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Influence of NO_3 and SO_4 on power generation from microbial fuel cells

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ABSTRACT

Potential competition in terms of electron transfer from bacteria to electron acceptors such as nitrate (NO_3) and sulfate (SO_4) or the anode of a microbial fuel cell (MFC) was investigated to determine how alternative electron acceptors would influence power generation in an MFC. The cell voltage was not initially affected when these electron acceptors were introduced into the MFCs. However, the presence of NO_3 decreased the CE of the MFC compared to the injections of SO_4 or control salt (sodium chloride). This suggests that the growth of nitrate-reducing bacteria independent of the microbial populations on the MFC anode were not utilizing the anode as an electron acceptor, rather, they were consuming organic carbon in the anodic chamber of the MFC, resulting in a decrease of the CE of this MFC with no immediate impact on power output. This suggests that the bacterial consortium in the nitrate-MFC still preferred the anode over nitrate as the electron acceptor, although the theoretical reduction voltage of nitrate (+0.74 V) is higher than the reduction voltage in an MFC air cathode (as high as +0.425). These results are useful when considering whether MFC technology can be applied in situ to enhance biodegradation of organic contaminants in the presence of alternative electron acceptors.

1. Introduction

A microbial fuel cell (MFC) is a device that generates electrical power by harnessing the microbial oxidation of various substrates in which bacteria pass electrons through a circuit containing a solid-state anode, an external load, and a cathode where the electrons are consumed in the reduction of oxygen and protons to water [1]. It is clear that the solution in an MFC must be anaerobic to promote electron transfer to the anode because in the presence of oxygen any facultative bacteria would use oxygen as a terminal electron acceptor (TEA) rather than the anode. What is not clear is whether the bacteria will utilize the anode in an MFC in the presence of alternate electron acceptor (AEA) that usually dominate under anoxic conditions, such as nitrate (NO_3) or sulfate (SO_4). If bacteria in an MFC use electron acceptors other than the anode, the power output of the MFC will decrease and/or stop. This is useful information for researchers investigating MFC applications for wastewater treatment or environmental remediation because the presence of AEAs may hinder power production and waste/contaminant degradation. Furthermore, the effects of NO_3 and SO_4 on MFC power generation may indicate where the anode falls in terms of thermodynamic favorability to bacteria.

Passive oxidation can be achieved using MFC technology, which could be both economically and environmentally beneficial because it would eliminate the need for air-sparging or injecting AEAs [2]. Furthermore, using MFC technology to enhance bioremediation of organic contaminants in sediment could replace expensive dredging practices that may release toxic compounds into the water column. The main expense of using MFC technology to enhance natural degradation of contaminants in groundwater or sediments is the cost of the anode and cathode materials and installation of the MFC system. While anodes are commonly constructed with relatively inexpensive carbon cloth, fiber [1], or even stainless steel [2,3]; cathodes often are coated with expensive catalysts such as platinum or other platinum group metals. However, in marine environments it has been shown that no cathode catalyst is required in a sediment MFC scenario (e.g., [3–5]) as it appears that a biofilm forms on the cathodes in this environment which acts as a biocatalyst for electron transfer from the cathode to oxygen [3,6].

The purpose of this experiment was to determine if AEAs interfere with power production in an MFC and shed light on where the anode falls in terms of realized affinity by bacteria as opposed to theoretical redox voltage. In other words, do our data suggest that an MFC could enhance degradation in an anaerobic environment similarly to electron acceptor (e.g., O_2 and NO_3) amendments? It is important to understand if the presence of AEAs in contaminated environments interferes with the ability of MFC technology to enhance contaminant biodegradation before MFC systems are installed for in situ remediation.

