A monetary comparison of energy recovered from microbial fuel cells and microbial electrolysis cells fed winery or domestic wastewaters

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A B S T R A C T

Microbial fuel (MFCs) and electrolysis cells (MECs) can be used to recover energy directly as electricity or hydrogen from organic matter. Organic removal efficiencies and values of the different energy products were compared for MFCs and MECs fed winery or domestic wastewater. TCOD removal (%) and energy recoveries (kWh/kg-COD) were higher for MFCs than MECs with both wastewaters. At a cost of $4.51/kg-H2 for winery wastewater and $3.01/kg-H2 for domestic wastewater, the hydrogen produced using MECs cost less than the estimated merchant value of hydrogen ($6/kg-H2). 16S rRNA clone libraries indicated the predominance of Geobacter species in anodic microbial communities in MECs for both wastewaters, suggesting low current densities were the result of substrate limitations. The results of this study show that energy recovery and organic removal from wastewater are more effective with MFCs than MECs, but that hydrogen production from wastewater fed MECs can be cost effective.

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1. Introduction

Activated sludge (AS) systems are often used to treat winery and domestic wastewaters [1], but the energy requirements for this process is becoming a concern. An AS process typically uses 1 kWh of electricity and produces ~0.4 kg of sludge per kg of oxidized COD [2]. To alleviate the cost of solids disposal, excess sludge is usually processed in an anaerobic digester to produce methane gas [3] at a rate which in some cases could sustain the energy demand of an AS-based wastewater treatment plant [4]. While the sustainable operation of wastewater treatment is an admirable benchmark, the high energy content of wastewater presents the potential for even greater energy recovery in the form of other energy products such as electrical power and hydrogen.

Different bioelectrochemical systems (BES) are being examined to make wastewater treatment facilities net energy producers by capturing electrical energy directly from the wastewater. BES such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) are engineered to capture electrical current produced by exoelectrogenic microbes that oxidize soluble organic matter present in wastewater [5,6]. These systems contain anodes where microbes grow and release electrons, and a cathode, where electrons are consumed to form different products. Exoelectrogenic bacteria transfer electrons to the anode of a BES either through direct contact (via highly conductive nanowires or membrane associated proteins) [7,8] or by using soluble electron shuttles [9]. Electrons flow from the anode through an external circuit and reduce O2 to H2O in an MFC [10], and
protons to hydrogen in an MEC [11]. The open circuit potential of a BES anode is typically ~300 mV at standard conditions [12], which is not sufficient to overcome the minimum potential for hydrogen evolution (~414 mV). As a result, hydrogen production from an MEC requires supplemental voltage from an external power source [11].

MFCs have been used to generate electricity from a variety of wastewaters including brewery, chocolate, food processing, meat packing, and paper recycling wastewaters [13]. MFCs fed pure compounds such as acetate [12] and glucose [14] under laboratory conditions generally produce more power than those using wastewaters primarily as a result of the higher solution conductivity and buffer capacity of laboratory media (typically containing high concentrations of phosphate buffers) [15,16]. In addition to the low solution conductivity of many wastewaters (~1 mS/cm), the conversion of complex organics in wastewater to fermentation end products can limit coulombic efficiency and overall energy recovery [15,17].

MECs have also been used to produce hydrogen from cellulose and to treat wastewaters, although overall fewer studies have been conducted with wastewater in MECs than in MFCs. Hydrogen production using the effluent from cellulose fermentation in a well buffered medium with a high solution conductivity was 0.96 ± 0.16 L/L-d, with an overall energy recovery of 220 ± 30% based on the energy value of the hydrogen compared to the electrical energy input [18]. Hydrogen was produced at rate of 0.9–1.0 m3 H2/m3-d using a high-COD (12–17 g-COD/L) swine wastewater without any amendments (Conductivity unknown) [19]. However, the batch cycle time required to remove 72 ± 4% of the influent COD was long (184 h), resulting in a lower overall efficiency of energy recovery as hydrogen relative to electrical energy input (91 ± 6%). Higher hydrogen recoveries (0.74 m3 H2/m3-d) and COD removal (79%) were obtained using a potato chip wastewater [20].

