Chemical Engineering Journal 146 (2009) 161–167

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Review

Microbial fuel cell in enhancing anaerobic biodegradation of diesel

Jeffrey M. Morris[a], Song Jin[b,c,*], Barbara Crimi[d], Amy Pruden[d]

[a] Western Research Institute, 365 North 9th Street, Laramie, WY 82072, USA
[b] MWH Americas Inc., 3665 JFK Parkway, Bldg 1, Suite 206, Fort Collins, CO 80525, USA
[c] University of Wyoming, Laramie Wyoming 82072, USA
[d] Colorado State University, Fort Collins, CO 80523, USA

ARTICLE INFO

Article history:
Received 14 March 2008
Received in revised form 16 May 2008
Accepted 19 May 2008

Keywords:
Anaerobic biodegradation
Bioremediation
Diesel
Groundwater
Microbial fuel cell
MFC
Petroleum hydrocarbons

ABSTRACT

Microbial fuel cell (MFC) technology can potentially be applied to enhance subsurface bioremediation of contaminants such as petroleum hydrocarbons by providing an inexhaustible source of terminal electron acceptors to a groundwater environment that is likely depleted in thermodynamically favorable electron acceptors such as oxygen and nitrate. Results indicate that anaerobic biodegradation of diesel range organics (compounds eluting with *n*-alkane markers ranging in size from C-8 to C-25) was significantly enhanced ($P = 0.007$) in an MFC (82% removal) as compared to an anaerobically incubated control cell (31% removal) over 21 days at 30 °C, meanwhile, as much as 31 mW/m^2 cathode of power was generated during diesel degradation (as measured during a polarization curve experiment). The microbial consortium on the anode of a diesel-degrading MFC was characterized by cloning and sequencing 16S rRNA genes. The majority of the clone sequences showed >98% similarity to bacteria capable of denitrification, such as *Citrobacter* sp., *Pseudomonas* sp., and *Stenotrophomonas* sp. The remaining clone sequences showed high similarity with organisms capable of using a wide range of electron acceptors, including sulfate, arsenate, and chlorinated inorganics. In particular, *Shewanella* sp. and *Alishewanella* sp. were found, which are typically capable of using multiple electron acceptors. This study suggests that MFC technology may be used for enhancing biodegradation of petroleum contaminants in anoxic environments, thus, eliminating the need to amend terminal electron acceptors such as oxygen.

1. Introduction

A microbial fuel cell (MFC) is a device that generates electrical power by harnessing the oxidizing potential of anaerobic bacteria. Anaerobic bacteria in an MFC oxidize various substrates and electron donors that include glucose, sewage sludge, petroleum hydrocarbons, and sulfide [1–4] and use the anode in the MFC as a solid-state intermediate electron acceptor. Electrons that the anode accepts flow through a circuit to the cathode, where they are used in the reduction of oxygen (terminal electron acceptor) and protons to water. Therefore, MFCs allow bacteria to breakdown various substrates while simultaneously generating power.

Petroleum hydrocarbons are widespread contaminants of concern in groundwater systems that are biodegradable and have been shown to generate power in MFCs [3]. This is because petroleum hydrocarbons, such as diesel range organics (DRO; compounds eluting with *n*-alkane markers ranging in size from C-8 to C-25) are highly reduced organic molecules, which can serve as a carbon source and electron donors for microorganisms to support microbial metabolism. Under anaerobic conditions, electron acceptors other than oxygen are utilized for microbial respiration and during this process hydrocarbons are oxidized to intermediate molecules and eventually carbon dioxide while terminal electron acceptors are reduced. Microbial degradation of DRO has been identified under various anaerobic conditions, both in pure cultures in the laboratory and mixed cultures in the field. Microbial populations responsible for DRO degradation include denitrifying [5], sulfate-reducing [6–8]; and methanogenic organisms [9,10]. Lovley and Lonergan [11] and Lovley et al. [12] determined that iron-reducing bacteria were capable of degrading aromatic hydrocarbons; however, no information is available regarding DRO degradation by iron-reducing bacteria.

Bond et al. [13] determined that electricity could be harvested from marine sediments and suggested using a sediment-type MFC for enhancing bioremediation of contaminants. Based on our work with MFCs utilizing hydrocarbon waste as an electron source [3], we hypothesized that providing bacteria with a solid-state electron acceptor (anode), as in an MFC, may enhance anaerobic degradation of certain contaminants. For instance in an anaerobic groundwater system, if indigenous terminal electron acceptors such as nitrate or

* Corresponding author. Tel.: +1 970 212 2766; fax: +1 970 377 9406.
E-mail address: sjin@uwyo.edu (S. Jin).

1385-8947/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2008.05.028

sulfate have been depleted, then applying MFC technology in situ would allow bacteria to breakdown substrates near the anode without amending the system with terminal electron acceptors (i.e., nitrate, or sulfate). In a typical air cathode MFC, electrons collected by the anode are eventually transferred to the cathode and subsequently consumed by oxygen and protons to produce water. If this electron pathway is equally or more thermodynamically favorable than using anaerobic terminal electron acceptors such as nitrate or sulfate and, therefore, increases the metabolic rate of anaerobic bacteria in a system with sufficient anaerobic terminal electron acceptors, this could result in faster degradation of contaminant substrates. In this case the effects on contaminant degradation rates might be similar to subsurface aeration or amendment with oxygen releasing compounds. Furthermore, even if the anode in an MFC is less thermodynamically favorable than nitrate, for instance, as an electron acceptor, the physical presence of the anode ensures a high concentration of electron acceptors, which would not need to be replenished in the same way nitrate amendments or oxygen sparging would. We hypothesized that DRO degradation would be enhanced in MFCs with a complete circuit because the anode could provide a higher availability or concentration of electron acceptors through the non-exhaustible source of solid-state electron acceptors (anode) and (or) the anode would provide a more favorable electron pathway than using sulfate and, thus, increase the metabolism of anaerobic bacteria. To our knowledge, it has not been determined whether the anode in an MFC using oxygen at the cathode is a more favorable electron acceptor than anaerobic electron acceptors such as nitrate or sulfate.

