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ORIGINAL RESEARCH PAPER

Microbial fuel cell technology for measurement of microbial respiration of lactate as an example of bioremediation amendment

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Abstract Microbial fuel cell (MFC) based sensing was explored to provide for the development of an in situ bioremediation monitoring approach for substrate concentrations and microbial respiration rates. MFC systems were examined in column systems where *Shewanella oneidensis* MR1 used an external electron acceptor (an electrode) to metabolize lactate (a bioremediation additive) to acetate. Column systems were operated with varying influent lactate concentrations (0–41 mM) and monitored for current generation (0.01–0.39 mA). Biological current generation paralleled bulk phase lactate concentration both in the influent and in the bulk phase at the anode; current values were correlated to lactate concentration at the anode ($R^2 = 0.9$), The electrical signal provided real-time information for electron donor availability and biological activity. These results have practical implications for efficient and inexpensive real-time monitoring of in situ bioremediation processes where information on substrate concentrations is often difficult to obtain and where information on the rate and nature of metabolic processes is needed.

Keywords Anaerobic respiration · Bioremediation · In situ sensing · Microbial fuel cell

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Introduction

Monitoring of groundwater contaminant plumes undergoing natural attenuation or bioremediation is often costly and time consuming (Bureau 2002). It normally requires routine monitoring of various contaminants, biogeochemical indicators, and water quality analytes to derive information regarding the processes that are central to bioremediation (Salanitro et al. 2000; Semprini and McCarty 1991; Wu et al. 2006). Reducing the cost of site monitoring, while meeting information needs, requires the development of innovative sensor technologies. Ideally, remote sensor technologies should be developed based on: in situ operation; real-time data acquisition; minimal power requirements; incorporation into existing monitoring networks without significant infrastructure modification; and, for the case of subsurface

monitoring, sensors should provide information regarding relevant biogeochemistries (Turner 2000). Recent advances in microbial fuel cells (MFC) suggest that this technology has potential for remote sensing applications. In this research, we examined the ability of MFCs to provide useful information about the microbial activity in porous media systems as a first step towards the development of MFC based technologies for in situ groundwater monitoring applications.

MFCs exploit the capacity of certain bacteria to donate electrons to a solid electrode to support oxidation of organic electron donors such as lactate, glucose, and acetate (Liu et al. 2005; Logan et al. 2006; Lovley 2006; Rabaey et al. 2003; Ringeisen et al. 2006). Microorganisms in MFC systems actually respire using the solid electrode, thus electricity generated is a measure of microbial respiration in the MFC system. Current produced in MFCs is easily quantified, thus, MFC's have the potential to be a direct, quantitative sensor for microbial respiratory activity. Because there is a wide range of substrates that bacteria can use as either electron donors or electron acceptors, the potential of MFCs may extend to a variety of contaminant monitoring applications. Importantly, many bacteria that are active in MFCs (e.g., *Geobacter* spp. and *Shewanella* spp.) are also known to be involved in contaminant detoxification (e.g., heavy metals, radionuclides, and chlorinated solvents).

This research serves as a first step in the development of MFC technology for remote sensing in groundwater bioremediation applications. While this concept is potentially applicable in numerous systems where remote-sensing is needed, results presented focus on the development of an in situ technology that could be placed within existing groundwater monitoring well networks to provide real-time information regarding biological activity related to electron donor consumption. Flow-through porous media systems fitted with electrodes, and inoculated with *Shewanella oneidensis* strain MR-1, were used to detect and quantify lactate availability and rate of lactate oxidation via biological current generation. Bulk phase concentrations of lactate, both in the influent and in close proximity of the anode, were correlated with the current produced. Results presented herein are the first to our knowledge that demonstrate the use of MFC based sensors for in situ bioremediation strategies.

