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# Electricity generation and microbial community structure of air-cathode microbial fuel cells powered with the organic fraction of municipal solid waste and inoculated with different seeds

Joline El-Chakhtoura<sup>a,1</sup>, Mutasem El-Fadel<sup>a</sup>, Hari Ananda Rao<sup>b</sup>, Dong Li<sup>b</sup>,  
Sophia Ghanimeh<sup>a</sup>, Pascal E. Saikaly<sup>b,\*</sup>

<sup>a</sup>American University of Beirut, Department of Civil and Environmental Engineering, Beirut, Lebanon

<sup>b</sup>King Abdullah University of Science and Technology, Biological and Environmental Sciences and Engineering Division, Water Desalination and Reuse Research Center, Thuwal 23955-6900, Saudi Arabia

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## ABSTRACT

The organic fraction of municipal solid waste (OFMSW), normally exceeding 60% of the waste stream in developing countries, could constitute a valuable source of feed for microbial fuel cells (MFCs). This study tested the start-up of two sets of OFMSW-fed air-cathode MFCs inoculated with wastewater sludge or cattle manure. The maximum power density obtained was  $123 \pm 41 \text{ mW m}^{-2}$  in the manure-seeded MFCs and  $116 \pm 29 \text{ mW m}^{-2}$  in the wastewater-seeded MFCs. Coulombic efficiencies ranged between  $24 \pm 5\%$  (manure-seeded MFCs) and  $23 \pm 2\%$  (wastewater-seeded MFCs). Chemical oxygen demand removal was  $>86\%$  in all the MFCs and carbohydrate removal  $>98\%$ . Microbial community analysis using 16S rRNA gene pyrosequencing demonstrated the dominance of the phylum Firmicutes (67%) on the anode suggesting the possible role of members of this phylum in electricity generation. Principal coordinate analysis showed that the microbial community structure in replicate MFCs converged regardless of the inoculum source. This study demonstrates efficient electricity production coupled with organic treatment in OFMSW-fueled MFCs inoculated with manure or wastewater.

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## 1. Introduction

In recent years, there has been an increased interest in harvesting energy from biomass (e.g. cellulosic biomass and residual biomass from human activities) [1,2]. The organic fraction of municipal solid waste (OFMSW), which constitutes a mixture of carbohydrates, proteins, lipids and fibers

(cellulose, hemicellulose and lignin), represents an attractive source of biomass energy, particularly in developing countries where the organic fraction can exceed 60% of the waste stream [3]. The anaerobic digestion (AD) of OFMSW has succeeded in many regions at both research and commercial scales, but lack of suitable inocula has hindered reliable start-up of this technology in developing nations [4]. Furthermore, despite the environmental and economic benefits of AD, the

\* Corresponding author. Tel.: +966 12 8084903.

E-mail address: [pascal.saikaly@kaust.edu.sa](mailto:pascal.saikaly@kaust.edu.sa) (P.E. Saikaly).

<sup>1</sup> Present address: King Abdullah University of Science and Technology, Biological and Environmental Sciences and Engineering Division, Water Desalination and Reuse Research Center, Thuwal 23955-6900, Saudi Arabia.

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biogas produced is often poor in quality, difficult to store and must be combusted for electric energy generation [5].

A microbial fuel cell (MFC) is a bioelectrochemical system inoculated with microorganisms that mediate the conversion of chemical energy stored in organic matter into electricity [6,7]. In MFCs, exoelectrogenic bacteria (pure or mixed cultures) catalyze the degradation of organic matter and transfer electrons to the anode, producing an electric current [8,9]. The electrons flow through an electric circuit containing a load onto a cathode where they combine with electron acceptors such as oxygen. A wide variety of substrates supports bioelectricity generation in MFCs, ranging from soluble, simple substrates such as acetate [10,11] and glucose [12] to particulate, complex substrates such as chitin [13] and cellulose [14,15]. Residual, low-value biomass has also been tested extensively as a feed, e.g. wheat or rice straw hydrolysate [6,16,17], straw [18], raw corn stover [19] and algae powders [20]. The OFMSW contains easily biodegradable organic matter with a lignin mass fraction <2.4% [3] and may represent a valuable source of feed for electricity production in MFCs. However, bioelectricity generation in MFCs using the OFMSW has been rarely reported [21].

With complex substrates, a synergistic consortium of hydrolytic, fermentative microorganisms and fermentation product-utilizing, electrochemically active bacteria is usually needed [14,22]. Different inoculum sources have been used to start-up MFCs treating complex substrates. Wastewater microorganisms have been efficient in starting up MFCs treating complex substrates such as carbohydrates and proteins [6,20]. Manure sludge was shown to generate electricity in an MFC [23] indicating the presence of certain exoelectrogenic bacteria, although power output was low (5–10 mW m<sup>-2</sup>). To the best of the authors' knowledge, the effect of different inoculum sources on the start-up, performance and microbial community structure in MFCs fed with the OFMSW has not been previously reported.

The objectives of this study were to (i) test different inoculum sources (wastewater sludge or cattle manure) for the start-up and performance (voltage, power density, Coulombic efficiency and organic matter removal) of replicate single-chamber air-cathode MFCs fed with the OFMSW; and (ii) characterize the microbial community structure that develops on the anodes, cathodes and in suspension. Bacterial communities were examined using high-throughput 16S ribosomal RNA (16S rRNA) gene pyrosequencing.