The purpose of our study was to determine if anaerobic degradation of DRO could be enhanced in an MFC. We ultimately used a double-cell MFC design to maintain anaerobic conditions in the anode chamber and compared DRO degradation rates in MFCs with complete and incomplete (control) electrical circuits. We determined that DRO degradation is significantly enhanced in an MFC when compared to anaerobic controls containing sulfate.

2. Materials and methods

The study was initially conducted using a single-cell MFC design, which showed apparent power generation from the degradation of DRO that was used as the sole substrate [3]. A double-cell design was implemented in this study to eliminate the potential for oxygen diffusion across the air/water exposed cathode as in a single-cell design and, thus, improved overall data interpretation.

2.1. Double-cell MFC design

Six double-cell MFCs were constructed using two 450-mL glass jars with a silicone-coated lid on the anode chamber and no lid on the cathode chamber [14]. A 12.5-g stainless steel anode (a Chore Boy® stainless steel scrubber composed of a mass of ~1-cm width stainless steel ribbons, Reckitt Benckiser Inc., Parsippany, NJ, USA) was placed inside the anode chamber and attached to a stainless steel wire using a stainless steel nut and bolt. The wire was passed through a hole in the lid, which was sealed with silicone. The two chambers were connected with a 45-cm proton bridge with a 1.3-cm inner diameter [3], inserted through a hole in the lid of the anode chamber and sealed with silicone to maintain anaerobic conditions. The anode was connected externally to a 1 kΩ resistor, which was connected to a 4.5-cm^2 piece of a commercially available cathode material (E-TEK Division, A3STDSI, 0.35 mg Pt/cm^2) with a 20-gauge copper wire that was connected to the cathode with conductive silver epoxy that was covered in silicone.

Table 1
Average electrical potential (mV; 1 kΩ) of the six double-cell MFCs for the 3 weeks prior to the start of this experiment (n = 298 for each cell) and the last reading before the test started

ID	Average	S.D.	Last reading
Individual control cells			
Cell 1	43	2	34
Cell 2	39	2	30
Cell 3	87	11	64
Individual MFCs			
Cell 4	25	2	19
Cell 5	82	5	64
Cell 6	65	4	54
All controls (n = 894)	56	23	43 (avg)
All MFCs (n = 894)	57	24	46 (avg)

The circuit of the three control cells was disconnected at time 0.

2.2. Cell media and inoculum

The cathode chamber of each MFC was filled with sterile phosphate buffer [3] and aerated to maintain an oxygenated environment near the cathode. The anode chamber was filled with a 1:1 mixture of phosphate buffer and diesel contaminated groundwater collected from a local refinery (Cheyenne, WY, USA) as inoculum, as described in Morris and Jin [3]. Surfactant (Palmolive® dish soap) was mixed into the phosphate buffer/diesel mixture at a final concentration of 2 mL/L to ensure a homogenous distribution of diesel spiked into each anode chamber. The anode chamber was refreshed every 2 weeks for 1.5 months until all six cells were performing relatively evenly (Table 1).

2.3. Hydrocarbon degradation experiments

The anode chamber of each of 6 MFCs was filled with 450 mL of phosphate buffer containing a nominal concentration of 300 mg diesel/L and 2 mL surfactant/L that was purged with nitrogen gas and mixed thoroughly for 2 h. The six MFCs were divided into one control and one experimental group each with three replicates. In the experimental group, the external circuit of the cell was connected normally and in the control treatment the cathode was removed from the cathode chamber to disconnect this circuit. All anode chambers were incubated in a water bath for 21-day at 30 °C to maintain high microbial activity. Twenty-milliliter water samples were collected from each anode chamber on days 1, 5, 15, and 21 and analyzed for DRO and major anions. We conducted statistical comparisons of DRO and sulfate among treatments with ANOVA (α = 0.05) using Minitab™ Version 13.31 (Minitab Statistical Software, Minitab Inc.). An initial water sample was not collected on day 0 to allow the DRO concentration in each cell to approach equilibrium because DRO concentrations actually increased after the initial sample was taken on day 0 in a preliminary test (data not shown) due to incomplete dissolution of the diesel. Cell potential (mV) was measured every 10 min with a data logger (ADC-16; Pico Technologies Limited, UK) connected to a computer.

Power density and polarization curves were generated for one of the connected MFCs by measuring cell potential at resistances ranging from 500 to 50,000 Ω and the internal resistance was calculated using the slope of the polarization curve as described in Logan et al. [15].

2.4. Microbial characterization

We characterized the microbial community of the biofilm growing on the anode of a single-cell MFC used during preliminary hydrocarbon degradation testing for this experiment using 16S

rRNA gene sequencing. The single-cell MFC microbial community was considered representative considering that the two MFCs performed comparably in terms of current density and biodegradation rates. 16S rRNA gene sequences provide direct information about the phylogenetic identity of microorganisms and some insight into potential metabolic functions, depending on how well conserved a function is within a phylogenetic group. DNA was extracted using the MO BIO UltraClean™ Soil DNA Kit (Carlsbad, CA, USA) according to the manufacturer's protocol. Bacterial 16S rRNA genes were PCR-amplified using universal primers 8F and 1492R [16]. Amplification conditions were as described by Weisburg et al. [16]. PCR products were cloned with the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Clones were screened for the presence of the gene inserts using PCR with vector-specific M13 primers, generating a library of 58 total clone inserts. Amplified rDNA restriction analysis (ARDRA) was performed visually from MspI restriction enzyme (Promega, Madison, WI, USA) digested products containing an insert. One clone was sequenced for each unique ARDRA pattern, or about half of clones from patterns observed four or more times. Sequencing was performed by Macromolecular Resources (Colorado State University, Fort Collins, CO, USA).

