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Optimizing the performance of microbial fuel cells fed a combination of different synthetic organic fractions in municipal solid waste

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ABSTRACT

The objective of this study was to establish the impact of different steam exploded organic fractions in municipal solid waste (MSW) on electricity production using microbial fuel cells (MFCs). In particular, the influence of individual steam exploded liquefied waste components (food waste (FW), paper–cardboard waste (PCW) and garden waste (GW)) and their blends on chemical oxygen demand (COD) removal, columbic efficiency (CE) and microbial diversity was examined using a mixture design. Maximum power densities from 0.56 to 0.83 $W\,m^{-2}$ were observed for MFCs fed with different feedstocks. The maximum COD removed and minimum CE were observed for a GW feed. However, a reverse trend (minimum COD removed and maximum CE) was observed for the FW feed. A maximum COD removal (78%) accompanied with a maximum CE (24%) was observed for a combined feed of FW, PCW plus GW in a 1:1:1 ratio. Lactate, the major byproduct detected, was unutilized by the anodic biofilm community. The organic fraction of municipal solid waste (OFMSW) could serve as a potential feedstock for electricity generation in MFCs; however, elevated protein levels will lead to reduced COD removal. The microbial communities in cultures fed FW and PCW was highly diversified; however, the communities in cultures fed FW or a feed mixture containing high FW levels were similar and dominated by *Bacteroidetes* and *β-proteobacteria*.

1. Introduction

The availability of inexpensive electron donor feedstocks is an important factor in developing an economical microbial fuel cell (MFC) technology. Low value organic substrates in municipal solid waste (MSW) are potential sources of electron donors for MFCs. The global quantity of MSW produced annually in 2010 of approximately 1.3 billion tonnes is expected to increase to 2.2 billion tonnes by 2025 (Gardner, 2002). In Canada, the quantity of MSW generated annually in 2008 reached 34 million tonnes (Statistics Canada, 2008) whereas in the United States (U.S.), the quantity generated in 2011 was estimated at 250 million tonnes (USEPA, 2011). Based on the organic fraction content, approximately 110–170 million tonnes of organic municipal solid waste (OMSW) is produced annually in Canada and the U.S. (37–55 million tonnes of paper and cardboard waste (PCW), 53–80 million tonnes of food waste (FW) and 21–32 million tonnes of garden waste (GW)) (Statistics Canada, 2008; USEPA, 2011).

Because of the large quantities of MSW generated globally, efficient management practices must be implemented to protect human health, reduce environmental impacts and preserve natural resources. Common practices used to manage MSW include materials or energy recovery by recycling, composting, land filling, anaerobic digestion and combustion with energy recovery. Negative impacts caused by the disposal of MSW in landfills include leachate production and uncontrolled greenhouse gas (methane) emissions. According to the United States Environmental Protection Agency (USEPA), the annual global quantity of landfill methane produce in Asia, Latin America and Africa is equivalent to 37 million tonnes of carbon dioxide equivalent (USEPA, 2002).

An alternative approach to manage MSW is to develop and/or improve existing technologies which can produce value added products from the organic fraction of municipal solid waste (OFMSW). Existing treatment processes include heat, combustion, gasification, pyrolization, landfill gas (LFG) recovery and anaerobic

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digestion (UNEP, 2005). Commercially available technologies such as combustion and anaerobic digestion (AD) are widely used to produce electricity and biogas, respectively, from OFMSW. In both processes, steam and biogas intermediates are converted into electricity using a steam/biogas powered electric generator. An alternative technology, which does not produce biogas nor require biogas combustion to produce electricity, is a microbial fuel cell (MFC) (Harnisch and Freguia, 2012). In MFCs, organic matter degradation can occur at temperatures below 20 °C and at lower substrate concentration; however, AD operating these conditions usually fails because of slow reaction rates (Nimje et al., 2012). In comparison to AD, MFCs are able to convert biodegradable substrates directly into electricity. Based on work reported by Venkata Mohan et al. (2010), Jia et al. (2013) and El-Chakhtoura et al. (2014), the percent COD removed from MSW fed to MFCs can range from approximately 60–85%. According to Jia et al. (2013), single-chamber air cathode MFCs fed a food waste were able to achieved a current density and CE of 556 mW m^{-2} and 23.5%, respectively.

Electricity production from renewable organic biomass using MFCs is a rapidly growing research area which has received widespread attention as an alternative energy producing technology. Converting the biodegradable fraction of OFMSW into bioelectricity is a novel waste-to-energy approach which is carbon neutral because the carbon as CO_2 produced from biomass undergoing degradation is recycled and converted into biomass via photosynthesis.

MFCs are able to produce electricity using pure and mixed microbial cultures. Using a mixed microbial community as a culture source in MFCs is advantageous for many reasons. Mixed consortiums are adaptable to a variety of complex substrates. In addition, these microorganisms interact syntrophically, they exhibit stress resistance and they are able to produce higher currents and power densities when compared to pure cultures (Nimje et al., 2012). Mixed microbial cultures are robust and able to degrade a wide variety of substrates (Ren et al., 2007) such as short chain fatty acids (Kumar et al., 2008), starch and cellulose (Spets et al., 2008), organic wastes (Moqsud et al., 2013), corn stover (Zuo et al., 2006) and organic chemicals in wastewater effluents (Harnisch and Freguia, 2012; Velasquez-Orta et al., 2011).

Organic municipal solid waste (OMSW) could serve as a potential feedstock because it is available in large quantities and it contains easily degradable substrates. Typically OMSW has an energy value of approximately 11.6 GJ tonne^{-1} (Wise, 1994). Assuming the total quantity of OMSW generated in the U.S. and Canada is approximately 280 million tonnes, the annual energy content of this waste is 3.25 EJ (3.25×10^{18} J) or 531 MBOE (million barrel of oil equivalent). Assuming the energy output from an MFC of 8700 MJ tonne^{-1} (2425 kW h tonne^{-1}) of waste (Goud et al., 2011), approximately 190 terawatt hours can be produced from 280 million tonnes of waste.

