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Complete List of Authors:	Mahmoud, Mohamed; Arizona State University, Swette Center for Environmental Biotechnology at the Biodesign Institute Parameswaran, Prathap; Kansas State University, Department of Civil Engineering Torres, César; Arizona State University, Rittmann, Bruce; Biodesign Institute at Arizona State University, Center for Environmental Biotechnology
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Relieving the fermentation inhibition enables high electron recovery from landfill leachate in a microbial electrolysis cell

Mohamed Mahmoud [*] [§], Prathap Parameswaran [§] [†], César I. Torres [§], and Bruce E. Rittmann [§]

[§] Swette Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, P.O. Box 875701, Tempe, AZ 85287–5701, USA

[†] Department of Civil Engineering, Kansas State University, 2118 Fiedler Hall, Manhattan, KS 66506

Abstract

The energy value of the organic matter in landfill leachate can be captured with a microbial electrolysis cell (MEC), which oxidizes organic compounds at an anode and generates H_2 gas at a cathode. Due to the low biodegradability of the organic matter in landfill leachate, MEC performance typically is characterized by low current density (*j*), Coulombic efficiency (CE), Coulombic recovery (CR), and organic-matter removal. Here, we evaluated whether or not Fenton oxidation of landfill leachate could enhance MEC performance compared to control MEC fed with raw leachate. Fenton pre-treatment significantly improved the leachate's biodegradability, leading to much higher MEC performance: 52 ±10% BOD_5 removal, 29±3 % CE, and 1.42±0.27 A/m^2 as *j*, compared to 3±0.3% BOD_5 removal, 1.8±0.5% CE, and 0.11±0.06 A/m^2 as *j* for the raw leachate. This higher performance of the MEC fed treated leachate was associated with an ~ 5-fold increase in the biofilm accumulation compared to the control MEC. Acetate-spike experiments in the control MEC revealed that inhibition of fermentation, not anode respiration, was the main factor causing poor COD removal and current density with raw leachate. The microbial community in the biofilm anode with treated leachate was enriched in anode-respiring *Geobacteraceae*: ~40% of the sequences, compared to only ~20% for MEC fed with raw leachate. *Bacterioidetes*, *Firmicutes*, *Spirochaetes*, and *Actinobacteria* were among the other abundant phyla of fermenting bacteria in the biofilm anode fed with treated leachate. This study provides proof-of-concept that Fenton oxidation of landfill leachate enhanced MEC performance by accelerating fermentation, allowing more biofilm accumulation, and establishing a syntrophic relationship between fermenters and ARB.

Keywords: Microbial electrolysis cells; Landfill leachate; Fermentation inhibition; Anode-respiring bacteria; Microbial community management.

1. Introduction

[*] Corresponding author. Tel.: +1 480 727 7596; fax: +1 480 727 0889.
E-mail address: mmali4@asu.edu (M. Mahmoud).

The organic substrates fed to microbial electrochemical cells (MXCs) used for wastewater treatment usually are complex, which leads to a diverse microbial community.[1,2] The biodegradation of these complex wastewater must take place through a cascade of anaerobic reactions, including hydrolysis, fermentation, homoacetogenesis, and anode respiration; undesired processes, such as methanogenesis, also can occur in parallel. Fermentation appears to be the rate-limiting step in an MXC utilizing complex, but mostly soluble organic substrates, such as in landfill leachate. In an MXC, the fermenters must first break down the complex organic matter into simple substrates that can be efficiently consumed by anode-respiring bacteria (ARB).[3–6]

Although some of the fermentation can be carried out separately from anode respiration in a pre-fermentation reactor, other fermentations must occur along with anode respiration in the MXC due to the need for syntrophic relationships between fermenters and ARB. For example, syntrophic coupling of fermenters and ARB was required during the degradation of cellulose in a microbial fuel cell (MFC).[7] The maximum power density of 143 mW/m^2 (anode area) and Coulombic efficiency (CE) of 47% were obtained with a co-culture of *Clostridium cellulolyticum* and *Geobacter sulfurreducens*, whereas neither pure culture generated electric current. Likewise, a microbial electrolysis cell (MEC) fed with ethanol involved a three-way syntrophic interaction among fermenters, homoacetogens, and ARB.[6,8] So far, nothing is known about the syntrophic interactions among ARB and other microbial community members in MXCs fed with landfill leachate.