The National Center for Biotechnology Information GenBank database and the BLAST alignment tool [17] were used to determine the closest matches of DNA sequences to known microorganisms. All 16S rRNA gene matches were verified using the Sequence Match tool of Ribosomal Database Project II (RDP) [18]. A literature survey was performed to assign putative functional properties based on those observed for the closest matches. All DNA sequences have been submitted to GenBank and are available under accession numbers EF619929–EF619958.

2.5. Chemical analyses

Diesel range organics were extracted using methylene chloride (CH_2Cl_2) and analyzed with a gas chromatograph-flame ionization detector (GC-FID) equipped with a Tekmar #8 trap and 30 M × 0.53 mm DB-5 column [19]. Nitrate (NO_3^-) and sulfate (SO_4^{2-}) concentrations were analyzed on a DIONEX DX-100 Ion Chromatograph equipped with a 4-mm × 250-mm IonPac AS14 anion exchange column. Dissolved oxygen could not be measured without exposing the anode chamber of each MFC to ambient air, therefore, resazurin (1.0 mg/L) was used to determine the redox potential in the anode chamber of a representative MFC that was setup the same as the 6 cells used in this study, resazurin was not added to any of the 3 MFCs or 3 controls used in this study.

3. Results

3.1. Hydrocarbon degradation

The DRO concentrations in the MFCs decreased by 31% in the control (from 241 to 167 mg DRO/L) and by 82% in the working MFCs (from 176 to 31 mg DRO/L) from day 1 to day 21 (Fig. 1a). The DRO concentrations in MFCs were not significantly different than in the controls on day 1. The percent decrease in DRO in the working MFCs was significantly greater ($P = 0.007$) from day 1 to day 21 compared to controls. Conversely, sulfate concentrations increased by 18% (from 68 to 80 mg SO_4^{2-}/L) in the controls and by 40% (from 67 to 94 mg SO_4^{2-}/L) in the working MFCs from day 1 to day 21 (Fig. 1b). The percent increase in sulfate in the working MFCs was also significantly ($P = 0.026$) higher than in the controls. Nitrate concentrations were below detection limit (0.08 mg/L) throughout the experiment. Maximum power generation in the MFC was

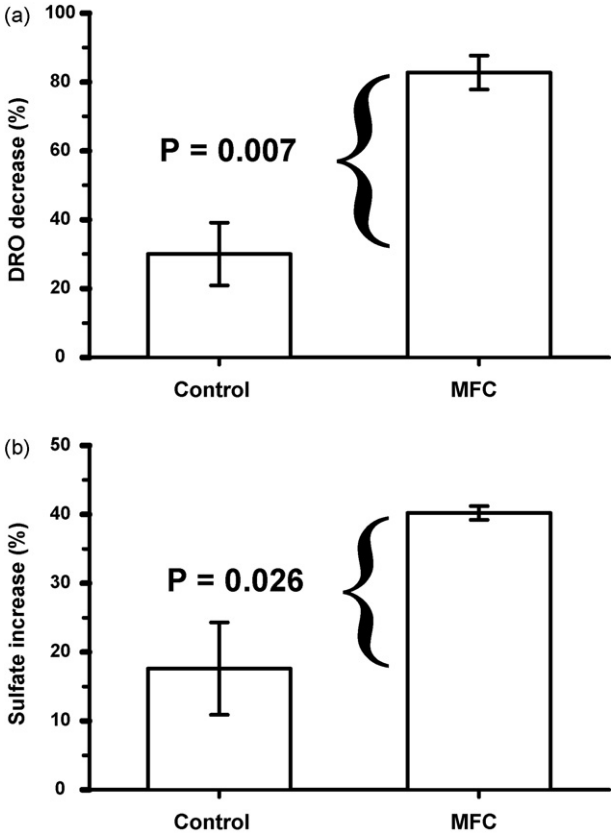

Fig. 1. Decrease in diesel range organics (DROs; (a) and increase in sulfate and (b) in double-cell microbial fuel cells (MFCs) and control MFCs (Control) with a disconnected external circuit (i.e., no power generation) during a 21-day experiment. Error bars are ± the standard error of the mean of replicate experiments ($n = 3$). Significant differences between treatments are indicated with brackets.

32 mW/m^2 and the internal resistance determined from the power density curve was 3213 Ω (Fig. 2). The average cell potential (mV) was elevated initially (160 mV) immediately after it was refilled, but quickly decreased to 10 mV as the system came to equilibrium after 5 h. The cell potential then increased over the first 4–5 days of the test (peaking at 60–65 mV) then began to gradually decrease and became stable at ~15 mV for the remainder of the 21-day experiment (Fig. 3).

The resazurin added to the representative MFC turned a trace pink to clear color. This indicates that this anode chamber was

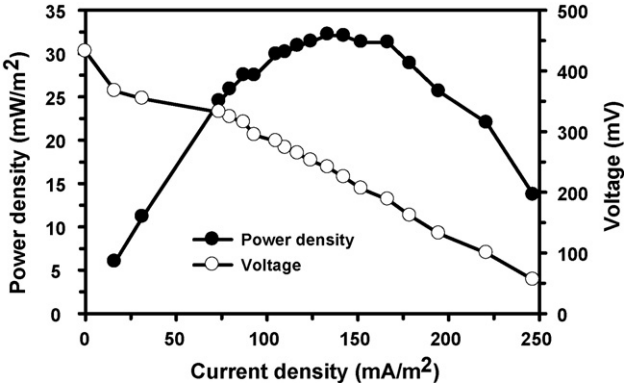

Fig. 2. Power density (mW/m^2 cathode; ●) and polarization (mV; ○) curves measured in a double-cell MFC using a resistor substitution box with resistances ranging from 500 to 50,000 Ω.

