

Decentralized wastewater treatment using a bioelectrochemical system to produce methane and electricity

Cynthia J. Castro, Varun Srinivasan, Joshua Jack and Caitlyn S. Butler

ABSTRACT

Biological electrochemical systems (BESs) have the potential for decentralized treatment in developing countries. A 46 L, two-chamber, hydraulically partitioned microbial fuel cell (MFC) was designed to replicate low-flow scenarios leaving a composting toilet. The co-evolution of electricity and methane in this MFC was evaluated by testing two distinct waste streams: synthetic feces (Case F) and municipal primary effluent (Case W). Oxidation of organic matter was $76 \pm 24\%$ during Case F and $67 \pm 21\%$ during Case W. Methanogenesis was dominant in the anode, yielding potential power of $3.3 \pm 0.64 \text{ W/m}^3$ during Case F and $0.40 \pm 0.07 \text{ W/m}^3$ during Case W. Electrical power production was marginal, Case F = 4.7 ± 0.46 and Case W = $10.6 \pm 0.39 \mu\text{W/m}^3$, although potentially useful in energy-limited areas. Complimentary batch cultivations with anode inocula yielded greater methane production in the presence of graphite. $74 \pm 11\%$ more methane was produced with graphite than suspended growth enrichments and $58 \pm 10\%$ more than enrichments with non-conductive plastic beads. The co-production of methane and electricity in an MFC may have utility in decentralized treatment. Further work is needed to optimize power from both electricity and methane.

Key words | hydraulically partitioned, methanogenesis, microbial fuel cell, sanitation, scale-up, wastewater treatment

Cynthia J. Castro
 Varun Srinivasan
 Joshua Jack
 Caitlyn S. Butler (corresponding author)
 Department of Civil and Environmental
 Engineering,
 University of Massachusetts Amherst,
 130 Natural Resources Way,
 Amherst,
 MA 01003-9284,
 USA
 E-mail: cbutler@ecs.umass.edu

INTRODUCTION

The majority of people who lack access to sanitation systems live in developing countries (WHO/UNICEF 2015). As of 2015, only 68% of the world population used an improved sanitation facility (WHO/UNICEF 2015), and the majority of people who do practice open defecation live in rural areas. Although the most widely used systems are ventilated improved pit latrines, efforts have been made to develop incentivized sanitation systems. Examples of incentivized systems include composting latrines that produce a natural fertilizer and anaerobic digesters to capture methane gas for heating and cooking purposes (Mihelcic 2009; Surendra *et al.* 2014). Although anaerobic digesters and biogas toilets seem feasible in promoting sustainable means for energy recovery, high nitrogen species present in anthropogenic

wastewaters have been documented to inhibit the anaerobic microbial degradation process (Fricke *et al.* 2007).

Bioelectrochemical systems (BESs) and, more specifically, microbial fuel cells (MFCs) have generated significant interest for energy-efficient or energy-yielding wastewater treatment approaches. MFCs decouple the electron donor and the electron acceptor, allowing for anaerobic organic degradation by microorganisms using the anode as an electron acceptor. Electrons are transported to a cathode via an electrical load, where the reduction of an electron acceptor occurs. Castro *et al.* (2014) previously designed and implemented a unique three-chamber MFC system that oxidized organic matter from feces in the anode chamber and utilized a biological cathode to reduce

nitrate-rich effluent from a nitrification chamber fed urine. It was designed for retrofitting composting latrines. The MFC latrine incentivizes sanitation by producing compost, electricity, and treated effluent water as the three main outputs. Although the system produced all three products, electricity recovery was low.

One explanation for the reduced power production at larger scale MFC is alternative, anaerobic microbial metabolisms in the anode compartment. Some anode-respiring bacteria (ARB), like many anaerobic, chemotrophic bacteria, prefer to oxidize acetate because of its low oxidation state (-1) (Thauer *et al.* 1989). However, the degradation of complex organic matter requires hydrolyzation of large organic macromolecules, followed by fermentation, producing simple organic acids and hydrogen. Symbiotic relationships between ARB and fermenters have been linked to efficient conversion of organic substrates to electricity (Parameswaran *et al.* 2009). In contrast, methanogenesis is often cited as a barrier to full-scale implementation of MFC technologies, as methanogens and ARB are often in direct competition for end products of fermentation. Methane has been observed to be a major contributor to inefficiencies at the anode even when simple substrates, such as glucose and sucrose, are used (He *et al.* 2006; Jung & Regan 2011).