Obtaining a well-defined uniform OMSW as a feedstock for MFCs is impractical because temporal and spatial changes cause non-uniform compositions. The composite nature of OMSW containing carbohydrates, proteins and fats can influence the treatment efficiency of MFCs. If OMSW is to be considered as a feedstock for MFCs, then it is critical to understand the impact of compositional variations on microbial structural and functional diversity as well as electricity production. Hence, the objectives of this study are to prepare different feedstock compositions using a mixture design and to predict the impact of feeding different mixtures on electricity production using an air cathode MFC. The third objective is to interpret the relative contribution, possible interactions between microbial diversity, columbic efficiency and substrate consumption (chemical oxygen demand (COD) removal)) using response surface methodology (RSM).

2. Materials and methods

2.1. Preparation of different feedstocks

A synthetic food waste (FW) was used to minimize compositional variation in the feedstock. Following the Canada Food Guide (Health Canada, 2011), vegetables, carbohydrates, protein and fat were the major components in the synthetic FW. The main constituents (wt% wet solids) were cooked rice (13), cooked pasta (13), bread (10), ground lean meat (18), potato (10), lettuce (8), broccoli (8), apple (10) and banana (10). The FW slurry was prepared by homogenizing the components. The PCW slurry and GW slurries were prepared by homogenizing mixtures containing 50 g paper plus 50 g corrugated cardboard in 1.5 L water and 50 g grass and 250 g wood shavings in 3.0 L water, respectively. The slurries were heat treated in a steam explosion reactor operating in batch mode with a working volume of 2 L. Based on the materials characteristics, the steam explosion time was varied (0.5–1 h) to minimize the formation of furans and to achieve higher COD levels. The FW slurry was heated at 160 °C for 0.5 h while the PCW and GW slurries were heated for 1 h at 160 °C.

2.2. MFC construction

Single-chamber MFCs were constructed in accordance with work reported by Liu and Logan (2004). The cylindrical Plexiglas MFCs (empty bed volume 28 mL) were 4 cm long and 3 cm in diameter. The graphite fiber brush anodes (2.5 cm diameter × 2.5 cm long; fiber type PANEX 33 160 K, ZOLTEK) were heat treated at 450 °C for 30 min to enhance the power production (Liu and Logan, 2004; Feng et al., 2010). The cathodes were constructed using wet proofed carbon cloth (7 cm^2) containing a Pt catalyst (0.5 mg cm^{-2}) on the water side (inside) and four polytetrafluoroethylene (PTFE®) layers (4 mg cm^{-2} of PTFE per coating) on the air side (outside) (Cheng et al., 2006).

2.3. MFCs operation

The MFCs were inoculated with flocculated anaerobic inoculum (10 g VSS L^{-1}) which was obtained from a municipal wastewater treatment plant (Chatham, ON). The reactors were fed a medium containing 2 g COD L^{-1} feedstock as per Table 1 plus the following (per liter): 310 mg NH_4Cl; 130 mg KCl; 4.97 g $NaH_2PO_4 \cdot H_2O$; 2.75 g $Na_2HPO_4 \cdot H_2O$ and a mineral solution (10 mL) plus a vitamin solution (10 mL) (Lovley and Phillips, 1988). The MFCs were operated repeatedly by decanting and filling with media until a biofilm was formed on the carbon brush anode and a maximum stable voltage was observed. During repeated operation, the MFC was refilled with media and substrate when the voltage decreased to less than 50 mV (1 KΩ resistor). The MFCs were operated for 70 cycles (feed, reaction and decant) to achieve stable conditions. All experiments were conducted in duplicate at 37 °C and an initial pH of 7.1.

2.4. Data acquisition, calculations and electrochemical analysis

The voltage (V) across an external resistor (1 KΩ) in the MFCs circuit was monitored at 10 min intervals using a data logger (Agilent Instruments, OH) configured to a personal computer. Maximum current densities were evaluated by measuring the current per m^2 of the cathode using linear sweep voltammetry (LSV) at a scan rate of 0.1 mV s^{-1} from open circuit voltage (OCV) to 100 mV (Logan et al., 2006). The maximum power density was calculated using Eq. (1).

$$P = IV/A. \tag{1}$$

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Table 1
Mixed design for coded and actual values together with responses (COD removal and CE).

Run No.	FW		PCW		GW		COD removal (%)	CE (%)
	Coded	Actual (g COD L^{-1})	Coded	Actual (g COD L^{-1})	Coded	Actual (g COD L^{-1})		
1	1	2	0	0	0	0	68.5	24.6
2	0	0	1	2	0	0	75.5	21.4
3	0	0	0	0	1	2	83.8	20.1
4	0.5	1	0.5	1	0	0	76.4	23.1
5	0.5	1	0	0	0.5	1	78.6	21.8
6	0	0	0.5	1	0.5	1	79.3	20.9
7	0.333	0.667	0.333	0.667	0.333	0.667	77.5	23.5
8	0.667	1.334	0.167	0.333	0.167	0.334	73.3	23.3
9	0.167	0.334	0.667	1.333	0.167	0.334	78.7	22.0
10	0.167	0.334	0.167	0.333	0.667	1.334	81.5	21.2

Note:
1. FW = food waste; PCW = paper–cardboard waste; GW = garden waste.

where P is the power density, V is the voltage, I is the current and A is the (m^2) surface area of the cathode. The power densities calculated from the LSV method were compared with the results obtained by the 'single cycle' method which consisted of varying the resistance (between 7500 and 50 Ω) from the OCV (Heilmann and Logan, 2006). The columbic efficiency (CE) was calculated using the following equation (Logan et al., 2006)

$$\mathrm{CE} = \frac{M\int_0^{t_b} I\,dt}{Fb\vartheta_{\mathrm{An}}\Delta\mathrm{COD}} \tag{2}$$

where $M = 32\ \mathrm{g\ mol^{-1}}$ (molecular weight of oxygen), $b = 4$ electrons transferred per mole of oxygen, $F = 96485.4\ \mathrm{C\ mol^{-1}}$ (Faraday's constant), υ_{An} is the liquid volume in the anode chamber and ΔCOD is the difference in COD consumed over the reaction period.

Cyclic voltammetry (CV) studies were performed using an electrochemical analyzer (CH 7420d, CH Instrument, TX). The CV studies were carried out with and without biofilm exposed to medium containing the substrate using the anode as a working electrode, the cathode as counter electrode and the $Ag/AgCl_2$ electrode (CH Instruments, TX) as a reference electrode. CVs profiles were obtained at a scan rate of $1\ \mathrm{mV\ s^{-1}}$ between −0.5 V and +0.2 V *vs* Ag/AgCl.