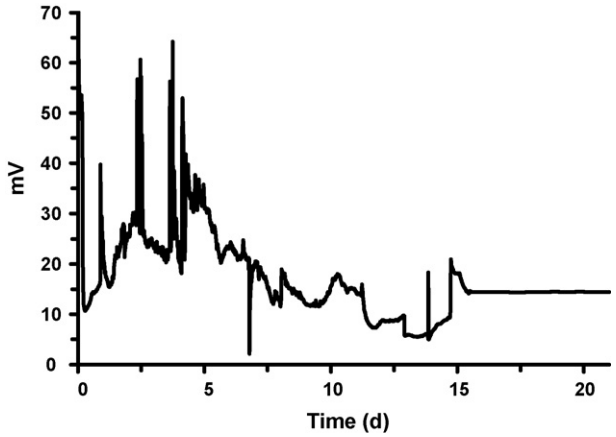

Fig. 3. Average ($n = 3$) cell potential (mV) of the double-cell MFCs during the 21-day experiment. Each cell had a 1 kΩ external resistance and a 4.5-cm^2 cathode. Measurements were taken automatically with a data logger every 10 min.

well sealed and anaerobic (reducing) conditions were maintained [20].

3.2. Microbial characterization

Rarefraction analysis of the ARDRA patterns indicated that the clone library provided good representation of the total bacterial community present on the anode of the single-cell MFC (Fig. 4). Based on ARDRA patterns and DNA sequence analysis, γ-proteobacteria was the most dominant class (81% of the clone library), and *Citrobacter* (20% of the clone library) was the most common genus (Table 2). A literature review of corresponding matches indicated that facultative γ-proteobacteria capable of nitrate-reduction, including *Citrobacter* sp., *Pseudomonas* sp., and *Stenotrophomonas* sp., were dominant on the anode. High similarity (≥95%) with organisms capable of using alternative electron acceptors such as arsenate and chlorinated inorganics were also found, in addition to at least one sulfate reducer. A bacterium with 99% similarity to *Shewanella* sp. ANA-3, belonging to a genus well known for its versatile electron acceptor utilization capabilities [21], was identified as 10% of the total library. Specifically, *Shewanella* sp. ANA-3 is capable of using nitrate, thiosulfate, ferric iron, permanganate, fumarate, succinate, disulfonate and arsenate as electron acceptors [22]. Its ability to degrade DRO has not been reported.

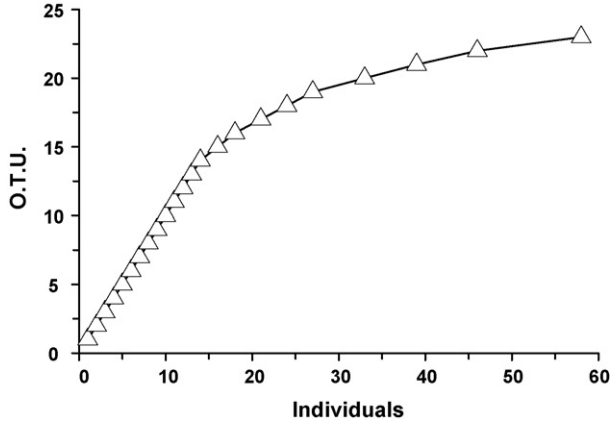

Fig. 4. Rarefraction analysis of amplified ribosomal DNA restriction analysis (ARDRA) patterns. The *x*-axis represents the number of clones screened and the *y*-axis represents how many unique operational taxonomical units (O.T.U.) were observed.

4. Discussion

Prior to conducting this study with a double-cell MFC design, we conducted an initial study using a single-cell MFC design, which generated power by degrading DRO as the sole substrate [3]. Our data also indicates an enhanced DRO degradation in single-cell MFCs over anaerobically incubated controls (56% vs. 21% DRO degradation, respectively, over 18 days; unpublished data). The single-cell design is determined not feasible for this study, because it may allow bacteria growing on the inside of the cathode to utilize oxygen as a terminal electron acceptor while possibly degrading hydrocarbons in the cell and, thus, improving the overall degradation rate of the cell. In order to further confirm the results, a double-cell design was subsequently implemented. The double-cell design eliminated the potential for oxygen diffusion across the air/water exposed cathode as in a single-cell design and, thus, improved overall data interpretation. The double-cell design is also more practical for field application due to the separation of the anode and cathode, which makes this design more feasible for in situ degradation of contaminated groundwater as described in Morris and Jin [3].

Results from the experiment demonstrated that DRO degradation was significantly enhanced in the MFCs compared to controls (Fig. 1a). Day-1 DRO concentrations were higher in the controls compared to the working MFCs (241 and 176 mg/L, respectively), but this difference was not significant and the working MFCs degraded significantly more DRO from day 1 to day 21. The difference in the day-1 DRO concentrations may be due to different rates of DRO degradation prior to sampling on day 1 and (or) the desorption of DRO from residual refinery sludge (190 mg DRO/L; [3]) that partially coated the inside of the anode chambers in the weeks leading up to this experiment while the MFCs were being inoculated with contaminated groundwater. However, since the same sludge was used throughout the experiment in both the controls and experimental treatments, it is not expected that toxicity would have played a role in the different extents of biodegradation observed.