Methanogens have recently been documented to work synergistically with ARB. In the presence of conductive surfaces, direct interspecies electron transfer (DIET) has been reported between known anode-respiring species, *Geobacter* sp., and the methanogens *Methanosaeta* sp. and *Methanosarcina* sp. (Liu *et al.* 2012; Zhao *et al.* 2015). Furthermore, enhanced methane production has been documented in anaerobic digester aggregates in the presence of conductive material such as graphite (Morita *et al.* 2011). This suggests that the conductivity of the anode could offer a pathway to divert electrons from electricity production in favor of methanogenesis.

Although not appropriate for all MFC applications, co-evolving methane with electricity is practical for developing countries where methane could be used for heating or cooking and electricity could be stored and used for lighting. Nearly three billion people still use cooking methods that involve burning locally available biomass such as firewood, animal excreta, and kitchen waste, producing harmful

indoor air pollution (Surendra *et al.* 2014). MFCs employed to generate both biogas and electricity at ambient temperatures could allow for on-demand electricity production and storable energy as methane.

This study evaluates the potential for methane production in MFC anode communities and the co-evolution of methane and electricity in a lab-based pilot MFC that is a one-to-one representation of the MFC paired with a composting latrine in Ghana (Castro *et al.* 2014). Methane production, electricity production, and treatment performance were evaluated for two different wastewater conditions: synthetic feces and municipal wastewater. Microbial enrichments from the operating pilot-scale MFC anode were incubated under ambient conditions to explore the role of conductive material on methane production. This BES delivers two additional outputs that a conventional anaerobic digester or biogas toilet cannot produce: direct electricity production and nitrogen removal.

METHODS

MFC construction and startup

A hydraulically partitioned, two-chamber MFC was constructed (Figure 1). The laboratory MFC was designed to emulate operational conditions when paired with a composting latrine (Castro *et al.* 2014). Each chamber consisted of a capped 56.8 L polypropylene tank, containing two baffle walls evenly spaced within the tank to promote passive mixing. Effluent from the anode chamber directly flowed into the cathode, where a separate nitrate medium was fed to a cathode-oxidizing, nitrate-reducing biofilm. Nitrate was added to the cathode to simulate the conditions of the field-deployed MFC, which contained an additional aerobic nitrifying chamber for the conversion of ammonia in human urine to nitrate. We chose to eliminate the nitrifying chamber and feed a constant concentration of nitrate to allow focus on the anode chamber in this study. In this design, no proton exchange membrane was used to simplify the design and reduce costs associated with building MFCs in the developing world. Packed synthetic granular graphite (EC 100 3/8 × 10, Graphite Sales) was used as the electrode material within the baffled anode and cathode. Each tank

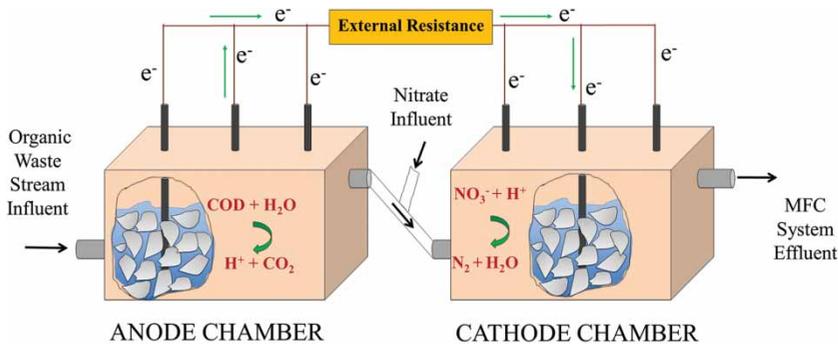


Figure 1 | Schematic of the MFC design (not to scale). The wastewater and the nitrate media were each pumped directly into the anode and cathode chambers, respectively. Both chambers contained granular graphite as their electrode material.

was filled with 45.5 L of the granular graphite and fully saturated with anode media. The estimated liquid volume of each electrode chamber was 23 L and the surface area of each electrode was 29.2 m². Three graphite rods were placed in each chamber as current collectors. Wires connected to the graphite rods of each tank were linked together via an external resistor box. The anode and cathode chambers were both inoculated with 4.0 L of primary wastewater obtained from the Amherst Wastewater Treatment Plant (WWTP) (Amherst, MA) and 1.0 L of pond water and sediments from the campus pond at the University of Massachusetts (Amherst, MA). The MFC initially operated under 1,000 mg/L acetate growth media in a 16 mM phosphate solution.