## 2. Materials and methods

### 2.1. Reactor construction

Six single-chamber, cube-shaped, air-cathode MFCs (4 cm long by 3 cm in diameter; empty bed volume of 28 cm<sup>3</sup>) were constructed from Plexiglas as previously described [10]. Two sampling ports were drilled on top of the MFCs and a plastic cap fitted in one of them to create a headspace for gas accumulation. The ports were sealed with rubber stoppers. The anodes were made of non-wet-proofed carbon cloth (type A; E-TEK, USA). The cathodes were made of wet-proofed (30% mass fraction) carbon cloth (type B-1/B; E-TEK, USA), treated

with four polytetrafluoroethylene (PTFE) diffusion layers and a platinum catalyst (5 g m<sup>-2</sup>) [12]. The anode and cathode were pressed by rubber gaskets on opposite sides of the chamber with a solid plate covering the anode side while the cathode side was covered by a flat plate with a 3 cm diameter hole.

### 2.2. Feed preparation

Source-sorted OFMSW was collected from households. It was moistened with distilled water and ground using a blender to augment biodegradation surfaces [3]. It was then sieved to obtain a feed with particles of size 300–850 μm to avoid reduced power densities with large particles [13]. The OFMSW was analyzed for relevant parameters: pH, conductivity, total solids (TS), total volatile solids (TVS), chemical oxygen demand (COD), ammonia nitrogen (NH<sub>3</sub>-N), phosphorus, total Kjeldahl nitrogen (TKN), proteins and carbohydrates. The homogenized waste was then stored at –20 °C until used in order to maintain its stability. Prior to use, the OFMSW was diluted to a COD concentration of 1.178 kg m<sup>-3</sup>. The OFMSW was diluted with (per liter of deionized water) a medium consisting of 5 cm<sup>3</sup> vitamins, 12.5 cm<sup>3</sup> trace minerals [24] and phosphate buffer (Na<sub>2</sub>HPO<sub>4</sub>, 4.56 kg m<sup>-3</sup>; NaH<sub>2</sub>PO<sub>4</sub>, 2.45 kg m<sup>-3</sup>; NH<sub>4</sub>Cl, 0.31 kg m<sup>-3</sup>; KCl, 0.13 kg m<sup>-3</sup>) [22]. The characteristics of the OFMSW before dilution are presented in Table 1.

### 2.3. Seed preparation

Two different seeds were tested. Wastewater sludge was collected from the aeration basin of a local (Baalbek, Lebanon) municipal wastewater treatment plant and stored at 4 °C until used. Fresh, moist manure was procured from a local (Beit-Chabab, Lebanon) cattle-rearing farm, transported in a sealed plastic bag and added to the reactors within a few hours without any modification.

### 2.4. Reactor operation

The reactors were first fed with glucose (1 kg m<sup>-3</sup>) as the sole substrate for approximately 10 days to enrich for exoelectrogens and fermentative bacteria. During the enrichment phase, two sets of MFCs (triplicate MFCs per set), inoculated

**Table 1** – Characteristics of the ground and sieved OFMSW used as substrate.

Parameters	Value
pH (at 25 °C)	4.12
Conductivity (mS cm <sup>-1</sup> at 25 °C)	4.38
TS (g kg <sup>-1</sup> )	78.00
TVS mass fraction of TS (%)	97.40
Total COD (kg m <sup>-3</sup> )	128.60
Soluble COD (kg m <sup>-3</sup> )	39.30
Ammonia nitrogen (g m <sup>-3</sup> NH <sub>3</sub> -N)	165.00
Total phosphorus (g m <sup>-3</sup> PO <sub>4</sub> <sup>3-</sup> )	870.00
Soluble phosphorus (g m <sup>-3</sup> PO <sub>4</sub> <sup>3-</sup> )	598.00
TKN (% mass fraction)	0.27
Proteins (% mass fraction)	1.66
Total carbohydrates (kg m <sup>-3</sup> )	21.91
Soluble carbohydrates (kg m <sup>-3</sup> )	13.14

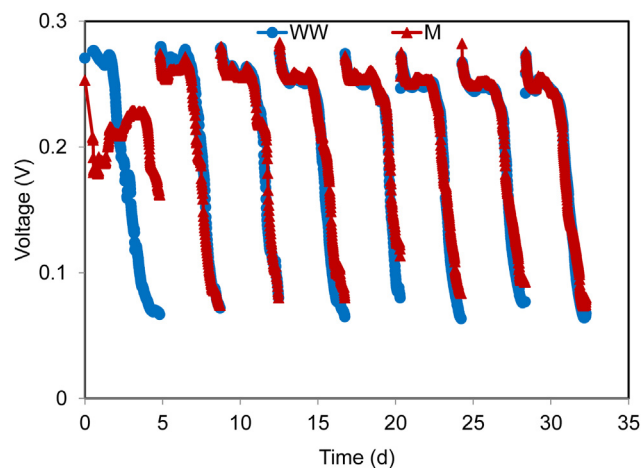
with different seeds, were operated in parallel. One set of MFCs (referred to as the wastewater MFCs) was inoculated with wastewater sludge (volume fraction of 10%). The second set of MFCs was inoculated with manure (volume fraction of 10%) (referred to as the manure MFCs). The glucose feed was replaced every time the voltage dropped to <50 mV, forming one complete batch cycle. After each cycle, 3 cm<sup>3</sup> of solution was removed with a pipette and added into a fresh medium/substrate so that some bacteria could be used as an inoculum for the following cycle, and the remaining solution was discarded [19]. Inoculation and feeding were conducted in an anaerobic glove box flushed with 100% N<sub>2</sub>. This phase was considered complete when reproducible voltage profiles were obtained over three consecutive cycles [19].