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2. Methods

2.1. MFC design & start-up

Three single-cell MFCs were constructed similarly to those described in Morris and Jin [7] except that we used clear PVC pipe (105 ml total volume) and 75 cm^2 of carbon cloth (E-TEK Division, Somerset, NJ, USA) for the anode, which extended out of the top of the cell for connection to the external circuit containing a 1 KΩ resistor. The MFCs were inoculated with sludge from a municipal wastewater treatment plant and filled with anaerobic growth media [7] containing 500 mg glucose/L. Each cell was refilled with fresh growth media containing the same concentration of glucose every 7–10 days for approximately 30 days until each cell was producing between 395 and 440 mV.

2.2. Electron acceptor competition

One of three MFCs was used to test power output in the presence of either NO_3 (treatment A), SO_4 (treatment B), or chloride (Cl; treatment C; osmotic control) as competitive AEAs. To begin the experiment at hour 0 (day 0), each MFC was filled with fresh media containing 500 mg glucose/L; at 24 h (day 1) AEA salts were injected into each MFC; at 95.3 h (day 4) each MFC was emptied and refilled with fresh media containing 500 mg glucose/L and no AEA salts; finally, at 196 h (day 8) each MFC was emptied and refilled with fresh media containing 500 mg glucose/L and AEA salts. The experiment ran for a total of 310.5 h (13 days) and water samples were drawn from each MFC periodically at 12- to 24 h intervals and analyzed for chemical oxygen demand (COD), dissolved organic carbon (DOC), and anions. On days when the MFCs received AEA salts, A received a nominal NO_3 concentration of 750 mg/L as $NaNO_3$ and 325 mg NaCl/L, B received a nominal SO_4 concentration of 725 mg/L as Na_2SO_4, and C received a nominal NaCl concentration of 790 mg/L. The NO_3 and SO_4 concentrations were based on the stoichiometric concentration needed for complete metabolism of 500 mg glucose/L using a 5:4 or 2:1 molar ratio of carbon to NO_3 or SO_4, respectively. The additional NaCl in A and C were added so that all three MFCs had similar electrical conductivity (EC) values during the experiments. At the first injection of AEA on day 1, the compounds were dissolved in 30 ml aliquots of water drawn out of each MFC and then re-injected. Following re-injection and before every sampling event, the water in each MFC was mixed by repeatedly drawing and re-injecting 60 ml of water in and out of the MFC with a plastic syringe. The AEAs were premixed with the growth media and injected as one solution on day 8. The beginning ECs for treatments A, B, and C were 16.6, 16.1, and 16.3 mS/cm, respectively.

2.3. Analyses and calculations

A subsample of each water sample was filtered (0.2 μm) and analyzed for anions on a DIONEX DX-100 Ion Chromatograph equipped with a 4 × 250 mm IonPac AS14 anion exchange column. A second subsample was filtered (0.45 μm) and analyzed for COD (HACH EPA-approved method 8000, using a DR/890 colorimeter; HACH Company, Loveland, CO). The final subsample was filtered (0.45 μm), acidified to pH 2–3 using 2N HCl, and analyzed for DOC on a Shimadzu TOC-V CSN total organic carbon analyzer. Voltage (mV) across the resistor on each cell was measured and recorded every 10 min with a data logger (ADA6; Pico Technologies Limited, UK) connected to a computer. Coulombic efficiency (CE) was calculated as $E_C = C_P/C_{Ti}100\%$ based on COD reduction in each cell according to Liu and Logan [8].