Over the past few years, current density (*j)*, CE, and Coulombic recovery (CR) of MXCs fed with a variety of complex organic substrates have significantly improved (Pant et al., 2010). For example, early MXC experiments had CE < 3% and maximum current density (j_{max}) < 0.2 A/m^2 when real wastewaters were used as the sole electron donors.[9–11] Subsequently, the performance has improved by applying pre-treatment technologies – such as pre-fermentation, microwave treatment, sonication, acid treatment, and alkaline treatment – that increase the bioavailability of the organic matter.[12–15] For example, when Mahmoud et al.[13] pre-treated a poorly biodegradable landfill leachate with mixed-culture fermentation, the degree of conversion to volatile fatty acids increased by 4-fold, and this led to a 68% increase in CE and a j_{max} up to 23 A/m^3 (or 1.7 mA/m^2)

in an MEC. Nevertheless, *j* values achieved so far with landfill leachate remain well below the target current density of ~140 A/m^3 needed to achieve an organic removal rate of ~ 1 kg 5-day biochemical oxygen demand (BOD_5)/m^3.d, as observed in anaerobic digesters treating landfill leachates.[13,16] Better pre-treatment approaches are needed for landfill leachate.

Among pre-treatment options, advanced oxidation processes (AOPs), through the strong, but non-selective action of hydroxyl free radicals ($OH^{\bullet}$), have promise to transform a variety of recalcitrant organic contaminants into forms that are more readily biodegradable.[17,18] One common AOP is the Fenton reaction, which relies on electron transfer between hydrogen peroxide (H_2O_2), the initiating oxidant, and Fe^{2+}, a homogenous catalyst, to yield $OH^{\bullet}$, which attacks the recalcitrant organic matter. The Fenton process occurs through a cascade of reactions that are summarized in Table S1 in Supporting Information.

Here, we use the Fenton reaction as pre-treatment to improve the biodegradability of organic matter in landfill leachate that is subsequently fed to an MEC. Previous work with the same leachate showed that, although the raw leachate had a relatively high absolute BOD_5 concentration, the *j*, CE, and CR values were very low, mainly due to toxicity in the influent organics.[13] Thus, we evaluated the feasibility of the Fenton process to improve the biodegradability of leachate before energy capture in a downstream MEC. Specifically, we evaluated if partial oxidation of organic matter in landfill leachate increased the fermentation kinetics of the organics for downstream electron recovery by ARB at an MEC anode. For this proof-of-concept effort, we used fixed ratios of $[H_2O_2]$:$[Fe^{2+}]$ and H_2O_2:COD. We investigated (1) the degree to which Fenton pre-treatment of leachate enhanced *j*, CE, CR, and organic-matter removal in an MEC; (2) what step of the biodegradation process that Fenton pre-treatment affected, and (3) how Fenton pre-treatment altered the microbial community in ways that explain the enhanced performance.

2. Materials and Methods

2.1. Landfill leachate

We collected landfill leachate from the Northwest Regional Landfill (Surprise, AZ) and kept it refrigerated at 4°C prior to use. The leachate samples had a dark brownish-black color, a relatively high concentration of organic matter (chemical oxygen demand (COD) = 2594 ± 94 mg/L; BOD_5 = 802 ± 10 mg/L; total organic carbon (TOC) = 663 ± 15 mg/L; and volatile fatty acids (VFAs) = 283 ± 73 mg/L), and good buffering strength (total alkalinity = 4068 ± 464 mg as $CaCO_3$/L with a pH = 8.1 ± 0.3). The leachate would be classified as a medium-age leachate based on its BOD_5/COD ratio of ~ 0.31.[19] The nitrogen content was relatively high, with most of nitrogen in inorganic forms (734 ± 4 mg TN-N/L and 645 ± 8 mg NH_3–N/L). Finally, the leachate had a high aromatic content, measured as absorbance at 254 nm normalized to TOC concentration (specific ultraviolet absorption at 254 nm (SUVA 254) = 1.23±0.06 L/mg TOC with 5-fold dilution), as well as to a high conductivity from the high concentration of chloride (3100 ± 20 mg/L) and sulfate (74.6 ± 1 mg/L). Throughout this study, we used the leachate samples without dilution.