2.5. Analytical methods

The chemical oxygen demand (COD) for the initial and final MFC liquid samples was determined in accordance with *Standard Method* (APHA, 1999). The total carbohydrate content was estimated by the anthrone method (Loewus, 1952). The quantity of free reducing sugars was determined using the dinitro-salicylic acid method (Miller, 1959). The detection limit for the reducing sugars was $100\ \mathrm{mg\ L^{-1}}$. Furfural and HMF were analyzed using high performance liquid chromatography (HPLC) (Pendyala et al., 2013). The detection limit for furfural and HMF was $0.5\ \mathrm{mg\ L^{-1}}$ using a 10 μL injection volume. The total amount of phenolic compounds was determined using the Folin–Ciocalteu (FC) reagent using catechol as a standard (Singleton and Rossi, 1965). The detection limit for phenols was $20\ \mathrm{mg\ L^{-1}}$. The protein content of the initial and final samples was estimated by the Lowry method (Lowry et al., 1951). The detection limit for the protein content (Bovine Serum Albumin) was $0.02\ \mathrm{mg\ mL^{-1}}$. Liquid samples were removed at the end of the cycle when the voltage attained a value less than 50 mV. At the end of the cycle, the volatile fatty acids (VFAs) metabolites were analyzed using ion chromatography (IC) (Chowdhury et al., 2007). The detection limits for formate (MP Biomedicals, CA), acetate (Merck KGaA, CA), propionate (JT Baker, CA) and butyrate (Lancaster Synthesis Inc., USA) was $0.5\ \mathrm{mg\ L^{-1}}$.

2.6. Mixture design for substrate optimization

In order to establish the optimum mixture substrate composition for maximum COD removal and maximum CE, the different feedstock compositions examined were based on varying the fractions of FW, PCW and GW in the feed. An augmented-simplex centroid mixture design was employed in this study (Goupy and Creighton, 2007). Single and interaction concentrations were optimized using a mixture design. In this design, the sum of the proportions for the different substrate components (FW, GW and PCW) was 100% (equivalent to $2\ \mathrm{g\ COD\ L^{-1}}$). The selected mixture design consisted of 10 experiments, three utilizing pure substrates (FW, GW and PCW), three with binary, three with tertiary blends and one at the center point (Table 1). The experimental conditions following the mixture design model as well as the response variables for COD removal and CE are shown in Table 1.

Linear, quadratic, special cubic and full cubic models (Design expert 8.0) shown as Eqs. (3)–(6) were used to model the mixture design responses (COD removal and CE).

Linear

$$Y = \sum_{i=1}^{p}\beta_i X_i \tag{3}$$

Quadratic

$$Y = \sum_{i=1}^{p}\beta_i X_i + \sum\sum_{i<j}^{p}\beta_{ij}X_iX_j \tag{4}$$

Special cubic

$$Y = \sum_{i=1}^{p}\beta_i X_i + \sum\sum_{i<j}^{p}\beta_{ij}X_iX_j + \sum\sum\sum_{i<j<k}^{p}\beta_{ijk}X_iX_jX_k \tag{5}$$

Full Cubic

$$Y = \sum_{i=1}^{p}\beta_i X_i + \sum\sum_{i<j}^{p}\beta_{ij}X_iX_j + \sum\sum\sum_{i<j<k}^{p}\beta_{ijk}X_iX_jX_k + \sum\sum_{i<j}^{p}\delta_{ij}X_iX_j(X_i - X_j) \tag{6}$$

where Y is a response function, p represents number of constituents in the system, β_i, β_{ij} and β_{ijk} are linear, quadratic and special cubic coefficients, respectively, and δ_{ij} is a parameter of the cubic model. The β_iX_i, $\beta_{ij}X_iX_j$, $\beta_{ijk}X_iX_jX_k$, $\delta_{ij}X_iX_j(X_i - X_j)$ terms represents linear, square, special cubic and cubic terms, respectively, and X is the proportion of the constituent. The β_{ij} parameter represents either synergic or antagonistic blending.

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2.7. Microbial analysis

After 120 days, microbial samples removed from the MFCs operating under the different experimental conditions (Table 1) were analyzed for microbial diversity using the terminal restriction fragment length polymorphism (T-RFLP) procedure. Samples of the carbon brush fibers were detached from the anode and then rinsed with sterile distilled water to remove microbial samples. Biofilm was separated from the fibers by vortexing and sonication. DNA was isolated from the microbial samples, quantified and PCR amplified using dye labeled universal bacteria and Archaea PCR primers for the 16S rRNA gene targeting the V1–V3 region (Chaganti et al., 2012). The purified PCR product was digested with two restriction enzymes (*Hae* III and *Hha* I) to generate terminal restriction fragments (T-RFs). The enzyme digested PCR products were separated using DNA analyzer (LiCor) using polyacrylamide gel electrophoresis.

2.8. Statistical analysis of microbial profiles

Duplicate T-RF profiles were aligned on the basis of the T-RF lengths and individual peak areas using the moving average algorithm in the T-Align software (Smith et al., 2005). For the T-RF profiles of the 16S rRNA genes, only terminal fragments with >1% of the peak height were considered to minimize band variation among the samples. T-RF profiles obtained from the restriction enzyme *Hae* III were analyzed using a non-parametric multivariate cluster analysis (MCA). The Kulczynski similarity procedure was used to assess the impact of different conditions (Table 1) on the microbial profile based on the presence or absence of T-RFs (Anderson et al., 2011). The MCA was performed using the PAST software package (Hammer et al., 2001). The T-RF profiles of *Hae* III and *Hha* I generated by digestion of the PCR-amplified 16S rRNA genes of MFCs microbial samples were formatted for Phylogenetic Assignment Tool (PAT; https://secure.limnology.wisc.edu/trflp/) and analyzed online using the default fragment bin tolerance window settings. Phylogenetic assignment was performed using a modified database consisting of the default database generated from MiCA (http://mica.ibest.uidaho.edu/) plus experimentally determined T-RFs for microorganisms identified from the 16S rRNA gene clone library analysis (Chaganti et al., 2012).

3. Results

3.1. Characterization of steam exploded liquors

The COD concentration as well as the quantity of free reducing sugars, furfurals and phenolic compounds in the steam exploded and untreated samples are shown in Table 2. In comparison to PCW, higher COD levels were detected in the steam exploded FW and GW samples. Diluting the steam exploded liquors to a final concentration of 2 g L^{-1} COD was performed to decrease the furans (furfural and hydroxy methyl furfural (HMF)) and phenolics levels in the feed streams to the MFCs (FW (24.1 mg L^{-1}), PCW (168.4 mg L^{-1}) and GW (101.9 mg L^{-1})) (Table 2).

