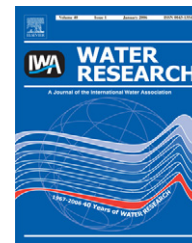


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Evaluation of energy-conversion efficiencies in microbial fuel cells (MFCs) utilizing fermentable and non-fermentable substrates

Hyung-Sool Lee*, Prathap Parameswaran, Andrew Kato-Marcus,
César I. Torres, Bruce E. Rittmann

Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, 1001 S. McAllister Avenue,
Tempe, AZ 85287-5701, USA

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ABSTRACT

We established the first complete electron-equivalent balances in microbial fuel cells (MFCs) fed with non-fermentable (acetate) and fermentable (glucose) electron donors by experimentally quantifying current, biomass, residual organic compounds, H₂, and CH₄ gas. The comparison of the two donors allowed us to objectively evaluate the diversion of electron flow to non-electricity sinks for fermentable donors, leading to different behaviors in energy-conversion efficiency (ECE) and potential efficiency (PE). Electrical current was the most significant electron sink in both MFCs, being 71% and 49%, respectively, of the initial COD applied. Biomass and residual organic compounds, the second and third greatest sinks, respectively, were greater in the glucose-fed MFC than in the acetate-fed MFC. We detected methane gas only in the glucose-fed MFC, and this means that anode-respiring bacteria (ARB) could out-compete acetoclastic methanogens. The ECE was 42% with acetate, but was only 3% with glucose. The very low ECE for glucose was mostly due to a large increase of the anode potential, giving a PE of only 6%. Although the glucose-fed MFC had the higher biomass density on its anode, it had a very low current density, which supports the fact that the density of ARB was very low. This led to slow kinetics for electron transfer to the anode and accentuated loss due to the substrate-concentration gradient in the anode-biofilm. The large drop of PE with low current, probably caused by a low ARB density and electron (e⁻) donor concentration, resulted in a poor maximum power density (9.8 mW/m²) with glucose. In contrast, PE reached 59% along with high current for acetate and the maximum power density was 360 mW/m².

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1. Introduction

Approximately 86% of world energy production comes from fossil fuels today (Energy Information Administration, 2005), but fossil fuels, especially petroleum, are being exhausted, leading to an energy crisis in the near future (Rifkin, 2002). Furthermore, the combustion of fossil fuels adds CO₂ to the

atmosphere and causes global climate change (IPCC, 2007). To mitigate the adverse effects of an energy crisis and global climate change, society needs to develop carbon-neutral, sustainable energy sources as alternatives to fossil fuels.

Biomass is proposed as one of the future energy sources, since it is carbon-neutral. However, biomass energy is used today mostly through combustion, which emits local air

*Corresponding author. Tel.: +1 480 727 0849; fax: +1 480 727 0889.

E-mail addresses: hyungsool@asu.edu (H.-S. Lee), Prathap.Parameswaran@asu.edu (P. Parameswaran), Andrew.Marcus@asu.edu (A. Kato-Marcus), Cit@asu.edu (C.-I. Torres), Rittmann@asu.edu (B.E. Rittmann).

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pollutants that threaten human and ecological health (EIA, 2006). A means to capture the energy values in biomass without combustion would provide sustainable energy for global society without causing local pollutant problems.

The energy value of biomass comes about because its carbon molecules contain high-energy electrons. Microorganisms can channel the electrons and their energy to forms that human society can use readily: methanogenesis to CH₄, biohydrogen to H₂, and microbial fuel cells (MFCs) to electricity. Among these alternatives, MFCs are attractive because they produce society's most widely useful energy form—electricity—directly without combustion. Due to their potential advantages, MFCs have gained much attention in recent years (e.g., Logan, 2004; Rabaey and Verstraete, 2005; Rittmann, 2006; Buckley and Wall, 2006; Lovley, 2006).

Power, the rate of energy generation, is an essential gauge of the electricity-supplying capacity for an MFC. Despite important scientific advances, the power density of MFCs (expressed as W/m³ or W/m²) is still insufficient for practical energy supply. Rabaey et al. (2003) reported the highest volumetric power density, 275 W/m³, using glucose, but others reported power densities one or two orders of magnitude less using glucose (Liu and Logan, 2004; Min and Logan, 2004; Chaudhuri and Lovley, 2003; Bond and Lovley, 2003). The maximum power density was increased up to 1 kW/m³ using acetate at the bench scale (Fan et al., 2007). For comparison, the power density from MFCs is smaller than what is generated from methanogenesis: 1–5 kW/m³ in full-scale anaerobic digestion (Van Lier, 2007). For MFCs to become a practical energy source, their power density must be improved by 10- to 100-fold.

Power density is not the only evaluation criterion for MFCs. Another criterion is the energy-conversion efficiency (ECE), or the fraction of energy in a fuel that is captured in the electricity output. Heat engines typically have an ECE of ~33% (Sun et al., 2004), while hydrogen fuel cells have about 55% (Larminie and Dicks, 2003). Rabaey et al. (2003) reported a 65% ECE in a batch MFC, but an ECE as low as 0.3% was reported for a continuous MFC (Min and Logan, 2004). One reason for a low ECE is that large amounts of residual organics are discharged from the MFC. When the MFC is used as part of waste treatment, this form of inefficiency means that the MFC has failed in its treatment role, as well as in its role of an energy producer.