Batch enrichment studies

To assess enhanced methane production at room temperature in the presence of conductive surfaces, enrichment studies were performed with anode effluent from the pilot MFC. In the enrichment studies, acetate served as the carbon source in a 16 mM phosphate buffer solution. Enrichment bottles were prepared in the presence of graphite granules, non-conductive plastic beads, or with no attachment surface present (suspended). The study was conducted in 12 sealed 150 mL serum bottles, with five replicate cultivations for bottles containing graphite granules, duplicate cultivations for bottles containing plastic beads and five replicates with no additional attachment surfaces present (suspended growth). Each bottle was capped and purged with nitrogen gas prior to inoculation. The growth media consisted of a 16 mM phosphate buffer containing

1,000 mg/L of acetate and 1 mL/L of a calcium-iron and trace mineral solutions. All bottles were autoclaved and cysteine (31.5 mg/L) was added to remove any residual oxygen. All bottles were covered in foil to prevent phototrophic growth, incubated at 22 °C, and continuously shaken for 31 days.

MFC operation

Simultaneous electricity production and methanogenesis was evaluated using the pilot MFC reactor while treating two types of organic wastewaters: synthetic feces wastewater (Case F – 54 day operation), and municipal wastewater (Case W – 50 day operation). Case F media consisted of 8.0 g of starch, 2.50 g of casein, 4.34 g of KH₂PO₄, 1.09 g of Na₂HPO₄, 0.310 g of NH₄Cl, 0.130 g of KCl, and 5.0 g of oleic acid per liter of reverse osmosis (RO) water (Du *et al.* 2011). The pH was 6.3 ± 0.003 and conductivity was 4,700 ± 150 μS/cm. For Case W, effluent was obtained from the primary clarifier of the Amherst WWTP and used as the influent to the anode. The pH was 7.2 ± 0.08 and conductivity was 640 ± 30 μS/cm. During both conditions, room temperature of 20–22 °C was maintained, unless otherwise noted. The cathode chamber was fed with nitrate in a 16 mM phosphate buffer with the following recipe: 0.710 g Na₂HPO₄, 1.50 g KH₂PO₄, 0.050 g MgSO₄, and 0.605 g NaNO₃ per liter of RO water. All anode and cathode media were purged with nitrogen gas for at least 30 minutes before being introduced into their respective compartments. Media was pumped continuously, with a hydraulic retention time (HRT) of 8 days for the anode chamber and 4 days for the cathode chamber to reflect operation when connected to

a composting latrine superstructure similar to a ventilated improved pit latrine (Castro *et al.* 2014). Both the anode and cathode biofilms were established prior to operation under both conditions, and the MFC has been in operation for the past four years. At the completion of each wastewater scenario, visual inspection of the anode and cathode was performed by opening each of the chambers. During Case F, minimal precipitated starch at the top layer was removed when visible but the interior remained intact so as to not disturb the biofilms around the granular graphite electrodes.

Chemical analysis

Samples were collected from the inlets and outlets of the anode and cathode. All samples were filtered through 0.45 μm syringe filters prior to analysis. An ion chromatograph (850 Metrohm) was used to measure nitrite and nitrate. Chemical oxygen demand (COD) was measured according to standard methods using Hach kits (Hach Method 8000). An Agilent gas chromatograph (GC) (7890A model) was used to measure the following short-chain fatty acids (SCFAs): acetic, propionic, isobutyric, n-butyric, isovaleric, n-valeric, isocaproic, n-caproic, and heptanoic acids (standards from Matreya LLC, Pleasant Gap, PA). Liquid samples for SCFA analysis were filtered with a 0.45 μm syringe filter and acidified with 6 N of sulfuric acid for large sample quantities, or 12 N of HCl for small sample quantities, before analyzing. The GC was also used to measure methane and carbon dioxide gases using an HP-PLOT-Q column. Gas samples for methane were collected from the headspace of the anode chamber and stored in gas-tight bags before analysis. Duplicate injections were made for each sample.