After the enrichment phase the substrate was switched from glucose to the diluted OFMSW. The feed was replaced every time the voltage dropped to <80 mV. Also, as was conducted during the enrichment phase, 3 cm<sup>3</sup> of solution was added to fresh feed for each subsequent batch cycle.

All MFCs were run in fed-batch mode at room temperature: 25 ± 1 °C. The fuel cells were operated in a closed-circuit mode using an external circuit of titanium wires containing a 1000 Ω resistor.

## 2.5. Chemical analysis

Analysis of COD was carried out at the end of three reproducible voltage cycles (cycles 5–7 in Fig. 1) according to Standard Methods for the Examination of Water and Wastewater [25] using the Hach DR/2010 portable data-logging spectrophotometer (Hach Co., Loveland, CO). Carbohydrates were measured by means of a colorimetric method based on an anthrone reagent. Briefly, 1 cm<sup>3</sup> of sample was digested with 2 cm<sup>3</sup> of a chilled 75% sulfuric acid solution and then vortexed with 4 cm<sup>3</sup> of a chilled anthrone solution. Samples were then boiled in a water bath at 100 °C for 15 min, cooled to room temperature and then analyzed at 578 nm using a Hach DR/4000U spectrophotometer (Hach Co., Loveland, CO). Absorbance values (in ABS units) were reported and the carbohydrate concentration was



**Fig. 1** – Voltage generation in the wastewater (WW) and manure (M)-seeded MFCs fed with the OFMSW.

determined based on standard curves generated using glucose solutions. The concentrations of volatile fatty acids (VFAs), intermediates of anaerobic degradation of OFMSW, were measured over the course of a batch cycle. Samples collected from the MFCs were filtered (0.45 μm-pore syringe filters), acidified by adding 11 μl of phosphoric acid to each sample and stored at –20 °C before analysis. Individual VFAs (acetate, propionate and butyrate) were analyzed by a gas chromatograph (TRACE GC Ultra, Thermo Electron Corp., Italy) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 μm capillary GC column (TRACE TR-FFAP, Thermo Fisher Scientific Inc., USA). The heating protocol was adopted from Zhang et al. [26]: injector temperature of 250 °C, initial oven temperature of 70 °C for 3 min followed with a ramp of 293.15 K min<sup>-1</sup> for 5.5 min and a final temperature of 180 °C for 3 min. Nitrogen was used as a carrier gas with a flow rate of 2.6 cm<sup>3</sup> min<sup>-1</sup>. Calibration stock solution and dilutions were carried out according to Standard Methods [25].

## 2.6. Electrical analysis

The cell voltage (V) was recorded every 30 min across a fixed load (1000 Ω) using a multimeter with a data acquisition system (Model 2701; Keithley Instruments Inc., Cleveland, OH). Current (I, A) was calculated according to Ohm's Law ( $I = V/R$ ) where V (V) is the voltage measured and R (Ω) is the external resistance applied. The power density (P, mW m<sup>-2</sup>) was calculated as  $P = (1000IV)/A$  where 1000 is to convert to mW and A (m<sup>2</sup>) is the cathode surface area. Coulombic efficiency (CE) was determined at the end of a batch cycle based on COD and was calculated as [9]:

$$CE = \frac{8 \int_0^t I dt}{Fv\Delta COD} \quad (1)$$

where 8 is a constant used for COD, F is Faraday's constant (96,485 C mol<sup>-1</sup> of electrons), v is the volume of liquid in the reactor (L), ΔCOD is the change in COD concentration (g L<sup>-1</sup>) over time t (s) and I is the current (A) [9]. Polarization and power density curves were generated using the single-cycle method by measuring the open-circuit voltage (OCV) over a 2 h period and then varying the external resistance from 10,000 Ω to 100 Ω (20 min for each resistor).

## 2.7. Microbial community analysis with 16S rRNA gene pyrosequencing

Biomass samples for bacterial analysis were collected from the anode, cathode and suspension at the end of the experiment (day 42) and genomic DNA was extracted using the PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Genomic DNA was also extracted from each of the two seed samples. Triplicate PCR reactions were performed for each extracted DNA sample as described in Sayess et al. [27]. Detailed analysis of 16S rRNA gene pyrosequencing is provided in the supporting information.

### 3. Results and discussion

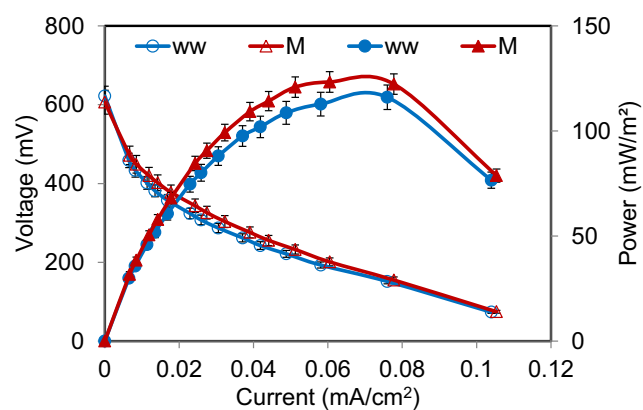
#### 3.1. MFC performance

Following glucose feeding and inoculation with wastewater sludge or cattle manure, the voltage generated was almost nil for a period of 48 h. Exoelectrogens require a lag period to colonize the anode and to synthesize the enzymes and/or shuttles needed to transfer electrons outside their cells [8]. After a lag period of 48 h, an immediate increase in voltage (100 mV) was observed in the second batch cycle for the two sets of MFCs, peaking at  $141 \pm 32$  mV after 14 h.