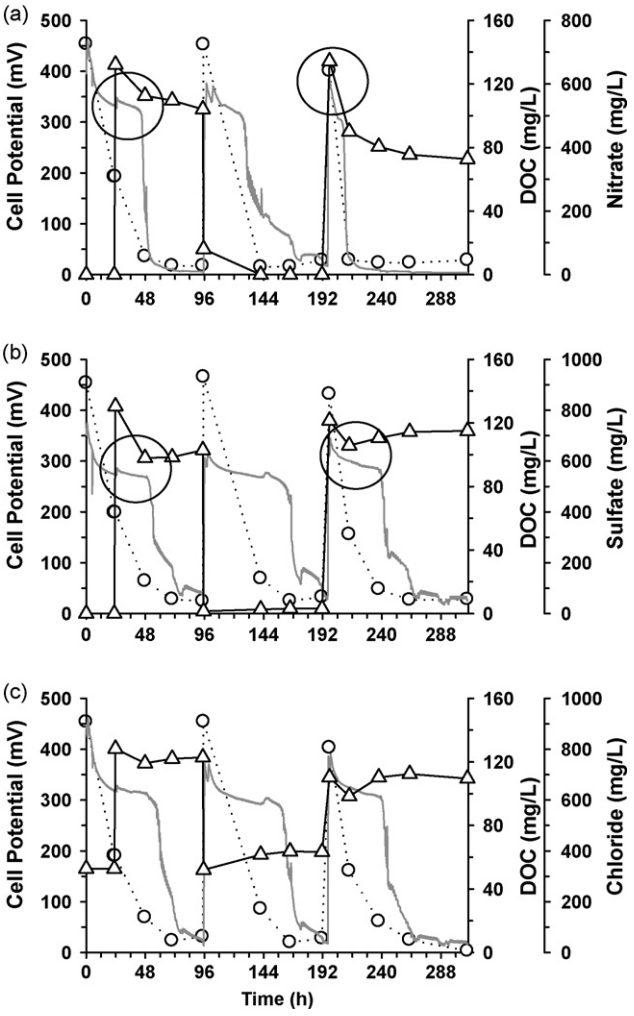

Fig. 1. Cell voltage (1 KΩ; gray line), dissolved organic carbon (DOC; • with dotted line), and specific anion concentrations (△ with solid line) in three single-cell MFCs injected with nitrate (a), sulfate (b), or chloride (c; control). The circles in panels a and b indicate points during the experiment where high power was maintained in the presence of the anions, indicating bacteria utilizing the anode as a electron acceptor did not switch to the respective anions introduced into the MFC.

3. Results

The rate at which the voltage and DOC concentrations decreased after each refill was higher in A than in B or C, although the initial voltage produced in each MFC after each refill was similar (Fig. 1). There was no sudden decrease in voltage after AEA injection in any of the MFCs during the first cycle, overall voltage decreased faster in A than in B and C, and initial voltage was not affected by the presence of AEAs in the last refill (Cycle 3); however, the voltage and DOC concentrations decreased noticeably faster in A during the 3rd cycle than in B or C (Fig. 1), and the CE was also substantially lower in A than in B or C during the 3rd cycle.

3.1. Cycle 1 – AEA injection after refill with growth media and glucose: hours 0–95.3

The average (± s.d., $n = 13$) voltage (mV; 1 KΩ) over the first 2 h of the experiment in A, B, and C was 438 (± 17), 360 (± 8), and 442 (± 12), respectively. The voltage readings in A, B, and C immediately before the first AEA injection at 24 h were 332, 274, and 319 mV, respectively. The voltage in all three cells increased slightly (10–15 mV) following AEA injection and then continued to steadily

Table 1
Coulombic efficiencies of each MFC during three cycles with and without alternative electron acceptor salts (NO_3 or SO_4) or control salt (Cl) additions.

	CE (%)		
	C-1 (NO_3)	C-2 (SO_4)	C-3 (Cl)
1st cycle: (hours 0–95.3)	11	13	14
1st cycle: pre salt injection (hours 0–24)	8	7	7
1st cycle: post salt injection (hours 24–95.3)	17	28	29
2nd cycle: no salts added (hours 95.5–196)	10	14	14
3rd cycle: salts added at beginning of cycle (hours 196.2–310.5)	4	13	15