Although MFCs and MECs can both be used to treat wastewaters, there has been no previous comparison of the economic value of the two different energy products (electrical power versus hydrogen gas) for treating wastewater. Previous studies have generally focused on energy recovery and organic removal only one process or from a single wastewater. In this study, we therefore examined treatment efficiency in terms of COD removal of two different types of wastewater (winery or domestic) using both MFCs and MECs. We then compared the energy recovered on the basis of the value of the hydrogen produced for the electrical energy input in the MEC, to the value of the electrical power produced by the MFC. Following operation of the systems as MFCs and then MECs, the anode communities were characterized to explore possible links between reactor performance and the relative abundance of known exoelectrogenic microorganisms such as Geobacter species.

2. Methods

2.1. MFC/MEC reactor construction and operation

Four single-chamber, cubic-shaped MFC and MEC reactors were constructed as previously described [21]. The anode and cathode were positioned at opposite ends of the cylindrical chamber 3-cm in diameter and 4-cm long (empty bed volume 28 mL). An anaerobic tube (Bellco, 10 mL) with the bottom cut off was glued to the top of the reactor to collected biogas produced during MEC operation. Anodes were graphite fiber brushes (PANEX33 160K, Gordon Brush, OD = 2.5 cm, L = 2.5 cm) pretreated using an ammonia gas process [22]. Reactors operated as MFCs contained air-cathodes [23] with a platinum catalyst (0.5 mg/cm2) applied to the water facing side of carbon cloth (Type B-1B, E-TEK, 3.8 cm diameter, 7 cm2 of exposed surface area), and four diffusion layers (PTFE) applied to the air facing side. Cathodes used in MECs contained the same loading of catalyst (0.5 g/cm2 Pt) applied to 30% wet-proofed carbon cloth, but no diffusion layers [21]. To convert MFCs to MEC mode, the air-cathodes were replaced with MEC cathodes, and sealed with a solid plate.

Prior to each batch-fed cycle reactors were sparged with N2 gas for 15 min. Anodes were enriched and evaluated in MFCs before being used in MECs. MFCs were operated at ambient temperature (23 ± 3 °C) on a laboratory bench, while MEC were operated under controlled conditions (30 ± 1 °C) in order to keep the density of hydrogen gas produced constant.

2.2. MFC/MEC operational analysis

Reactor voltage (E) was measured across an external resistor (MFC: R = 1 kΩ, MEC: R = 10 kΩ) every 20 min using a multimeter (2700; Keithley, United States) connected to a desktop personal computer. Current was calculated using I = E/R. Coulombic efficiency (CE) was based on total current generation and change in measured chemical oxygen demand (COD) over a complete batch cycle as previously described [18]. Electricity production in the MFC (kWh/kg-COD) was based on power production (kWh/m3) and estimated COD removal (kg-COD/m3) during the peak current production of each fed-batch cycle. The peak currents (Ip) were obtained from the highest ten voltages for each batch experiment, or over a period of time (tp) = ~2.3 h. COD removal (g/L) during peak current production (ΔCODp) was estimated with Faraday’s constant and average CE (based on the complete batch cycle) [18] as:

$$\Delta \text{COD}_p = \frac{8 \int_0^{t_p} I_p \, dt}{F \, CE \, v_{An}}$$  

(1)

where dt (s) the time interval, Ip (A) the peak current generated during each time interval, F (C/mol e−) Faraday’s constant, CE coulombic efficiency over the fed-batch cycle, and vAn (L) the reactor volume.

The value of electricity produced in MFC ($/kg-COD) was based on the national average price of electricity in the USA ($0.096/kWh) [24]. Electricity saved was estimated using a typical value for activated sludge of 1 kWh/kg-COD removed [2].

Volume and composition of gas produced in the MEC [21], and the hydrogen yield (YH2), production rate (Q) and electrical efficiency (NE) were calculated as previously described [25]. The net monetary value of wastewater treatment in MEC ($/kg-COD) was calculated by subtracting the cost of electricity consumed ($/kg-COD) during each MEC batch from the value of hydrogen.
produced. This analysis did not include capital costs or for the MEC the cost of gas treatment and compression. Energy recovered as hydrogen was determined by multiplying $Y_{H_2}$ by the lower heating value (LHV) of hydrogen (33.3 kWh/kg-$H_2$) [26]. The value of hydrogen produced was determined by multiplying $Y_{H_2}$ (kg-$H_2$/kg-COD) by the market value of hydrogen ($/kg-$H_2$).