3.2. Feeding diluted OFMSW steam exploded liquor to MFCs

Stable levels of current production using steam exploded liquors from the different waste materials were reproducible after 5 feeding cycles. The voltage, current density and power density produced with liquors from the different waste materials are shown in Fig. 1. The polarization data revealed that the current density and power density ranged from 2.14 A m^{-2} to 2.67 A m^{-2} and 0.56 W m^{-2} to 0.83 W m^{-2}, respectively. A maximum power density of 0.83 W m^{-2} at a current density of 2.57 A m^{-2} ($R_{ext} = 180\ \Omega$) was observed for GW (run 3) (Fig. 1A). In case of a dual mixture in a 1:1 ratio, a maximum power density of 0.78 W m^{-2} at a current density of 2.59 A m^{-2} ($R_{ext} = 167\ \Omega$) was observed for the FW plus GW fed MFCs (run 5) (Fig. 1B). However, for the 3 component mixture fed MFCs, a maximum current density of 2.67 A m^{-2} ($R_{ext} = 153\ \Omega$) with a power density of 0.76 W m^{-2} was achieved with a feed containing three different substrates in equal proportion (run 7) (Fig. 1C). Power overshoot was observed in MFCs fed three different substrates in equal proportion (run 7). According to Hong et al. (2011), power overshooting in MFCs with higher current densities leads to under estimating the performance.

Cyclic voltammetry was employed to characterize the electron transfer mechanisms between microorganisms and between microbial biofilms and electrode surfaces. The oxidation and reduction peaks obtained from the MFCs fed three different substrates were distinct based on their shape (Fig. 2). A maximum current (2.42 mA) was observed for MFCs fed with GW followed by FW (2.19 mA) and PCW (2.0 mA). A first-order derivative analysis of the cyclic voltammograms revealed the potentials for the redox systems were in the range of −0.4 to −0.2 V.

3.3. Mixture design analysis of organic matter consumption (COD removal) and coulombic efficiency (CE) for diluted feeds

The COD removal rate is influenced by the composition of bacterial communities as well as the composition of the feed (Velasquez-Orta et al., 2009). The COD removal and CE were compared for the different substrates and mixtures. The results showed the COD removed and CE for all the feed conditions ranged from 69% to 84% and 20% to 25%, respectively (Table 1). The maximum COD removed (84%) was obtained for the GW substrate (run 3) and the maximum CE (25%) was detected for the FW substrate (run 1). However, a maximum CE (24%) accompanied with a

Table 2
Various characterization parameters of steam exploded filtrate samples for different waste materials.

Sample	COD (g L^{-1})	Free reducing sugar (g L^{-1})	Furfural (mg L^{-1})	Hydroxy methyl furfural (mg L^{-1})	Phenolic compounds (mg L^{-1})
FW	110	27 (0.49)	35 (0.64)	440 (8.0)	851 (15.5)
PCW	4.2	1.6 (0.76)	4.1 (1.95)	32.4 (15.4)	318 (151)
GW	24	6.9 (0.58)	16.5 (1.38)	138 (11.5)	1069 (89)
UFW	61	16	ND	ND	ND
UPW	1.9	1	ND	ND	ND
UGW	9.1	2.6	ND	ND	ND

Notes:
1. FW = Food waste; PCW = Paper cardboard waste; GW = Garden waste; U = Untreated.
2. Values in parenthesis represent the initial concentration when normalized to a total concentration of 2 g COD L^{-1}.
3. ND = Not detectable.

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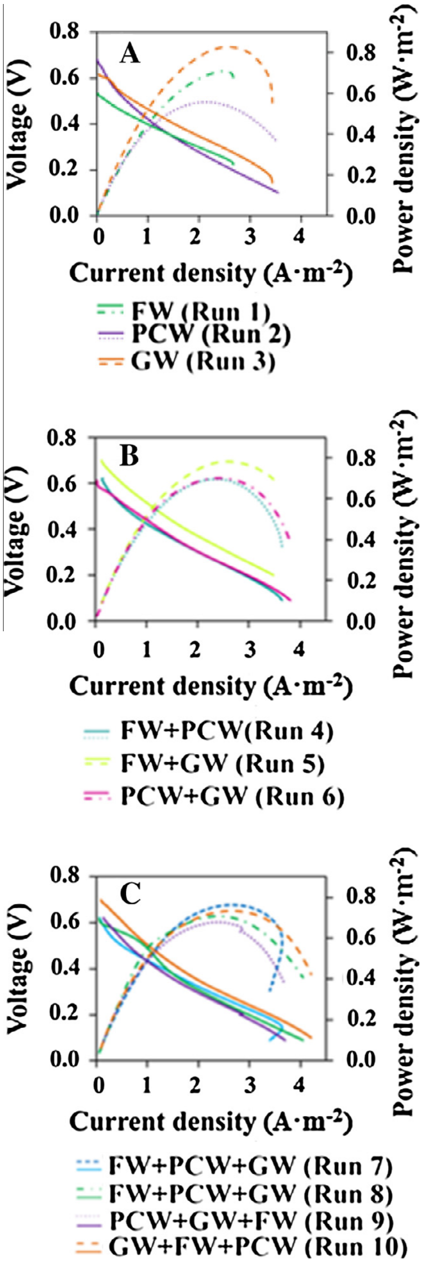

Fig. 1. Power density (dashed lines) and whole cell polarization curves (solid lines) of single chamber microbial fuel cells for (A) Individual substrates FW, PCW and GW. (B) Combination of two substrates at a 1:1 ratio FW + PCW, FW + GW and PCW + GW. (C) Combinations of three substrates (FW, PCW and GW) are according to Table 1.

maximum COD removal of 78% was also observed for a feed containing the three substrates in equal proportions (run 7).