Desorption and dissolution of residual refinery sludge that contained as much as 122 mg sulfate/L [3] may also explain why sulfate concentrations increased throughout this experiment. Interestingly, the sulfate concentrations in the working MFCs increased significantly more than in the controls (Fig. 1b), which suggests that although sulfate concentrations were increasing in both treatments, more sulfate may have been consumed in the controls than in the MFCs. This could possibly be due to the preferential use of the anode in the MFCs as a terminal electron acceptor over sulfate due to the higher thermodynamic favorability of the anode in MFCs. We have determined that a mixed microbial community growing on the anode of a single-cell MFC using glucose as the electron donor and cathode exposed to oxygen prefer the anode as the terminal electron acceptor in the presence of sulfate or nitrate (unpublished data). Therefore, the sulfate-reducing activity in the controls in this DRO-degradation experiment was likely higher than in the MFCs, which may explain why sulfate concentrations were lower in the controls and why there was the 31% decrease in DRO concentrations in these treatments. Another indication for sulfate reduction in the controls was the formation of a black precipitate, that appeared to be some kind of metal sulfide. Other anaerobic pathways such as fermentation and methanogenesis might also be responsible for DRO degradation in the controls. Finally, 16S rRNA gene sequencing identified the presence of a well-known genus of sulfate reducers, *Desulfovibrio*, in the single-cell MFC that was inoculated with the same material. A sulfate-reducing bacterium related to *Desulfovibrio* was previously reported to degrade DRO [7].

Table 2
Clones isolated from the anode of a single-cell microbial fuel cell operating on a hydrocarbon substrate

Highest Match (GenBank accession number)/[Highest Cultured Match]/# clones with same restriction pattern	Class	% Similarity	Terminal electron acceptor				Reference
			NO_3^-	SO_4^{2-}	O_2	Other	
Citrobacter diversus strain CDC 3613 (AF025372), *Citrobacter amalonaticus* (AF025370), *Citrobacter* sp. SAF (AM401577)/12	γ-Proteobacteria	99, 99, 98	+		+	#	Huang and Tseng [30]; Bardiya and Bae [31]; Anderson and Lovley [9]
Uncultured bacterium clone rRNA381 (AY959154)/[*Acinetobacter junii* (AM184300)]/7	γ-Proteobacteria	97 [97]			[+]		
Shewanella sp. ANA-3 (CP000469)/6	γ-Proteobacteria	99	+	–	+	^	Saltikov et al. [22]
Pseudomonas fluorescens strain PC37 (DQ178234), *Pseudomonas stutzeri* strain 24a51 (AJ312176)/6	γ-Proteobacteria	99, 98	+		+		Lalucat et al. [32]
Pseudomonas sp. MACL12A (EF198249)/3	γ-Proteobacteria	99	+		+		
Acinetobacter junii (AF417863)/3	γ-Proteobacteria	99			+		
γ-Proteobacterium CW-KD (EF154515)/3	γ-Proteobacteria	99					
Pseudomonas sp. KBOS 04 (AY653221)/2	γ-Proteobacteria	99	+		+		Ciesielski et al. [33]
Stenotrophomonas sp. LMG 19833 (AJ300772)/2	γ-Proteobacteria	99	+		+		
Uncultured bacterium clone EV818BHEB5102502DRLWq27f072 (DQ256336)/[*Alishewanella fetalis* (AF144407)]/1	γ-Proteobacteria	99 [98]	[+]	[+]	[+]		Vogel et al. [34]
Stenotrophomonas maltophilia {Previous Pseudomonas maltophilia} (DQ465007)/1	γ-Proteobacteria	99	+		+		Su and Kafkewitz [35]
Uncultured eubacterium clone IAFR510 (AF270959)/1	γ-Proteobacteria	99				1	Beaulieu et al. [36]
Uncultured bacterium clone EV818SWSAP61 (DQ337085)/1	γ-Proteobacteria	97				2	
Uncultured bacterium clone 661220 (DQ404770)/1	α-Proteobacteria	97				3	Abulencia et al. [37]
Bosea minatitlanensis (AF273081)/1	α-Proteobacteria	99	–		+		Ouattara et al. [38]
Uncultured clone GZKB1 (AJ853496)/[*Dechloromonas aromatica* RCB (CP000089)]/1	β-Proteobacteria	95 [93]	[+]		[+]	*, #	Coates et al. [39]
Acidovorax sp. JS42 (CP000539)/1	β-Proteobacteria	98	±		+		Williams et al. [40]
Uncultured bacterium clone FW003-A-E01 (DQ407460)/[*Brachymonas petroleovorans* strain CHX (AY275432)]/1	β-Proteobacteria	98 [98]	[±]		[+]		Rouvière and Chen [41]
Corynebacterium variabile (AB116137)/1	Actinobacteria	99	+		+		Takeuchi et al. [42]
Desulfovibrio intestinalis strain KMS2 (Y12254)/1	δ-Proteobacteria	98		+	–		Fröhlich et al. [43]
Uncultured Bacteroidetes bacterium clone CLi112 (AF529321)/[*Flexibacter* sp. CF 1 (AF361187)]/1	Sphingobacteria	96 [93]	[±]	[–]	[+]		
Uncultured bacterium clone aaa99f09 (DQ816804)/[*Clostridium aminobutyricum* (X76161)]/1	Clostridia	96 [93]	[–]	[–]	[–]		Grabowski et al. [44]
Sedimentibacter sp. B4 (AY673993)/1	Clostridia	97		±	–		

+, function is present; –, function is absent; ±, function might be present. *, Chlorate; #, perchlorate ~ thiosulfate and sulfite. ; Couples the oxidation of lactate to acetate with the reduction of arsenate to arsenite. Also capable of using soluble ferric iron, oxides of iron and manganese, fumarate, the humic acid functional analog 2,6-anthraquinone disulfonate, and thiosulfate as electron acceptors. 1. Found in pentachlorophenol contaminated soil slurry. 2. Found in subsurface water from Kalahari Shield, South Africa. 3. Found in sediments contaminated with nitrate, heavy metals, radionuclindes, and halogenated organics.