The ECE value in an MFC is the product of its coulombic efficiency (CE) and potential efficiency (PE) for the electrons captured as electrical current. Specifically, CE is the fraction of electrons transferred to the MFC anode from e⁻ donor applied, while PE is the fraction of the potential difference captured as useful electrical energy in comparison to the potential difference between the e⁻ donor and acceptor. Thus, ECE can be expressed mathematically by

$$\begin{aligned} \text{ECE} &= \frac{P\Delta t}{e_{\text{donor}}^- (-\Delta G_{\text{rxn}}^{\circ})} = \frac{I\Delta t V_{\text{meas}}}{e_{\text{donor}}^- (-\Delta G_{\text{rxn}}^{\circ})} \\ &= \frac{e_{\text{trans}}^-}{e_{\text{donor}}^-} \frac{V_{\text{meas}}}{V_{\text{reac}}} = \text{CE} \times \text{PE}, \end{aligned} \quad (1)$$

where P is the power (J/s), Δt the reaction time (s), e_{donor}^- the electron equivalents of initial substrate, $\Delta G_{\text{rxn}}^{\circ}$ the change in standard Gibb's free energy at pH 7 between the e⁻ donor and

acceptor (J/e⁻ eq), I the current (C/s), e_{trans}^- the electron equivalents transferred to the anode during the reaction time, V_{meas} the measured voltage output (J/C), V_{reac} the $-\Delta G^{\circ}/F$ (J/C), and F the Faraday constant (96,485 C/e⁻ eq).

ECE values reach a maximum only when CE and PE are high together. However, PE is negatively correlated with current density, which is a factor needed to optimize the power density (Liu and Logan, 2004; Rabaey et al., 2005a; Jong et al., 2006; Logan et al., 2007). For this reason, the best way to have high ECE and high power density is to maximize CE at the optimum power density.

CE seems to depend on substrate type. Acetate appears to be the best substrate, with CEs ranging from 65% to 84% (Min and Logan, 2004; Liu et al., 2005; Rabaey et al., 2005a; Torres et al., 2007). The CE dropped to 14–20% for glucose (Min and Logan, 2004; Liu and Logan, 2004) and to 8% for wastewater (Min et al., 2005). The sharp decline in CE for the more complex organics suggests that competing electron sinks are important. Biomass, soluble organic products, H₂, and CH₄ are possible electron sinks in MFCs if exogenous e⁻ acceptors, particularly oxygen, do not enter the anode compartment. Electron equivalents for the gases can be considerable for MFCs fed with fermentable substrates, as compared to non-fermentable substrates, since H₂ is normally produced in fermentation. And, H₂ is a good electron donor for methanogens to produce CH₄, a factor that is supported by CH₄ detection in MFCs fed with glucose, but not in acetate-fed MFCs (Freguia et al., 2007; Torres et al., 2007). Furthermore, fermentation is performed by diverse microorganisms that have higher growth yields than do anode-respiring bacteria (ARB) (Esteve-Núñez et al., 2005; IWA, 2002), and their high yield can make biomass a significant electron sink that reduces CE. Despite the significance of tracking the fate of all electron equivalents in MFCs, no experimental studies have quantified electron sinks and completed an electron-equivalent balance.

In conventional fuel cells, the PE is normally decreased by three kinds of energy losses, or over-potentials: ohmic, concentration, and activation (Larminie and Dicks, 2003; Logan et al., 2006). Ohmic losses occur due to the current resistance in the electrodes and interconnection circuit, as well as resistance to ion flow in the electrolyte and through the cation exchange membrane (CEM). Concentration losses occur when the oxidation of fuel at the anode or the reduction of oxidant at the cathode is much faster than the transfer rates of fuel or oxidant to the electrode. Generally, concentration losses become important with high current density, for which the concentration of fuel or oxidant must be much lower at the electrode to drive the high rate of mass transfer. Rabaey et al. (2005b) argued that MFCs do not generate high enough current density to cause significant concentration losses. Activation losses occur during electron transport from the e⁻ donor to the anode or from the cathode to the oxidant to gain a certain current in a fuel cell. For conventional fuel cells, activation loss is a means to include the local concentration at the electrode without needing to determine the local concentration.

For an MFC, energy losses associated with the anode (i.e., the anode over-potential) cannot be described only with the conventional energy losses. Formation of the anode-biofilm leads to a different environment from a conventional fuel cell.

First, the biofilm has its own ohmic resistance for electron conduction from the cells to the anode surface (Kato-Marcus et al., 2007). Second, bacterial metabolism has a potential loss, since the bacteria must capture energy from potential difference between its electron donor and its terminal electron carrier, such as a cytochrome. From the point of view of PE, the desirable situation has the potential of the terminal electron carrier close to that of the electron-donor substrate, since this minimizes the potential that is claimed by the ARB. At the same time, such a low potential difference should make the biomass yield low (Rittmann and McCarty, 2001), which also helps to keep CE high. Third, the donor substrate can have a large concentration gradient between the bulk fluid and a location within the biofilm due to mass-transfer resistance (Rittmann and McCarty, 2001; Wanner et al., 2006; Kato-Marcus et al., 2007). Therefore, the local concentration of the substrate in the biofilm is lower than its bulk concentration, and this creates a concentration over-potential described by the Nernst equation (Bard and Faulkner, 1980). Kato-Marcus et al. (2007) showed that concentration losses can be large if the biofilm on the anode becomes thick or inert biomass accumulates over time.

Although anode over-potentials associated with the biofilm-anode in MFCs are not understood well, past MFC research shows that proper acclimation of ARB lowers the anode over-potential and is aligned with a relatively high power density (Rabaey et al., 2003, 2004, 2005a). Rabaey et al. (2003, 2004, 2005a) obtained the highest volumetric power densities to date (172–275 W/m³) through lengthy pre-selecting of ARB: they scratched biofilm from the anode, re-suspended it in the anode chamber, grew more biofilm with the target substrate, and repeated the procedure for several months. Their results suggest the significance of enriching ARB able to oxidize the target substrate rapidly and also maintain a high PE. Even though the enrichment of proper ARB seems essential for a high PE, quantitative assessment of ARB effects on PE, ECE, and power density is lacking.