The net monetary value of wastewater treatment in MEC ($/kg-COD$) was calculated by subtracting the cost of electricity consumed ($/kg-COD$) during each MEC batch from the value of hydrogen produced.

Total chemical oxygen demand (TCOD) was measured in duplicate, according to Standard Methods [28] (TNT plus COD Reagent; HACH Company). Samples used for SCOD and high-performance liquid chromatography (HPLC) analysis were filtered using 0.22-μm pore-diameter syringe filter. The concentrations of volatile fatty acids and alcohols were determined by gas chromatography as previously described [15].

2.3. Wastewater collection

Winery wastewater was collected from a sump prior to its discharge to aerated lagoons at the Napa Wine Company in Oakville CA. Samples were placed on ice and shipped overnight to the H2E center and stored at 4 °C. Domestic wastewater was collected from the primary clarifier of the Pennsylvania State University Wastewater Treatment Plant and stored at 4 °C. Wastewater served as both inoculum and substrate in all experiments.

2.4. Bacterial community and phylogenetic analysis

DNA extraction from bacteria on the anode biofilm, gene cloning and 16S rRNA gene sequencing for community analysis were conducted previously described (Call et al., 2009). DNA was extracted from anode fiber samples, with a PowerSoil DNA isolation kit (MIO BIOSOLUTIONS). 16S rRNA gene fragments of the extracted DNA were amplified by PCR using universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCT-3′) and 1541R (5′-AAGGAGGTGATCCAGCC-3′) [29]. PCR products were purified using QiAquick PCR Purification Kit (Qiagen) and then ligated and cloned using a TOPO TA cloning kit (Invitrogen) according to the manufacturer’s instructions. Appropriate colonies were plated in a 96-well format on LB plates (AmpR 50 μg/ml). Plasmid extractions were carried out on these colonies using the E-Z 96 Fastfilter® Plasmid Kit® (www.omegabiotek.com/). Once extracted, plasmids were sequenced with the M13R primer using an ABI 3730XL DNA sequencer (Applied Biosystems). The nucleotide collection (nr/nt) of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) was used to search using the BLAST algorithm to analyze the sequences. Mega 4.0.2 [30] was used to align these sequences and generate a phylogenetic tree using the neighbor-joining method with a bootstrap test (500 replicates) of phylogeny.

2.5. Statistical analysis of microbial communities

Rarefaction curves were developed as previously described for each microbial community to determine whether sufficient representative clones had been sequenced [31]. 16S rRNA fragments of clones representing distinct phylotypes were plotted against the total number of clones. Sampling coverage was calculated as previously described [32]. Community diversity was determined by calculating Shannon Diversity Index values for each reactor [33] as follows: $H = -\sum (p_i \ln p_i)$, where $p$ represents the proportion of a phylotype relative to the sum of all phylotypes.

3. Results

3.1. Performance of MFCs fed winery and domestic wastewaters

Winery wastewater fed MFCs produced a maximal voltage of 441 ± 17 mV (1 kV) and electrical energy at 31.7 ± 2.1 Wh/m³, compared to MFCs fed domestic wastewater that produced 381 ± 10 mV and 22.5 ± 1.9 Wh/m³ (Fig. 1a, b). Batch cycle time was six days for winery wastewater due to the high-COD (2,200 mg/L), compared to only one day for domestic wastewater (345 mg-COD/L) (Table 1). TCOD removal was 83 ± 10% using domestic wastewater, and 65 ± 7% for the winery wastewater, with both wastewaters having a coulombic efficiency of 18% (Table 2).

