation capability to complex LPW than SFW-S, which had not been applied to the treatment of such raw materials before.

Prior to use in MFCs, the anaerobic sludges were sieved by 1 mm mesh to get rid of larger particles. To characterize and compare these inocula sources

149 from a microbiological point of view, initial population structures of both were
150 examined as detailed later on in the Results and Discussion section.
151 As for the substrate, high organic-strength municipal liquid pressed waste
152 (abbreviated as LPW) was applied to feed and adapt the mixed culture MFCs.
153 The technology to produce raw LPW was detailed in our previous publication
154 (Rózsenberszki et al., 2015) and in brief, it includes consecutive shredding, metal
155 separation and trommeling, leading to a so-called biofraction of municipal solid
156 waste, from which LPW is obtained by mechanical pressing. Prior to use, in this
157 study, LPW was pre-filtered through 0.22 μm pore size membrane discs
158 (Sartorius Stedim Biotech GmbH, Germany) in order to remove its natural
159 microflora and hence, avoid possible cross-effects and interactions with microbial
160 communities in the inoculum.
161
162 **2.2. Microbial fuel cell set-up**
163
164 In this study, batch experiments (at 35 ^{o}C) were carried out in cylindical
165 two-chambered MFCs applying Nafion N115 proton exchange membrane
166 (Sigma-Aldrich, USA) with diameter of 4.5 cm to separate the (anaerobic) anode
167 and (continuously aerated) cathode chambers (each having 60 mL total volume).
168 Before use, the membrane underwent an activation treatment as referenced in
169 our previous papers (Koók et al., 2017ab). Carbon fibers with 36 cm^2 surface
170 area (serving as anodes to be colonized by exoelectrogenic strains during biofilm
171 formation) were fixed on a central Ti wire (current collector; Sigma – Aldrich,
172 USA). As for the cathode material, Pt-coated carbon cloth (with 12.5 cm^2
173 apparent surface area) (Cloth GDE - 0.3 mg cm^{-2} Pt/C 40 %, FuelCellsEtc) was
174 employed and connected to the external electric circuit by Ti wire. For inoculation
175 of anode, 10 mL of either SFW-S or MWW-S was added to 45 mL phosphate
176 buffer (pH = 7; 50 mM). At the same time, 55 mL of KCl solution (pH = 7; 0.1 M)
177 was loaded to the cathode compartment. To feed the MFCs, LPW as substrate

was injected in various quantities for successive cycles (**Fig. 1A**). Before LPW additions, equal volumes of spent anolyte (1, 2 or 4 mL) were drawn. Control MFCs without LPW supplementation were run to be able to take into account the electricity generation that originates from the degradation of residual organic matter contained in the sludge inocula.

2.3. Electrochemical assessment

To follow electricity generation of MFCs in operation, cell voltage (the actual potential between the anode and cathode electrodes) (**Fig. 1A**) was measured via a 150 Ω external resistor. The reactors were running in duplicate and results presented thoroughly are derived as arithmetic averages of those. According to Ohm's law and based on the (closed-circuit) voltage profiles recorded (**Fig. 1A**), current data and consequently, electrical power (*P*) were computed. Thereafter, by integrating the time (t) dependent power curve, cumulative energy yield (E) was calculated (Eq. 1) and is presented in **Fig. 1B**.

$$E = \int_0^{\tau} \mathrm{P(t)dt} \qquad \text{(Eq. 1)}$$

where τ is the operation time (h) for a given batch feeding cycle.

2.4. Microbial structure assessment – DNA extraction, PCR amplification, sequencing and bioinformatics analysis

Bacterial DNA was extracted from 15 mg matrix per sample using the AquaGenomic Kit (MoBiTec) and further purified using KAPA PureBeads (Roche) according to the manufacturer's protocols. The concentration of genomic DNA was measured using a Qubit 3.0 Fluorometer with Qubit dsDNA HS Assay

Kit (Thermo Fisher Scientific). Bacterial DNA was amplified with tagged primers (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) covering V3–V4 region of the bacterial 16S rRNA gene (Klindworth et al., 2013). Polymerase chain reactions (PCR) and DNA purifications were performed according to Illumina's demonstrated protocol (Part #15044223 Rev. B, to be accessed at: https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).

The PCR product libraries were quantified and qualified by using High Sensitivity D1000 ScreenTape on TapeStation 2200 instrument (Agilent). Equimolar concentrations of libraries were pooled and sequenced on an Illumina MiSeq platform using MiSeq Reagent Kit v3 (600 cycles PE).