In this study, we intentionally stimulated different biofilms using non-fermentable (acetate) and fermentable substrates (glucose) in order to understand the relationships among CE, PE, ECE, and power density caused by different substrates and biofilm characteristics. Based on a complete electron-equivalent balance, we quantified the losses to CE for both systems and quantified the effects of non-electricity sinks. We also computed the PE and ECE, related them to over-potentials in the biofilm-anode, and analyzed the effects of PE and CE on power density.

2. Materials and methods

2.1. Inoculum

We obtained the inoculum from an MFC-fed acetate in the continuous mode for 3 weeks; the inoculum of the mother reactor to the acetate-fed MFC was a mixture of activated sludge and anaerobic digester sludge. We transferred 10 mL of effluent from the acetate-fed MFC to the new MFC reactor. Analysis of 16S-rRNA gene clone libraries (described below) on the inoculum showed the presence of 46 *Geobacter sulfurreducens* clones out of 48 total clones.

2.2. Clone library

We built a clone library to evaluate dominant bacterial species in the inoculum. We scratched biofilm from the anode of the acetate-fed MFC that provided the inoculum for our studies using sterile pipette tips. We extracted DNA with an Ultraclean soil DNA isolation kit (Mo Bio Laboratories, Inc.), amplified the 16S-rRNA genes from the DNA with primers 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AAGGAGGT-GATCCAGCGCA-3') by PCR, and cloned them using TOPO TA cloning[®] kits (Invitrogen). We inserted the amplified genes into chemically competent *Escherichia coli* according to the manufacturer's instructions, spread 10–50 µL to the LB agar plates having 50 µg/mL ampicillin, and incubated the plates at 37 °C overnight. We picked white-isolated colonies from the plates to other LB agar plates, with streaked rods after touching the colonies, and incubated them again under the same condition. We transferred each colony into LB broth containing 100 µg/mL ampicillin and incubated it at 37 °C overnight. We amplified the plasmid with 16S-rRNA genes by PCR using TA primers provided by the manufacturer, isolated the plasmids with Ultraclean[™] 6 min Mini Plasmid Prep Kit (Mo Bio Laboratories, Inc.), and sent them out to the DNA Laboratory at Arizona State University, which sequenced the amplicons using an Applied Biosystems 3730 DNA analyzer (Applied Biosystems).

2.3. Batch experiments

We carried out experiments in the batch mode with an H-type MFC (Fig. 1) having a working volume of 300 mL in each compartment. The electrodes were graphite rods having an outer diameter of 0.79 cm and a surface area of 20.3 cm², and we bundled together three rods in the anode chamber (total anode surface area = 60.9 cm²). We connected the anode to one graphite rod in cathode compartment through a copper wire and external resistor of 100 Ω and inserted a CEM (CMI 7000, Membranes International Inc.) between the two chambers to permit only cation transport to maintain electroneutrality. The CEM surface area was 12.6 cm², and it had been soaked in 3 M NaCl for 3 days before use.

Glucose and acetate were electron donors present in each anode chamber initially at 48 e⁻ meq/L (384 mg/L as COD) in mineral medium. The composition of the mineral medium was (per L of deionized water) KH₂PO₄ 1680 mg, Na₂HPO₄ 12,400 mg, NaCl 1600 mg, NH₄Cl 380, 5 mg EDTA, 30 mg MgSO₄·7H₂O, 5 mg MnSO₄·H₂O, 10 mg NaCl, 1 mg CO(NH₃)₂, 1 mg CaCl₂, 0.001 mg ZnSO₄·7H₂O, 0.001 mg ZnSO₄·7H₂O, 0.1 mg CuSO₄·5H₂O, 0.1 mg AlK(SO₄)₂, 0.1 mg H₃BO₃, 0.1 mg Na₂MoO₄·2H₂O, 0.1 mg Na₂SeO₃, 0.1 mg Na₂WO₄·2H₂O, 0.2 mg NiCl₂·6H₂O, and 1 mg FeSO₄·7H₂O. The initial pH in the anode chamber was 7.8 ± 0.2.

We filled the cathode chamber with 100 mM ferricyanide (corresponding to 100 e⁻ meq/L) and phosphate buffer at 100 mM in which the initial pH was adjusted at 7.5 ± 0.2 with 0.1 N NaOH. The 100 e⁻ meq/L potassium ferricyanide was more than twice the number of electron equivalents available at the anode, which ensured that the terminal acceptor concentration did not limit the process. In addition, the sufficient