2.2. Fenton reaction

We carried out batch Fenton-reaction experiments using 250-mL glass vessels mixed with a magnetic stir bar at a constant mixing speed of 150 rpm at ambient temperature (25 ± 2 °C). We used a $[H_2O_2]$:$[Fe^{2+}]$ molar ratio of 4.0 and a H_2O_2:raw leachate COD ratio of 1.1 (*w:w*) at an initial pH value of 3.5. First, we continuously and manually adjusted the reaction medium's pH to 3.5 ± 0.1 using 10 N NaOH or 50% H_2SO_4. After initial pH adjustment, we added measured amounts of ferrous sulfate ($FeSO_4.7\ H_2O$) to reach the targeted ferrous ion (Fe^{2+}) concentration. Then, we added H_2O_2 in one step to reach the designated H_2O_2 concentration. Just before the addition of H_2O_2 and Fe^{2+}, we collected a sample (set as reaction time = 0) to measure COD and TOC. Aliquots of treated leachate were taken every 30 min with a syringe, and the experiment was carried out for 3 hours. We split the samples into two portions. The first portion was used to measure residual H_2O_2 and COD after filtering the sample through a 0.22-μm filter membrane. The second portion was neutralized to ~ pH 9.0 with 10 N NaOH and then mixed in a beaker for

30 min with a magnetic stirring bar. We centrifuged the second sample for 10 min at 4000 rpm to collect the supernatant to analyze COD, TOC, and H_2O_2. In order to eliminate any possibility of H_2O_2 interference with COD measurements, we corrected the COD by subtracting the COD value equivalent to residual H_2O_2 from the measured COD. We repeated this experiment 6 times.

2.3. Microbial electrolysis cells

We used two dual-chamber, H-type MECs with a liquid volume of 320 mL in each chamber.[13] Anodes were two square graphite electrodes having a total surface area of 22 cm^2 (each 6.1 cm-long and 0.45 cm-width). We had treated the graphite-rod anodes by soaking them in 1 M H_2SO_4 for 12 h followed by soaking in 1N NaOH overnight. Following the treatments, we washed the graphite rod anodes 4 times with distilled water before placing them in the MEC. A 0.8-cm outer diameter (OD) graphite rod was the cathode, and the pH of the cathode was maintained at 12 by addition of 10 N NaOH. The cathode chamber was separated from the anode chamber by an anion exchange membrane (AMI 7001, Membranes International, Glen Rock, NJ). An Ag/AgCl reference electrode (BASI Electrochemistry, west Lafayette, IN) was placed about 0.5 cm away from the anode to control the anode potential at – 0.3 V vs. Ag/AgCl (– 0.046 V vs SHE) using a VMP3 digital potentiostat (Bio-Logic USA, Knoxville, TN). The temperature was controlled at 30°C in a temperature-controlled room, and the liquid in both chambers was mixed at 220 rpm using a magnetic stirrer.

Prior to the MEC start-up, we seeded the anode chamber with a mixture of effluent of an MEC supplemented with acetate (150 mL) and anaerobic digester sludge (3 mL) as the inoculum. For the initial formation of biofilm on the anode, we fed each MEC with acetate as a sole substrate and operated MEC in batch mode for about 2 days. After achieving a stable current density, we changed the operation mode to continuous feeding (hydraulic retention time (HRT) = 17.8 h) with acetate and then with a mixture of volatile fatty acids (VFAs; acetate, 20.4 mM; propionate, 11.1 mM; and butyrate, 1.8 mM) for about 12 days. Following the start-up period, we fed both MECs with raw leachate and Fenton-treated leachate, neutralized with 10 N NaOH to pH ~ 7.6, in continuous mode with an HRT of 17.8 h to reflect the anode biofilm that can be used for real

applications of MECs. We calculated CE and CR by normalizing the recovered electrons as measured current to the COD removal and to total influent COD, respectively.

In order to investigate whether fermentation or anode respiration was the main cause for poor organic-matter consumption and *j* generation with raw leachate, we performed acetate-spike experiments on the control MEC. Before performing the spike experiments, we stopped the continuous flow of raw leachate, and then acetate was introduced to the MEC's anode using a syringe to a final concentration of ~25 mM or ~21 mM. After the acetate spike, we operated the MEC in batch mode for about 10 days and monitored the *j* generation. We repeated these experiments twice.

