

Power Production in MFCs Inoculated With *Shewanella oneidensis* MR-1 or Mixed Cultures

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ABSTRACT: Power densities and oxidation–reduction potentials (ORPs) of MFCs containing a pure culture of *Shewanella oneidensis* MR-1 were compared to mixed cultures (wastewater inoculum) in cube shaped, 1-, 2-, and 3-bottle batch-fed MFC reactor configurations. The reactor architecture influenced the relative power produced by the different inocula, with the mixed culture generating 68–480% more power than MR-1 in each MFC configuration. The mixed culture produced the maximum power density of $858 \pm 9 \text{ mW m}^{-2}$ in the cubic MFC, while MR-1 produced $148 \pm 20 \text{ mW m}^{-2}$. The higher power by the mixed culture was primarily a result of lower internal resistances than those produced by the pure culture. Power was a direct function of ohmic resistance for the mixed culture, but not for strain MR-1. ORP of the anode compartment varied with reactor configuration and inoculum, and it was always negative during maximum power production but it did not vary in proportion to power output. The ORP varied primarily at the end of the cycle when substrate was depleted, with a change from a reductive environment during maximum power production (approximately -175 mV for mixed and approximately -210 mV for MR-1 in cubic MFCs), to an oxidative environment at the end of the batch cycle ($\sim 250 \text{ mV}$ for mixed and $\sim 300 \text{ mV}$ for MR-1). Mixed cultures produced more power than MR-1 MFCs even though their redox potential was less negative. These results demonstrate that differences between power densities produced by pure and mixed cultures depend on the MFC architecture.

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Introduction

Bacteria in microbial fuel cells (MFCs) convert chemical energy produced by the oxidation of organic or inorganic compounds (potentially obtained from waste streams) to electrical current (Logan et al., 2006). Two performance indicators that are commonly used to compare fuel cells are power density and coulombic efficiency (CE). The materials utilized and their configuration within the reactor (MFC architecture), as well as the bacteria that produce current, are factors that affect the MFC performance (Logan, 2009; Logan et al., 2006). Variations in these operating conditions therefore affect power output.

Wastewater is often used as a rich source of exoelectrogenic bacteria for inoculating an MFC. Reports of power densities by these mixed culture MFCs generally tend to be higher than those using pure cultures, but direct comparisons of power densities are not possible unless other conditions are kept exactly the same (Logan, 2009). There have been relatively few such direct comparisons of pure and mixed cultures under exactly identical conditions. Power densities by two isolates (*Ochrobactrum anthropi* YZ-1 and *Enterobacter cloacae* FR) were lower than those produced by the original mixed cultures (Rezaei et al., 2009; Zuo et al., 2008), and we have found that numerous other isolates obtained from MFCs fail to produce much power (unpublished results). Power production by *Geobacter sulfurreducens* PCA was compared to mixed cultures in an MFC that used a ferricyanide catholyte and a cathode eight times larger than the anode, and it produced more power (1.9 W m^{-2}) than the mixed culture (1.6 W m^{-2}) (Nevin et al., 2008). In a different type of MFC with an air cathode, however, *G. sulfurreducens* produced less power ($461 \pm 8 \text{ mW m}^{-2}$) than the mixed culture ($576 \pm 25 \text{ mW m}^{-2}$) (Ishii et al., 2008). The difference in performance in the air-cathode MFC was attributed to the lower anode potential (-350 mV) produced by the mixed culture than that achieved by *G. sulfurreducens* (-285 mV) (Ishii et al., 2008). In another study, using an air-cathode MFC with a high surface area brush anode and close electrode spacing ($< 4 \text{ cm}$), a pure culture of *Rhodospseudo-*

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monas palustris DX-1 produced more power (2.7 W m^{-2}) than a mixed culture biofilm from which it was isolated (1.7 W m^{-2}) (Xing et al., 2008).

Despite a large number of MFC studies with *Shewanella*, there have been no previous studies comparing power production by *S. oneidensis* to mixed cultures, or any parallel studies examining power production by pure and mixed cultures in different reactors under conditions where all other factors (such as solution chemistry) are kept constant. Previous research with *S. oneidensis* MR-1 has examined the anode potential produced at different current densities (Manohar et al., 2008), the abundance of planktonic and attached biofilms (Lanthier et al., 2008), the use of nanowires as a method of electron transport (Gorby et al., 2006), mediator secretion (Marsili et al., 2008; von Canstein et al., 2008), and power production and metal reduction by deletion mutants (Bretschger et al., 2007). *S. oneidensis* DSP10 has been studied in MFCs (Biffinger et al., 2007; Ringeisen et al., 2006) in relation to effects of oxygen exposure on power production (Ringeisen et al., 2007) and substrate utilization (Biffinger et al., 2008). However, the reactor architecture and the solution chemistry have varied among these studies, and there have been no direct comparisons of power production by *S. oneidensis* to mixed cultures. Such comparisons are needed to provide insight into the effects of various architectures and operating conditions on power production by pure and mixed cultures in order to more fully understand the differences between reactor design limitations and microbial limitations.

One factor that is thought to be important in the performance of different MFC architectures is oxygen leakage into the system. Low dissolved oxygen concentrations, and the potential of anaerobic aqueous environments, are usually monitored using oxidation–reduction potential (ORP) probes. ORP measurements (reported in mV) indicate whether an environment is aerobic/oxidizing (positive value) or anaerobic/reducing (negative value). Variations in ORP are known to affect bacterial growth and metabolism rates which impact the operation of biological systems (Lee and Oleszkiewicz, 2003). Systems with a variable ORP can select for certain microbial species over others, and may cause bacteria to alter their metabolic pathways (Tempest and Neijssel, 1984). ORP-based screening methods have been developed to select for bacterial strains/mutants that perform better than the wild-type strain (Du et al., 2007). ORP can have a significant impact on nitrification rates in sequencing batch reactors under aerobic and alternating anoxic/aerobic conditions (Lee and Oleszkiewicz, 2003). In studies using ORP for real time control of aeration tanks in wastewater treatment systems, ORP values were strongly related to COD removal, and were different during organic substrate oxidation than when nitrification occurred. The researchers concluded that ORP measurements of the effluent could be used for online aerator control when combined with other parameters such as temperature, pH, and dissolved oxygen concentration (Li and Bishop, 2004). So far, no studies have examined MFC performance in relation to ORP.