Data from all experimental conditions (Table 1) were further analyzed using a multiple-linear regression with the COD removal and CE designated as the response variables. Sequential selection of the component terms (linear to full cubic) was used in assessing the different models (Table 3). Sequential sampling is a non-probability sampling method in which a single or a group of samples are selected in a given time period and the data is subsequently analyzed. Next, another series of samples are selected, the data analyzed and the process repeated (Goupy and Creighton, 2007). The analysis showed that the quadratic model was a suitable fit for the COD removal response with an R^2 value of 0.9686 and a p-value of 0.0002 (Table 3). However, for the CE response,

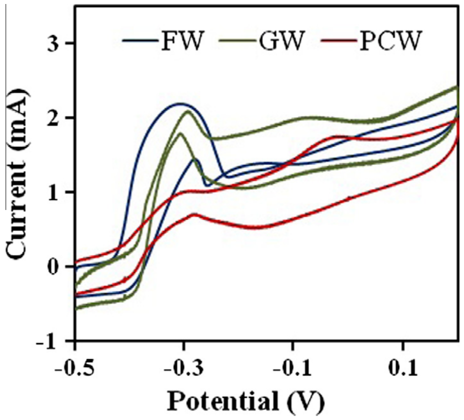

Fig. 2. Cyclic voltammograms for anodic biofilms. Notes: The CV's were recorded for MFCs fed with individual substrates.

a special cubic model with a large R^2 (0.9652) and low p-value (<0.0066) was identified as the best-fit model. For COD removal, the special cubic and full cubic model order terms with p-values > 0.05 indicate these models were insignificant. In case of the CE cubic model, p-values for the terms were >0.05 and hence, the cubic model was insignificant and aliased. Aliased terms are those in which the effects are undistinguishable (Urdhwareshe, 2012).

An analysis of variance (ANOVA) was used to establish the adequacy and significance of the best fit model quadratic (Eq. (4)) and special cubic (Eq. (5)) for the COD removal and CE responses, respectively ($p < 0.05$) (Tables S1A and S1B). For the COD removal response, the linear and quadratic terms (FW × PCW and FW × GW) were significant. Similarly, in the case of the CE response, linear terms, quadratic term (FW × GW) and special cubic term (FW × PCW × GW) were significant. In both cases, the model term PCW × GW was insignificant. In both of these feeds, the carbohydrate content was ⩾90% (Run #2 (PCW) and #3 (GW) Table 4). Due to the similar nature of the substrate composition for PCW and GW, their combination did not show any significant influence on either CE or COD removal. Note all the terms in combination with FW were significant because the FW contain only 56% carbohydrate and a significant amount of protein (23%).

The empirical relationship between the responses (COD removal and CE) and substrate variables in coded units (Table 1) were quantified in using the quadratic expression (Eq. (7)) and a special cubic expression (Eq. (8)), respectively (Table 3).

$$\begin{aligned}\text{COD removal }(\%) = {} & 67.7 * \text{FW} + 76.2 * \text{PCW} + 83.5 * \text{GW} \\ & + 16.1 * \text{FW} * \text{PCW} + 9.52 * \text{FW} * \text{GW} \\ & - 3.23 * \text{PCW} * \text{GW}\end{aligned} \tag{7}$$

$$\begin{aligned}\text{CE}(\%) = {} & 24.4 * \text{FW} + 21.3 * \text{PCW} + 20 * \text{GW} + 0.47 * \text{FW} * \text{PCW} \\ & - 2.6 * \text{FW} * \text{GW} + 0.47 * \text{PCW} * \text{GW} \\ & + 32.8 * \text{FW} * \text{PCW} * \text{GW}\end{aligned} \tag{8}$$

The models were further analyzed using the Anderson–Darling (AD) statistic to establish if the residuals are normally distributed (Stephens, 1974). In both cases, the AD statistics indicated the residuals are normally distributed (data not shown).

The impact of the feedstock composition on the COD removal and CE are shown in surface 3D contour plots (Fig. 3A and B). The steep COD removal contour indicates greater sensitivity to variation in the mixture feedstock proportion. Notice, the COD removed increased with increasing GW and decreasing with the FW fraction (Fig. 3A). However, in case of CE, an opposite trend was observed. Between CE values of 20% to 23%, the steep contour

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Table 3
Regression coefficients of the different models for the mixture design responses (COD removal and CE).

Source	COD removal (%)			CE (%)		
	Sequential *p*-value	Adjusted R^2	Predicted R^2	Sequential *p*-value	Adjusted R^2	Predicted R^2
Linear	<0.0001	0.8464	0.7914	<0.0001	0.9052	0.8986
Quadratic	0.0002	0.9686	0.9520	0.4253	0.9063	0.7876
Special Cubic	0.5374	0.9666	0.9464	0.0066	0.9652	0.8553
Cubic	0.0651	0.9803	0.9435	0.9349	0.9526	Aliased

Note:
1. Underlined values indicate best fit model.

Table 4
Initial, final concentration and percentage removal of the carbohydrates and proteins in different MFCs in the mixture design.

Run No.	CHO (mg L^{-1})		CHO removal (%)	Protein (mg L^{-1})		Protein removal (%)
	Initial	Final		Initial	Final	
1	1120	135	88	520	450	13
2	1925	432	78	ND	ND	-
3	1810	260	86	130	70	46
4	1520	280	82	260	166	36
5	1461	196	87	325	243	25
6	1869	347	81	65	0	100
7	1621	272	83	217	127	41
8	1370	201	85	368	258	30
9	1772	349	80	108	47	57
10	1710	268	84	173	85	51

Notes:
1. CHO = carbohydrate.
2. ND = Not detected.
3. Conditions for the Run # (1–10) are shown in Table 1.

indicates that increasing CE values were associated with increasing the proportion of FW and reducing the proportion of GW and PCW (Fig. 3B). A feed comprising >17% FW and <67% GW showed a negative impact on the COD removal while a decreasing CE trend was observed under conditions with a feed consisting >17% GW and <67% FW. The results indicated a counterintuitive negative correlation between COD removal and CE (Fig. 4).

A numerical optimization method was employed to optimize the FW, PCW and GW ratio which resulted in maximum COD removal and maximum CE. The optimization routine showed a desirability contour with a single optimal solution (Fig. 3C) for a feed containing 40% (0.8 g COD^{-1}) FW, 34% (0.68 g COD^{-1}) PCW and 26% (0.52 g COD^{-1}) GW. Under this feed condition, the MFC removed approximately 78% of the COD and the CE reached 24%. Notice, the predicted optimal feed composition is close to the centroid point of the mixture design shown in Table 1 (Run 7).