decrease at the same rate (2–3 mV/h). A steep decrease in cell voltage began in A, B, and C approximately 20, 30, and 37 h after AEA injection, at rates of 33, 27, and 12 mV/h (Fig. 1). The NO_3 and SO_4 concentrations in all cells prior to AEA injection were below instrument detection limits (IDLs) of 0.31 and 0.12 mg/L, respectively. The NO_3 concentration in A decreased from 661 to 520 mg/L and the SO_4 concentration in B decreased from 816 to 643 mg/L from injection at hour 24 to the first refill at hour 95.5 (Fig. 1). The Cl concentrations in A, B, and C prior to injection were 321, 322, and 330 mg/L, respectively. The Cl concentrations in A and C increased to 543 and 802 mg/L after injection and decreased to 532 and 768 mg/L by hour 95.3, respectively (Fig. 1c). The DOC concentrations in A, B, and C decreased by 140, 137, and 135 mg/L (Fig. 1) and the COD concentrations decreased by 450, 416, and 438 mg/L by hour 95.3, respectively. The CEs calculated for A, B, and C during this first cycle were 10.9%, 12.5%, and 14.4%, respectively (Table 1).

3.2. Cycle 2 – refill with glucose and growth media (no AEA salts): hours 95.5–196

Following a refill of growth media with glucose at hour 95.5, the voltage in A, B, and C increased to 371, 315, and 367 mV and steadily decreased (1–2 mV/h) with steep declines in voltage beginning at 128, 163, and 158 h, at rates of 10, 19, and 15 mV/h, respectively (Fig. 1). The residual NO_3 concentration in A, following a rinse with growth media and a refill of growth media and glucose, was 80 mg/L and this decreased to below IDL by hour 142 (Fig. 1a). The residual SO_4 concentration in B increased from ranged from 9 to 19 mg/L during this cycle (Fig. 1b). The Cl concentration in B remained relatively constant at ~290 mg/L during this cycle. The DOC concentrations in A, B, and C decreased by 136, 139, and 137 mg/L (Fig. 1) and the COD concentrations decreased by 454, 436, and 468 mg/L by hour 196, respectively. The CEs calculated for A, B, and C were 10.4%, 14.1%, and 13.7%, respectively (Table 1).

3.3. Cycle 3 – refill with growth media, glucose, and AEA salts: hours 196.2–310.5

The cell voltage in A, B, and C increased to 388, 338, and 382, respectively, following injection of growth media, glucose, and salts at hour 196.2. The voltage in A began decreasing after about 2 h at a rate of 7.5 mV/h for 11 h, then the rate increased to 54.2 mV/h for 5 h at which time the voltage was 34 mV and the voltage slowly approached 3 mV by the end of the test at hour 310.5 (Fig. 1a). The voltage in B and C decreased at slower rates between 1.5 to 2.0 mV/h for the first 41 h, then the rates increased to between 7.6 and 9.9 mV/h for the next 28 h while the voltages remained relatively constant between 20 and 30 mV until hour 310.5 when the test was completed (Fig. 1b and c, respectively). The DOC concentrations in A, B, and C decreased by 119, 129, and 128 mg/L (Fig. 1) and the COD concentrations decreased by 354, 377, and 353 mg/L by hour 310.5, respectively. The CEs calculated for A, B, and C were 4.3%, 13.1%, and 14.7%, respectively (Table 1).

4. Discussion

Biological and abiotic nitrate reduction at the cathode of an MFC has been investigated as an alternative approache for nitrate removal in wastewater treatment and other environmental remediation applications (i.e., [9] and [10]). However, it is also important to understand how the presence of electron acceptors such as nitrate and sulfate near the anode of an MFC affect its performance in terms of electricity generation and CE. The purpose of this study was to determine if the introduction of nitrate or sulfate (TEAs) caused an immediate decrease in MFC power generation, which would indicate that the microbial consortium on the anode switched from using the anode as a solid-state AEA to using a chemical TEA. The results indicate that the consortium did not switch to nitrate or sulfate and, therefore, there was no direct competitive effect of the introduced TEAs. However, the presence of nitrate did decrease the CE of the MFC, which suggests that a disjointed microbial population was growing in the MFC independent of the anode as an AEA.