3.2. Performance of MECs fed winery and domestic wastewater

After stable operation in MFC mode, reactors were converted to MEC operation. At an applied voltage of −0.9 V, winery wastewater fed MECs produced ~0.9 mA of electrical current and hydrogen at a maximum flow rate of 0.17 ± 0.01 m³-H₂/m³-d (Fig. 3c), with a coulombic efficiency of 50 ± 8% and TCOD removal of 47 ± 3% (Table 2). The gas composition for the winery wastewater was 70 ± 8% hydrogen, 26 ± 7% carbon dioxide, and 4.3 ± 3% methane. The average energy consumption for MECs fed winery wastewater was 1.4 ± 0.01 kWh/kg-COD.

MECs fed domestic wastewater briefly produced more than 1 mA of electrical current (applied voltage of −0.9 V), but current steadily declined for the remainder of the batch cycle (Fig. 1d). The coulombic efficiency was 64 ± 9% with a TCOD removal of 58 ± 3% (Table 2). The total biogas production rate was $Q_{biog} = 0.28 ± 0.04 m³-H₂/m³-d$ over one day, which was higher than the winery wastewater fed MECs. The gas produced by the domestic wastewater fed MECs (70 ± 1% hydrogen, 28 ± 2% carbon dioxide, and 2.5 ± 0.6% methane) was similar to that produced using winery wastewater. The average energy consumption rate for MECs fed domestic wastewater was 2.0 kWh/kg-COD.

3.3. Value of energy products produced using winery and domestic wastewaters

Electrical energy recovery was 0.26 ± 0.01 kWh/kg-COD (based on peak voltage) for winery wastewater fed MFCs, and 0.22 ± 0.01 kWh/kg-COD for domestic wastewater fed MFCs (Table 2). The net value of electricity recovered from MFCs was therefore $0.026/kg-COD$ for winery wastewater and $0.021/kg-COD$ for domestic wastewater. If the energy “saved” by
eliminating aeration needed for AS is included in this analysis, the net worth was $0.10/kg-COD for both the winery and domestic wastewaters.

The hydrogen yield from winery wastewater was $0.026 \pm 0.004 \text{ kg-H}_2/\text{kg-COD}$, with an electrical efficiency of $78 \pm 20\%$, resulting in a net energy input $-0.32 \pm 0.3 \text{ kWh/kg-COD}$ (Table 2). The high value of H$_2$ ($\$6/\text{kg-H}_2$) produced however, offset the electrical energy requirement resulting in a net monetary gain of $0.06 \pm 0.05/\text{kg-COD}$ of winery wastewater (Table 2). The hydrogen yield of domestic wastewater fed MECs was $0.050 \pm 0.01 \text{ kg-H}_2/\text{kg-COD}$ and the electrical efficiency was $104 \pm 10\%$ resulting in a net energy recovery of $0.14 \pm 0.04 \text{ kWh/kg-COD}$, and a net gain of $0.19 \pm 0.02/\text{kg-COD}$ based on the value of the hydrogen gas produced (Table 2).

### 3.4. Bacterial communities in MECs

The microbial community that developed on the anode of the MECs fed winery wastewater was a mixed consortium dominated by *Geobacter sulfurreducens* (~44% of all 16S rRNA clones). Roseivivax, a gram negative bacterium, belonging to the Rhodobacteraceae family, comprised ~14% of the anodic community. Other significant microbes represented by the MEC clone library included: *Pelobacter propionicus* (7.7%) and the phototroph *Rhodopseudomonas palustris* (2.6%). The genus level Shannon Diversity index for the winery wastewater fed MEC anode communities was 1.56.

The anodic microbial community in MECs fed domestic wastewater similarly was dominated by *Geobacter species* (Fig. 2). The 16S rRNA clone libraries indicate four *Geobacter* strains comprise a total of 52.6% of the community: *Geobacter metallireducens* (23%), *G. sulfurreducens* (14%), *Geobacter lovleyi* (14%), and *Geobacter uraniireducens* (1.6%). *P. propionicus*, a fermentative member of the *Geobacter* species family accounted for an additional 4.7% of isolates. A genus of green sulfur bacteria, Chlorobium, known for oxidizing hydrogen sulfide comprised 9.4% of the anodic community. The genus level Shannon Diversity index for the winery wastewater fed MECs was 1.95.