2.4. Chemical analyses

COD, total nitrogen (Total–N), VFAs, ammonia, alkalinity, and sulfate were measured, in duplicate, using HACH kits (HACH, Ames, IA). BOD_5 was measured according Method 5210 in *Standard Methods*.[20] We measured TOC using a TOC analyzer (TOC-VCSH, Shimadzu Scientific Instruments, Columbia, MD) equipped with combustion catalytic oxidation/non-dispersive infrared (NDIR) gas analyzer, the chloride concentration using ion chromatography (ICS 2000, Dionex Corporation, CA) after filtration through a 0.22-μm membrane filter, the Fe^{2+} concentration using the 5-sulfosalicylic acid (SSA) colorimetric method,[21] and the H_2O_2 concentration using the starch-iodine colorimetric method, in which a mixture of potassium iodide, ammonium molybdate, and starch reacted with H_2O_2 in acidic medium forming a blue peroxo-complex.[22] We assessed the aromatic content by the specific ultra-violet absorbance (SUVA), in which the absorbance reading at 254 nm is divided by the TOC concentration.[23] All spectrophotometric analyses were carried out using either a UV-Vis spectrophotometer (Varian Cary 50 Bio, Varian Inc., Walnut Creek, CA) or a Genesys 20 spectrophotometer (Thermo Spectronic, MA).

We quantitatively estimated the biomass concentration, as mg/cm^2 of anode surface area, by harvesting the entire biofilm at the end of each run from the MEC anode and suspending it in sterilized deionized water. After centrifuging the entire content at 10,000*g* (Eppendorf Centrifuge

5414 D, USA) for 10 min, we measured the dry-weight of the pellets gravimetrically and normalized it to the anode surface area.

2.5. Microbial community analyses

At the end of each experiment, we harvested the entire biofilm biomass from the MEC anode by scraping it off with a sterilized pipette tip and suspending the biomass sample in sterilized deionized water.[6] We extracted the DNA from a fraction of biomass (~ 0.125 g) using the MOBIO Powersoil DNA extraction kit according to manufacturer's instructions and determined the quality and quantity of the extracted DNA using a nanodrop spectrophotometer (ND 1000, Thermo Scientific) by measuring absorbance at 260 and 280 nm. The DNA samples were sent to the Microbiome Analysis Laboratory at Arizona State University (Arizona, USA) for amplicon pyrosequencing of the V4 region of the 16S rRNA gene with the barcoded primer set 515f/806r designed by Caporaso et al.[24] and following the protocol by the Earth Microbiome Project (EMP) (http://www.earthmicrobiome.org/emp-standard-protocols/) for the library preparation. PCR amplifications for each biofilm sample were performed in triplicate, and sequencing was performed in a MiSeq Illumina sequencer (Illumina Inc., USA) using the chemistry version 2 (2 x 150 paired-end).

We analyzed data received from the Microbiome Analysis Laboratory using QIIME software version 1.8[25] after discarding sequences shorter than 25 bp, longer than 450 bp, or labeled as chimeric sequences. The forward and reverse reads of each sequence were paired before downstream data analysis. After screening, primer sequences were trimmed off, and taxonomic classification was performed using the RDP classifier at the 80%-confidence threshold.[26] The total number of sequence reads for each sample after screenings were: raw leachate biofilm = 69472 and treated leachate biofilm = 141650.

3. Results and discussion

3.1. Effects of the Fenton reaction on the biodegradability of leachate organic matter

Table 1 summarizes the effects of the Fenton reaction. The most important trends are that the BOD_5/COD ratio and VFAs increased significantly, although the absolute concentrations of COD and TOC declined. The organic material in the leachate was partially oxidized: ~ 53% loss of COD and TOC. The treated leachate also had less aromatic organic content, as the SUVA declined by about 30%. The BOD_5/COD ratio, VFAs, and SUVA findings support that Fenton oxidation significantly improved the biodegradability of leachate organics by converting refractory organic matter into more biodegradable organic matter.

Table 1.

3.2. MEC performance

We operated two MECs – one of them fed with raw leachate (control) and the other fed with treated leachate in a continuous mode with an HRT of 17.8 h – to achieve quasi-steady state conditions following an ~ 2-months start-up and acclimation period. The performance of each MEC was stable and reproducible over repeated HRTs. Figure S1 reports the current density during the initial period of biofilm formation on the anode. Throughout the entire operating period, we maintained constant organic loading rates for the MECs fed with raw leachate and treated leachate, ~ 1.08 ± 0.01 kg BOD_5/m^3.day (~ 3.5 ± 0.11 kg COD/m^3.day) and ~ 0.82 ± 0.06 kg BOD_5/m^3.day (~ 1.7 ± 0.07 kg COD/m^3.day), respectively. The performance of both MECs was evaluated in terms of j at a fixed anode potential (– 0.3 V vs. Ag/AgCl or – 0.046 V vs. SHE), which led to the stable and clearly distinct performance patterns that can be seen in Figure 1A.