Considering that the single-cell MFC performed comparably to the double-cell MFC, with similar DRO removal, it is expected that the microbial communities on the anode of the single-cell MFC would be representative. Both had the same electron donor and acceptor availability, the same inoculum, and were incubated in the same medium. Though intrusion of trace amount of oxygen at the cathode proximity was not confirmed in the single-cell MFC, the double-cell MFC did provide better control overall. Thus, one potential difference between the microbial communities may be tolerance of dissolved oxygen. However, a biofilm community did flourish on the single-cell MFC anode and several of the clones sequenced were highly similar to bacteria, such as *Shewanella*, recently found to play a major role in other MFCs [23,24]. The dominance of several bacteria on the anode with high similarity to bacteria capable of nitrate-reduction and the absence of nitrate in the groundwater sample suggest that the electron accepting potential of the solid electrode is close to that of nitrate, though further studies are warranted to verify it. The dominance of several bacteria on the anode with high similarity to bacteria capable of nitrate-reduction and the absence of nitrate in the groundwater sample suggest that the electron accepting potential of the solid electrode may be similar to that of nitrate. Therefore, denitrification may be a default pathway for organic waste degradation at the anode of an MFC, provided no competing electron acceptors are present. At the same time, organisms such as *Shewanella*, *Dechloromonas* and others capable of utilizing alternative electron acceptors, including chlorinated inorganics, were found on the anode. Interestingly, *Shewanella oneidensis* was recently reported by others to play a major role in MFC electron shuttling [23]. *Shewanella* sp. has been demonstrated to possess nanowires, which may facilitate utilization of the anode as an electron acceptor [24].

The diversity of microbes present on the electrode is encouraging evidence that MFC technology may prove useful for remediating a wide array of xenobiotics. It is also important to note that in a mixed-culture MFC, it is not necessarily the case that all microbes utilize the electrode as an electron acceptor. Rather, a mixed culture allows for potential beneficial interactions among microbes to further enhance biodegradation. For example, fermenters have been detected in this study (e.g., Clostridia, Actinobacteria, and Bacterioidetes, Table 1) and have been noted to thrive in MFCs by others, potentially because the MFC aids in disposing of inhibitory fermentation by-products [25]. Methanogens were not analyzed in this study and have been noted to be present in other MFCs, though a recent comparative study demonstrated that methanogens were inhibited by the closed-circuit arrangement in a cellulose-degrading MFC [26]. Because 16S rRNA gene sequences are not ideal for characterizing less conserved metabolic functions, such as denitrification, future research targeting functional genes directly involved in nitrate reduction or reduction of other electron acceptors may be of interest. Nonetheless, the fact that multiple clones with high similarity to bacteria capable of denitrification provides a significant line of evidence of the redox preference of the electrode community and supports the hypothesis that denitrification is a default metabolic pathway in this MFC.

Interestingly, one sulfate-reducer that was found, *Desulfovibrio intestinalis*, which appears to be in conflict with the redox preference of the remaining MFC electrode bacteria detected. Considering the discrepancy in thermodynamic potential, *D. intestinalis* may have been utilizing the sulfate that was present as an electron acceptor, rather than the anode. Also, *D. intestinalis* happens to be an aerotolerant sulfate-reducer, which may have enabled its survival in the elevated redox conditions near the electrode. Thus, overall we would hypothesize that *D. intestinalis* are located near the interface of the electrode, where sulfate is available. Further study using specific electron acceptors is currently ongoing in our laboratory to determine the electron accepting potential of the solid electrode and involvement of key microbial populations in MFCs under different operational conditions.

The mixture of diesel hydrocarbons, their degradative intermediates, and different solubility of each constituent prohibited dissolved organic carbon from being measured. The degradation observed in this study obviously cannot be assumed as "complete degradation", which results in CO_2 and stoichiometric balance. However, three separate microbial fuel cells produced power while DRO concentrations decreased by ~80% while three separate control cells with disconnected circuits experienced only ~30% decrease in DRO. This result fully supports the main conclusion that MFC technology enhances degradation of DRO, in which the intermediates are known to be more amenable to further microbial pathways to achieve complete biodegradation. Due to the fact that diesel is a mixture of hydrocarbon compounds (C8–C25), we assumed an approximate molecular weight of 200 g/mol as recommended by the U.S. Department of Energy [27] for #2 diesel in the following calculations. Anaerobic degradation of DRO in the controls was equivalent to a degradation rate of 3.5 mg DRO/(L day) (or 17.5 μmol/day), which is similar to the rate of sulfidogenic degradation of other hydrocarbons reported by Edwards et al. [28]; e.g., up to 15 μmol/day for toluene. The degradation rate of DRO in the MFCs was 6.9 mg DRO/(L day) (or 34.5 μmol/day), which suggests that utilizing a similar MFC design in the field may significantly enhance DRO degradation rates. Degradation of DRO could be particularly enhanced when using MFC technology in anaerobic environments such as groundwater with depleted natural supplies of terminal electron acceptors (e.g., nitrate or sulfate). Thus, installing an MFC system in situ could be a low-cost alternative to aerating or adding other electron donors to boost aerobic metabolism.