Materials and methods

Columns

Upflow columns were constructed from transparent poly-vinyl chloride. Columns were 15 cm long with a 2.6 cm innerdiameter and filled with 3.5 mm glass beads, densely packed (Supplementary Fig. 1). Pore volume (PV) was measured to be 36.4 ml, calculated by weight differential for air dried and water saturated columns. Columns were fitted with two graphite cloth electrodes (d = 2.5 cm, 9.8 cm^2 accessible area; Quintech, Göppingen, Germany). Electrodes were connected to wires using silver epoxy and sealed with Marine Adhesive Sealant. Prior to use, electrodes were soaked overnight in 0.5 M HCl, and then stored in deionized water until use. The anode was placed 4.4 cm from the base and a Pt-embedded/graphite cloth cathode (contained 0.5 mg/cm^2; 32 cm^2 to enhance reduction of O_2 at the cathode) was placed at the top of the column (14.5 cm from base) where the column was open to the atmosphere to allow O_2 to reach the cathode. Columns were loosely covered with sterile aluminum foil to prevent contact of extraneous materials with the cathode. A source of 400 mV was applied to the circuit to provide the appropriately reduced conditions at the working electrode and reduce activation time. Electrodes were allowed to equilibrate for more than 24 h prior to inoculation, and background values

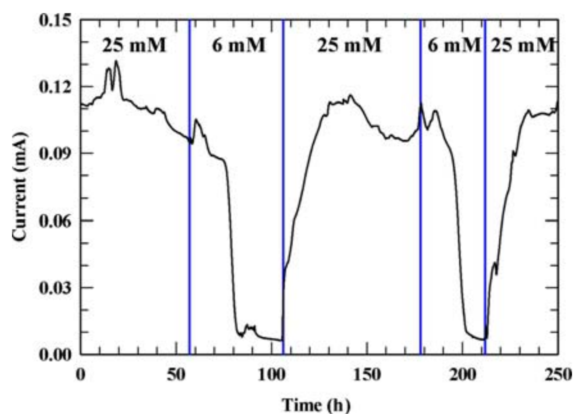

Fig. 1 Electricity output for column system with varying influent lactate concentrations (flow rate = 1.8 ml/h). Changes in influent concentration are indicated by vertical lines and text above the data designates the new concentration

were established (<0.01 mA). A syringe pump fitted with 25 ml gas-tight syringes was used to control column influent, with flow rates of either 1.8 ml/h or 3.6 ml/h depending on the experiment. The influent port was at the bottom of the column and media flowed up the column, and effluent flowed over the edge at the top the column, where it was discharged into a waste-basin. All media was a minimal salts media referred to as shewanella media or SM media (Bencheikh-Latmani et al. 2005) prepared as described with the substitution of 4.77 g HEPES/l, 0.1 g K_2HPO_4/l · $12H_2O$, the addition of resazurin (1 µmol/l) and 20 min of intensive N_2 gas bubbling in lieu of boiling prior to autoclaving. Influent was SM media (pH 7.1) prepared with varying concentrations of sodium lactate (0–41 mM) in 1 l Scott-Duran flasks fitted with gas-tight septa to facilitate refilling syringes and to prevent introduction of O_2. Media, tubing, electrodes, and glass beads were autoclaved prior to column initiation and the column and end pieces were treated with ethanol (3×) and UV exposure (30 min, 3×) to minimize bacterial presence. Column systems were operated at room temperature (22 ± 2°C).

A pure culture of *Shewanella oneidensis* strain MR-1 was used in all experiments. Organisms for columns without a side-arm sampling port were grown overnight in LB media and then washed twice before re-suspension in 0.5 ml SM media containing 20 mM lactate. Columns without a side-arm sampling port were inoculated using a 20 cm stainless steel needle (sterile) which was inserted from the top of the column until the tip reached the anode, and growth was established by flowing SM media containing 25 mM lactate at 0.36 ml/h. Flow rate was increased gradually for 4 d until 1.8 ml/h was reached. For columns with side-arm sampling ports, organisms were grown overnight in LB media, washed twice, transferred to SM media, grown overnight in SM media, then washed three times, and re-suspended in 0.5 ml SM media (wash and re-suspension media contained 20 mM lactate and no fumarate). Column inoculation was achieved by direct injection of cells at the anode via the side sampling port. All results reported were confirmed independently, however as a biofilm development was as a part of each column experiment, they were considered to be independent systems and statistical comparison was not performed.