The MEC fed with the treated leachate had nearly 13-fold higher j, with an average value of 1.42 ± 0.27 A/m^2 vs. 0.11 ± 0.06 A/m^2 for the untreated leachate. This increase was much more dramatic than the increase in the BOD_5/COD ratio: to 0.56 ± 0.04 from 0.31 ± 0.01. Treated

leachate also gave remarkably enhanced removals of COD, BOD_5, and TOC, as well as CE and CR values, all shown in Figure 1B.

The higher MEC performance supports that significant organic matter removal is possible if toxic components in the influent are substantially reduced. Likewise, Mahmoud et al. (2014) saw that pre-treatment of leachate (by fermentation) led to substantially better MEC performance. Thus, while direct oxidation of leachate organics by ARB in MECs may not be feasible, pre-treatment has a positive impact on MEC performance in terms of *j*, CE, CR, and organic matter removal.

Approximately 5.9% of the influent COD ended up as biomass in the biofilm of the MEC fed treated leachate, corresponding to much higher total biofilm accumulation (i.e., ~ 0.70±0.01 mg/cm^2) compared to the control MEC (i.e., ~ 0.66% of influent COD and biofilm accumulation of ~ 0.16±0.02 mg/cm^2). The higher biofilm accumulation for the MEC fed treated leachate is consistent with its greater rate of organic-matter consumption.

Figure 1.

The likely cause for the poor MEC performance and low biofilm accumulations with raw leachate was inhibition caused by the complexity and aromaticity of its organic matter. The Fenton reaction reduced the leachate's SUVA value by ~ 30% and increased the VFA/COD ratio by ~ 1.6–fold, both consistent with the hypothesis that the Fenton reaction relieved inhibition related to aromatics. Zhang et al.[10] detected leachate inhibition to anode respiration in a membraneless air-cathode microbial fuel cell (MFC) with even higher leachate biodegradability (BOD_5/COD ratio ~ 0.40). A recent study by Cheng et al.[27] supports that eliminating aromatics relieved inhibition to fermentation and anode respiration in an MFC. They observed that anaerobic biodegradation of aniline, a typical recalcitrant aromatic organic matter, was sluggish compared to aerobic biodegradation. Air sparging of the biofilm anode caused an ~ 5-fold increase in power density and an ~ 6-fold increase in aniline removal, suggesting that aerobic biodegradation of aromatics relieved inhibition for fermentation and anode respiration.

To test whether fermentation or anode respiration was the inhibited step, we spiked the anode chamber of the control MEC with a known amount of 1-M acetate medium to yield a final acetate concentration in the anode-chamber of ~25 mM. Figure 2 shows a rapid increase in *j* upon addition of acetate, and the CE was ~ 80% based on the COD change. Given that acetate is the preferred electron donor for ARB,[3,4] the rapid response to acetate indicates that fermentation was the inhibited step. After the added acetate was consumed, *j* decreased to less than 0.05 A/m^2 for 36 days, at which time we added a second acetate spike (~ 21 mM). The second spike gave trends consistent with the first spike experiment, again showing that the biofilm was capable of rapid anode respiration if a readily available donor were present.

Figure 2.

Since a goal of MXC technology is to treat wastewater, it is important to have a high removal efficiency for organic matter, along with maximizing *j,* CE, and CR. The BOD_5 concentration in the effluent of MEC fed with treated leachate was 270 mg/L, which represents ~ 70% BOD_5 reduction for the integrated treatment system (i.e., Fenton oxidation and MEC). This residual BOD_5 concentration is close to the discharge limits for landfill leachate (220 mg BOD_5/L) imposed by the USEPA.[28]

Thus, several pieces of evidence support that complexity of the biodegradable organic matter and the presence of inhibitors led to minimal fermentation and low *j* with the raw leachate. Pre-treating the leachate with the Fenton reaction overcame both bottlenecks to fermentation, and this allowed the syntrophy of fermenters and ARB to function more robustly: higher organic-matter removal, CE, and *j*.