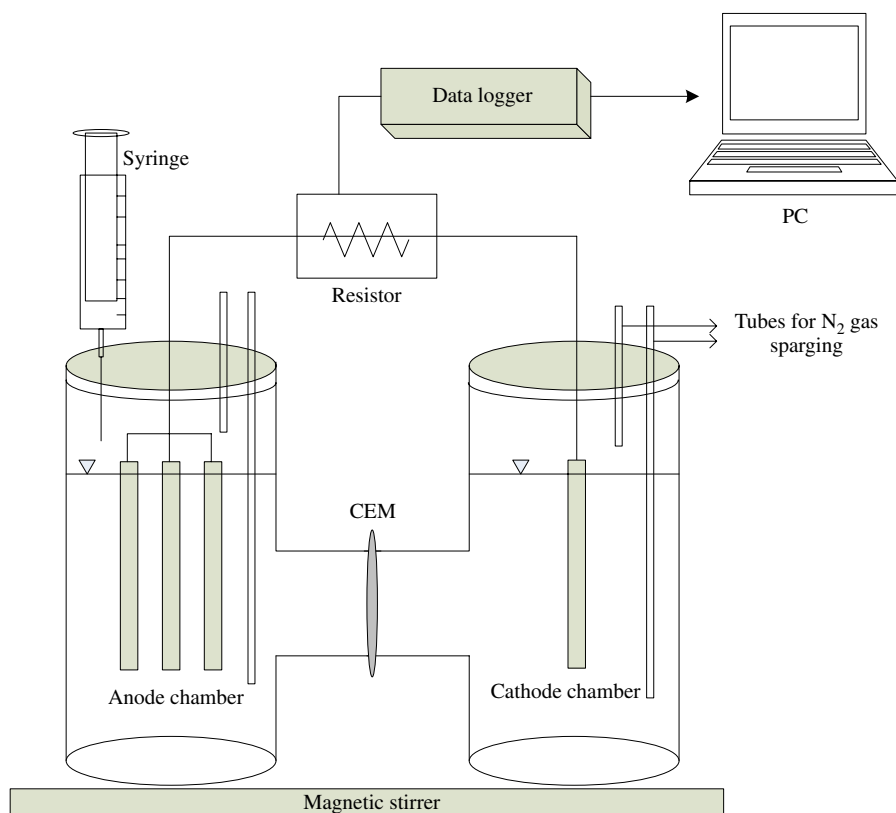


Fig. 1 – Schematic diagram of the H-type MFC reactor used in this study. CEM is cationic exchange membrane.

ferricyanide kept the cathode potential constant during the experiments, which we confirmed by preliminary tests.

In an anaerobic glove box, we sparged the anode chamber with N_2 gas for 1 min and vacuumed the MFCs at 0.67 atm, repeating the vacuum-and-release procedure five times. We kept the MFCs in the anaerobic glove box chamber for 2 h before adding inoculum to the anode; the procedure of vacuum and release in the globe box completely removed O_2 in the anode and cathode chambers, which makes electron loss by O_2 negligible in experiments. In addition, we used ferricyanide as the electron acceptor in the cathode to eliminate the possibility that O_2 became an electron sink in the anode. We then operated the MFCs in a constant-temperature room at 30 °C, with the reactors placed on a magnetic stirrer plate for mixing. We operated the MFCs without an acclimation period for the target e^- donors, which enabled us to evaluate CE, PE, and ECE for an inoculum that had been pre-selected for anode reduction with acetate. We continuously monitored the voltage every 2 min with a data logger (National Instruments, TX) and computed currents with $I = V_{meas}/R_{ext}$ ($R_{ext} = 100\ \Omega$) and inserted a friction-free syringe of 10 mL (Popper & Sons, Inc.) in the anode chamber to quantify cumulative gas production over the reaction time.

2.4. Analyses

We measured gas percentages of H_2 , CH_4 , and CO_2 in samples taken with a gas-tight syringe (SGE 500 μ L, Switzerland) using

a gas chromatograph (GC 2010, Shimadzu) equipped with a thermal conductivity detector. We used a packed column (ShinCarbon ST 100/120 mesh, Resteck Corporation) for separating sample gases. N_2 was the carrier gas fed at a constant pressure of 5.4 atm and a constant flow rate of 10 mL/min, and the temperature conditions for injection, column, and detector were 110, 140, and 160 °C, respectively. Analytical grade H_2 , CH_4 , and CO_2 were used for standard calibration curves. We carried out gas analyses in duplicate and averaged the data. Detection limits of H_2 and CH_4 were 0.5%.

We took liquid samples for quantifying intermediate compounds and suspended biomass after the MFC stopped generating detectable current (<0.024 mA). Intermediate compounds analyzed by high-performance liquid chromatography (HPLC; Model LC-20AT, Shimadzu) were pyruvate, formate, acetate, *n*-butyrate, iso-butyrate, propionate, valerate, succinate, fumarate, lactate, malate, maleate, acetone, ethanol, butanol, propanol, and residual glucose. We used an Aminex HPX-87H (Bio-Rad, 1997) column for separating the simple acids and solvents. Sulfuric acid at 5 mM was the eluent fed at a flow rate of 0.5 mL/min. We detected chromatographic peaks using photodiode-array (210 nm) and refractive index detectors. The total elution time was 60 min, and the oven temperature was constant at 40 °C. We established a new calibration curve with standard solutions for all the compounds for every set of analyses, performed duplicate assays, and report data as average concentrations. We determined total and soluble COD in the anode chamber

using HACH COD vials (concentration range 10–1500 mg/L), using a 0.2- μm membrane filter (PVDF GD/X, Whatman) to remove particulate matter.

We separately quantified suspended and biomass attached to the anode. We used COD for the quantification of suspended biomass according to

$$\text{Suspended biomass} = (\text{TCOD} - \text{SCOD})_{\text{final}} - (\text{TCOD} - \text{SCOD})_{\text{initial}} \quad (2)$$

where TCOD and SCOD are total and soluble COD, respectively. We quantified biomass attached on the electrodes by protein content, assuming that proteins comprise 50% of biomass by dry weight (Rittmann and McCarty, 2001), using the bicinchoninic-acid assay (BCA) for protein analysis (Brown et al., 1989). We extracted the protein from the attached biomass on the electrodes by heat treatment at 90 °C with 0.1N NaOH for 30 min, removed the electrodes from the centrifuge tubes, centrifuged the lysate, and used 0.1 mL of the supernatant for the BCA assay. We performed the assay in duplicate using a BCA protein assay kit (Sigma Aldrich BCA-1/B 9643), measured the absorbance at a wavelength of 562 nm using a spectrophotometer, and developed a standard calibration curve with bovine serum albumin at each time of protein quantification.