The objective of our study was to examine power production by *S. oneidensis* MR-1 compared to that of a diverse consortium of microbes that develops (from wastewater inocula) in four different MFC environments. We monitored ORP in the anode chamber over a full cycle to evaluate redox conditions, and overall MFC performance was assessed in terms of maximum power based on polarization and power density curves, internal resistance, and CE.

Materials and Methods

MFC Reactor Construction

Four different reactor configurations (1-, 2-, and 3-bottle, and cubic) were used for comparison of MR-1 to mixed cultures in different MFC conditions. The 1-bottle MFC (Fig. 1A) was constructed as previously described (Logan et al., 2007). The total anode chamber volume was 325 mL. The brush anode was constructed from carbon fibers (PANEX[®]33 160K, ZOLTEK, Bridgeton, MO) wound into a titanium wire core (overall dimensions: 5 cm diameter, 7 cm length, 1.06 m^2 surface area) (Logan et al., 2007) and treated with ammonia gas (Cheng and Logan, 2007). The Pt catalyst air cathode (projected surface area of 4.91 cm^2) was constructed from 30% wet proofed carbon cloth (Type B-1B, E-TEK, Somerset, NJ) with PTFE diffusion layers, and 0.5 mg-Pt/cm^2 (Cheng et al., 2006a). The cathode side port was protected from contamination using a foam plug (VWR). The anode/cathode spacing was 7 cm (center of the anode to the surface of the cathode).

The 2-bottle MFC (Fig. 1B) was a standard “H” design that was constructed from two crimp-top anaerobic bottles with side ports (1.8 cm^2 cross section; 225 mL total anode volume) separated by a cation exchange membrane (CEM) (Nafion 117, DuPont, Wilmington, DE) (Rezaei et al., 2008). The anode was plain Toray carbon paper (E-TEK) ($1.5 \text{ cm} \times 7.5 \text{ cm}$; projected surface area 22.5 cm^2) that was ammonia treated (Cheng and Logan, 2007) and connected to the circuit using titanium wire. The carbon paper cathode (E-TEK, $1.5 \text{ cm} \times 7.5 \text{ cm}$, projected surface area of 12.25 cm^2) contained a Pt catalyst ($0.5 \text{ mg Pt cm}^{-2}$) and was connected with titanium wire. The cathode chamber was sparged with air using an aquarium air stone. The anode/cathode spacing was 12.5 cm.

In order to reduce oxygen leakage between the anode and cathode chamber, we developed a new type of 3-bottle MFC (Fig. 1C) that consisted of three crimp-top anaerobic bottles connected in series with a CEM separating each bottle. The same electrodes and cathode aeration methods were used for the 2- and 3-bottle MFCs. However, the middle chamber of the MFC was bubbled with nitrogen gas (99.9%) to remove dissolved oxygen that leaked from the cathode chamber into the middle chamber, eliminating the possibility of oxygen transfer into the anode chamber. The electrode spacing was 26.5 cm.

The fourth reactor was a cube-shaped (cubic) MFC (Fig. 1D) constructed as described previously (Liu and Logan, 2004). The anode chamber was a 28 mL cylindrical