3.3. Microbial community analysis

Since we operated both MECs in continuous mode with a relatively short HRT (~ 17.8 h), which is shorter than the minimum solids retention time for fermenting bacteria (≥ 1.5 day) and acetoclastic methanogens (≥ 3 days),[29] we performed microbial community analysis only on the biofilms, as most of microbial community was washed out from the suspended phase. Figure 3A presents the microbial community analyses at the phylum level, and Figure S2 gives the family-level information. At the phylum level, *Proteobacteria* dominated the microbial community with treated leachate (~ 66% of the sequences), followed by *Bacterioidetes* (~ 16% of the sequences) and *Firmicutes* (~ 12% of the sequences). Earlier studies revealed that *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were among the most abundant phyla in the anode of MXCs successfully treating different waste streams.[14,30,31] *Bacteroidetes* and *Firmicutes* have members responsible for polysaccharide hydrolysis and fermentation, whereas many members of *Proteobacteria* are known to perform anode respiration.[8,32] Predominance of *Proteobacteria* after Fenton treatment supports that the anode respiration was enhanced due to greater bioavailability of the partially oxidized recalcitrant organic matter into compounds that could readily be transformed into the simple substrates used by ARB.[1,5,6] Since the Fenton reaction produced little or no acetate directly (data not shown), the Fenton products had to be fermented, which is why fermenters (*Bacteroidetes, Firmicutes, Spirochaetes,* and *Actinobacteria*) had to be present along with ARB to create the necessary syntrophy.

In contrast for the biofilm anode fed raw leachate, *Deferribacteres* (~ 23% of the sequences) became the second abundant phylum after *Firmicutes* (~ 45% of the sequences), and *Proteobacteria* were only ~ 23%. *Deferribacteres*, which, like *Proteobacteria*, are Gram-negative and can respire iron, were among the most abundant phyla in anaerobic digesters treating complex organic wastes, such as brewery wastewater and leachate,[33,34] but previously have not been associated with anode respiration.

Figure 3B presents that community breakdowns at the genus level within the *Proteobacteria*. Notable is the dominance of *Geobacter* in both biofilms, which reflects that the metabolic core of the biofilm was anode respiration. For the treated leachate, *Arcobacter* and *Pseudomonas* also

became important. This probably reflects the high diversity of organic substrates available to the community after Fenton pre-treatment. The low fraction of *Proteobacteria* in the biofilm fed raw leachate is consistent with a recent phylogenetic and metagenomic analysis[35] showing that introducing leachate to an acetate-fed MFC caused a significant decline (~ 10-fold) in the relative abundance of *Geobacter*-affiliated phylotypes that was accompanied by a 50% decrease in CE.

Figure 3.

3.4. Evaluation of the MEC and the integrated treatment system

Table 2 summarizes results from a range of studies on treating landfill leachate in an MEC or MFC. It is obvious that landfill leachate without pre-treatment led to low *j,* CE, and CR in almost all the studies; a main cause was the low BOD_5 content due to the largely recalcitrant organic matter in the leachate's COD and the presence of inhibitory materials. An exception was with diluted leachate, which may have relieved inhibition.[11] The lack of microbial-community analysis in these studies makes it impossible to determine the relative impacts of recalcitrance versus inhibition. Our results after Fenton treatment gave the highest (and usually much higher) values of *j,* CE, and CR, confirming that Fenton pre-treatment of leachate was able to enhance the biodegradability of the organic material in the MXC setting. Here, our data suggest that relieving the fermentation inhibition may have been the more important factor.

This work establishes the fundamental proof of concept that pre-treatment by the Fenton's reaction can make recalcitrant organics in landfill leachate much more biodegradable in an MEC. This greatly enhanced *j*, CE, and CR, and final effluent quality. Further research is required to determine the optimal conditions of Fenton oxidation process to improve leachate biodegradability before energy capture in a downstream MEC. Optimization will be essential for making large-scale application economically feasible, since H_2O_2 might be costly. Recent studies by our team (and others) are showing that H_2O_2 can be produced sustainably in MXCs in high concentration (up to ~ 74 mM H_2O_2) via partial reduction of O_2 using inexpensive carbon cathode materials.[39, 40] Combining the possibility of energy capture from recalcitrant landfill leachate with

H_2O_2 production in MXCs may offer a truly sustainable means of enhancing treatment and energy capture from recalcitrant landfill leachate.

Table 2.