Electrochemical analysis

Voltage production was monitored using a Keithley Model 2700 Multimeter with a 7700 Switching Module (Keithley Instruments Inc., Cleveland, OH, USA). Readings were collected every 10 minutes across the external resistance. Polarization curves for determining internal resistance were conducted by linear sweep voltammetry (LSV) using a Gamry Series G750 Potentiostat/Galvanostat/ZRA (Gamry, USA). LSV was run for three cycles at a scan rate of 1 mV/s from zero to the open circuit potential. Current

was determined using Ohm's law, $I = V/R$, where I is the current in amps (A), V is the voltage in volts (V), and R is the resistance in ohms (Ω). Power was determined using $P = I^2R$, where P is power in watts (W). Power densities were normalized to the anode surface area or anode liquid volume, where specified, and reported with standard errors. Reported power density from the literature was converted to the appropriate units using the ratio of reported anode surface area to anode liquid volume.

Mass balances and power production

In order to compare the alternative end-products produced by the continuous flow MFC anode, all influent and effluent products in and out of the anode chamber were converted to mass rates of electron equivalents as COD. Average current produced by the MFC over the operational period was converted to mg COD/min using the following relationship:

$$\text{Electron equivalents as COD} \left[\frac{\text{mg COD}}{\text{min}} \right] = \frac{I}{Fn} \times MW_{O_2}$$

where I is current (C/min), F is Faraday's constant (96,485 C/e⁻ eq), n is the electron equivalent for COD (O₂), which is 8 e⁻ eq/mol COD, and MW is the molecular weight of oxygen (32 g/mol).

The mass rate of soluble methane production was calculated by the following relationship:

$$m_{\text{CH}_4,s} \left[\frac{\text{mg COD}}{\text{min}} \right] = C_{\text{CH}_4,s} \times Q \times 64 \frac{\text{g COD}}{\text{mol CH}_4}$$

where $C_{\text{CH}_4,s}$ is the soluble methane concentration in mol/L determined by Henry's law using K_H , 1.4×10^{-5} mol/m³-Pa (Sander 2014), and the measured partial pressure of methane in the headspace, Q , is the flowrate into the reactor (2 mL/min), and 64 g COD per mole of CH₄ assuming complete oxidation of methane by oxygen. The rate of methane production in the headspace was determined by collecting 1 L of gas from the headspace on consecutive days and determining the amount of methane produced between days. Conversion factors for mg of each SCFA and methane to mg COD were obtained from Pitter & Chudoba (1990). The estimated power from methane production was determined by using

the average net heating value of methane, 1,000 BTU/ft³ (EPA 1995), and the conversion factor of 3.41 BTU·h⁻¹/W (EPA 1995). Power values were presented in two ways: normalized to the COD consumed during each wastewater treatment scenario or by the liquid volume of the anode.

RESULTS AND DISCUSSION

Methanogenesis within communities enriched from the anode

In consideration of methanogenesis within the anode environment, batch cultivations were used to assess the role of graphite granules in methanogenesis at ambient temperature. Microbial communities obtained from the previously operating pilot MFC anode were transferred into one of three batch enrichments: acetate growth media only, acetate growth media with graphite granules, or acetate growth media with plastic beads. Methane production was observed in all three enrichments. Methane production comprised $19 \pm 9.5\%$ of the headspace gas when granular graphite was present, which was greater than the suspended growth and plastic bead enrichments. The methane concentration in suspended growth cultures was $5.0 \pm 1.6\%$ in the headspace (Figure 2). The plastic bead enrichments were most similar to the suspended growth enrichment, with $8.1 \pm 0.65\%$ headspace methane. This suggests that the conductivity of graphite may have led to the greater methane production. Our findings are supported by other studies

which have determined that conductive surfaces, such as activated carbon, can support DIET between *Geobacter* species, associated with anode respiration and methanogens (Liu et al. 2012; Zhao et al. 2015).