Fig. 1 – Voltage generated in duplicate studies of MFCs fed (a) winery and (b) domestic wastewaters (1 kΩ resistance), and current produced in MECs fed (c) winery and (d) domestic wastewaters (10 Ω resistance).

Fig. 2 – Anodic bacterial communities based on cloned 16S rRNA gene sequence distribution for MECs fed (a) winery and (b) domestic wastewaters.
4. Discussion

4.1. Winery and domestic wastewater as substrate for MECs

Hydrogen was successfully generated, although at low rates compared to pure compounds in well buffered solutions, using winery and domestic wastewaters. The rate of hydrogen production from winery wastewater fed MECs ($0.17 \pm 0.09 \text{ m}^3\text{H}_2/\text{m}^3\text{-d}$) was lower than that obtained with domestic waste fed MECs ($0.28 \pm 0.04 \text{ m}^3\text{H}_2/\text{m}^3\text{-d}$) but similar to values previously reported for reactors fed cellulose ($0.11 \text{ m}^3\text{H}_2/\text{m}^3\text{-d}$) and valeric acid ($0.14 \text{ m}^3\text{H}_2/\text{m}^3\text{-d}$) [34]. This similarity in production may be a result of the high particulate fraction ($77\%$) of total COD of the winery wastewater. Hydrolysis of particulates and fermentation of complex substrates are required prior to utilization by exoelectrogens, slowing the rate of substrate utilization and resulting in low current production (Fig. 1c, Table 2). Previous MEC tests using domestic wastewater in a two-chamber MEC resulted in hydrogen yields and electrical efficiencies [35] similar to those obtained here, although at a lower CE (26% compared to 64% here).

4.2. MFC/MEC comparison

Conversion from MFC to MEC resulted in higher CEs and current densities, but lower COD removals. MECs have an anaerobic cathode, which minimizes oxygen leakage into the system and increases CEs relative to those for MFCs (Fig. 3b). Overall COD removal was 25% higher in MFCs than MECs, suggesting that oxygen leakage into the MFC is responsible for greater COD removal. Batch cycle times were similar for MFCs and MECs fed domestic wastewater, suggesting that the remaining COD in the MECs would not contribute to additional current generation. It should be noted, however, that MFCs were operated at room temperature ($23 \pm 14 \text{ C}$) compared to $30 \text{ C}$ used to keep gas pressures constant for the MECs. Recent data using acetate as a substrate suggests that use of a 7°C higher temperature for the MFC could have increased power by 15–20% [36], although it is not known what the temperature would be when using more complex substrates such as wastewaters.

Energy was successfully recovered from winery wastewater in both MFC and MEC modes. The electrical energy consumption rate for MECs fed winery ($1.4 \pm 0.02 \text{ kWh/kg-COD}$) wastewater was higher than the estimated energy demand of activated sludge ($1 \text{ kWh/kg-COD}$). However, 78% of energy invested while treating winery wastewater in MECs

| Table 1 – Comparison of domestic and winery wastewaters. |
|-----------------|-----------------|-----------------|
| Parameter       | Domestic         | Winery          |
| pH              | $7.4 \pm 0.2$    | $7.2 \pm 0.1$   |
| S (mS/cm)       | $1.2 \pm 0.1$    | $1.3 \pm 0.2$   |
| TCOD (mg/L)     | $345 \pm 47$     | $2200 \pm 510$  |
| SCOD (mg/L)     | $160 \pm 30$     | $510 \pm 280$   |
| Volatile Acidity (mg/L of sCOD) | $7.9 \pm 5$ | $132 \pm 23$ |