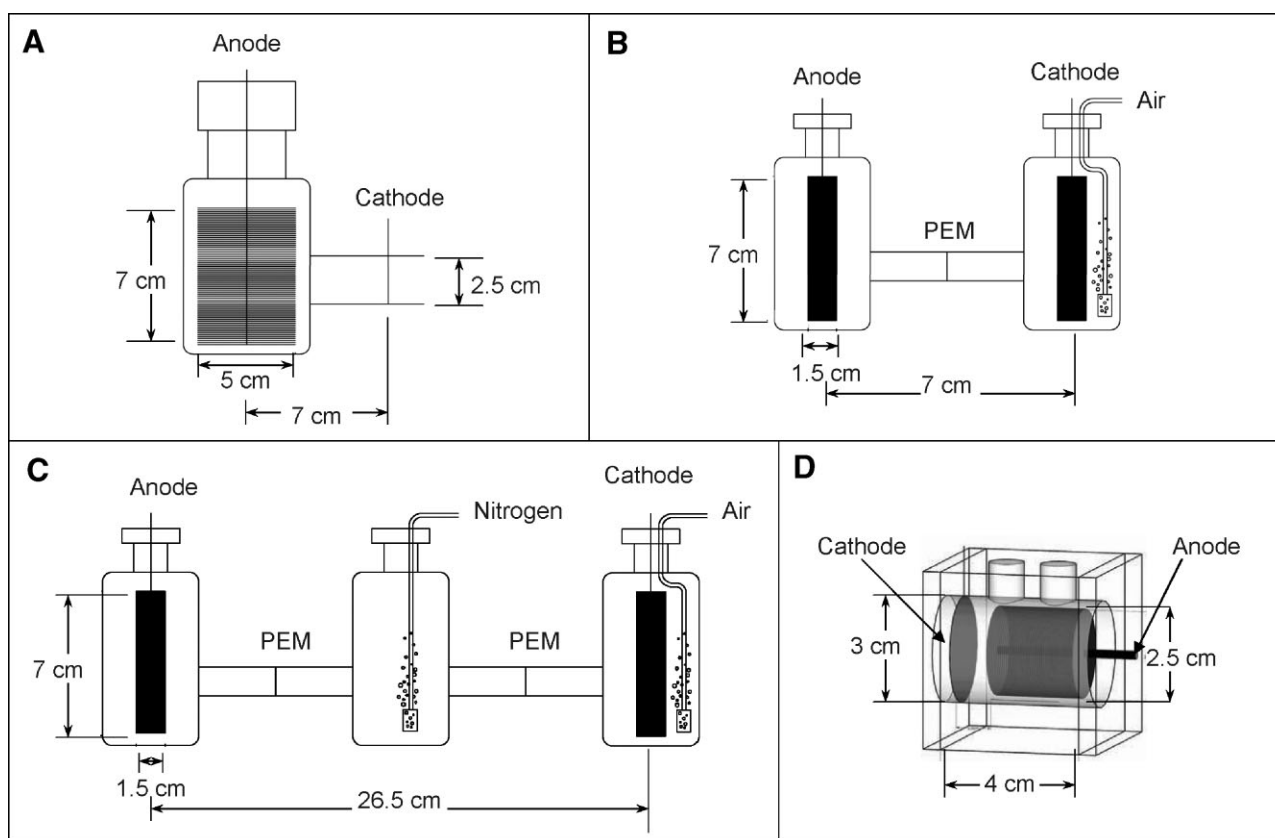


Figure 1. MFC reactor configurations (A) 1-bottle, (B) 2-bottle, (C) 3-bottle, and (D) cubic.

chamber (7.07 cm^2 cross section) bored into solid Lexan, with an ammonia treated carbon fiber (PANEX[®]33 160K, ZOLTEK) brush anode (2.5 cm diameter, 2.5 cm length, 0.22 m^2 surface area) placed horizontally in the center of the cylinder. The cathode was constructed as describe above for the 1-bottle reactor, but it had a projected surface area of 7.07 cm^2 . The electrode spacing was 2.5 cm (center of the anode to the cathode).

Inocula

Primary clarifier effluent from the Pennsylvania State University Wastewater Treatment Plant was used for the mixed culture inoculum. *S. oneidensis* MR-1 was obtained from Orianna Bretschger at the University of Southern California. MR-1 was grown in standard Luria-Bertani Medium for 3 days at 30°C before inoculation of the MFCs. The medium used for mixed culture and MR-1 tests was the same as the growth medium used by Bretschger et al. (2007) with 18 mM lactate from lactic acid. After addition of the lactic acid, the medium was adjusted to pH 7, and then autoclaved. Vitamins (also adjusted to pH 7) were added by sterile filtration ($0.2 \mu\text{m}$ pore diameter sterile syringe filter; VWR) after autoclaving. The cathode chambers (2- and 3-

bottle reactors) and the center bottle (3-bottle MFC) were filled with growth medium lacking vitamins, minerals, amino acids, and substrate. All reactors were autoclaved before inoculation, with all tests conducted in duplicate.

Startup and Operation

MFCs were inoculated with a 10% (v/v) (bottle reactors) or 50% inoculum (cubic reactors) of wastewater or *S. oneidensis* MR-1 and medium. Mixed culture reactors were covered with aluminum foil to exclude light. MFCs were connected using a $1,000 \Omega$ resistor, except as noted. Once an MFC produced $\geq 100 \text{ mV}$, only fresh medium was added over subsequent fed batch cycles. Medium replacement was performed in a laminar flow hood (Class II Biosafety Cabinet) for cubic and 1-bottle *Shewanella* inoculated MFCs, and in an anaerobic glove box (Coy Scientific, Grass Lake, MI) for 2- and 3-bottle MFCs using sterile techniques. All MFCs were operated at ambient temperatures ($23 \pm 3^\circ\text{C}$). The MFCs were considered enriched and ready for testing once they achieved the same maximum voltage for three consecutive batch cycles (i.e., $\sim 12\text{--}14$ days for cubic, $\sim 60\text{--}70$ days for 1-bottle, $\sim 60\text{--}100$ days for 2-bottle, and $\sim 80\text{--}100$ days for 3-bottle MFCs).

Analysis

The voltage across the resistor was measured every 15–30 min using a multimeter (model 2700 Keithley Instruments, Cleveland, OH) connected to a computer. Polarization and power density curves were obtained by varying the external resistance applied to the circuit and using the voltage obtained after stabilization (2- to 3-h intervals). Medium in the cubic reactors was replaced before each new resistance was applied to avoid substrate limitation. Current density was calculated using $I = E/R$, where I is the current, E the measured voltage, and R the applied resistance, and normalized to the projected cathode surface area. Power densities were calculated using $P = IE$, and normalized by the projected cathode surface area (Logan et al., 2006).

Impedance was measured by electrochemical impedance spectroscopy (EIS) using a potentiostat (PC 4/750, Gamry Instrument, Inc., Warminster, PA). The test was run at open circuit voltage (OCV) over a frequency of 100,000–0.1 Hz with sinusoidal perturbation of 10 mV amplitude, and the internal ohmic resistance was obtained from the response at high frequency excitation (Cheng et al., 2006b; He et al., 2006). Total internal resistance was estimated as the slope of the linear portion of the polarization curve as $R_{\text{int}} = -\Delta E/\Delta I$ (Logan et al., 2006).

CE was calculated using the ratio of the total coulombs produced during the experiment (at 1,000 Ω) to the theoretical amount of coulombs available from the oxidation of lactate to acetate (MR-1) (Tang et al., 2007), or lactate to carbon dioxide (mixed cultures), using $CE [\%] = (C_{\text{Ex}}/C_{\text{Th}}) \times 100$, where $C_{\text{Ex}} = \sum_{i=1}^T (E_i t_i)/R$, $C_{\text{Th}} = FbMv$, F is the Faraday's constant (96,485 C/mol e^-), b the number of moles of electrons available per mole of substrate (4 mol e^- /mol lactate for MR-1 and 12 mol e^- /mol lactate for mixed cultures), M the lactate concentration (mol L⁻¹), and v the volume of liquid in the anode chamber (L) (Logan et al., 2006).

The ORP in the anode chamber was measured using a combination redox electrode (InLab[®] Redox Micro, 3 mol L⁻¹ KCl, AgCl saturated, Mettler-Toledo, Columbus, OH) calibrated using a standard buffer solution (220 mV, Mettler-Toledo). The redox electrode was inserted into the anode chamber and left to stabilize for 30 min before a measurement was recorded. Measurements were recorded as mV versus Ag/AgCl and adjusted to the standard hydrogen electrode (SHE; +207 mV) (Nordstrom and Wilde, 1998).