Analytical methods

Organic acid concentrations were measured using a Dionex IC20 with an EG40 eluent generator. A 4 mm Ionpac AS11-HC anion exchange column was used in conjunction with an ASRS Ultra 11 detector. A gradient of 0.5 mM to 1 mM NaOH was used over 33 min at 1.5 ml/min. Injection volume was 7.5 µl. Total current flow in the circuit is monitored in real time to quantify the electron consumption by the microorganisms. The electrodes were placed in series with a 100 Ω resistor, and voltage drop across the resistor was measured every second using a Hewlett Packard 6280 M series multifunction DAQ measurement system. Each recorded data point was the average of 300 voltage-drop measurements. Current values were calculated based on voltage drop across the 100 Ω resistor based on Ohm's law (V = IR, where I is the current in amperes, V is the potential difference between two points of interest in volts, and R is the resistance). A diagram presenting the overview of the circuit in the MFC-biosensor system is presented in Supplementary Fig.2.

Results

Column systems

Flow through column systems fitted with electrodes were used to evaluate the potential for a correlation

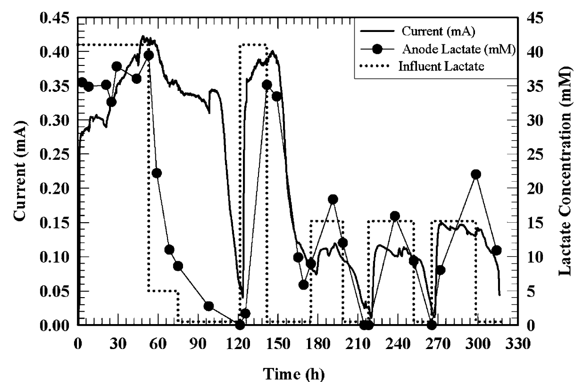

Fig. 2 Electricity output for column system with varying influent lactate concentrations, where influent lactate concentrations are indicated by a dashed line. Data points represent discrete values of bulk phase lactate concentration directly at the anode

between electricity production and electron donor concentration under dynamic flow conditions in porous media. Control columns, operated for 500 h without inoculation, showed a constant current output (<0.01 mA) regardless of influent lactate concentration, thus establishing background current level. Data shown in Fig. 1 presents results from a column system where the influent lactate concentration was varied between 25 mM and 6 mM after growth was initiated (flow rate = 1.8 ml/h). The column was operated for 3 d prior to inoculation and current output recorded (<0.01 mA), thus establishing background current level. After inoculation, the magnitude of electricity production was observed to be a function of the lactate concentration. Increased current production was observed when influent lactate concentration was higher (i.e., 0.10–0.12 mA, 25 mM lactate) and lower levels of current were observed when the influent lactate concentrations were lower (<0.01 mA, 6 mM). When lactate concentrations were decreased from 25 to 6 mM, a lag period of approximately one pore volume (17–19 h; 35–39 ml) was observed before the corresponding drop in electricity production was observed. Conversely, when lactate concentration was increased from 6 to 25 mM, the corresponding increase in electricity production was observed almost immediately.