Increased methane production from anode microbial communities in the presence of graphite granules has implications for most MFC applications. Methane is well documented as a competing metabolism for anode-respiring communities, but the results from this study suggest that anode environments may be even more favorable to methanogenesis than conventionally thought. For optimization of energy production in MFC technologies, there are two approaches to consider. Methanogenesis could be suppressed in the anode to increase electricity production, which may be appropriate in large-scale industrial or municipal applications. Methane could also be captured as a co-evolved, value-added product, which may be useful for applications in developing areas where resources are limited and simplified reactor design and operation are required.

COD removal and nitrate reduction in the MFC

In order to evaluate the potential for decentralized wastewater treatment and energy recovery, a pilot-scale MFC was operated continuously through two sequential, influent wastewater conditions: synthetic feces wastewater (Case F) and municipal wastewater (Case W). The membraneless, hydraulically partitioned MFC was designed for direct human waste treatment (Castro et al. 2014), resulting in a relatively long, 8-day HRT in the anode chamber. During both cases, nitrate was fed separately to the cathode for biocathodic denitrification. Treatment performance was evaluated based on COD removal in the anode and in the system, and nitrate removal in the cathode. Organic matter was removed in the anode chamber when treating influent waste streams (Figure 3). COD removal efficiencies were $76 \pm 24\%$ ($8,200 \pm 2,000$ mg/d) for Case F and $67 \pm 21\%$ (290 ± 56 mg/d) for Case W. These COD removal efficiencies are similar or higher than other large-scale MFC reactors (Jiang et al. 2011; Alzate-Gaviria et al. 2016; Ge & He 2016). Removal is also similar to bench-scale MFCs treating similar substrates. Between 60 and 98% COD removal has been reported for batch MFCs fed starch (Lu et al. 2009) and 48–93% when fed municipal wastewaters in

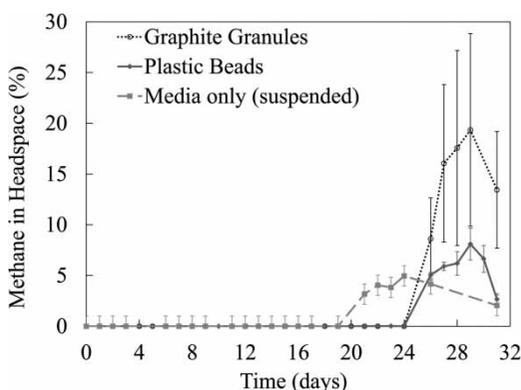


Figure 2 | Methane production in the headspace of batch reactors enriched with microbial communities from the large-scale MFC, containing either graphite granules, plastic beads, or neither (suspended).

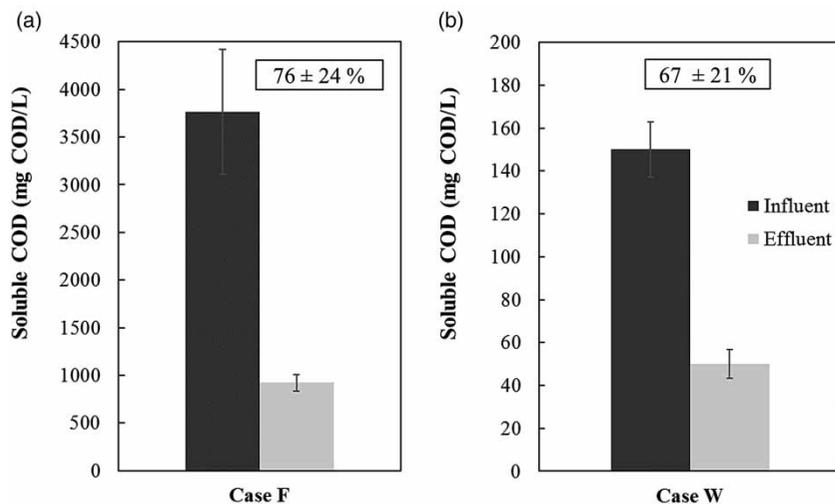


Figure 3 | Average anode chamber influent and effluent COD concentrations for (a) Case F and (b) Case W with standard errors. The percentages above the bars represent average removal efficiencies during each operational period.

continuous flow MFCs (Liu *et al.* 2004; Kim *et al.* 2015). The HRTs presented in these cited studies were significantly shorter than this MFC. The 8-day HRT used in this study was purposely designed to replicate low liquid flows when used as a sanitation system in the developing world, and likely contributed to the significant COD removal.