Results

Anode Enrichment

In all four MFC reactor configurations, MR-1 produced power <1 day after inoculation (Fig. 2). In general, the cubic and 1-bottle MR-1 MFCs produced a maximum cell potential sooner (14 days and 6 days respectively) than the 2- and 3-bottle MFCs (~50 days). MR-1 produced power before the mixed cultures, but the mixed cultures

eventually produced higher cell potentials than MR-1 in all four configurations. Long enrichment times in the MFCs along with complete media replacement should have allowed the microbial community to develop viable, active, anodic biofilm, minimizing the effect of differences in cell amounts present in inocula on power production.

There were substantial differences in the time required to reach the maximum voltage in the different reactors with strain MR-1 when the solution was replaced (data not shown). The voltage in the cubic and the 1-bottle MFCs with MR-1 returned to the maximum value within minutes (<30 min), while the voltage in the 2-bottle reactor usually recovered in about 12 h when the solution was replaced with fresh lactate medium. The cell potential of the 3-bottle reactor reached 60% of its maximum value within a few hours, but it required 5–10 days to return to the previous maximum potential. All reactors were run for at least 80 days, and in some cases for up to 180 days without substantial changes in voltage generation.

Voltage and Power Generation in MFCs

Polarization and power density curves were obtained for both the mixed culture and MR-1 inoculated MFCs in each of the four reactor configurations (Figs. 3 and 4). In most cases, the OCVs in the MR-1 MFCs were higher than in MFCs inoculated with mixed cultures. The only exception was the 3-bottle MFCs where the OCVs were the same (0.780). The OCVs for MR-1 ranged from 0.751 V (1-bottle reactor) to 0.800 V (cubic reactor), while the mixed culture OCVs ranged from 0.658 V (cubic reactor) to 0.780 V (3-bottle reactor) (Fig. 3).

The mixed culture produced more power than the MR-1 culture in all four reactors. The mixed culture in the cubic reactor produced the largest maximum power density of $858 \pm 9 \text{ mW m}^{-2}$ (cathode basis) (Fig. 4). Maximum power densities for the mixed culture decreased in the order: cubic MFC > 1-bottle > 2-bottle > 3-bottle (Table I). Tests with MR-1 generally produced the same trend in power with reactor type observed for the mixed cultures, except the maximum power was larger in the 1-bottle reactor ($332 \pm 21 \text{ mW m}^{-2}$) compared to the cubic reactor ($148 \pm 20 \text{ mW m}^{-2}$) (Fig. 4). This finding of lower power in the cubic reactor with MR-1 was unexpected because previous experiments using mixed cultures have shown that the cubic MFCs used here produce higher power densities than the 1-bottle MFCs (Logan et al., 2007), and the ability of MR-1 to scavenge the oxygen at the cathode before it reached the anode would be similar to that of a mixed culture. This result of larger power generation in bottle compared to cubic reactors using MR-1 was verified in additional experiments (also run in duplicate).

Ohmic and Internal Resistances

The ohmic resistances measured using EIS in each of the four different reactor configurations were the same whether

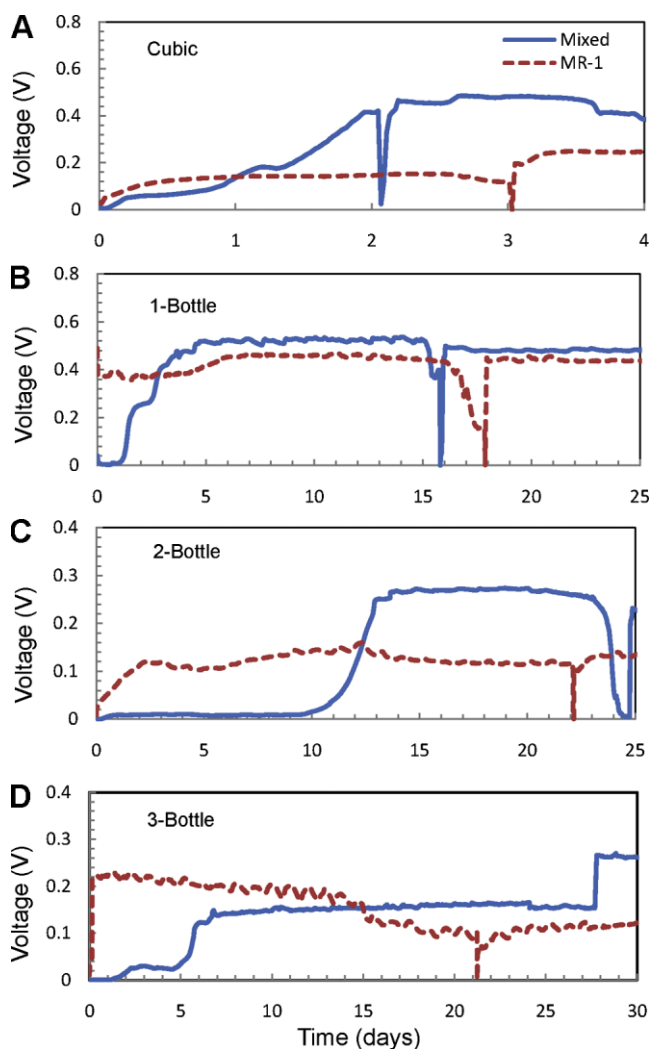


Figure 2. Cell voltage measurements following reactor inoculation in (A) cubic, (B) 1-bottle, (C) 2-bottle, and (D) 3-bottle MFCs. (Note: Sharp drops in voltage represent media exchanges.) [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

the reactors were inoculated with MR-1 or a mixed culture. A lack of a change in the ohmic resistance for the two different inocula is expected as the ohmic resistance should only reflect the electrode spacing and solution chemistry. The ohmic resistances increased in the order expected based on decreased maximum power densities measured for the mixed cultures: cubic, $12 \pm 1 \Omega$; 1-bottle, $52 \pm 1 \Omega$; 2-bottle, $401 \pm 16 \Omega$, and 3-bottle, $745 \pm 20 \Omega$ (Table I). For MR-1, power density versus ohmic resistance did not follow this same trend.