3.4. Volatile fatty acids formation

The quantity of VFAs produced was determined at the end of each cycle for all the experimental conditions examined (Table 5). The data revealed that lactate was the major byproduct under all the feedstock treatments. Acetate, propionate, formate and butyrate were also detected in low quantities. The COD content of the residual VFAs together with COD removal accounted for approximately >90% of the COD mass balance for all conditions except for those where pure FW or PCW themselves were fed to the MFCs (Table 5).

3.5. Microbial characterization

The T-RFs profiles show considerable diversity in the bacterial community structure for the different experimental conditions. In MFCs fed FW, GW or PCW (Run 1, 2, 3 (Table 1)), the total number of T-RFs observed were 33, 33 and 18, respectively. A large number of T-RFs reaching 38 bands and 35 bands were detected in MFCs fed FW plus PCW (1:1 ratio) and FW plus GW (1:1 ratio), respectively. However, in MFCs fed PCW plus GW in a 1:1 ratio, only 18 T-RFs were detected. The lowest T-RF diversity (13 bands) was detected for a feed containing FW, GW plus PCW (1:1:1). Cluster analysis of the bacterial T-RFLP profiles for different anodic biofilms was conducted to compare the similarities in communities developed with different mixture substrates (Table 1). A cluster analysis revealed microbial populations from conditions designated as runs 1 (FW), run 8 (FW:PCW:GW), run 4 (FW:PCW) and run 5 (FW:GW) (Table 1) were grouped together with the highest similarity (66–72%) (Fig. 5). The microbial clustering observed is likely related to the large fraction of FW ($\geqslant$50%) in the feeds for runs 1, 4, 5 and 8 (Table 1). Less similarity was detected between FW ($\geqslant$50%) and GW or PCW with a similarity of $\geqslant$66% (run no. 2, 3, 9 and 10 (Table 1)) (Fig. 5). The Kulczynski index indicated that less similarity was observed among microbial communities fed different substrates (Fig. 5).

T-RFs were assigned to their associated microorganism by blasting against the modified MiCA database for the 16s rRNA gene. The blast results indicated the dominant T-RF bands in the FW-fed microbial communities were associated with *Bacteroidetes* and *β-proteobacteria*. However, for cultures fed GW, the major groups detected were *Clostridia*, *Bacteroidia* and *β-proteobacteria*. In contrast, PCW fed cultures were dominated by *Synergistia*, *α-proteobacteria* and *β-proteobacteria*.

4. Discussion

In this study, individual and mixtures of FW, PCW and GW were used as feedstocks for MFCs. The maximum power densities observed (0.56–0.83 W m^{-2}) were greater than values reported for a variety of wastewaters (<0.5 W m^{-2}) (Harnisch and Freguia, 2012; Spets et al., 2008; Ahn and Logan, 2010; Hays et al., 2011) and comparable to data (0.98 W m^{-2}) for *Chlorella vulgaris* algae powder as a substrate (Velasquez-Orta et al., 2009). However, in comparison, higher power densities of 1.2 W m^{-2} and 2.4 W m^{-2} were reported for MFCs fed a short chain fatty acid fermentation liquid and pure acetate, respectively (Logan et al., 2007; Chen et al., 2013). The power produced by different substrates is unpredictable because of variability in chemical structure and the energy released during degradation. During the degradation of complex macromolecules, a fraction of energy is consumed by hydrolysis and fermentation into simple compounds. In this study, the greater power density associated with the GW feed (Fig. 1) is likely due to the presence of higher carbohydrate levels and less amounts of furfurals and phenolic compounds.

Cyclic voltammetry can be used to detect the presence of redox molecules which are produced or consumed by microorganisms and also to sense the transfer of electrons to and from the electrode (Fricke et al., 2008). The method has been used in resolving the electron transfer mechanism governing catalytic processes at

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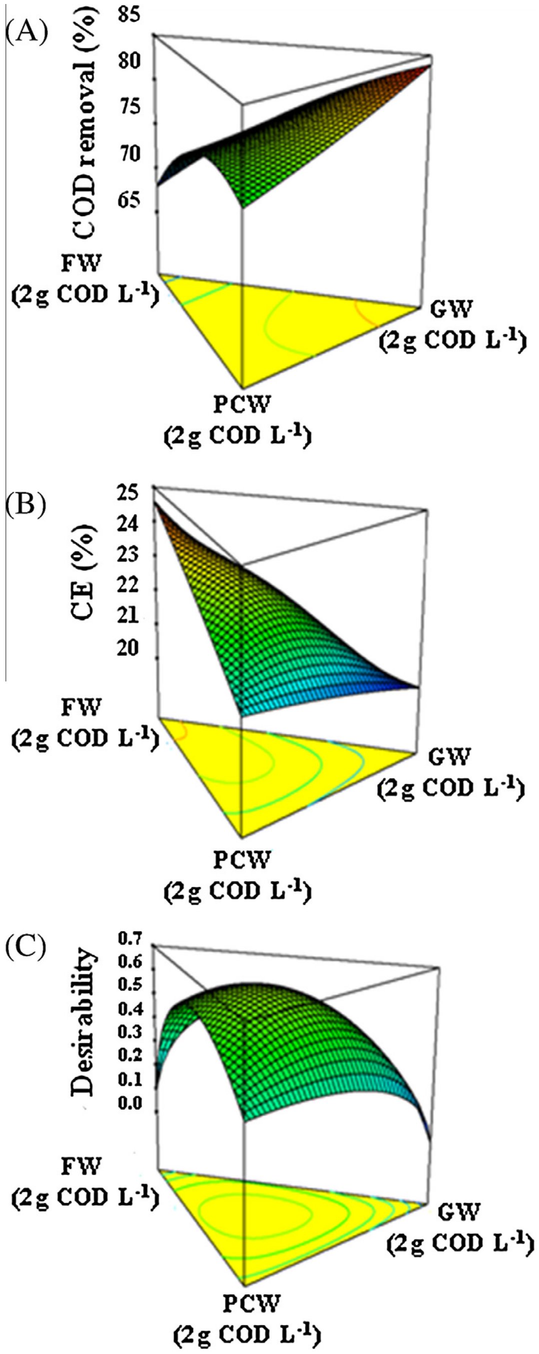

Fig. 3. Response surface plots for optimizing the feed (FW, PCW and GW) ratio using a mixture design. (A) COD removal; (B) CE; (C) Desirability plot for maximum COD removal accompanied with maximum CE. Notes: 1. Orange indicates a high response; 2. Green indicates a medium response; 3. Blue indicates a low response 4. The ratios of the substrate composition used in the mixture design are presented in Table 1.