Lactate was oxidized to acetate by electricity-producing microorganisms in the column system, and our hypothesis was that lactate concentration directly at the anode could be correlated to electricity produced. To demonstrate that biological electricity production is directly related to the lactate concentration surrounding the anode and associated biofilm, a side sampling port was installed on the column directly at the anode. Columns were operated with a flow rate of 3.6 ml/h, the influent concentration of lactate was manipulated (0–41 mM lactate) and 0.1 ml samples were periodically withdrawn from the side sampling port. Data from a representative column operated with a side sampling port are shown in Fig. 2. The lactate concentration at the anode dictated the magnitude of electricity production in the flow through system. Increased quantities of current were observed when influent lactate concentrations were elevated (i.e., 0.35–0.40 mA, 41 mM lactate), intermediate quantities of current were observed for transitional concentrations (0.1–0.2 mA, 10–25 mM) and current levels similar to background were observed when the influent lactate concentration was below the detection limit (<0.01 mA, <0.03 mM). Lactate concentrations for samples taken directly at the anode were mirrored by the current values, demonstrating that the electricity produced and the lactate concentration at the anode were linked.

Similar to data shown in Fig. 1, there was a delay in the decrease in electricity production after the lactate concentration was decreased. The lag time between concentration decrease and attenuation of electricity production was 0.5–1 PV. However a more significant lag was observed with the first manipulation of influent concentration for the experiment shown in Fig. 2. The lactate concentration was changed from 41 mM to 5 mM (Δ 36 mM) at 53 h and then decreased to 0.5 mM at 75 h; however, the electricity production remained high until around 108 h, when a rapid decrease in current was observed. Thus, before the first decrease in electricity was observed, 1.5 PV of 5 mM lactate and 2 PV of 0.5 mM lactate had passed through the column. The delay in attenuation of electricity production after lactate concentration was decreased was possibly a result of biofilm-diffusion-limitations and non-ideal-plug flow conditions, among other factors. In addition, recent work from Freguia et al. (2007) showed that organisms producing electricity in microbial fuel cells are able to temporarily store carbon within cells during high substrate conditions for use under starvation conditions. Thus, the lag-phase observed was possibly attributable to bacterial storage behavior. In contrast, almost no lag in electricity production was observed after lactate concentration was increased, indicating that there was an active-biofilm on the electrode and that growth was not necessary for electricity production after the microbial population was established.

It is important to note, resazurin, a colorimetric redox-indicator, was used in all media. Influent media in syringes was O_2-free although not chemically reduced (i.e., media was pink (Guerin et al. 2001)). However, media in columns was clear, and therefore reduced (Guerin et al. 2001). The fact that columns maintained reduced conditions despite a minimal, background electrical signal indicates that the electricity production was not an abiotic byproduct of differential redox conditions at the anode cathode. The inoculation and corresponding metabolism of lactate by *S. oneidensis* MR1 was necessary for electricity production in the flow through system.

Electricity production is correlated to electron donor concentration

Lactate concentration at the anode controlled electricity production in the flow through systems, as shown in Figs. 2 and 3. To develop a relationship between the two parameters (i.e., electricity production and electron donor concentration) bulk phase lactate concentrations at the anode were compared with current values at discrete time points corresponding to aqueous phase sampling. In Fig. 3, the magnitude of current was correlated with bulk phase lactate concentration for the experiment shown in Fig. 2. Current increased as lactate concentration at the anode increased, and a linear regression was applied to data shown in Fig. 3 [y = 0.009x + 0.017; R^2 = 0.90; y = current (mA), x = lactate concentration (mM)]. As previously described, a lag time in electrical response was observed after lactate concentration was decreased (i.e., Fig. 2, time = 53–108 h). Data associated with the lag period were not included in the linear regression.

Discussion

Delivery of the electron donor ranks among the most problematic challenges engineers face in field implementation of anaerobic bioremediation and complex