The internal resistance varied with the inoculum, with larger internal resistances obtained for MFCs with MR-1 than those with mixed cultures (Table I). On the basis of the type of inoculum, the internal resistance in MFCs with MR-

1 was 1.7–5.8 times more than that measured in the mixed culture MFCs. The larger internal resistance with MR-1 compared to the mixed cultures resulted from higher anode overpotentials at the same working current. The internal resistance relative to the ohmic resistance varied for the different MFC configurations, with internal resistances that were 1.5 (3-bottle, mixed) to 60 times (cubic, MR-1) more than the ohmic resistance.

Coulombic Efficiencies

The CEs of the MFCs ranged between 15% and 35% (Fig. 5). Since MR-1 oxidizes lactate to acetate ($4 \text{ mol e}^-/\text{mol lactate}$) in anaerobic conditions, and has not been shown to produce power from acetate oxidation, and mixed cultures can degrade lactate and acetate ($12 \text{ mol e}^-/\text{mol lactate}$) for power production, the CE for MR-1 was typically less than the mixed culture CE when compared on a $12 \text{ mol e}^-/\text{mol lactate}$ basis. In the 3-bottle and cubic reactors, the CEs calculated for the MFCs using mixed cultures on the basis of complete lactate oxidation, were similar to those with MR-1 calculated on the basis of lactate oxidation to acetate. Larger differences in CEs were observed for 1- and 2-bottle MFCs with MR-1 compared to mixed cultures, with the MR-1 culture having a higher CE in the 1-bottle reactor and the mixed culture having a higher CE in the 2-bottle reactor.

Oxidation–Reduction Potentials in MFCs

The ORP of the anode solution in each MFC configuration was measured several times during individual cycles in all reactors, and over multiple batch cycles. In general, the ORP was negative in all reactors, for both types of inocula, when the reactors were producing their highest voltages ($>100 \text{ mV}$) and power densities (Fig. 6). The ORP measured with MR-1 averaged $-217 \pm 17 \text{ mV}$ when the cell potential was $>100 \text{ mV}$, and thus was more negative than that of the mixed culture which averaged $-161 \pm 44 \text{ mV}$ over the same voltage range. In the 1-bottle and cubic reactors, however, several positive redox potentials were measured even though the MFC was still producing substantial voltages. For example, positive ORP were obtained with cell voltages of $81\text{--}100 \text{ mV}$ in the 1-bottle MFC, and at $30\text{--}47 \text{ mV}$ in the cubic MFCs. In contrast, the redox potential in the 2- and 3-bottle MFCs maintained negative ORPs (except for one measurement) when the reactors were producing a measurable current ($>0.02 \text{ mA}$). It is difficult to compare the redox potentials measured in the 1-bottle MFCs to those measured in the other configurations because the reference probe in the 1-bottle MFC could not be placed between the anode and cathode and was instead located in the solution above the anode. Therefore, the redox potentials in the 1-bottle MFC may be measuring a different anode environment relative to the cathode than in the other reactors.