Conclusions

While landfill leachate gave poor MEC performance – 3±0.3% BOD_5 removal, 1.8±0.5% CE, and 0.11±0.06 A/m^2 *j* – pre-treating the leachate with the Fenton reaction greatly improved all aspects of performance: 52±10% BOD_5 removal, 29±3 % CE, and 1.42±0.27 A/m^2 *j*. Inhibition of fermentation, not anode respiration, was the main cause of poor MEC performance when treating landfill leachate. Fenton pre-treatment of landfill leachate overcame fermentation bottlenecks by decreasing the complexity of the biodegradable-organic matter and the presence of inhibitors. Feeding the MEC with pre-treated leachate led to an ~5-fold increase in biofilm dry weight and to a microbial community enriched in phyla known to contain strains able to hydrolyze and ferment in complex organic matter – *Firmicutes*, *Bacterioidetes*, *Spirochaetes*, and *Actinobacteria* – along with known ARB within the *Proteobacteria*.

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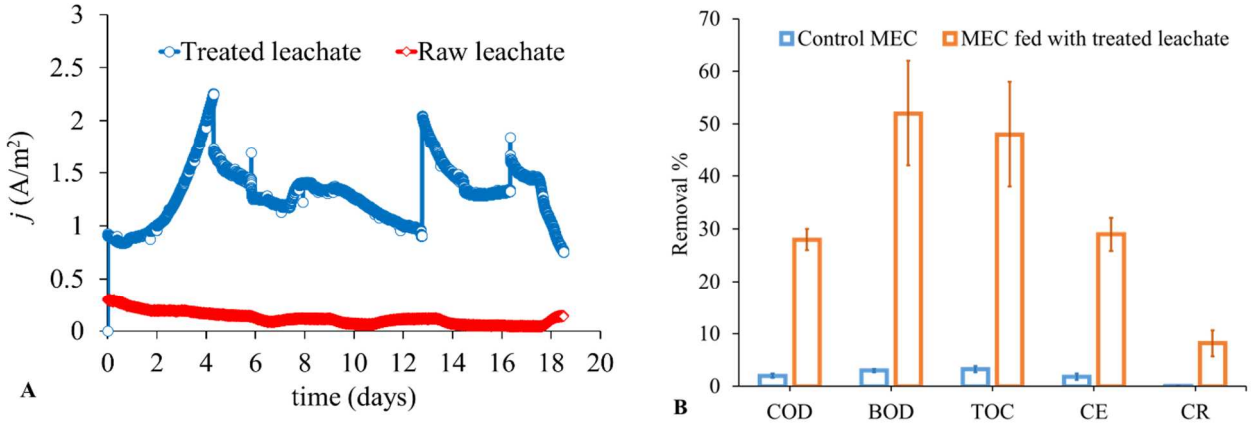
Figures captions

Figure 1. (A) Quasi-steady state current generation versus time for MECs fed with treated and raw leachates during continuous operation at an HRT = 17.8 h. (B) Summary of MEC performance parameters.

Figure 2. Current density in response to acetate spikes in the MEC fed with raw leachate. The dashed blue arrows indicate acetate spikes of 25 or 21 mM.

Figure 3. (A) Microbial community distribution for biofilms at phylum level, and (B) the composition of phylum *Proteobacteria* for biofilms at the genus level.



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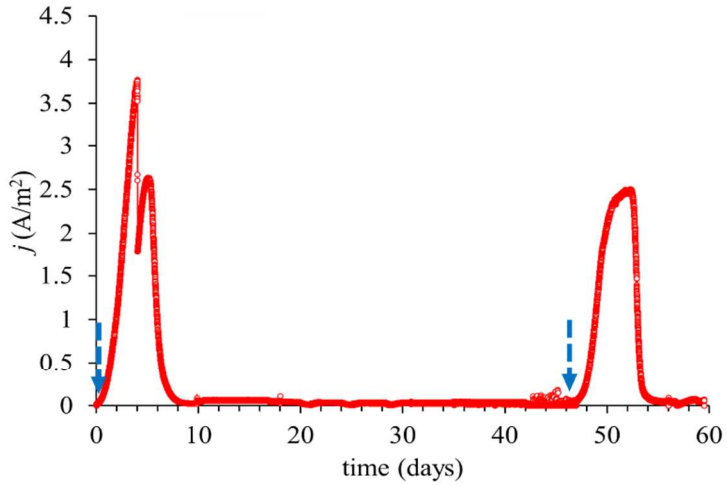