2.5. Calculations

We converted voltage to power (P in W) according to $P = \text{current} \times \text{voltage}$. We estimated the CE by dividing cumulative coulombs collected by the electron equivalents of glucose or acetate consumed (Eq. (1)). We express all electron equivalents as COD ($1 e^- \text{ eq} = 8 \text{ g COD}$) to establish mass balance according to

$$\text{COD}_{\text{ini}} = \text{COD}_{\text{current}} + \text{COD}_{\text{biomass, sus}} + \text{COD}_{\text{biomass, att}} + \text{COD}_{\text{gases}} + \text{COD}_{\text{fin}} + \text{COD}_{\text{unknown}} \quad (3)$$

where COD_{ini} is the initial COD in the anode chamber, $\text{COD}_{\text{current}}$ the COD for current generation over the incubation time, $\text{COD}_{\text{biomass, sus}}$ the COD for suspended biomass net accumulation over the incubation time in the anode chamber, $\text{COD}_{\text{biomass, att}}$ the COD for attached biomass net accumulation on the anode over the incubation time, $\text{COD}_{\text{gases}}$ the COD for cumulative gas productions of CH_4 and H_2 over the incubation time in the anode chamber, COD_{fin} the soluble COD in anode chamber at the end of current generation, and $\text{COD}_{\text{unknown}}$ the unaccounted for COD in the liquid of the anode chamber. Units are mg.

We converted units and computed electricity energy with the following relationships:

$$1 \text{ mA} = 8 \text{ mg COD} \cdot e^- \text{ eq} / 96,485 \text{ C}$$

$$1 \text{ mL CH}_4 = \frac{1 \text{ mmol CH}_4}{22.4 \text{ mL}} \cdot \frac{273.15 \text{ K}}{303.15 \text{ K}} \cdot \frac{8 \text{ meq } e^-}{\text{mmol CH}_4} \cdot \frac{8 \text{ mg COD}}{\text{meq } e^-} = 2.57 \text{ mg COD}$$

$$1 \text{ mL H}_2 = \frac{1 \text{ mmol H}_2}{22.4 \text{ mL}} \cdot \frac{273.15 \text{ K}}{303.15 \text{ K}} \cdot \frac{2 \text{ meq } e^-}{\text{mmol H}_2} \cdot \frac{8 \text{ mg COD}}{\text{meq } e^-} = 0.64 \text{ mg COD}$$

$$\text{Electricity energy} = \int P dt \approx \sum P_i (t_i - t_{i-1}),$$

where P_i is power at t_i and $(t_i - t_{i-1})$ is 120 s.

3. Results and discussion

3.1. Current generation

Fig. 2 shows that electricity began to be generated after approximately 3 and 6 days for acetate and glucose, respectively; we present the results as current evolution over time, instead of voltage, since current is directly related to substrate-utilization rates of ARB. Current generation then increased, reached a maximum, and declined, eventually becoming undetectable, below 0.024 mA. Despite the generally similar trends, Fig. 2 illustrates that the fermentable and non-fermentable substrates had distinctly different patterns of current generation with time. In the acetate-fed MFC, current rapidly reached a maximum value of 4.66 mA between 5 and 6 days, and then the current sharply declined by 7 days. In the glucose-fed MFC, current was generated for 24 days, but with a lower maximum current of 0.76 mA.

3.2. Electron equivalent balances

Table 1 describes the distribution of electron equivalents as COD at the end of the experiments. The closures of the COD mass balances for the glucose- and the acetate-fed MFCs were 97% and 98%, respectively. In both cases, electricity was the largest electron sink at the end of the experiment. The acetate-fed MFC obtained a CE of 71%, while it was 49% in the glucose-fed MFC. The relatively low CE in the glucose-fed MFC probably occurred because glucose was not used directly by the ARB. *G. sulfurreducens*, the major bacterial species in the clone library of our inoculum, cannot utilize glucose as an electron donor for reducing insoluble forms of Fe(III) (Caccavo et al., 1994). Then, glucose needs to be fermented to simple organic compounds for electricity generation. The long acclimation found in the glucose-fed MFC supports the fact that the glucose fermenters needed to grow and produce simple organic acids (e.g., acetate) for ARB to generate

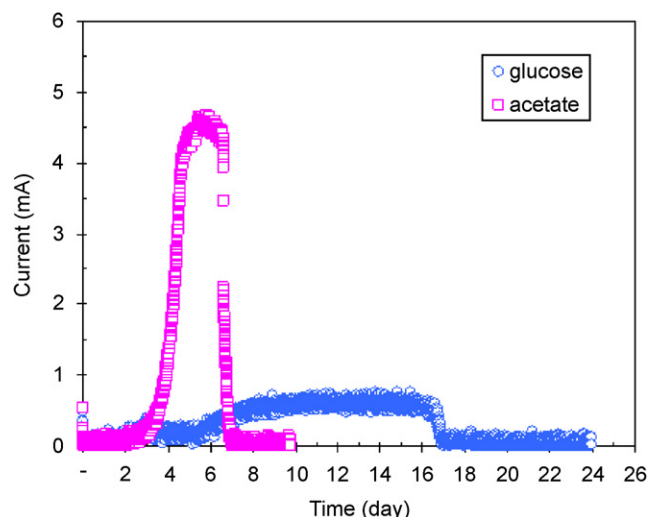


Fig. 2 – Current generation with time in batch MFCs fed with acetate and glucose.