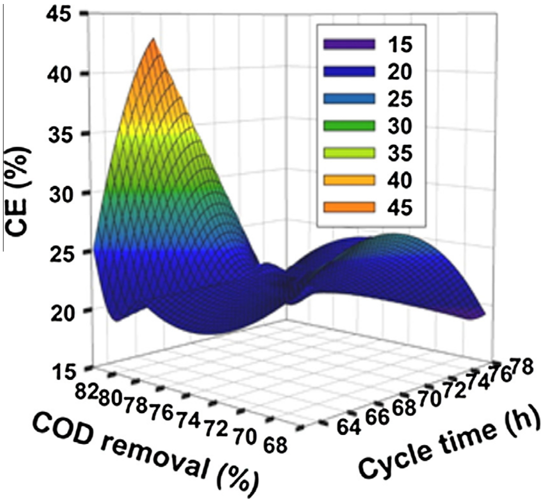

Fig. 4. 3D-mesh plot for optimizing the CE (%) for different substrates fed (Table 1) using the data format *XYZ* triplet, where *X* = cycle time (h); *Y* = COD removal (%); *Z* = CE (%).

biofilm-electrode interfaces. However, caution must be used when comparing CVs of biofilms enriched from diverse inocula such as those from wastewater treatment facilities. Note it is reasonable to compare similar CV profiles as a means to propose similar electron transfer mechanisms between microbial biofilms and electrodes. In this study, the sigmoidal shaped CV profiles observed are typical for *Geobacter* sp. (Fricke et al., 2008), an electroactive bacteria whose electrochemical activity is associated with outer membrane cytochromes. The range of redox values (−0.4 to −0.2 V) are characteristic for cytochromes which are involved in the direct electron transfer of electrons between microorganisms and anodic electrode surfaces (Fricke et al., 2008; Inoue et al., 2010). Based on the CV data for the microbial suspension, the generation of no significant current (results not shown) indicate that electron transport is mainly due to the surface confined redox bacterial biofilms developed over the carbon brush. This suggests a direct electron transfer mechanism is involved.

For MFCs fed with single waste components (FW, PCW or GW), the COD removal trend was as follows: GW > PCW > FW. A similar trend was observed for MFCs fed with mixture substrates based on their presence and proportion. This difference in the quantity of COD removed could be attributed to the carbon structure of chemicals in the different feedstock components (FW, PCW or GW). Notice higher levels of carbohydrate in PCW (96%) and GW (90%) compared to FW (56%) could be attributed to this trend (Table 4).

Beyond a protein threshold level range of approximately 65–108 mg L^{-1}, the percent protein removed decreased drastically. High COD removals were likely attributed to a feedstock containing a low level of protein coupled with an elevated quantity of carbohydrate (Velasquez-Orta et al., 2009). Data for pure protein substrates and substrates containing high protein levels showed lower COD removal (Heilmann and Logan, 2006; Velasquez-Orta et al., 2009). This trend would be due to biodegradable nature of complex protein compounds and unbalanced carbon to nitrogen (C/N) ratio. Similarly in the present study, a waste containing a high fraction of carbohydrates such as in the PCW and GW lead to elevated COD removals. However, the lower COD removal detected in PCW in comparison to the GW was likely attributed to the presence of a higher quantity of furfurals and phenolic compounds. Data from this study are in agreement with the maximum COD removals (80–90%) reported using wastewater effluents, landfill leachate, municipal solid waste substrates (Harnisch and Freguia, 2012; Spets et al., 2008; Liu et al., 2004; You et al., 2006) (Table 6) and higher than primary effluent of municipal waste water (Zhang et al., 2013). The high carbohydrate, low protein content and the absence of phenols and furans likely contributed to the high COD removal for wastewater feedstocks.

The CE values (20.1–24.6 (Table 1)) are less than those reported for pure carbon substrates such as acetate (86%) and ethanol (49%) as well as propionate (41%) (Torres et al., 2007). However, the CE values are in the range of values reported for simple, complex wastewater and municipal solid waste substrates (0.8–30%) (Liu et al., 2004; Cercado-Quezada et al., 2010; He et al., 2005;

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Table 5
Average percent COD removed and residual volatile fatty acids (VFAs) with different combinations of MFCs following the mixture design.

Run #	Lactate (mg L^{-1})	Acetate (mg L^{-1})	Propionate (mg L^{-1})	Formate (mg L^{-1})	Butyrate (mg L^{-1})	VFA COD (%)	COD removal (%)	COD balance (%)
1	208	0.03	0.03	0.57	11.5	11.0	68.5	79.5
2	108	0.20	0.29	0.15	3.0	5.6	75.5	81.0
3	114	0.06	0.61	0.29	5.8	6.0	83.8	89.8
4	338	0.36	1.81	0.06	0.0	17.0	76.4	93.4
5	251	0.01	0.03	0.04	0.0	12.6	78.6	91.2
6	313	0.14	0.09	0.20	0.0	15.7	79.3	95.0
7	389	0.36	0.24	1.51	0.0	19.6	77.5	97.1
8	338	0.25	0.30	0.15	0.0	17.0	78.7	95.7
9	191	0.03	0.12	0.15	3.0	9.8	81.5	91.2
10	318	0.00	0.36	0.21	0.0	16.0	73.3	89.2

Notes:
1. Conditions for the Run # (1–10) are shown in Table 1.
2. Average for duplicate samples.

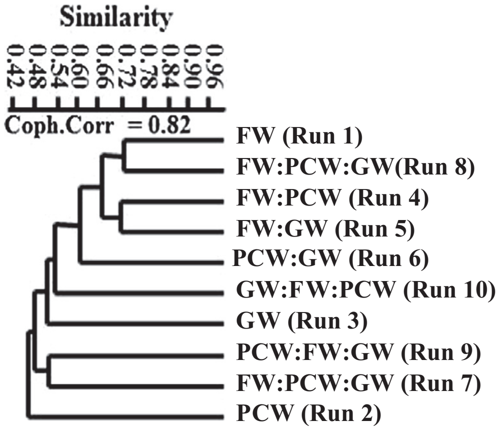

Fig. 5. Dendrogram for terminal restriction fragments (T-RFs) of the 16S rRNA for bacterial communities in the anode biofilm cultures fed with different composition of FW, GW and PCW (mixture composition is shown in Table 1).