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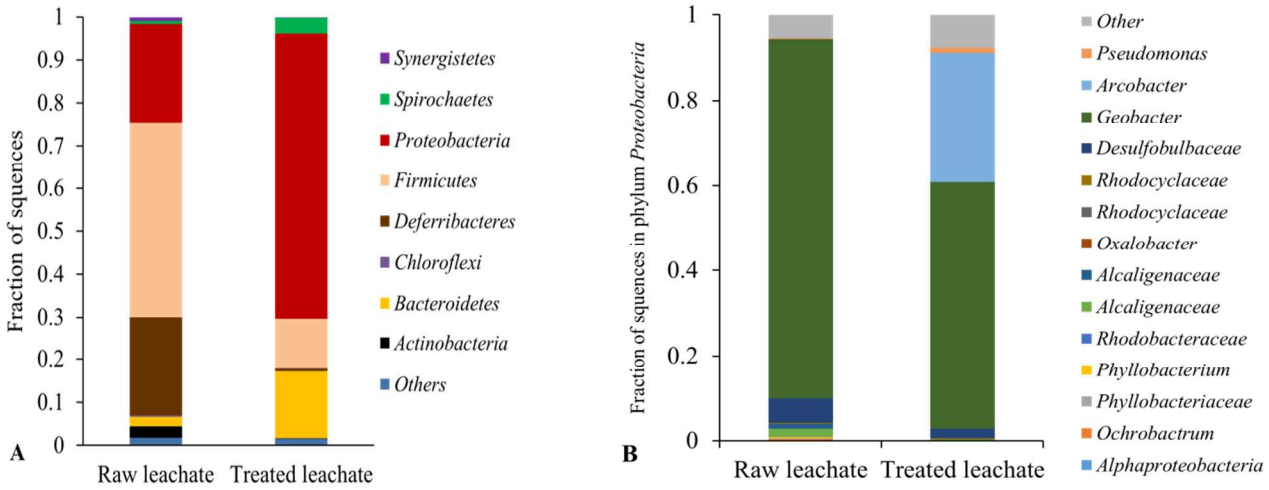

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List of Tables

Table 1. Effects of Fenton oxidation with a $[H_2O_2]$:$[Fe^{2+}]$ molar ratio of 4.0, a H_2O_2:COD *w/w* ratio of 1.1, pH 3.5, and time = 3 h

Parameter	Unit	Raw leachate	Fenton-treated leachate
COD	mg/L	2594±94	1227±93
BOD_5	mg/L	802±10	608±50
BOD_5/COD ratio	–	0.31±0.01	0.56±0.04
TOC	mg/L	663±15	317±4
VFAs	mg/L as CH_3COOH	283±73	340±4.6
SUVA*	L/mgC.m	1.23±0.06	0.88±0.01

* With 5-fold dilution.

Table 2. Summary of landfill-leachate treatment in microbial electrochemical cells

Reactor configuration	Influent COD (influent BOD_5) [d]	Organic matter removal %	j (A/m^2)	CE (%)	CR (%)	Reference
MFC [a]	12,033 (898)	28.6 [e]	0.102	1.27	~ 0.36 [h]	Ganesh and Jambeck [36]
MFC [a]	2386(305)	16 [e]	0.07	17.4	~ 4.7 [h]	Damiano et al. [37]
MFC [b]	1257–1612 (572)	12.5 [f]	3.79×10^{-3}	~ 0.3 [h]	~ 0.03 [h]	Greenman et al. [38]
MFC [b]	1960 (823)	~ 70 [e]	~ 1	6.6	~ 4.6 [h]	You et al. [11]
MFC [a]	3,400 (1,360)	60–90 [e]	~ 0.16 – 1.3	1.2 – 14.4	~ 0.7 – 13 [h]	Zhang et al. [10]
MEC [a]	2594 (802)	2 [e] (3 [f])	0.11	1.8	0.04	This study
MEC [c]	1227(608)	28 [e] (52 [f])	1.42	29	8.2	This study

[a] Experiment was performed with raw leachate; [b] Experiment was performed with diluted leachate; [c] Experiment was performed with Fenton-treated leachate; [d] Unit is mg/L; [e] COD removal %; [f] BOD removal %; [h] Not reported in the original study, but calculated based on their data.