Table 1 – Distribution of electron equivalents in the MFCs expressed as COD at the end of the batch experiments

	Glucose-fed MFC		Acetate-fed MFC	
	COD (mg)	Fraction (%)	COD (mg)	Fraction (%)
Initial COD	103	100	115	100
Final SCOD	19	18	12	11
Current	50	49	82	71
Biomass _{sus} ^a	10	10	6	5
Biomass _{att} ^b	17	16	12	10
CH ₄ gas	3.8	3.7	ND	–
H ₂ gas	ND	–	ND	–
Sum of final COD sinks	99.8	97	112	98
Unknown	3.2	3	3.0	2

ND: not detected.
^a Suspended biomass.
^b Attached biomass.

current. Some fermentation products (e.g., H₂) may not be good substrates for ARB (Torres et al., 2007), in which case they either become electron sinks or are converted to CH₄. If glucose is fermented only into acetate and H₂, then two-thirds of the electron equivalents of the initial glucose are routed to acetate (69 mg COD), while one-third go to H₂ (34 mg COD). If the ARB in the glucose-fed MFC had the same CE for acetate as in the acetate-fed MFC (71%), then the electron equivalents from acetate should be 49 mg COD (69 mg COD × 0.71), making the CE 48% (49 mg COD/103 mg COD). This CE is almost the same as the observed CE of 49% for the glucose MFC (Table 1) and supports the fact that H₂ was a poor electron donor for ARB.

Biomass was the largest non-electricity sink in both cases: 26% of the initial COD (COD_{ini}) for the glucose-fed MFC and 15% for the acetate-fed MFC. Attached biomass was slightly more than suspended biomass in both reactors, which supports the fact that biofilm on the anode was more significant for substrate utilization in MFCs, even though the MFCs were operated in a batch mode. The observed biomass yields were 0.26 and 0.15 mg COD biomass/mg COD removed for the glucose- and acetate-fed MFCs, respectively. The net yield for the glucose-fed MFC is close to 0.21 mg COD biomass/mg COD removed observed by Rabaey et al. (2003). The higher biomass yield in the glucose-fed MFC means that the accumulation of fermenter biomass was a significant reason for its lower CE.

SCOD accounted for the second largest fraction of non-electricity sinks. SCOD concentrations were 63 and 40 mg/L, respectively, for the glucose- and acetate-fed MFCs, and these correspond to net COD-removal efficiencies of 82% and 90%. We detected none of the typical acids and alcohols (C1–C5) by HPLC. Fig. 3 compares the chromatographic peaks for standards and the unknown compounds detected by UV at the wavelength of 210 nm. The retention time for the unknown peak ranged from 7.2 to 7.3 min, which did not correspond to any of the organic acids or alcohols we

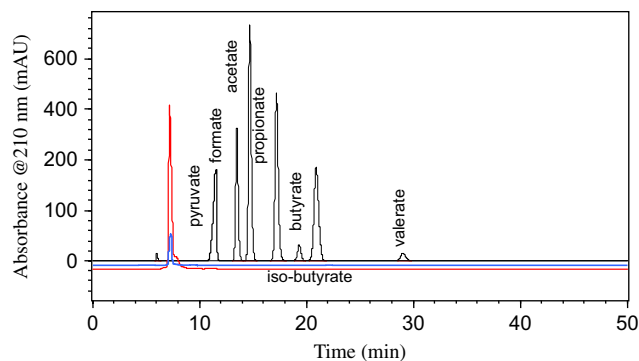


Fig. 3 – Chromatogram of the standard mixture of organic acids compared to the observed soluble end products for MFCs. Black line—standard mixture, red line—chromatogram of glucose-MFC, blue line—chromatogram of acetate-MFC.

evaluated with HPLC. The maximum UV absorbance for the unidentified soluble product was approximately 10 times higher in the glucose-fed MFC than in the acetate-fed MFC, and this is consistent with the higher SCOD concentration in the glucose-fed MFC. It is likely that at least some of the unidentified soluble organics were soluble microbial products (Lapidou and Rittmann, 2002), which are known to be produced more in glucose-based methanogenesis than in acetate-based methanogenesis (Noguera et al., 1994; Kuo et al., 1996). High SCOD concentration and intensive UV absorbance in the glucose-fed MFC over the acetate-fed MFC are in accord with the finding that more SMP was accumulated with glucose feeding than with acetate feeding in anaerobic chemostats (Noguera et al., 1994; Kuo et al., 1996). The SCOD comparison between the glucose- and acetate-fed MFCs suggests that residual COD can be significant in MFCs using complex wastes or wastewaters as fuel, even if common fermentation products are absent. This is reinforced by the findings of Liu et al. (2004), who achieved COD removal efficiencies as low as 50–70% in an MFC using domestic wastewater. Liu and Logan (2004) also reported that the final SCOD was 25% of the initial SCOD in a batch MFC fed with glucose, even though glucose removal efficiency was 98%. In such cases, MFCs may require post-treatment before effluent discharge to receiving water.

We detected CH₄ gas only in the glucose-fed MFC, where it was 3.7% of COD_{ini}; CH₄ was below the detection limit in the acetate-fed MFC. Negligible CH₄ in the acetate-fed MFC indicates that acetoclastic methanogens were out-competed by ARB. Acetoclastic methanogens have a half-maximum rate concentration (K_s) of 177–427 mg COD/L for acetate (Lawrence and McCarty, 1969; Finney and Evans, 1975; Rittmann and McCarty, 2001). *G. sulfurreducens*, apparently the dominant bacteria in our inoculum, has a K_s value of 0.64 mg COD/L (Esteve-Núñez et al., 2005) for acetate. This large difference may explain why ARB out-competed methanogens for acetate.

In contrast, CH₄ production in the glucose-fed MFC likely occurred because hydrogenotrophic methanogens oxidized and grew on H₂ produced in fermentation. It was reported that *G. sulfurreducens* prefer acetate over H₂ as their electron

donor (Cord-Ruwisch et al., 1998; Bond and Lovley, 2003). If H₂ is poorly utilized by ARB (Torres et al., 2007), CH₄ production may not be avoidable in MFCs fed by fermentable substrates, which are inevitable with complex biomass inputs, such as agricultural residues, activated sludge, and anaerobic digestion sludge (Kim et al., 2005; He et al., 2006). When fermentation occurs, H₂ can become a considerable electron sink. H₂ competition between ARB and other H₂ consumers can have a strong impact on the CE. While it may be possible to suppress H₂ oxidation by excluding electron acceptors such as O₂, NO₃⁻, and SO₄²⁻, it is not possible to exclude HCO₃⁻. Thus, methanogenesis is always a possibility with MFC oxidation of complex organic compounds. Perhaps methanogenesis is the reason why MFC studies have not presented significant H₂ accumulation in the anode chamber when it has been measured: less than 500 ppmv H₂ gas in the anode (Bond and Lovley, 2003; Rabaey et al., 2003). On the other hand, others have observed methane in significant quantities when they have measured it (Kim et al., 2005; He et al., 2006; Freguia et al., 2007).