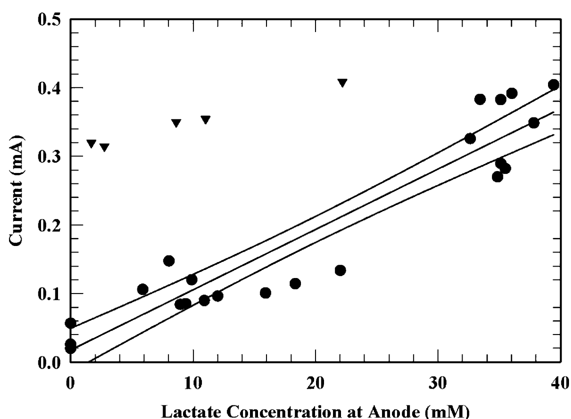

Fig. 3 Current is compared with lactate concentration at the anode for column data presented in Fig. 2. A linear regression is provided with 95% confidence interval (R^2 = 0.90; y = 0.009x + 0.017). Data points indicated by ▼ represent samples where lactate concentration was transitioning and resultant current decrease was delayed (i.e., t = 59, 68, 75, 98, 125 in Fig. 2) and were not included in the linear regression

strategies have been employed to avoid problems encountered with delivery of reducing equivalents (Dybas et al. 1998). Delivery of a non-specific electron donor often results in heterogeneous biomass growth that greatly reduces efficiency of delivery to critical populations (Chu et al. 2003; Semprini and McCarty 1991; Thullner et al. 2004). Although often difficult and costly to monitor, it is crucial in many scenarios to quantify the electron donor that is reaching the desired bacterial population thus stimulating contaminant reduction. This research conceptually establishes that MFC technology can be used to monitor biological activity in porous media and results indicate the potential for further development of this technology for real-time, quantitative measurements of electron donor which is biologically available in affected groundwater. Results presented correlate biologically catalyzed electrical current production with bulk phase (influent and at the anode) lactate concentration. It is likely that correlations developed for related systems can be used in a sensing capacity; however it is clear that additional research is needed to examine the influence of parameters such as temperature, flow rate, microbial ecology, microbial species or community used, non-specific electron donors, long-term biomass stability and system conductivity, among other factors. MFC-based sensor systems, developed for specific field applications based on results presented here, may provide additional insight into the rate and productivity of microorganisms in the subsurface. Properly engineered, the MFC-technology can detect and monitor bulk phase analytes in the subsurface, especially those critical in monitoring bioremediation processes.

Respiration requires coupling of electron donor oxidation with electron acceptor reduction, the electron acceptor utilized (i.e., energy produced) is stoichiometrically related to the electron donor oxidized by the bacteria. Previous work in batch systems has demonstrated that current generated by *Shewanella putrefaciens* IR-1 was related to lactate concentration in the electrochemical cell (Kim et al. 1999). Electric current produced is a signal of respiration by the microbial population capable of using the electrode as an electron acceptor. Thus, MFC-technology also measures of the respiration rates of bacteria using a solid electron acceptor, and is an indirect measure of electron donor oxidation. Therefore, the electrical signal observed in column systems presented herein is

a measure of metabolic *rates* for specific respiratory processes (reduction to an external electron acceptor in this case) at a fixed point in a porous media system. Additionally, because the MFC-biosensor provides information about in situ metabolic rates, it can possibly provide information about decreased metabolic rates caused by competing substrates and toxins, however further research is needed to demonstrate this functionality. Future development of sensor based on results presented here are promising because electricity producing organisms such as *Shewanella* spp., *Geobacter* spp. have been associated with reduction of a broad range of solutes including: heavy metals, chlorinated compounds and radionuclides (Anderson et al. 2003; Bencheikh-Latmani et al. 2005; Picardal et al. 1995; Sung et al. 2006) allowing for a possible broad range of sensing capacities. These organisms, and possibly other, previously unidentified, species and/or microbial communities that have evolved to fit into this ecological niche, and are capable of both electricity production and reduction of contaminants could be potentially applied in MFC-sensing systems. Furthermore, electricity produced during these processes could be further utilized for a variety of operations including: internal powering, other sensing application(s) or even temporarily stored, thus expanding MFC-based sensor applicability. These results support further research and development to identify requirements for the implementation of this technology for possible in-well use during groundwater monitoring (e.g., during engineered bioremediation and natural attenuation).

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