After three batch cycles of reproducible voltage generation with a peak of  $317 \pm 41$  mV (lasting for 48 h) during the enrichment phase (10 days), the glucose solution (COD = 1.219 mg/L) was replaced with the diluted OFMSW (COD = 1178 kg m<sup>-3</sup>). During the first batch cycle after switching the substrate, the two sets of MFCs generated voltage instantaneously with no lag phase (Fig. 1). By shifting the substrate to the OFMSW, the voltage decreased; wastewater:  $271 \pm 39$  mV; manure:  $253 \pm 46$  mV and the duration of the batch cycles increased to ~90 h (Fig. 1). This can be attributed to the complexity of the new feed. Biomass polymers and particulate material, unlike glucose, cannot be consumed directly by bacteria. They have slower degradation kinetics than soluble matter and require more energy for hydrolysis and fermentation [20].

The manure MFCs exhibited almost the same voltage behavior as the wastewater MFCs after the second feeding and throughout the rest of the experiment, producing a stable voltage of  $260 \pm 42$  mV compared to  $255 \pm 23$  mV for the wastewater MFCs (Fig. 1). The voltage produced for the wastewater and manure MFCs was comparable to Jia et al. [21] using a similar reactor configuration (i.e. single-chamber, air-cathode MFC) and substrate (buffered food waste) but higher COD concentrations (2, 3.2 and 4.9 kg m<sup>-3</sup> compared to ~1.2 kg m<sup>-3</sup> in this study). The voltage reported in their study ranged from 281 mV (COD = 2 kg m<sup>-3</sup>) to 322 mV (COD = 4.9 kg m<sup>-3</sup>) [21].

All the reactors displayed polarization curves typical of MFCs (Fig. 2). At high external resistance there was a swift



**Fig. 2** – Polarization and power density curves for the wastewater (WW) and manure (M)-seeded MFCs fed with the OFMSW.

drop in voltage as current flowed through the circuit, followed by a linear decrease in voltage. The maximum power density was  $123 \pm 41$  mW m<sup>-2</sup> and  $116 \pm 29$  mW m<sup>-2</sup> in the manure- and wastewater-seeded MFCs, respectively (Fig. 2). These values are much lower than what has been reported with simple substrates such as acetate (2400 mW m<sup>-2</sup>) [11] in identical MFCs using brush anodes, but are within the range of values reported for complex substrates, e.g. buffered cellulose (59 mW m<sup>-2</sup>) [14] and buffered wheat straw hydrolysate (124 mW m<sup>-2</sup>) [6]. In MFCs, power density is affected by several factors. These include type of substrate (simple versus complex; soluble versus particulate), concentration of substrate, inoculum source, reactor configuration, electrode material (whereby brush anodes generate higher power than carbon cloth anodes), solution conductivity and internal resistance [9,11]. Thus, direct comparison of power density between this study and previous MFC studies is not feasible. However, the results obtained in this study indicate the feasibility of using buffered OFMSW as a feed to produce electric energy in single-chamber, air-cathode MFCs.

Coulombic efficiencies achieved in this study were  $22.7 \pm 1.9\%$  in the wastewater-seeded MFCs and  $24.0 \pm 5.4\%$  in the manure-seeded MFCs. These values are comparable to those reported in other studies utilizing complex substrates. Cellulose was tested in rumen-seeded MFCs and efficiencies achieved were dependent on the external resistance applied: CE reached 19% with a 20  $\Omega$  load versus 12% with a 1000  $\Omega$  load [28]. CEs ranging from 15.5% (COD = 2 kg m<sup>-3</sup>) to 37.1% (COD = 0.25 kg m<sup>-3</sup>) were obtained with wheat straw hydrolysate [6]. Coulombic efficiencies achieved in single-chamber, air-cathode MFCs are typically low. This could be due to several factors: 1) the absence of a proton exchange membrane between the cathode and anode where the substrate is distributed among the anode and cathode [29]; 2) the electrons released during substrate degradation are used for bacterial growth [7]; 3) COD loss by aerobic degradation due to oxygen diffusion into the cathode [30]. It should be noted that the cycle duration reported in this study was ~90 h, resulting in more oxygen diffusion into the system and increased loss of COD by aerobic degradation; and 4) COD loss due to methanogenesis [10].

Removal of COD was similar for the two sets of MFCs with  $88.0 \pm 1.1\%$  for the wastewater MFCs and  $86.6 \pm 2.6\%$  for the manure MFCs. These results indicate that as much as 62.6% to 65.3% of the COD removed (based on CE values) did not generate an electric current. Carbohydrate removal was almost complete with  $99.0 \pm 0.3\%$  for the wastewater MFCs and  $99.1 \pm 0.3\%$  for the manure MFCs. Jia et al. [21] tested three different concentrations of food waste (COD of 2, 3.2 and 4.9 kg m<sup>-3</sup>) in MFCs and obtained slightly lower removals of COD (77.2% to 86.4%) and carbohydrates (94.3% to 95.9%) than reported here. Collectively these results indicate the feasibility of using MFCs for simultaneously treating and generating electricity from the OFMSW using wastewater or manure as seeds. Multiple seeds are usually required for the startup of AD treating the OFMSW [31]. However, future studies are needed to test the competitiveness of MFCs for treating OFMSW with high COD loadings.