3.3. Energy-conversion efficiency (ECE) and potential efficiency (PE)

Table 2 summarizes the CE, PE, and ECE values for our experiments and compares them to pertinent literature values. Energy captured as electricity ($= \sum P_i \Delta t$) was 30 and 377 J, respectively, in the glucose- and acetate-fed MFCs, which correspond to ECEs of 3% and 42%, respectively. Considerable over-potentials (PE of only 6%) led to a sharp drop of ECE in the glucose-fed MFC, even though CE was 49% with glucose. In contrast, the PE was 59% with acetate.

Our ECE results are generally consistent with previously reported results using different inocula. In continuous MFCs, CE and PE values were relatively high with acetate, producing ECEs from 17% to 39%, but low with glucose, 0.3–20% (Table 2). This difference supports that the fact electron sinks associated with fermentation decrease CE. Because the PE values

in the glucose-fed MFCs were much lower than with acetate in our study and the study of Min and Logan (2004), the higher PE values with acetate were most responsible for its higher ECEs. Thus, an ECE can be negligible when the CE is acceptable, if the PE is low. High PE by itself does not improve ECE significantly, since a high PE decreases CE. For instance, Liu et al. (2005) reported only a small difference in ECE (<4%) at different voltages changed by external resistances.

In our experiments, we used the same electrode, CEM, external resistance, reactor configuration, and electrolyte composition. Thus, ohmic losses outside the biofilm should have been approximately the same in both MFCs. Furthermore, the MFCs had relatively low currents, especially in the glucose-fed MFC, which should have minimized the effect of ohmic losses in general.

It is likely that the different PE values between the two MFCs resulted, at least in part, from using a *G. sulfurreducens*-rich inoculum, which was one of the objectives of our experimental design. Our evidence for poor accumulation of ARB leading to large anode over-potential is consistent with other work with glucose-fed MFCs (Rabaey et al., 2004). Since the cathode potential is relatively constant when ferricyanide concentration is sufficient, the large PE drop for the glucose-fed MFC must be related solely to an increase in anode potential. As the ARB probably did not utilize glucose directly, glucose fermenters had to grow from a very small inoculum size in the glucose-fed MFC. This is evidenced by the much longer lag time observed in the glucose-fed MFC, and the low current over time in the glucose-fed MFC indicates poor catalysis of the transfer of electrons to the anode. We interpret that the growth and accumulation of fermenters led to space competition with ARB near the anode, making ARB density too low for rapid electron transport. This interpretation is supported by the higher biomass density on the anode (330 mg biomass/m²) with glucose, compared to the acetate (230 mg biomass/m²), even though the current was much lower. It indicates that biomass unproductive to current generation accumulated in the anode-biofilm.

Table 2 – Comparison of MFC efficiencies (CE, PE, and ECE) and the maximum power density for our study with two other studies that compared acetate to glucose

Maximum power density (W/m ³)	e ⁻ donor/e ⁻ acceptor (starting concentration)	CE (%)	PE (%) ^a	ECE (%)	References
90	Acetate/ferricyanide (0.30 g/L)	75	52	39	Rabaey et al. (2005a)
66	Glucose/ferricyanide (0.44 g/L)	43	47	20	Continuous
77	Acetate/O ₂ (0.94 g/L)	65 ^b	26	17	Min and Logan (2004)
55	Glucose/O ₂ (0.94 g/L)	14 ^b	2	0.3	Continuous
7.25	Acetate/ferricyanide (0.36 g/L)	71	59	42	This study
0.19	Glucose/ferricyanide (0.32 g/L)	49	6	3	Batch

CE: coulombic efficiency, PE: potential efficiency.

^a PE = ECE/CE.

^b CE = cumulative coulombs/e⁻ donor consumed.

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Furthermore, the relatively long time over which fermentation occurred allowed methanogens to grow and accumulate in competition with ARB.

Fermenters are normally not anode-respiring, and thus a high density of fermenters in the anode-biofilm can lead to low current. However, some fermenters (i.e., *Clostridium butyricum* EG3, *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, and *Enterococcus gallinarum*) can transfer electrons from a donor substrate to an electrode or are intimately involved in the electron transport (Park et al., 2001; Pham et al., 2003; Rabaey et al., 2004). The presence of anode-respiring fermenters would open up the possibility of having a high PE for a fermentable substrate. Unfortunately, the relative abundance of anode-respiring fermenters versus normal fermenters is unrevealed in previous MFC research. The normally lower PE with fermentable substrates (Min and Logan, 2004; Rabaey et al., 2005b), however, supports the fact that normal fermenters decrease ARB density whether or not anode-respiring fermenters are present. Our results are consistent with the normal situation, not with the presence of fermenting bacteria active in electron transport. The fact that glucose-fed MFCs often select for *Geobacter*, which utilize acetate, not glucose, bolsters our results (Torres et al., 2007). In addition, no studies have shown that ARB that directly utilize glucose became dominant in MFCs inoculated with mixed culture.