Herrero-Hernandez et al., 2013; Min et al., 2005; Wen et al., 2009) (Table 6). A maximum CE was correlated with a high COD removal and a short cycle time (Fig. 4). In comparison, lower CEs were associated with low COD removal and long cycle time. Note, higher COD removal was observed with the GW feed; however, the longer cycle time resulted in a low CE. Low CE values with long cycle times have been reported by Feng et al. (2008). As the cycle time increases, oxygen diffusion from the air cathode into the reactor is enhanced and this results in oxygen reduction rather than electron transfer to the anode (Feng et al., 2008; Nevin et al., 2011). Thus, aerobic COD removal decreases the CE by transferring electrons to oxygen instead of the anode (Feng et al., 2008).

In the steam exploded liquors, the presence of furans at threshold levels is likely another factor contributing to low CE values. The different CE values observed for MFCs treating carbon based substrates could be attributed to a variety of factors. CE values are dependent on factors controlling the flow of electron fluxes such as pH and temperature as well as the substrate concentration (Zuo, 2008), substrate type (Teng et al., 2010), oxygen concentration (Liu and Logan, 2004), reactor configuration (Teng et al., 2010; Rittmann et al., 2008), inoculum (Teng et al., 2010) and organic loading as well as substrate degradation rate (Juang et al., 2011).

High COD removals and CE values are critical for the application of MFCs on a commercial scale. The COD removed and CE response desirability analysis indicates that mixed substrates are likely more important in improving both the COD removal and CE when compared to pure substrates. These results also revealed that the optimum COD removal is related to the substrate composition and an optimum CE is dependent on the initial removable COD concentration for MFCs configured with an air cathode.

Analysis of the VFAs data suggested that with the exception of lactate, the remaining fatty acids were utilized by mixed microbial communities in the anodic biofilm (Table 5). Metal and sulfate reducing bacteria (SRB) are actively involved in lactate utilization (Lovley and Phillips, 1988; Nielsen et al., 2009). The low band intensity for *δ-proteobacteria* indicates the presence of metal reducers and SRB at low levels. Hence, the elevated lactate levels are likely linked to low SRB levels. Similar results have been reported by other researchers for MFCs fed lactate (Jung and Regan, 2007) and municipal wastewaters (Ishii et al., 2008; Zhang et al., 2009). Many exoelectrogenic bacteria are members of *δ-proteobacteria* (*Geobacter* sp.) or *γ-proteobacteria* (*Shewanella* sp.) (Richter et al., 2008; Watson and Logan, 2010). However, *Shewanella* sp. is rarely detected on microbial anodes (Lovley, 2011). Both of these microorganisms were detected at low levels in the PCW fed cultures.

Although all the MFCs were inoculated with the same mixed anaerobic culture, the T-RF cluster analysis indicated least

Table 6
Comparison of power density, COD removal and CE with different municipal solid waste feedstocks.

Feedstock	Power density (mW m^{-2})	COD[a] removal (%)	CE[b] (%)	References
Vegetable waste	57.4	62.9	–[c]	Venkata Mohan et al. (2010)
Canteen based food waste	556	86.4	23.5	Jia et al. (2013)
OFMSW	123 ± 41	>86	24 ± 5	El-Chakhtoura et al. (2014)
Kitchen garbage	60	–[c]	–[c]	Moqsud et al. (2014)
Food waste[d]	710	68.5	24.6	This study
Paper waste[d]	560	75.5	21.4	This study
Garden waste[d]	830	83.8	20.1	This study
OFMSW blend[d]	760	77.7	23.5	This study

[a] COD = Chemical oxygen demand.
[b] CE = Coulombic efficiency.
[c] Data not reported.
[d] Steam exploded simulated.

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microbial similarity between feeds containing FW and PCW and the highest similarity for feeds dominated with FW. Teng et al. (2010) also observed the shifts in bacterial community structures when different compositions of VFA mixtures (acetate, propionate and butyrate) were used as the electron donor. Results from this work suggest the microbial composition of the anode biofilm was a function of the feed composition. This study also revealed that non-uniform biomass derived from OFMSW can be used as a potential feedstock for MFCs. However, regulating the protein content and removing furfurals and phenolic compounds in feedstocks could increase the percent COD removed and the CE.

5. Conclusions

MFCs fed with steam exploded liquor derived from a synthetic municipal organic solid waste produced maximum power densities in the range of 0.56–0.83 W m^{-2}. The optimum COD removed and CE produced were 78% and 23.5%, respectively for MFCs fed equal proportions of FW, PCW and GW. Results from the mixture design analysis revealed that variation in substrate type and proportion in feedstock mixture affected the COD removal, CE and microbial diversity of the anodic biofilm. The T-RFs analyses showed that the large microbial diversity detected in cultures fed 100% FW or 100% GW were linked to either optimum CE or optimum COD removal. In contrast, cultures fed FW, PCW and GW in a 1:1:1 ratio exhibited least diversity with both CE and COD removal optimum. Although the microbial composition of the biofilms changed with the substrate type, *β-proteobacteria* was abundant under all the feed conditions examined. The CV results suggest multiple cytochromes from mixed anaerobic community actively participated in the electron transfer between the bacteria and the anode; however, cytochromes expression is dependent on the electron acceptor availability in the surrounding environment.

The data showed mixed anaerobic cultures in MFCs are suitable and capable of utilizing complex substrates for power generation. An optimum OFMSW blend containing FW, PCW and GW in a 1:1:1 ratio can be further examined as a substrate for electricity production in MFCs. The data suggest that maximum COD removal which was accompanied with an optimum CE was associated with threshold protein content in the feed. Based on the different factor ranges under consideration, OMFSW could serve as a potential feedstock for electricity generation in MFCs; however, elevated protein levels are linked to reduced COD removal. Hence, blending of different waste groups to adjust the protein content in the feed will be required to improve COD removal rate of OFMSW in MFCs.

The voltage generated per MFC is approx. 0.5–0.7 V. However, this low voltage can be magnified by stacking the number of MFCs, which enables the opportunity to generate 100 V or more. For large scale applications, the configuration (electrode spacing and packing per unit volume of the reactor) and fuel (chemical composition) remain the important factors in power production.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.wasman.2015.12.032.

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