In average ca. 755.000 raw sequencing reads per sample were generated, which were demultiplexed and adapter-trimmed by using MiSeq Control Software (Illumina). The high-quality sequences were aligned, and OTUs were generated by using Kraken software (Wood and Salzberg, 2014).

2.5. Statistical analysis

The statistical analysis is an important element of process evaluation. In this work, the comparison of SFW-S and MWW-S inoculated MFCS was carried out based on the widely-applied mathematical statistical tool, t-test (**Table 1**). For the analysis, the measured (closed-circuit) voltage values (**Fig. 1A**) were used as independent variables after being grouped in accordance with the LPW doses, representing the actual stage of operation.

3. Results and Discussion

3.1. Evaluation of initial period with different sludges (SFW-S and MWW-S) applied in MFCs

After some (2-3) days of starvation aiming the reduction of organic matter inherently contained in both sludge inocula (SFW-S and MWW-S), MFCs were supplemented with 2 mL LPW substrate, as to be noted in **Fig. 1A**. At that point, one particular difference in the behavior of the two MFC systems was observed. In case of MWW-S inoculated bioelectrochemical cells, a clearly detectable voltage signal (between approx. 3rd and 7th days of operation) could be registered unlike for SFW-S with quasi negligible response (**Fig. 1A**). This may be related with the different characteristics and history of the two inocula.

First of all, the SFW-S is delivered from an anaerobic digester that has been mainly processing mono-substrate (sugar beet solid residue) and was therefore inefficient to deal with the LPW, representing a substrate of higher complexity and remarkably different origin. Nevertheless, LPW would appear to be a more feasible feedstock in MFCs started-up with MWW-S since this seed source has been used to assist municipal waste water treatment plant continuously fed with influents of versatile composition. Thus, faster adaptation to this substrate could have taken place in this system. This step, the acclimation is an essential feature of the initial, start-up phase and can take an effect on the process performance (Boghani et al., 2013; Borjas et al., 2015; Kim et al., 2005; Kumar et al., 2017; Sato et al., 2009; Wang et al., 2010).

Second of all, it might be that the two sludges inherently contained different amounts of exoelectrogenic strains taking part in LPW decomposition in the anode chamber. For further elaboration and to be able to draw supportive conclusions, the initial microbial community structures were checked. As it can be inferred from **Fig. 2**, initial SFW-S contained nearly 20 % of representative exoelectrogenic phylum, namely *Firmicutes* (15 %), *Proteobacteria* (3 %) and

Actinobacteria (1 %) (Kiely et al., 2011; Liu et al., 2010; Sharma and Kundu, 2010; Sun et al., 2010). In contrast, at the beginning (**Fig. 3**), the proportion of same groups in the whole MWW-S population was 58 %, to be distributed in the following order according to their relative abundance as *Proteobacteria* (38 %), *Firmicutes* (14 %) and *Actinobacteria* (6 %).

Therefore, it can be deduced that because of reason such as (i) the higher portion of potential electroactive bacteria and (ii) probably more effective initial metabolic acclimation of the mixed community to LPW led together to better initial bioelectrochemical performance for MWW-S inoculated MFC, as reflected by cell voltage (**Fig. 1A**) as well as cumulative energy yield patterns (**Fig. 1B**).

3.2. Assessment of post-initial phase with different sludges (SFW-S and MWW-S) employed in MFCs

After the first operating phase (7^{th}-8^{th} days), 4 mL LPWs were injected (**Fig. 1A**). As a result, both MFCs produced clear voltage responses without significant lag time. This, for SFW-S, was a considerable improvement especially in comparison with the case of 2 mL LPW lacking any meaningful electricity generation. This could be taken as a positive feedback regarding the stepwise adaptation of the system, which, however, still performed less efficiently than its counterpart working with MWW-S seed source. This is well-expressed by cumulative energy yields (**Fig. 1B**), illustrating a more or less 3-fold difference for the two BES at that point of experiments (30-32 vs. 10-12 Joules). By delivering cumulative energy yield, the kinetics of the energy production can be also visualized as the increasing (steep) phases show the current generation (voltage peaks on **Fig. 1A**), while the stationary phases imply the depletion of substrate according to which no further increase can be observed. Additionally, it should be noticed for MWW-S-MFC that the higher substrate dose (4 mL) induced a markedly bigger cell voltage peak and corresponding area than the lower one (2

mL). This is a good indication that the exoelectrogenic strains had sufficient capacities to manage even larger organic matter loadings.