Specifically, although the voltage produced from A (NO_3) decreased at a faster rate than B (SO_4) or C (osmotic control) during cycle 1 (AEAs injected into each cell during high voltage output) and cycle 3 (AEAs added with refill media and glucose at the beginning of the cycle), the bacteria utilizing the anode as the electron acceptor in A and B did not appear to switch to NO_3 or SO_4 as an AEA because the cell voltage was not immediately affected by the presence of these anions (i.e., the voltage did not quickly decrease after AEA injection; circled areas in Fig. 1a and b). However, the presence of NO_3 in A did appear to increase the DOC consumption rate and immediately affect CE after the first salt injection in Cycle 1 and during the entire 3rd cycle compared to CEs calculated in MFCs B and C (Table 1). A plausible explanation for this is that the NO_3 addition to A during the 1st cycle facilitated the growth of bacteria (e.g., denitrifying populations) that were not utilizing the anode as an electron acceptor or not attached to the anode. The metabolic consumption of DOC by bacteria using nitrate as an electron acceptor aggravated the overall depletion of DOC in the MFC. Therefore, the presence of NO_3 decreased the CE of A during the 1st cycle following the salt injection relative to treatments B and C. The CEs in A, B, and C during the first cycle prior to salt injections were 8%, 7%, and 7%, respectively, following salt injection they were 17%, 28%, and 29%, respectively. This overall increase in CE in all treatments only reflects the steeper portion of the power curve during the later portion of Cycle 1 (Fig. 1). The interesting point here is that the CE in A was more than 10% lower than B and C following salt injection. Similarly, the CE in A was only 4% during the 3rd cycle where salts were added at the beginning of the cycle while the CE was 13% and 15% for treatments B and C, respectively. Alternatively, the CE was closest among all three treatments (A, B, and C) in the absence of additional salts during the first portion of the 1st cycle (11%, 13%, and 14%) and during the entire 2nd cycle (10%, 14%, and 14%, respectively). This demonstrates that the presence of AEAs decreased the overall CE but did not directly interfere with electron transfer to the anode (i.e., no direct competition). The CE in treatments B and C did not change substantially during the three cycles (B = 12.5%, 14.1%, and 13.1%; C = 14.4%, 13.7%, and 14.7%, respectively, Table 1). Sukkasem et al. [11] reached similar conclusions using single-cell MFCs exposed to lower NO_3 concentrations (~250–500 mg/L) during which they determined that the presence of NO_3 did not seem to affect MFC power generation but they did observe decreased CEs compared to controls with no NO_3. The lack of a sudden reduction in cell voltage in A suggests that the bacterial consortium established in this MFC prior to the AEA injection preferred the anode over NO_3 as an electron acceptor. This may indicate that although the theoretical standard reduction voltage of NO_3 (+0.74 V) is higher than the actual reduction voltage in an MFC with an air cathode (as high as +0.425;

[8]) the bacteria do not realize an energetic advantage to using one or the other in this environment and, therefore, electron transfer to the anode is not directly affected when a concentration of more thermodynamically favorable AEAs are available. This supports our working hypothesis that MFC technology can be utilized for passive oxidation of contaminated sediments and groundwater by a similar mechanism to air sparging or amending O_2^- or NO_3^- releasing compounds, and thereby enhance natural degradation of organic contaminants [2,7]. A recent study by Pham et al. [12] also suggested such a mechanism through which enhanced dichloroethane degradation in the anode chamber of an MFC was achieved. Although we have not yet directly compared the rate of enhanced degradation in groundwater or sediments treated with an air sparging system or AEA-releasing amendments to the application of MFC technology, the preferential electron transfer/acceptance by a solid electrode and the passive nature of the MFC design may offer a cost-effective in situ technology for enhanced bioremediation of organic contaminants.

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