In the cathode chamber, nitrate removal was observed in all cases. Nitrate removal was greater with high organic loading rates at the anode, observing $53 \pm 16\%$ removal in Case F and only $12 \pm 6.9\%$ in Case W. The anode and cathode were hydraulically linked in this MFC configuration and effluent COD, not oxidized in the anode, was allowed to flow to the cathode, resulting in heterotrophic denitrification. This served as a COD ‘polishing’ step. For Case F, a further $9.6 \pm 4.9\%$ of anode influent COD was removed in the cathode chamber, for a total COD removal in the system of $85 \pm 33\%$. For Case W, an additional $14 \pm 6.7\%$ was removed, for a total COD removal of $86 \pm 13\%$. Power production was lower than expected in all cases, and minimal autotrophic denitrification occurred in the cathode chamber from current delivery to the cathode.

Electrical power production in the pilot-scale MFC

Power production from electricity was observed and sustained during both media treatment cases. Operational power outputs for Case F and Case W were 4.7 ± 0.46 and

$10.6 \pm 0.39 \mu\text{W}/\text{m}^3$. Polarization curves were used to determine the internal resistances and maximum power densities for both cases ($42 \text{ k}\Omega$ for Case F and $214 \text{ k}\Omega$ for Case W). Case F had the greatest concentration of organic matter entering the system, at $3,800 \text{ mg COD/L}$, while yielding the lowest average operational power output. Low power output is frequently noted in bench-scale reactors treating complex waste streams. Starch processing waste has yielded $1.4 \text{ W}/\text{m}^3$ ($240 \text{ mW}/\text{m}^2$) (Lu *et al.* 2009), $4.3 \text{ W}/\text{m}^3$ ($170 \text{ mW}/\text{m}^2$) from swine wastewater (Min *et al.* 2005), and $1.74 \text{ W}/\text{m}^3$ ($26 \text{ mW}/\text{m}^2$) from domestic wastewater (Liu *et al.* 2004). Recent large-scale applications of MFCs for wastewater treatment and sanitation purposes have only achieved moderate power output even when utilizing numerous stacked or sequential liter-scale MFCs (Alzate-Gaviria *et al.* 2016; Ge & He 2016; Ieropoulos *et al.* 2016). In this study, large external resistances were used to match the internal resistances of the MFC system and to maximize the voltage drop across the resistor to support an LED light and to produce methane. The large external resistances may have limited the anode’s availability as an electron acceptor to ARB, which could lead to increased methanogenesis (Jung & Regan 2011).

SCFA and methane production in the MFC anode

Other microbial metabolisms in the anode were investigated. Dissolved and headspace methane gas accounted

for 8.4% of electrons obtained from the oxidation of COD in Case F and 18.9% in Case W. An accumulation of SCFAs was detected in Case F and accounted for a total of 3.1% of the electrons: acetic (1.1%), propionic (0.70%), isobutyric (0.16%), n-butyric (0.24%), isovaleric (0.34%), n-valeric (0.17%), isocaproic (0.11%), n-caproic (0.11%), and heptanoic acid (0.14%). SCFAs were below the detection limit in Case W. The major SCFAs found in the effluent of the anode for case F were acetic, propionic, and n-butyric acid, suggesting that large polysaccharides derived from starch were broken down to various sugar forms such as maltose and glucose and further fermented to the simplest SCFAs. Upon inspection of the anode chamber at the end of Case F, a minor amount of starch had precipitated in the anode and contributed to COD removal. Since it was difficult to separate the small amount of precipitated starch from other volatile solids in the packed bed of graphite granules, we were unable to quantify the accumulation or its contribution to COD removal; although qualitative inspection leads us to believe it was not a significant component of COD removal.