The two sets of MFCs exhibited a similar VFA profile over the course of a fed-batch cycle (Fig. 3). Acetate was the

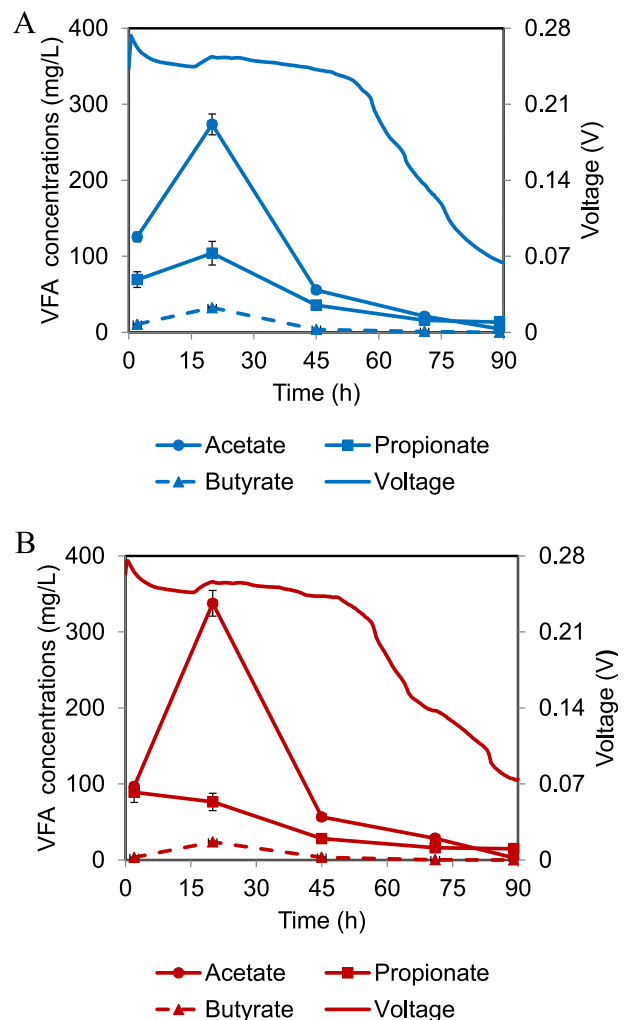
dominant intermediate followed by propionate and then butyrate. Acetate reached a maximum of  $274 \pm 23 \text{ mg L}^{-1}$  (WW MFCs) and  $338 \pm 36 \text{ mg L}^{-1}$  (manure MFCs) 20 h after feeding and then decreased to low levels ( $<10 \text{ mg L}^{-1}$ ) at the end of a batch cycle, in agreement with the drop in voltage generation (Fig. 3). Similarly, propionate, another key intermediate of anaerobic degradation of the OFMSW, increased to  $104 \text{ mg L}^{-1}$  (WW MFCs) and  $76 \text{ mg L}^{-1}$  (manure MFCs) at 20 h and decreased to  $<15 \text{ mg L}^{-1}$  at the end of the cycle (Fig. 3). Butyrate concentrations also peaked at 20 h but at lower levels ( $24\text{--}32 \text{ mg L}^{-1}$ ) compared to acetate and propionate. Others have shown that acetate followed by propionate were the dominant intermediates in MFCs fed with complex substrates such as wheat straw hydrolysate [6] and algae powders [20]. Acetate is known to be a favorable substrate for power generation in MFCs compared to other fermentation endproducts [10,22].

### 3.2. Microbial community analysis

Pyrosequencing was used in this study to characterize the bacterial communities of biofilm (anode and cathode) and suspension samples from six replicate MFCs operated under the same experimental conditions but seeded with different inoculum sources (wastewater sludge or cattle manure). Pyrosequencing allows identification of both the dominant and rare operational taxonomic units (OTUs) in a community [32], overcoming the limitations of commonly used molecular biology techniques in MFC studies such as denaturing gradient gel electrophoresis (DGGE) and clone library analysis of PCR-amplified 16S rRNA genes [6,17,20,22,33].

It has been reported that the inoculum source can influence the microbial communities that develop in MFCs and subsequently affect their performance [6]. To account for differences in microbial community structure due to inoculum source, we sampled the microbial communities at the end of the experiment (day 42) after the voltage had stabilized for several cycles. Alpha diversity measures (Table S1) in the six replicate OFMSW-fed MFCs showed that the cathodic community had higher diversity (Chao1:  $1275 \pm 197$ ; H:  $7.30 \pm 0.15$ ) than the anodic (Chao1:  $911 \pm 180$ ; H:  $3.80 \pm 0.55$ ) and suspension communities (Chao1:  $989 \pm 93$ ; H:  $5.37 \pm 0.19$ ). At the end of the experiment there was no difference in diversity (anodic, cathodic or suspension community) between replicate MFCs ( $P > 0.05$ ,  $n = 6$ ) seeded with wastewater or manure despite differences in diversity between the initial inoculum sources (Table S1). These results are consistent with those reported by Yates et al. [34] where no difference in diversity was observed among the anodic communities of nine replicate, single-chamber, air-cathode MFCs fed with acetate and seeded with different inoculum sources (wastewater or anaerobic bog sediment). Only the anodic community was characterized in their study. These results suggest that bacterial communities in replicate MFCs operated under the same experimental conditions will most likely evolve to the same bacterial diversity irrespective of differences in the initial inoculum source.