On the 15th and 19th days, 1 mL and 2 mL LPW was added to the microbial electrochemical cells, respectively (**Fig. 1A**). Overall, it can be drawn that over the time elapsed, differences in electrical performance became less notable between the MFCs using either SFW-S or MWW-S as inoculum. This assumes that though the adaption of MWW-S could be likely accomplished in faster way, in the end, by ensuring suitable time, the microbial consortia of SFW-S could also get used to the LPW feedstock and produce electricity with comparable performance. This is reflected by the similar increments of cumulative energy yields upon the 4th feedings in both MFCs (**Fig. 1B**). Moreover, by comparing the voltage profiles of the 2 mL (1st and 4th) LPW feedings and the related cumulative energy yields, it can be clearly seen that both MFCs were able to produce significantly higher amount of electricity from the equal amount of substrate. This observation matches well with the expectations regarding the adaptation process and hence, supports the statements above. The results of statistical analysis (**Table 1**) are also supportive regarding the system behaviors using SFWS and MWW-S as inocula. In conclusion, generated voltages in MFCs were found statistically different ($p<0.05$) during the first three stages of operation (2 mL, 4 mL and 1 mL LPW additions), while because of the adaptation of SWF-S over time, the values were not significantly distinguishable ($p>0.05$) in the fourth cycle when 2 mL LPW was added. In order to compare the results with literature data, current density values can be delivered. In this work with LPW, 65-306 mA m^{-2} was possible to achieve, depending on the experimental conditions i.e. the substrate loading and the souce of inoculum. Taken into account MFCs operated using complex, landfill-derived feedstock that show similarities with LPW, works such as Cercado-Quezada et al. (2010), Ganesh and Jambeck (2013), Tugtas et al. (2013) and previous work by Koók et al. (2016) can be referenced, reporting current densities of 209, 114, 418-548 and 152-218 mA m^{-2}, respectively. This

indicates that throughout studies the values fall to the same order of magnitude and the results of the present investigaton match well with the literature trends.

Generally, in case of complex organic matter with municipal origin, carbohydrates, proteins and lipids/oils as main constituents should be considered. In our previous papers, LPW was found as a feedstock characterized by high COD content and relatively lower quantities of proteins, polysaccharides and reducing sugars (Rózsenberszki et al., 2017; Zhen et al., 2016). As it has been demonstrated, the degradation of biopolymeric components by exoelectrogenic microorganisms can face challenges. Hence, solubilization and hydrolysis are essential, resulting in the release of amino acids, glucose, glycerol, fatty acids. These components, by the cooperative metabolism of fermentative strains, can be converted to acetic, butyric and propionic acid (Chen et al., 2013), which are among the primary carbon sources for electro-active microbes. Thus, the decomposition of organic matter in bioelectrochemical systems seems to be hierarchical, demanding the simultaneous involvement of various groups of microorganisms. To make an attempt for the description of such a process and follow the fate of feedstock, a generalized equation presented by Harnisch et al. (2009) can be referenced (**Eq. 2**), where, however, the exact composition of particular organic matter is a requirement (in this aspect, typical formulation of biomass was described by Ortiz-Martínez et al. (2015)).

$$C_xH_yO_z + (2x - z)H_2O \rightarrow xCO_2 + (y + 4x - 2z)H^+ + (y + 4x - 2z)e^- \qquad \text{(Eq. 2)}$$

where *x, y* and z are stoichiometric factors. It can be said that even the simplest molecules can be oxidized through different pathways and intermediates. For example, glucose can be converted to acetate, pyruvate, lactate, propionate, succinate as well as ethanol in BES, in addition to its direct oxidation to CO_2,

protons and electrons (Das, 2017). The other (mainly diverse and unknown) components and the microbiome present in the anode chamber of MFCs make the stoichiometric description difficult. In other words, a component-wise analysis can be quite laborious and rely on sophisticated analytical techniques. For instance, in the study by Wang et al. (2012) where the degradation of pretreated, algal organic matter in MFCs was investigated, 18 different amino acids had to be subjected to HPLC. Therefore, following the removal of proteins, lipids and carbohydrates can be proposed via the COD consumption of underlying microbial community. In the literature, COD conversion factors for above substances are available (Chen et al., 2013; Wang et al., 2012). Moreover, establishing COD balance to monitor the biotransformation can be a way forward (Mahmoud et al., 2014; Rózsenberszki et al., 2017; Su et al., 2013; Zhen et al., 2016), which, in an implicit manner, expresses the fate of compounds having contribution to measurable COD.

Overall, tracking the decomposition of LPW via a COD-based method in MFC can be an interesting aspect to continue this work in the future and more deeply elaborate the performance of the bioelectrochemical system.