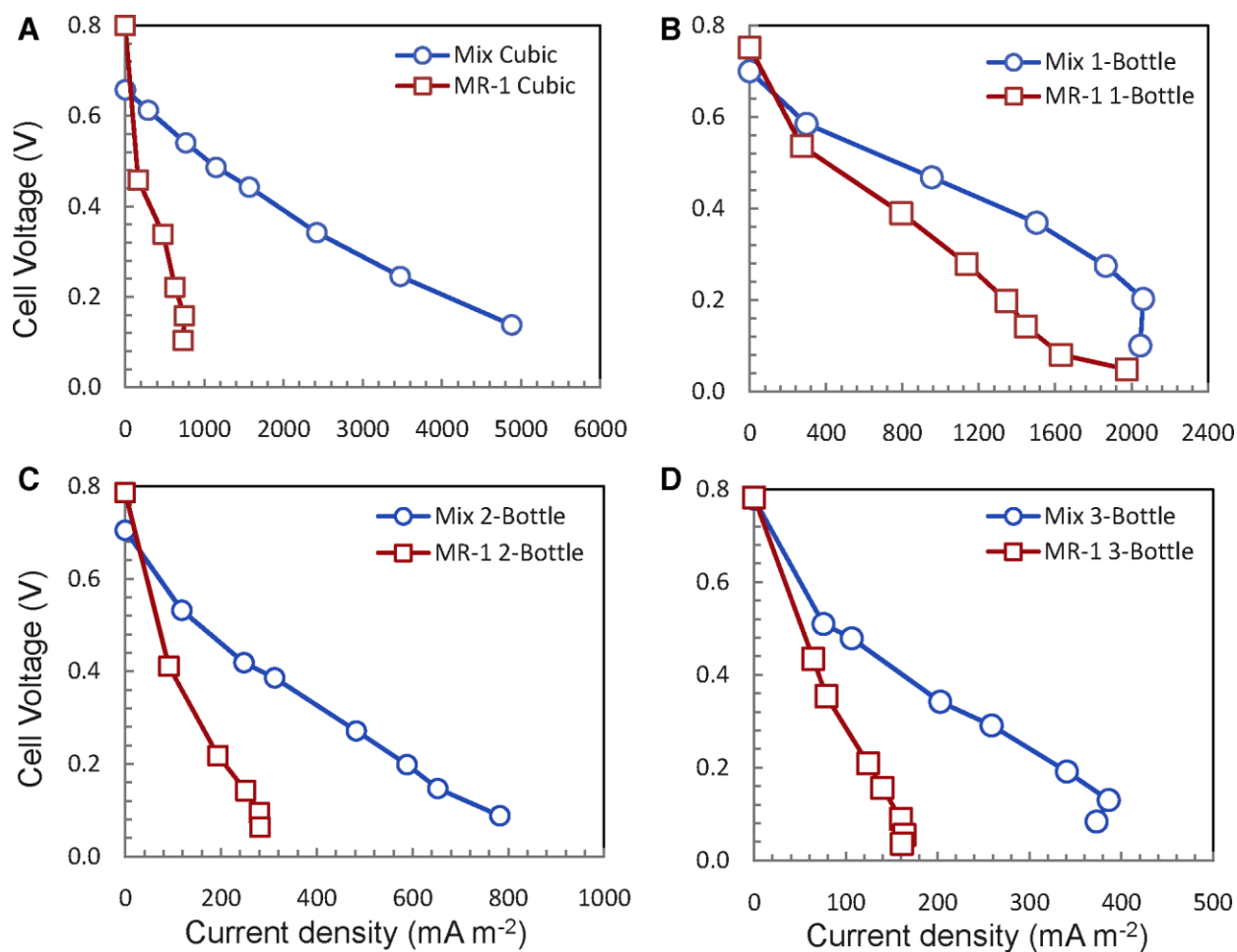


Figure 3. Polarization curves for *Shewanella oneidensis* MR-1 (□) versus a mixed culture (○) in (A) cubic, (B) 1-bottle, (C) 2-bottle, and (D) 3-bottle MFCs. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

Discussion

MFCs inoculated with the mixed culture (wastewater inoculum) consistently produced more power than MFCs inoculated with MR-1, with a difference that varied by 68–480% depending on the reactor configuration. Power production increased inversely with internal resistance (the resistance to electron flow), with the cubic reactor having the largest and the 1-bottle reactor having the least difference in power production (Fig. 7A). The changes in the power produced by the different inocula and reactor configurations were due to changes in the internal resistance. The differences in internal resistance and power production in each configuration could only have resulted from the different inocula and their interactions with the anode as the ohmic resistances measured in each reactor configuration were the same for both inocula. The MFCs with MR-1 had a higher OCV than the MFCs with mixed cultures, but there was a much larger drop in the working potentials for MR-1 than the mixed cultures over the

measured range in current. In MFCs, the OCV reflects the ability of the biofilm to accumulate charge. In one study where the ability of bacteria to accumulate charge was examined, it was found that *G. sulfurreducens* had enough electron-storage capacity to eliminate the need to respire over a period of 8 min when there was no electron acceptor available (Esteve-Núñez et al., 2008). The high OCV obtained for MR-1 suggests that *Shewanella* sp. can also accumulate charge, but the release of electrons is less efficient than that of a mixed culture, as the decrease in working voltage in the low current region indicates larger activation losses, and therefore greater energy losses, for MFCs with MR-1 than with mixed cultures.

Close electrode spacing had an adverse effect on power generation by strain MR-1 for reasons not fully understood. While power production with the mixed culture varied inversely with ohmic resistance as expected, there was no significant correlation of power with ohmic resistance for MR-1 (Fig. 7B). When the electrode spacing was decreased from 7 cm in the 1-bottle reactor to 2 cm using a cubic