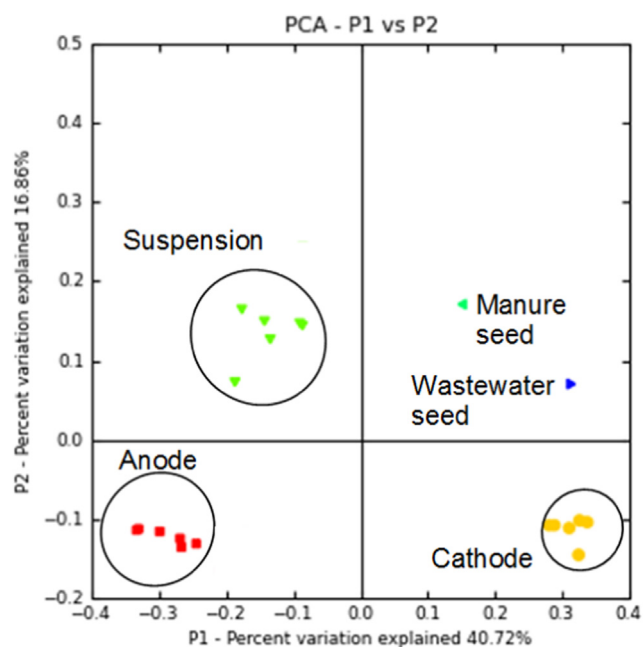
A PCoA plot (Fig. 4) based on weighted UniFrac distance matrix showed three clear clusters where samples collected from the anodes, cathodes or suspensions were clustered



**Fig. 3 – VFA concentrations and voltage generation over the course of a fed-batch cycle in the MFCs inoculated with (A) wastewater and (B) manure.**

together, indicating a clear distinction in the microbial community structure between the anode, cathode and suspension samples. This suggests that the unique environment present on the anode, cathode and suspension of single-chamber, air-cathode MFCs allowed for the development of different microbial communities. Also, PCoA revealed that the anodic, cathodic or suspension communities in the two sets of MFCs had evolved and converged at the end of the experiment irrespective of the inoculum source (Fig. 4). This finding is similar to a previous study where principle component analysis of DGGE band patterns and 16S rRNA gene pyrosequencing data indicated convergence of the anodic community (after 16 cycles) of nine replicate single-chamber, air-cathode MFCs fed with acetate and seeded with different inoculum sources [34]. These results imply that the inoculum source was not an important factor in shaping the anodic community with the MFC design used in this study.

Analysis of similarity (ANOSIM) based on Bray–Curtis similarity index was conducted to test the differences in the overall bacterial community between the two sets of MFCs.



**Fig. 4 – PCoA of weighted UniFrac distance matrix results showing the relatedness of bacterial communities between the seed samples and samples collected from the anode, cathode and suspension of the six MFCs.**

The global R value from ANOSIM was  $< -0.0264$  with  $P = 0.98$ , indicating that there was no significant difference in the bacterial community structure of replicate MFCs seeded with different inoculum sources. The average similarity at the end of the experiment between the anodic, cathodic and suspension communities of replicate MFCs was  $76.6 \pm 3.3\%$ ,  $74.7 \pm 1.9\%$  and  $73.5 \pm 4.4\%$ , respectively. These results confirm the high reproducibility of the bacterial communities in replicate MFCs irrespective of the initial inoculum source.

To classify the bacterial community that developed on the anode, suspension and cathode, qualified reads were assigned to known phyla and families/genera. The predominant phyla detected in the six MFCs belonged to *Firmicutes* (anode, 67%; suspension, 36%; cathode, 22%), *Bacteroidetes* (anode, 18%; suspension, 45%; cathode, 35%) and *Proteobacteria* (anode, 6%; suspension, 7%; cathode, 27%) (Fig. S1A). The highest percentage of the phylum *Firmicutes* was detected in the anode samples with *Bacteroidetes* and *Proteobacteria* being more dominant in the suspension and cathode samples, respectively. It should be noted that the manure seed was dominated by the phylum *Bacteroidetes* (25%) and *Firmicutes* (71%) whereas the wastewater seed was dominated by the phylum *Bacteroidetes* (55%) and *Proteobacteria* (24%) (data not shown).

The breakdown of the bacterial classes on the anode, suspension and cathode is presented in Fig. S1B. The predominant classes identified on the anode for the six MFCs by 16S rRNA pyrosequencing were *Clostridia* (66%), *Bacteroidia* (18%), *Gammaproteobacteria* (3%), *Deltaproteobacteria*, *Synergistia* and *Mollicutes* (2% each). In the suspension, the predominant class was *Bacteroidia* (45%) followed by

*Clostridia* (35%), *Gammaproteobacteria* (7%), *Synergistia* (3%) and *Mollicutes* (2%). As for the cathode, *Bacteroidia* also dominated (32%) followed by *Clostridia* (19%), *Betaproteobacteria* (10%), *Gammaproteobacteria* (8%), *Alphaproteobacteria* (4%), *Deltaproteobacteria* (4%), *Spirochaetes* and *Synergistia* (3% each). The higher phylogenetic diversity at the cathode could be due to the presence of dissolved oxygen gradient within the biofilm, allowing the coexistence of aerobic (members of the class *Betaproteobacteria*) and anaerobic bacteria (members of the class *Clostridia*), and the availability of electron donors from the suspension and electrons from the cathode electrode. These results portray the phylogenetic diversity of microbial communities in single-chamber, air-cathode MFCs fed with the OFMSW. This diversity may be attributed not only to the mixed cultures found in the original inocula but also to the complexity of the OFMSW and the variety of intermediates and metabolites derived from its degradation that can sustain different functional groups of bacteria including electricity-generating and fermentative bacteria (e.g. members of the phylum *Firmicutes* and *Bacteroidetes*).