3.3. Microbial community dynamics

To elucidate the progress observed based on population shifts taken place during the 3 weeks of operation, community structures were analyzed. The results are depicted in **Figs. 4 and 5** for MFCs driven by SFW-S and MWW-S, respectively. On one hand, according to **Fig. 4**, it can be concluded that in the former system, the portion of bacteria comprising likely of exoelectrogens increased to 27 % (14 % *Firmicutes*, 10 % *Proteobacteria* and 3 % *Actinobacteria*) from the initial 19-20 % (**Fig. 2**). On the other hand, as shown in **Fig. 5**, a similar enrichment process of predominant species seemed to occur in

376 the latter MFCs as demonstrated by the overall 75 % of potentially
377 exoelectrogenic phylum (38 % *Proteobacteria*, 25 % *Firmicutes* and 12 %
378 *Actinobacteria)*, which was 58 % initially in accordance with **Fig. 3**.
379 Moreover, literature surveys and studies – such as the work of Oh et al.
380 (2010) and Ki et al. (2008) – suggest the possible involvement of *Bacteroidetes*
381 in the electricity generation. Species from this phylum were found in remarkable
382 percentages (7-39 %) depending on the samples, as depicted in **Figs. 3-6**.
383 Besides, these strains can be usually found in anaerobic sludge and reportedly
384 participate in hydrolytic and acidogenic steps of anaerobic digestion (Delbes et
385 al., 2000).
386 Overall, based on the research outcomes detailed so far, it can be pointed
387 out that (i) the source of inoculum and its history, (ii) the adaptation time provided
388 as well as (iii) the microbial community dynamics are key-factors that will highly
389 affect the utilization efficiency of a feedstock, in particular LPW in this study. In
390 the concern of adaptation, it is noteworthy that Park et al. (2017) have also
391 emphasized the beneficial effect of pre-acclimation in MFCs treating waste water,
392 which coincides well with core of our conclusions in this subject. Therefore, it
393 seems to be that inoculum selection and its subsequent adaptation are critical for
394 adequate bioelectrochemical applications, however, both should be done
395 according to the conditions of the particular case. In other words, an inoculum
396 may fit better in one case and underperform in another, depending on the
397 environmental factors i.e. feedstock (substrate) properties. In future studies,
398 several questions have to be addressed. For example, the relationship between
399 the composition of the inocula and the type of adapting substrate should be
400 explored in order to suggest further implications for proper acclimation of
401 bioelectrochemical systems.
402

4. Conclusions

In this paper, the role of inoculum (using anaerobic sludge taken either from sugar factory or municipal wastewater treatment plant) on the electricity generation efficiency from municipal liquid waste feedstock using microbial fuel cells was addressed. It was found that the characteristics of seed source were able to demonstrate substantial effect on the process (>65% higher energy yield obtained for reactors inoculated with municipal waste sludge). Nonetheless, by ensuring proper adaptation time (during 3 weeks of operation) for adequate development of MFCs, initial differences in performances (due to various inocula) could be alleviated resulting in *Firmicutes-, Proteobacteria*- and *Actinobacteria*-dominant systems.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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Figure Legends

Fig. 1 – (A) Cell voltage and (B) Cumulative energy yield progress curves for MFCs. Red squares: using MWW-S as inoculum; Blue diamonds: using SFW-S as inoculum.

Fig. 2 – Initial microbial community profile of SFW-S used as MFC seed source (bacteria level)

Fig. 3 – Initial microbial community profile of MWW-S used as MFC seed source (bacteria level)

Fig. 4 – Microbial community structure in the end of experiments for MFC inoculated with SFW-S (bacteria level)

Fig. 5 – Microbial community structure in the end of experiments for MFC inoculated with MWW-S (bacteria level)

Table 1 – Statistical analysis of MFC voltage outputs.

Independent variable: Closed-circuit voltage	Mean (SFW-S)	Mean (MWW-S)	t-value	df	p-value	Valid N (SFW-S)	Valid N (MWW-S)	Std. Dev. (SFW-S)	Std. Dev. (MWW-S)
1 mL LPW	0.015892	0.020243	-6.10166	768	<0.000001	385	385	0.004245	0.013332
4 mL LPW	0.048828	0.071256	-7.81914	574	<0.000001	288	288	0.030588	0.037867
1 mL LPW	0.041049	0.050628	-3.02478	382	0.002656	192	192	0.028731	0.033165
2 mL LPW	0.082869	0.089376	-1.19790	296	0.231915	149	149	0.040917	0.052181

$p<0.05$ represenst statistical significance.