The focus of this research was on enhancing the biodegradation of diesel compounds in an MFC; therefore, the work did not intend to compare power generation from this study to other research aimed at optimizing MFC power generation using alternative cell designs and readily degradable substrates [1]. Results suggest that bacteria could utilize the anode as an electron acceptor with similar energetic gains as using nitrate; however, inadequate evidence is there to confirm due to the scope of this work. Further efforts are ongoing to systematically investigate the electron-accepting potential of solid electrodes. The type of in situ system proposed based on this study would consist of an anode in contact with a groundwater plume and a cathode at the air–water interface in the well if sufficient oxygen is present or a cathode on the surface with a proton bridge connecting the anode and cathode as described in Morris and Jin [3]. This system would require no auxiliary power and little maintenance. Furthermore, the modest amount of power generated by the MFC system could potentially be used to power small monitoring devices as suggested for sea-floor monitoring equipment by Reimers et al. [29] in addition to enhancing bioremediation.

Acknowledgments

Financial support for this research was provided by the United States Department of Energy (DoE) through Western Research Institute's (WRI's) Cooperative Agreement No. DE-FC26-98FT40322 with DoE. We thank Jeff Cooper and Joel Mason (WRI) for conducting chemical analyses. Any opinions, findings, conclusions, or recommendations expressed herein are those solely of the authors.

References

[1] S. Cheng, H. Liu, B.E. Logan, Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing, Environ. Sci. Technol. 40 (2006) 2426–2432.