A large proportion (~55%) of sequence reads retrieved from the reactors could not be classified at the genus level. The majority of sequences at the anode were classified down to the family level with the family *Eubacteriaceae* (phylum *Firmicutes*, class *Clostridia*) dominating (61%) (Fig. S1C). The relative abundance of *Eubacteriaceae* was lower in the suspension (24%) and cathode (4%) samples. In a study testing cellulose-fed MFCs inoculated with rumen microorganisms, *Firmicutes* was found to be the dominant phylum (59%) detected on the anode compared to 13% in suspension [2]. Recently, it has been reported that members of the phylum *Firmicutes* play a significant role in current production in thermophilic MFCs [35]. Collectively, these results suggest the possible role of members of the phylum *Firmicutes* in electricity generation.

The abundance of OTUs belonging to the genus *Geobacter* (phylum *Deltaproteobacteria*), a well-known exoelectrogenic bacteria, was low (1%) (Fig. S1C) compared to previous studies showing the dominance of *Geobacter* in single-chamber, air-cathode MFCs fed acetate [34,36]. The type of substrate used in our study, characterized by a high proportion of carbohydrates (Table 1), may have resulted in the prevalence of fermentative bacteria such as *Clostridia* (phylum *Firmicutes*). Previous studies testing complex substrates in MFCs also reported the lack of abundance of *Geobacter* on the anode [2,6,33].

In this study, both reactor performance (voltage and power density) and anodic microbial community structure were reproducible in replicate MFCs regardless of the initial inoculum source. These results are consistent with Yates et al. [34] who reported similar performance (voltage and power density) and similar anodic microbial community structure in replicate, single-chamber, air-cathode MFCs fed with acetate and seeded with different inoculum sources (wastewater or anaerobic bog sediment). Both studies reveal the dominance of a specific population on the anode. *Geobacter* was the dominant population in their study [34] compared to *Eubacteriaceae* in the current study. However, this similarity in reactor performance between replicate reactors does not

always suggest a similarity in anodic microbial community. For example, Beecroft et al. [17] showed similar levels of power in three replicate MFCs fed with sucrose and inoculated with anaerobic digester sludge, however, the similarity between the anodic biofilm communities was low (33–46%) and the dominant bacterial species varied over time. This may result from functional redundancy among the exoelectrogenic community (i.e. different species are capable of fulfilling the same function) where the loss of one species will be compensated by another species in the community resulting in similar reactor performance. Several factors could affect the anodic microbial community structure in MFCs including but not limited to inoculum source [6], electrode material [36], substrate used [22] and system architecture [8]. It should be noted that there is no systematic study that addresses all these factors at once. Therefore, development of a better understanding of the most important factor that shapes the anodic microbial community in MFCs remains a research need.

#### 4. Conclusions

The start-up of wastewater- or manure-seeded MFCs was efficient with power densities comparable to other MFC studies using complex substrates and high removals of COD and organics were achieved. High reproducibility in reactor performance and microbial community was achieved in replicate reactors regardless of the inoculum source. Although AD remains a competitive technology for the treatment of the OFMSW, start-up periods can be quite long [3], multiple seeds must be supplied [31] and mesophilic to thermophilic temperatures are required. On the contrary, MFCs treating the OFMSW could be started in a few days (~10 days) at room temperature using easily accessible seeds (wastewater or manure). Nevertheless, the two processes could be regarded as complementary where VFAs from AD could be treated in MFCs [10,22] or MFCs could function as a polishing step to further treat the effluent from AD treating the OFMSW.

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#### Appendix. Supplementary material

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.biombioe.2014.04.020.

#### REFERENCES

- [1] Rittmann BE. Opportunities for renewable bioenergy using microorganisms. *Biotechnol Bioeng* 2008;100(2):203–12.
- [2] Rismani-Yazdi H, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH. Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. *Biotechnol Bioeng* 2007;97(6):1398–407.
- [3] Maroun R, El-Fadel M. Start-up of anaerobic digestion of source-sorted organic municipal solid waste in the absence of classical inocula. *Environ Sci Technol* 2007;41(19):6808–14.
- [4] Ghanimeh S, El-Fadel M, Saikaly P. Mixing effect on thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste. *Bioresour Technol* 2012;117:63–71.
- [5] Pham TH, Rabaey K, Aelterman P, Clauwaert P, De Schampelaere L, Boon N, et al. Microbial fuel cells in relation to conventional anaerobic digestion technology. *Eng Life Sci* 2006;6(3):285–92.
- [6] Zhang Y, Min B, Huang L, Angelidaki I. Generation of electricity and analysis of microbial communities in wheat straw biomass-powered microbial fuel cells. *Appl Environ Microbiol* 2009;75(11):3389–95.
- [7] Sato C, Martinez RG, Shields MS, Perez-Gracia A, Schoen MP. Behavior of microbial fuel cell in a start-up phase. *Int J Environ Eng* 2009;1(1):36–51.
- [8] Logan BE. Exoelectrogenic bacteria that power microbial fuel cells. *Nature Rev Microbiol* 2009;7(5):375–81.
- [9] Logan BE, Hamelers B, Rozendal R, Schroder U, Keller J, Freguia S, et al. Microbial fuel cells: methodology and technology. *Environ Sci Technol* 2006;40(17):5181–92.
- [10] Liu H, Cheng S, Logan BE. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ Sci Technol* 2005;39(2):658–62.
- [11] Logan B, Cheng S, Watson V, Estadt G. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ Sci Technol* 2007;41(9):3341–6.
- [12] Cheng S, Liu H, Logan BE. Increased performance of single-chamber microbial fuel cells using an improved cathode structure. *Electrochem Commun* 2006;8(3):489–94.
- [13] Rezaei F, Richard TL, Logan BE. Analysis of chitin particle size on maximum power generation, power longevity, and Coulombic efficiency in solid-substrate microbial fuel cells. *J Power Sources* 2009;192(2):304–9.
- [14] Ren Z, Ward TE, Regan JM. Electricity production from cellulose in a microbial fuel cell using a defined binary culture. *Environ Sci Technol* 2007;41(13):4781–6.
- [15] Ahmad F, Atiyeh MN, Pereira B, Stephanopoulos GN. A review of cellulosic microbial fuel cells: performance and challenges. *Biomass Bioenergy* 2013;56:179–88.
- [16] Thygesen A, Poulsen FW, Angelidaki I, Min B, Bjerre AB. Electricity generation by microbial fuel cells fuelled with wheat straw hydrolysate. *Biomass Bioenergy* 2011;35(11):4732–9.
- [17] Beecroft NJ, Zhao F, Varcoe JR, Slade RC, Thumser AE, Avignone-Rossa C. Dynamic changes in the microbial community composition in microbial fuel cells fed with sucrose. *Appl Microbiol Biotechnol* 2012;93(1):423–37.
- [18] Wang CT, Yang CMJ, Chen ZS. Rumen microbial volatile fatty acids in relation to oxidation reduction potential and electricity generation from straw in microbial fuel cells. *Biomass Bioenergy* 2012;37(0):318–29.
- [19] Wang X, Feng Y, Wang H, Qu Y, Yu Y, Ren N, et al. Bioaugmentation for electricity generation from corn stover biomass using microbial fuel cells. *Environ Sci Technol* 2009;43(15):6088–93.