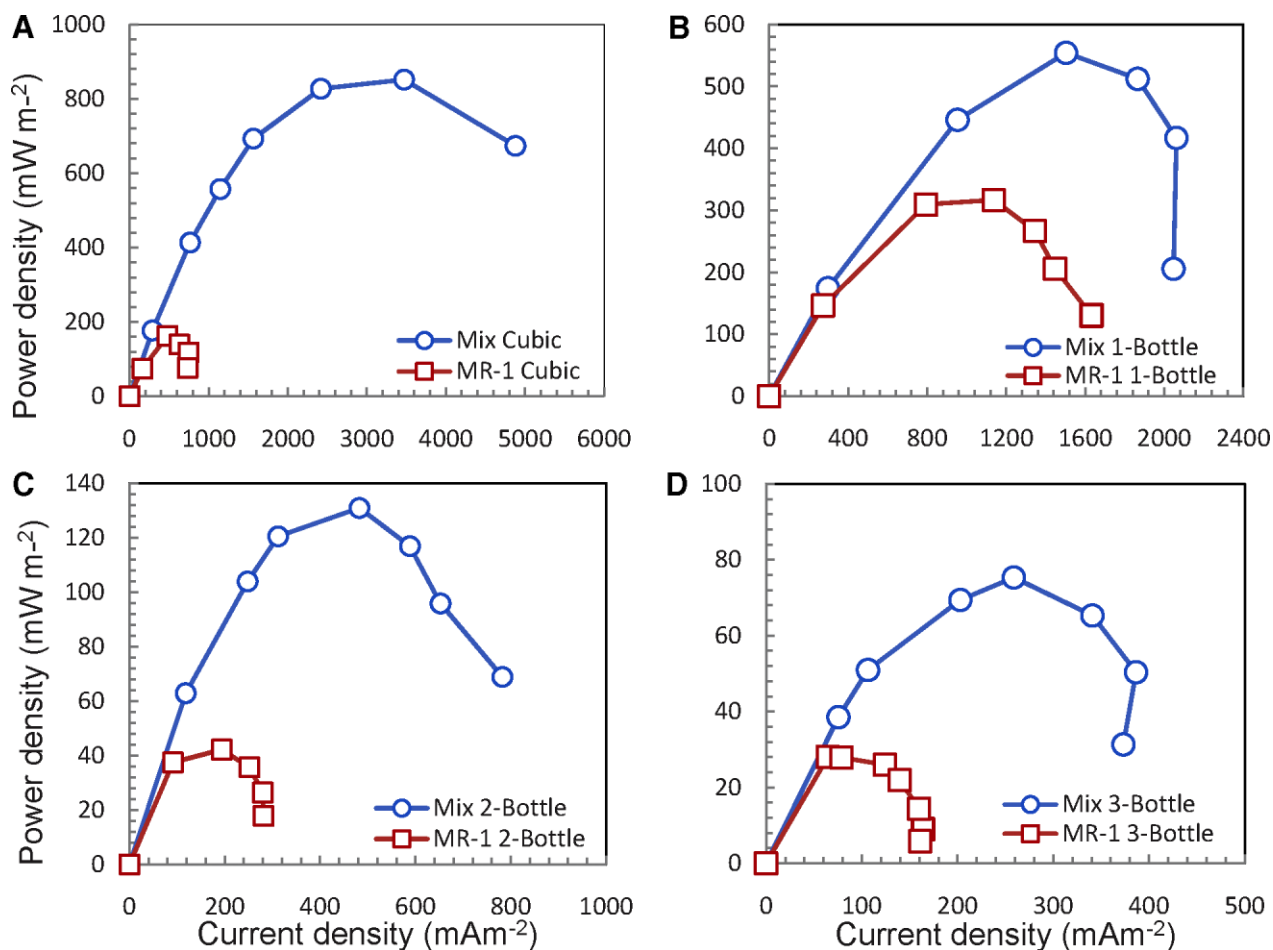


Figure 4. Power density curves for *Shewanella oneidensis* MR-1 (□) versus a mixed culture (○) in (A) cubic, (B) 1-bottle, (C) 2-bottle, and (D) 3-bottle MFCs. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

reactor, the ohmic resistance decreased from 52 to 12 Ω and the power density with the mixed culture increased from 559 to 858 mW m^{-2} . However, the power density with MR-1 decreased from 332 to 158 mW m^{-2} when the electrode spacing was decreased. One reason why power density could be adversely affected by close electrode spacing is leakage of

oxygen into the MFC. It has previously been observed using mixed cultures that as the electrode spacing is decreased, power density first increases but then decreases as the electrodes get too close (Cheng et al., 2006b). It has been suggested in these studies that this results from oxygen leakage into the anode solution, creating a more positive

Table I. Ohmic resistance (from EIS at OCV), internal resistance (from polarization), and power production for each MFC configuration.

Reactor	Inoculum	Ohmic resistance ^a (Ω)	Internal resistance (Ω)	% Increase R_{int} MR-1 compared to mixed	OCV (V)	Max power density ^b (mW m^{-2})
Cubic	Mixed	12 \pm 1	162		0.658	858 \pm 9
	MR-1		733	350	0.800	148 \pm 20
1-Bottle	Mixed	52 \pm 1	428		0.700	559 \pm 6
	MR-1		688	60	0.751	332 \pm 21
2-Bottle	Mixed	401 \pm 16	621		0.705	118 \pm 18
	MR-1		1,533	150	0.786	41 \pm 2
3-Bottle	Mixed	745 \pm 20	1,082		0.780	80 \pm 7
	MR-1		3,194	200	0.783	27 \pm 1

^aThe value listed represents both mixed culture and MR-1 MFCs. It is the average of two mixed culture and two MR-1 MFCs ($n = 4$).

^bThe values reported are the average maximum power obtained by each set of duplicates ($n = 2$).

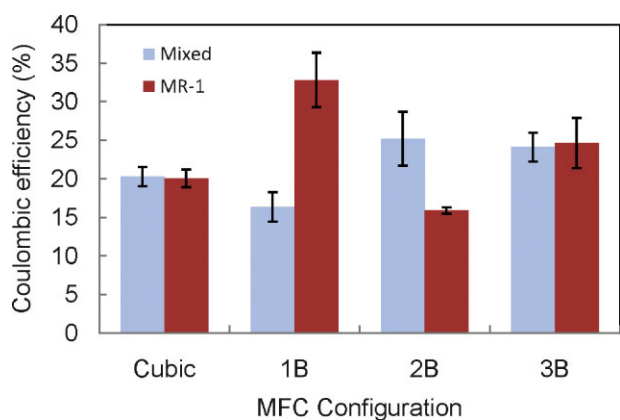


Figure 5. Coulombic efficiencies for *Shewanella oneidensis* MR-1 (lactate to acetate) (dark) and mixed cultures (lactate to CO₂) (light) in cubic, 1-bottle, 2-bottle, and 3-bottle MFCs. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

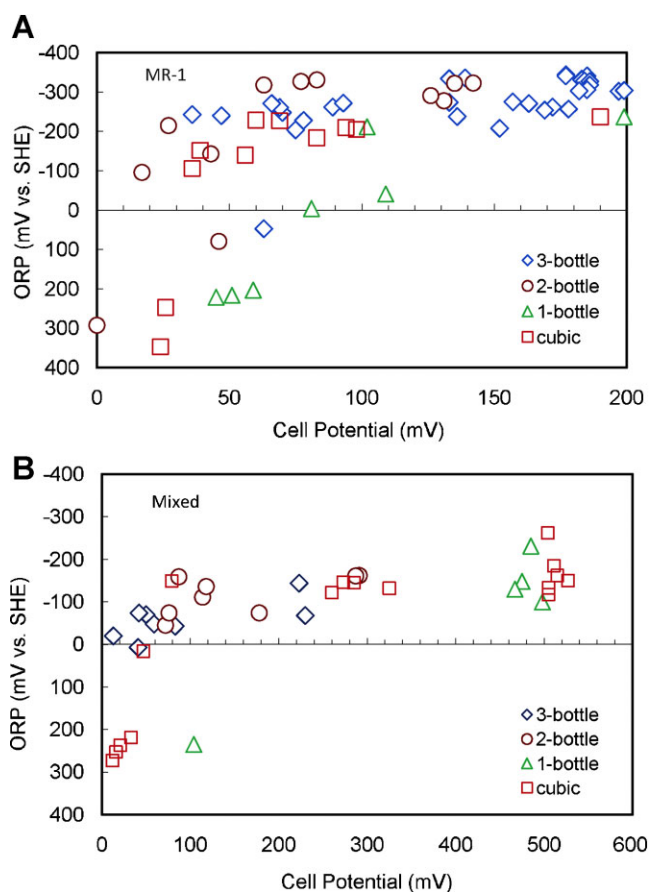


Figure 6. Oxidation–reduction potential versus measured cell potential in cubic, 1-bottle, 2-bottle, and 3-bottle MFCs inoculated with (A) *Shewanella oneidensis* MR-1 and (B) a mixed culture measured over multiple cycles. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