Methane as an alternative energy source

For large-scale applications of MFC technology, methane production seems inevitable without active suppression of methanogenesis, especially when treating wastewaters with multiple complex organics. The energetic value from an MFC is typically measured by the maximum electrical energy it can produce. Although electrical energy is lowered by competition with methanogens (Torres *et al.* 2007), an alternate perspective includes methane as a value-added product, particularly in developing areas where biogas can be used as a cooking and heating fuel.

When the MFC anode was fed synthetic feces wastewater (Case F) or municipal wastewater (Case W), it could support the production of 0.008 ± 0.001 and 0.001 ± 0.0002 L CH₄/L_{Reactor}-d, respectively. Power production from methane would yield 2.6 ± 0.31 mW/m² (3.3 ± 0.64 W/m³) for Case F and 0.31 ± 0.06 mW/m² (0.39 ± 0.07 W/m³) for Case W. When power recovered from methane was normalized to the COD removed in the anode, the MFC could produce 1.2 ± 0.53 mW/g COD during Case F and 3.9 ± 1.5 mW/g COD during Case W.

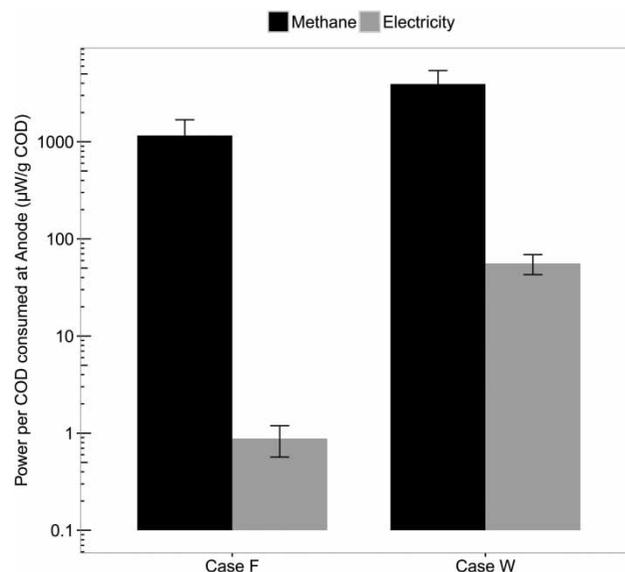


Figure 4 | Comparison of power recovery from methane and electricity normalized by the COD consumed in the anode and presented with standard errors.

Potential power from methane production exceeds the contribution from electrical power in the anode in this system (Figure 4). At ambient temperatures, both methane and electricity production were greater in Case W with low-strength municipal wastewater, which may allow for other household wastewater to be added to the MFC stream fed to the MFC anode. In this study, treating municipal wastewater yielded 2.6 times more power from methane and 64 times more electricity (normalized to COD removed) than treating concentrated synthetic feces wastewater. We note that the complexity of starch as the primary carbon source and the unaccountable fraction of starch lost to precipitation in Case F should also be considered when comparing the power output. Based on our results, the complexity of the organic source and concentration in the wastewater should be carefully considered when optimizing power for both methane and electricity.

CONCLUSION

Enhanced methane production in the presence of graphite granules was demonstrated within mixed cultures enriched from anode communities. A pilot-scale MFC produced energetic products in the form of methane and electricity when

treating complex wastewaters. The MFC was able to reliably remove greater than 70% of organics when treating two different wastewater streams: synthetic feces wastewater and municipal wastewater. Nitrate removal was also consistently observed in the cathode chamber, and was primarily due to heterotrophic denitrification. Energetic products resulting from anode respiration and methanogenesis were simultaneously observed, yielding a total energy production of $3.3 \pm 0.64 \text{ W/m}^3$ for Case F and $0.40 \pm 0.07 \text{ W/m}^3$ for Case W.

This study considers the potential for co-evolving methane and electricity in a single anode treating different strength wastewaters in a pilot MFC mirroring the operation parameters of a previous field demonstration (e.g., low and infrequent usage). For application in small rural communities, future work will evaluate the effects operational parameters have on treatment performance and energy recovery including HRT, temperature, and organic loading. Future work will also explore the major metabolic pathways for complex organic degradation to balance electrical power and methane production at the pilot scale. Simple and low-cost designs for pilot MFCs, coupled with optimization of energy recovery from all potential sources, is key to bringing this wastewater treatment technology to fruition for underserved communities.

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