- [20] Velasquez-Orta SB, Curtis TP, Logan BE. Energy from algae using microbial fuel cells. *Biotechnol Bioeng* 2009;103(6):1068–76.
- [21] Jia J, Tang Y, Liu B, Wu D, Ren N, Xing D. Electricity generation from food wastes and microbial community structure in microbial fuel cells. *Bioresour Technol* 2013;144:94–9.
- [22] Kiely PD, Rader G, Regan JM, Logan BE. Long-term cathode performance and the microbial communities that develop in microbial fuel cells fed different fermentation endproducts. *Bioresour Technol* 2011;102(1):361–6.
- [23] Scott K, Murano C. A study of a microbial fuel cell battery using manure sludge waste. *J Chem Technol Biotechnol* 2007;82(9):809–17.
- [24] Cheng S, Xing D, Call DF, Logan BE. Direct biological conversion of electrical current into methane by electromethanogenesis. *Environ Sci Technol* 2009;43(10):3953–8.
- [25] APHA/AWWA/WEF. Standard methods for the examination of water and wastewater. 21st ed.. In: Eaton AD, Clesceri LS, Rice EW, Greenberg AE, editors. Washington, DC: American Public Health Association/American Water Works Association/Water Environment Federation; 2005.
- [26] Zhang ML, Sheng GP, Mu Y, Li WH, Yu HQ, Harada H, et al. Rapid and accurate determination of VFAs and ethanol in the effluent of an anaerobic H<sub>2</sub>-producing bioreactor using near-infrared spectroscopy. *Water Res* 2009;43(7):1823–30.
- [27] Sayess RR, Saikaly PE, El-Fadel M, Li D, Semerjian L. Reactor performance in terms of COD and nitrogen removal and bacterial community structure of a three-stage rotating bioelectrochemical contactor. *Water Res* 2013;47(2):881–94.
- [28] Rismani-Yazdi H, Christy AD, Carver SM, Yu Z, Dehority BA, Tuovinen OH. Effect of external resistance on bacterial diversity and metabolism in cellulose-fed microbial fuel cells. *Bioresour Technol* 2011;102(1):278–83.
- [29] Yu CP, Liang Z, Das A, Hu Z. Nitrogen removal from wastewater using membrane aerated microbial fuel cell techniques. *Water Res* 2011;45(3):1157–64.
- [30] Shehab N, Li D, Amy GL, Logan BE, Saikaly PE. Characterization of bacterial and archaeal communities in air-cathode microbial fuel cells, open circuit and sealed-off reactors. *Appl Microbiol Biotechnol* 2013;97(22):9885–95.
- [31] Ghanimeh S, El-Fadel M, Saikaly P. Improving the stability of thermophilic anaerobic digesters treating SS-OFMSW through enrichment with compost and leachate seeds. *Bioresour Technol* 2013;131:53–9.
- [32] Saikaly PE, Oerther DB. Diversity of dominant bacterial taxa in activated sludge promotes functional resistance following toxic shock loading. *Microb Ecol* 2011;61(3):557–67.
- [33] Kim BH, Park HS, Kim HJ, Kim GT, Chang IS, Lee J, et al. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. *Appl Microbiol Biotechnol* 2004;63(6):672–81.
- [34] Yates MD, Kiely PD, Call DF, Rismani-Yazdi H, Bibby K, Peccia J, et al. Convergent development of anodic bacterial communities in microbial fuel cells. *ISME J* 2012;6(11):2002–13.
- [35] Wrighton KC, Agbo P, Warnecke F, Weber KA, Brodie EL, DeSantis TZ, et al. A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. *ISME J* 2008;2(11):1146–56.
- [36] Butler CS, Nerenberg R. Performance and microbial ecology of air-cathode microbial fuel cells with layered electrode assemblies. *Appl Microbiol Biotechnol* 2010;86(5):1399–408.