redox environment. In previous studies by Ringeisen et al. (2007), it was further shown that power generation by *S. oneidensis* DSP10 decreased by 33% when reactors were operated in an aerobic environment compared to an anaerobic environment. In order to examine the possible effect of oxygen accumulation in the reactor we measured the redox potential in the MFCs over the course of the batch cycle. When dissolved oxygen is present, the redox potential is positive. In the cubic MFCs, the ORP measurements showed positive redox potentials only at the end of the cycle when substrate was depleted. Under these conditions at the end of a cycle, bacteria in the mixed culture, or facultative MR-1, would have been unable to appreciably consume oxygen leaking into the system due to lack of an electron donor. However, the redox environment during significant power generation by MR-1 (>25 mV) was consistently

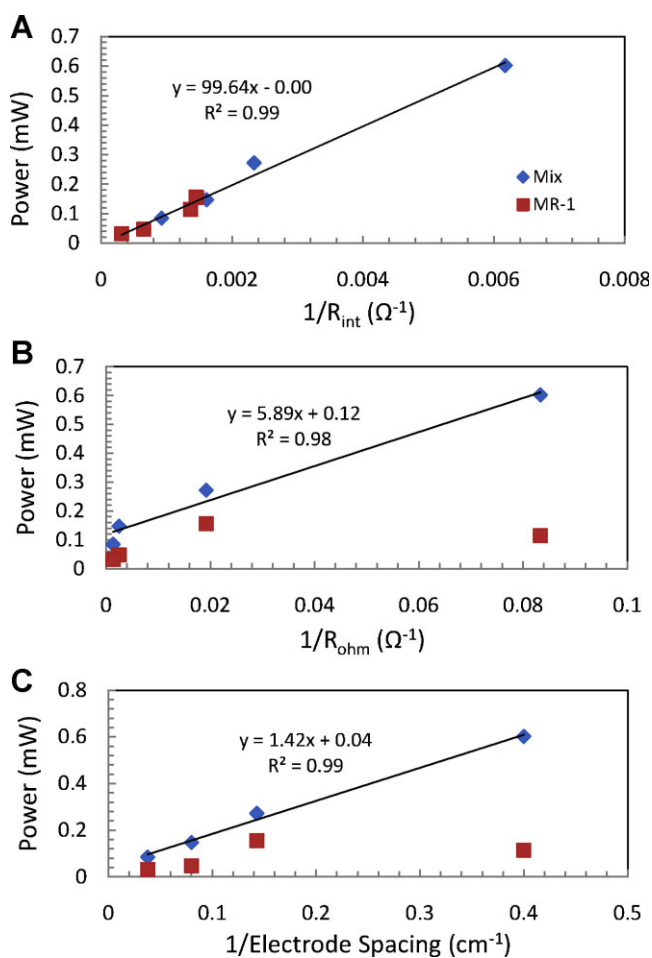


Figure 7. (A) Power generation is inversely related with the measured internal resistance, (B) power related to the inverse of ohmic resistance, and (C) power as a function of the inverse of electrode spacing for MR-1 (□) and mixed (○) culture MFCs. (Note: For (A) linear regression is based on both mixed culture and MR-1 data, for (B) and (C) linear regression is for mixed culture data only.) [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

negative indicating that dissolved oxygen did not accumulate in solution. The redox potential measured with MR-1 was even more negative than that measured with the mixed culture, indicating the absolute value of the redox potential was not a good predictor of power density. Because we did not observe a positive redox potential in tests with MR-1 in the cubic reactor during significant power generation, it is unlikely that the lower power production in the cubic reactor can be explained solely on the basis of oxygen leakage into the reactor.

The reasons for the lower power production by MR-1 compared to mixed cultures and the lack of high power generation by MR-1 in the cubic MFC are not clear. These differences in power production may be due to the electron transfer mechanisms used by MR-1 compared to mixed cultures, and the effects of the response of the microorganisms to variable redox environments. The mechanisms used by MR-1 for electron transfer to an anode vary, and the relative importance of these different mechanisms to electron transfer in MFCs remains controversial. Mechanisms proposed for electron transfer by *Shewanella* sp. include direct transfer by cell–surface contact, self-produced mediators/electron shuttles, and bacterial nanowires (Biffinger et al., 2007; Bretschger et al., 2007; Gorby et al., 2006; Lanthier et al., 2008; Marsili et al., 2008; von Canstein et al., 2008). It is not established whether these different pathways are used separately or in concert in an MFC, or if mechanisms change with reactor type and over time. The cyclic fluctuation of ORP in the cubic MR-1 MFC could certainly lead to a succession in changes in respiratory mechanisms used by this bacterium. The particularly rapid changes in redox environment in the cubic reactor may have precluded optimization of electron transfer by any single pathway. For example, the ORP of the solution was positive at the beginning of the cycle as fresh medium (without bacteria) was placed into the enriched MFC in a laminar flow hood, it became negative when power was produced, and then returned to a positive value at the end of the cycle (when the electron donor was depleted). In a cubic MFC, the complete cycle lasts only 2 days. In contrast, a single cycle for the 1-bottle reactor lasts 20 days. The short cycle time may therefore preclude optimization of electron transfer in the same manner possible in the longer batch cycle times in the other reactors. In mixed culture tests, this cycling of ORP may not be as detrimental to the operation of the biofilm due to bacteria able to rapidly remove oxygen at the top of the biofilm. Additional studies on how *Shewanella* use different mechanisms for respiration with the anode in an MFC, and the effect of different redox environments on respiration in MFCs (including anodic biofilm formation), will need further investigation. Future research on characterizing the mixed microbial communities in different MFC environments and analyzing the roles of the different microbial groups in substrate oxidation and power production is also needed.

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