[2] B. Min, J. Kim, S. Oh, J.M. Reagan, B.E. Logan, Electricity generation from swine wastewater using microbial fuel cells, Water Res. 39 (2005) 4961–4968.
[3] J.M. Morris, S. Jin, Feasibility of using microbial fuel cell technology in bioremediation of hydrocarbons in groundwater, J. Environ. Sci. Health, Part A: Environ. Sci. Eng. 43 (2008) 18–23.
[4] K. Rabaey, K. Van de Sompel, L. Maignien, N. Boon, P. Aelterman, P. Clauwaert, L. De Schamphelaire, H.T. Pham, J. Vermeulen, M. Verhaege, P. Lens, W. Verstraete, Microbial fuel cells for sulfide removal, Environ. Sci. Technol. 40 (2006) 5218–5224.
[5] T. Bregnard, P.-A. Hohener, Zeyer F P., Bioavailability and biodegradation of weathered diesel fuel in aquifer material under denitrifying conditions, Environ. Toxicol. Chem. 17 (1998) 1222–1229.
[6] F. Aeckersberg, F. Bak, F. Widdel, Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium, Arch. Mirobiol. 156 (1991) 5–14.
[7] P. Rueter, R. Rabus, H. Wilkes, F. Aeckersberg, F.A. Rainey, H.W. Jannasch, F. Widdel, Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria, Nature 372 (1994) 455–458.
[8] J. Kleikemper, M.H. Schroth, W.V. Sigler, M. Schmucki, S.M. Bernasconi, J. Zeyer, Activity and diversity of sulfate-reducing bacteria in a petroleum hydrocarbon-contaminated aquifer, Appl. Environ. Microbiol. 68 (2002) 1516–1523.
[9] R.T. Anderson, D.R. Lovley, Hexadecane decay by methanogenesis, Nature 404 (2000) 722–723.
[10] J.M. Salminen, P.M. Tuomi, A.-M. Suortti, K.S. Jorgensen, Potential for aerobic and anaerobic biodegradation of petroleum hydrocarbons in boreal subsurface, Biodegradation 15 (2004) 29–39.
[11] D.R. Lovley, D.J. Lonergan, Anaerobic oxidation of a toluene, phenol and *p*-cresol by the dissimilatory iron reducing organism GS-15, Appl. Environ. Microbiol. 56 (1990) 1858–1864.
[12] D.R. Lovley, J.C. Woodward, F.H. Chapelle, Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe(III) ligands, Nature 370 (1995) 128–131.
[13] R. Bond, D.E. Holmes, L.M. Tender, D.R. Lovley, Electrode-reducing microorganisms that harvest energy from marine sediments, Science 295 (2002) 483–485.
[14] J.M. Morris, S. Jin, J. Wang, C. Zhu, M.A. Urynowicz, Lead dioxide as an alternative catalyst to platinum in microbial fuel cells, Electrochem. Commun. 9 (2007) 1730–1734.
[15] B.E. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, Environ. Sci. Technol. 40 (2006) 5181–5192.
[16] W.G. Weisburg, S.M. Barns, D.A. Pelletier, D.J. Lane, 16S ribosomal DNA amplification for phylogenetic study, J. Bacteriol. 173 (1991) 697–703.
[17] National Center for Biotechnology Information, Basic Local Alignment Search Tool (BLAST). http://www.ncbi.nlm.nih.gov/BLAST/. Cited March 8, 2007.
[18] J.R. Cole, B. Chai, R.J. Farris, Q. Wang, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, A.M. Bandela, E. Cardenas, G.M. Garrity, J.M. Tiedje, The ribosomal database project (RDP-II): introducing *myRDP* space and quality controlled public data, Nucleic Acids Res. 35 (Sequence Match Tool): D169-D172 (2007), doi:10.1093/nar/gkl889, Cited 8 Mar 2007.
[19] U.S. Environmental Protection Agency, Non-Halogenated Organics using GC/FID, EPA Method SW8015, 1996.
[20] R.S. Twigg, Oxidation–reduction aspects of resazurin, Nature (Lond.) 155 (1945) 401–402.
[21] H.H. Hau, J.A. Gralnick, Ecology and biotechnology of the genus *Shewanella*, Annu. Rev. Microbiol. 61 (2007) 237–258.
[22] C.W. Saltikov, R.A. Wildman Jr., D.K. Newman, Expression dynamics of arsenic respiration and detoxification in *Shewanella* sp. strain ANA-3, J. Bacteriol. 187 (2005) 7390–7396.
[23] M. Lanthier, K.B. Gregory, D.R. Lovley, Growth with high planktonic biomass in *Shewanella oneidensis* fuel cells, FEMS Microbiol. Lett. 278 (1) (2008) 29–35.
[24] Y.A. Gorby, S. Yanina, J.S. McLean, K.M. Rosso, D. Moyles, A. Dohnalkova, T.J. Beveridge, I.S. Chang, B.H. Kim, K.S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D.A. Saffarini, E.A. Hill, L. Shi, D.A. Elias, D.W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K.H. Nealson, J.K. Fredrickson, Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 11358–11363.
[25] C.J. Sund, S. McMasters, S.R. Crittenden, L.E. Harrell, J. Sumner, Effect of electron mediators on current generation and fermentation in a microbial fuel cell, Appl. Microbiol. Biotechnol. 76 (2007) 561–568.
[26] S. Ishii, Y. Hotta, K. Watanabe, Methanogenesis versus electrogenesis: morphological and phylogenetic comparisons of microbial communities, Biosci. Biotechnol. Biochem. 72 (2008) 286–294.
[27] United States Department of Energy, General table of fuel properties. http://www.eere.energy.gov/afdc/pdfs/fueltable.pdf. Cited Janusry 15, 2007.
[28] E.A. Edwards, L.E. Wills, M. Reinhard, D. Grbić-Galić, Anaerobic degradation of toluene and xylene by aquifer microcosms under sulfate-reducing conditions, Appl. Environ. Microbiol. 3 (1992) 794–800.
[29] C.E. Reimers, L.M. Tender, S. Fertig, W. Wang, Harvesting energy from the marine sediment–water interface, Environ. Sci. Technol. 35 (2001) 192–195.
[30] H.K. Huang, S.K. Tseng, Nitrate reduction by *Citrobacter diversus* under aerobic environment, Appl. Microbiol. Biotechnol. 55 (2001) 90–94.
[31] N. Bardiya, J. Bae, Role of *Citrobacter amalonaticus* and *Citrobacter farmeri* in dissimilatory perchlorate reduction, J. Basic Microbiol. 44 (2004) 88–97.
[32] J. Lalucat, A. Bennasar, R. Bosch, E. García-Valdés, N.J. Palleroni, Biology of *Pseudomonas stutzeri*, Microbiol. Mol. Biol. Rev. 70 (2006) 510–5470.
[33] S. Ciesielski, A. Cydzik-Kwiatkowska, T. Pokoj, E. Klimiuk, Molecular detection and diversity of medium-chain-length polyhydroxyalkanoates-producing bacteria enriched from activated sludge, J. Appl. Microbiol. 101 (2006) 190–199.
[34] B.F. Vogel, K. Venkateswaran, H. Christensen, E. Falsen, G. Christiansen, L. Gram, Polyphasic taxonomic approach in the description of *Alishewanella fetalis* gen. nov., sp. nov., isolated from a human foetus., Int. J. Syst. Evol. Microbiol. 50 (2000) 1133–1142.
[35] J. Su, D. Kafkewitz, Utilization of toluene and xylenes by a nitrate reducing strain of *Pseudomonas maltophilia* under low oxygen and anoxic conditions, FEMS Microbiol. Ecol. 15 (1994) 249–258.
[36] M. Beaulieu, V. Becaert, L. Deschenes, R. Villemur, Evolution of bacterial diversity during enrichment of PCP-degrading activated soils, Microb. Ecol. 40 (2000) 345–355.
[37] C.B. Abulencia, D.L. Wyborski, J.A. Garcia, M. Podar, W.Q. Chen, S.H. Chang, H.W. Chang, D. Watson, E.L. Brodie, T.C. Hazen, M. Keller, Environmental whole-genome amplification to access microbial populations in contaminated sediments, Appl. Environ. Microbiol. 72 (2006) 3291–3301.
[38] A.S. Ouattara, E.A. Assih, S. Thierry, J. Cayol, M. Labat, O. Monroy, H. Macarie, *Bosea minatitlanensis* sp. nov., a strictly aerobic bacterium isolated from an anaerobic digester, Int. J. Syst. Evol. Microbiol. 53 (2003) 1247–1251.
[39] J.D. Coates, R. Chakraborty, J.G. Lack, S.M. O'Connor, K.A. Cole, K.S. Bender, L.A. Achenbach, Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of *Dechloromonas*, Nature 411 (2001) 1039–1043.
[40] S.T. Williams, M.E. Sharpe, J.G. Holt (Eds.), Bergey's Manual of Systematic Bacteriology, Williams & Wilkins, Baltimore, MD, 1989.
[41] P.E. Rouvière, M.W. Chen, Isolation of *Brachymonas petroleovorans* CHX, a novelcyclohexane-degrading L-proteobacterium, FEMS Microbiol. Lett. 227 (2003) 101–106.
[42] M. Takeuchi, T. Sakane, T. Nihira, Y. Yamada, K Imai, *Corynebacterium terpenotabidum* sp. nov., a bacterium capable of degrading squalene, Int. J. Syst. Bacteriol. 49 (1999) 223–229.
[43] J. Fröhlich, H. Sass, H. Babenzien, T. Kuhnigk, A. Varma, S. Saxena, C. Nalepa, P. Pfeiffer, H. König, Isolation of *Desulfovibrio intestinalis* sp. nov. from the hindgut of the lower termite *Mastotermes darwiniensis*, Can. J. Microbiol. 45 (1999) 145–152.
[44] A. Grabowski, O. Nercessian, F. Fayolle, D. Blanchet, C. Jeanthon, Microbial diversity in production waters of a low-temperature biodegraded oil reservoir, FEMS Microbiol. Ecol. 54 (2005) 427–443.