Because substrate gradients often occur in biofilms (Rittmann and McCarty, 2001; Wanner et al., 2006), the local concentration of the e^- donor in the biofilm can be lower than its bulk concentration, making the anode potential more positive (Bard and Faulkner, 1980). In the glucose-fed MFC, ARB in the anode-biofilm utilized the products of glucose fermentation. Even if glucose were completely converted to acetate by fermentation, acetate can diffuse out of biofilm or be consumed by methanogens, unless acetate consumption is very rapid by the ARB. When the acetate-consumption rate is slowed by ARB being “diluted” by fermenters and methanogens, concentration loss is accentuated. Based on the conduction model of the biofilm-anode (Kato-Marcus et al., 2007), the accumulation of the biomass unavailable for current generation intensifies mass-transfer resistance and lowers the concentration of e^- donor near anode surface, which accentuates the anode’s concentration over-potential. In contrast, local concentration effects can be mitigated at high ARB density in the anode-biofilm, based on the conduction model. Reguera et al. (2006) reported in an MFC inoculated with pure culture of *G. sulfurreducens* that current did not decrease as the biofilm thickness increased up to 50 μm with a constant anode potential.

In order to improve the ECE and power density in MFCs, we must keep the anode over-potential as low as possible. Achieving a high ARB density on anode-biofilm should be one way to achieve the goal. One approach is pre-selection of ARB by inoculating a new anode from the biofilm of a successful biofilm-anode; pre-selection surely works well on the small scale, although it may not be practical for field applications. A second approach is sustained acclimation with non-fermentable substrates (e.g., acetate), which should naturally select ARB in the anode-biofilm. A third approach is to suppress acetoclastic methanogens using a low concentra-

tion of acetate, which appears to favor ARB over methanogens. Another option for lowering anode over-potential should be to improve anode characteristics: e.g., increasing its specific surface area and improving mass-transport rates between bulk and anode. Recently, Logan et al. (2007) introduced a brush-anode that likely achieves the two criteria, and they reported a high power density of 73 W/m^2 . Finally, operation in a high-turbulence regime should increase substrate mass transport to the biofilm and biomass density in the biofilm (Chang et al., 1991).

3.4. Power density

The acetate-fed MFC had much higher power output, up to 2.2 mW (360 mW/m^2), compared to the glucose-fed MFC, only up to 0.06 mW (9.8 mW/m^2). The difference in power between the two MFCs was larger than the difference in current, since power depends on the square of current ($P = I^2R$). A higher power density in an acetate-fed MFC than a glucose-fed MFC was also found by Min and Logan (2004) and Rabaey et al. (2005a), which reinforces the fact that non-fermentable compounds, especially acetate, are more efficient for power generation in MFCs.

Power density is the product of voltage and current density. Experimentally, the maximum power density is typically achieved by manipulating circuit resistance, which creates a polarization curve (Liu and Logan, 2004; Jong et al., 2006; Logan et al., 2007). We did not generate a polarization curve for our experiments, and this means that the maximum voltage and power density we report may not be the maximum achievable with our system if we had manipulated the circuit resistance.

The maximum voltage in the acetate-fed MFC was 466 mV (maximum current density: 0.76 A/m^2), but it was only 76 mV (maximum current density: 0.12 A/m^2) in the glucose-fed MFC. Thus, the considerable voltage loss in the glucose-fed MFC (PE only 6%) significantly lowered the power density in the glucose-fed MFC.

Low current density was another factor for low power density in the glucose-fed MFC. Although current depends on the voltage, the substrate-utilization and electron-transfer rates of ARB are rate-limiting for current, especially when the current is saturated by the anode potential (Kato-Marcus et al., 2007; Torres et al., 2007). We saw a highly positive anode potential with a constant low current for glucose, and this suggests that current was saturated for the anode potential in the glucose-fed MFC. In this condition, the slow substrate-utilization rate probably was caused by a low density of ARB and perhaps also by a low e^- donor concentration in the biofilm.

Because CE is not a kinetic parameter, it is not directly related to power density. However, a low CE can be related to a low ARB density in the anode-biofilm due to space competition, and this contributes to low PE and power density. This connection helps explain why many high power densities have been associated with high CE (Rabaey et al., 2004; Jong et al., 2006). Because CE has only an indirect effect on power density, establishing very robust ARB in the anode-biofilm can lead to a relatively high power density despite a modest CE.

For example, Rabaey et al. (2005a) reported high power density of 49 W/m³ with a relatively low CE of 43% for glucose.

4. Conclusion

We provide the first complete electron-equivalent balance for MFCs fed by non-fermentable (acetate) and fermentable (glucose) donors. Electrical current was the most significant electron sink in both MFCs, being 71% and 49%, respectively, of the acetate- and glucose-COD applied. The second largest electron sink was biomass (acetate 15%, glucose 26%), and the third was residual organic compounds (acetate 11%, glucose 18%). Higher SCOD concentration in the glucose-fed MFC suggests that MFCs using complex materials might require post-treatment before effluent discharge. Methane gas (3.7%) was found only in the glucose-fed MFC, which indicates that ARB out-competes acetoclastic methanogens, but not H₂-oxidizing methanogens. For this reason, the control of CH₄ gas production will improve CE significantly when complex organics are used as fuel in MFCs.

The ECE was relatively high for acetate (42%), but negligible (3%) for glucose. The major reason for the very low ECE with glucose was the very low PE, 6%, compared to 59% for the acetate-fed MFC. It is likely that a low ARB density and a concentration gradient in the anode-biofilm caused the large anode over-potential with glucose. A much lower current density at the same time that the biomass density is higher supports the fact that the ARB density was small in the biofilm of the glucose-fed MFC, and this can accentuate local concentration effects. The maximum power density in the acetate-fed MFC was 360 mW/m², while it was only 9.8 mW/m² in the glucose-fed MFC. Low PE and low current density together caused the very poor power density with glucose.

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