| Table 2 – Performance Comparison of MFCs and MECs fed domestic and winery wastewaters. |
|-----------------|-----------------|-----------------|
| Mode            | Parameter       | Domestic         | Winery          |
| MFC TCOD Removal (%) | $83 \pm 10$    | $65 \pm 7$      |
| CE (%)          | $18 \pm 6$      | $18 \pm 4$      |
| Energy Recovery (kWh/kg-COD) | $0.22 \pm 0.006$ | $0.26 \pm 0.009$ |
| Monetary Value ($/kg-COD$) | $0.021$ | $0.025$ |
| MEC TCOD Removal (%) | $58 \pm 3$    | $47 \pm 3$      |
| CE (%)          | $64 \pm 5$      | $50 \pm 5$      |
| % H$_2$         | $70 \pm 1$      | $70 \pm 8$      |
| Q (m$^3$H$_2$/m$^3$-d) | $0.28 \pm 0.04$ | $0.17 \pm 0.09$ |
| n$_H$ (%)       | $104 \pm 10$    | $78 \pm 20$     |
| Y$_{H_2}$ (kg-H$_2$/kg-COD) | $0.050 \pm 0.01$ | $0.026 \pm 0.004$ |
| Energy Recovery (kWh/kg-COD) | $0.14 \pm 0.4$ | $-0.32 \pm 0.3$ |
| Monetary Value ($/kg-COD$) | $0.19 \pm 0.06$ | $0.06 \pm 0.05$ |

Fig. 3 – Performance parameters for winery and domestic wastewaters in MFCs and MECs: (a) TCOD and TCOD removal; (b) CE; and (c) gas composition and production (Q) of domestic and winery wastewaters in MECs.
was recovered as hydrogen gas. The negative energy balance of winery wastewater fed MECs resulted in the value of the gas ($4.15/kg-H_2) being above the DOE 2015 target price for hydrogen gas ($2–3/kg-H_2). When considering the estimated market value ($6/kg-H_2) (costs for gas purification and compression were not included), the monetary value of winery wastewater treatment by MEC is $0.06 ± 0.05/kg-COD. In comparison, the monetary value of energy recovered and saved for winery wastewater treatment in an MFC (1.26 ± 0.01 kWh/kg-COD, $0.10/kg-COD) was significantly higher, indicating that on this basis an MFC is a more economical approach for winery wastewater treatment.

Energy recovery from domestic wastewater was positive for both MFCs and MECs (Table 2). The energy saved and recovered was significantly higher during MFC treatment (1.22 ± 0.006 kWh/kg-COD) than MEC treatment (0.14 ± 0.4 kWh/kg-COD). COD removal was also significantly greater in MFCs (83 ± 10%) than MECs (58 ± 3%). While COD removal and energy recovery were greater in MFC, the value of energy recovered in MECs ($0.19 ± 0.06/kg-COD) was much higher than the value of energy saved and recovered from MFCs ($0.10 ± 0.006/kg-COD), indicating that, at a high hydrogen market price ($6/kg), MECs are the more economical choice for domestic wastewater treatment.

4.3. Microbial diversity of winery and domestic wastewater fed MEC anodes

The electrodes analyzed for microbial communities following MEC tests consistently contained a high proportion of Geobacter species. Anode communities from the MECs fed winery wastewater were dominated by 16S RNA clones with sequence similarity to two Geobacter species (G. sulfurreducens, 37%; G. metallireducens, 2.6%). The anodes of the domestic wastewater fed MECs contained these two species (37% total) as well and two others (G. lovleyi, 14% and G. uraniireducens, 1.6%). Both microbial communities also contained 16S RNA clones with sequence similarity to the fermentative microbe, P. propionicus (4.7% of clones, domestic; 7.7%, winery). The presence of clones with sequence similarity to P. propionicus is not surprising considering fermentation of complex organic matter would be needed for subsequent electricity generation by different Geobacter strains [37]. Based on previous studies the presence of these Geobacter strains should have allowed high current densities and high rates of hydrogen production [20,38]. This suggests that the reason for the relatively lower current densities obtained here was substrate limitations for exoelectrogens, i.e. the availability of acetate or other substrates that can be used by Geobacter to produce current [39].

5. Conclusions

Organic removal efficiencies and values of the different energy products were compared for MFCs and MECs fed winery or domestic wastewater. TCOD removal (%) and energy recoveries (kWh/kg-COD) were higher for MFCs than MECs with both wastewaters. At a produced cost of $4.51/kg-H_2 for winery wastewater and $3.01/kg-H_2 for domestic wastewater, hydrogen cost less than the estimated merchant value of hydrogen ($6/kg-H_2). These results show that energy recovery and organic removal from wastewater can be more effective with MFCs than MECs, but that hydrogen production from wastewater fed MECs can also be cost effective based on electrical energy requirements.

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