



Enhanced denitrification through microbial and steel fuel-cell generated electron transport

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ARTICLE INFO

Article history:

Received 7 July 2008

Received in revised form 27 May 2009

Accepted 27 May 2009

Keywords:

Denitrification

Electrochemical remediation

Iron oxidation

Nitrate reduction

Microbial fuel cell

ABSTRACT

Enhancement of nitrate reduction was studied in a two-chambered microbial fuel cell (MFC) and a similar abiotic fuel cell (steel fuel cell or SFC) with an oxidizable steel wool anode and catalyst-free stainless steel mesh cathode. In the MFC and SFC systems, nitrate was reduced in the cathode chamber at 11.4 or 40.0 mg nitrate/L/day, respectively. The MFC utilized petroleum compounds in refinery wastewater as the electron donor and the SFC utilized steel wool as the electron donor. Oxidation of the petroleum compounds in the MFC and steel wool in the SFC caused electron flow from the anode to the cathode, where nitrate was reduced. Nitrate reduction was significantly ($P < 0.001$) higher in SFCs with non-sterile groundwater in the cathode chambers and the flow of electrons to the cathode stimulated microbial growth. Our results suggest the both MFC and SFC designs could serve as electron source for nitrate reduction at the cathode. Particularly the SFC could be an innovative low-cost, low-maintenance alternative for in situ remediation of nitrate-contaminated groundwater.

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1. Introduction

Elevated nitrate (NO_3^-) contamination in groundwater is prevalent across the US and around the world due to non-point source contamination primarily from nitrogenous fertilizers and detergents. The maximum contaminant level (MCL) allowed by the US Environmental Protection Agency (US EPA) in drinking water is 10 mg NO_3^- /L. Infants who drink water containing higher concentrations of nitrate can become seriously ill or die (US EPA National Primary Drinking Water Regulations, www.epa.gov/safewater/contaminants/index.html, 2007). The main mechanisms of nitrate toxicity are physiological complications due to hypoxic conditions that occur when the body converts nitrate to nitrite, which oxidizes ferrous iron in hemoglobin and forms methemoglobin, which does not transfer oxygen as efficiently. Some estimates suggest that millions of families may consume water from wells that exceed the federal MCL for nitrate and additional families may be exposed to high levels in surface waters caused by municipal and agricultural discharges [1].

Current methods used to remove nitrate from water primarily involve reducing NO_3^- to nitrogen gas (N_2) or ammonium

(NH_4^+) [2], which is considered as one of the most favorable nitrogenous nutrient to be readily consumed by microbes. These methods include physicochemical processes, such as ion exchange and reverse osmosis (RO), electrochemical reduction, and bioremediation. Drawbacks to these technologies include high installation and maintenance costs, brine production (ion exchange and RO), membrane fouling (RO) [3], the need for a constant DC power supply (electrochemical reduction), and continuous amendments of electron donors (bioremediation). In this paper we present a new technique that may render electrochemical reduction more desirable than existing technologies by achieving remediation with a low-maintenance, low-cost design that can quickly reduce the majority of NO_3^- in solution to N_2 gas.

Removing nitrate using electrochemical reduction has received attention from a number of researchers, and studies have demonstrated successful removal of nitrate from water and saturated systems using this technique (e.g. [4–11]). Typical electroremediation systems utilize DC power to run an electrical current through a contaminated solution, in which certain contaminants are drawn toward the cathode and transformed [12] to NH_4^+ and/or N_2 gas [6–10,13].

In this study we investigated two innovative DC power sources to deliver electrons for nitrate reduction. The first power source was a two-chambered microbial fuel cell (MFC), which harnesses and enhance the anaerobic microbial oxidation of various substrates, ranging from glucose, municipal wastewater, phenol-containing wastewater to petroleum hydrocarbons [14–17], and transports

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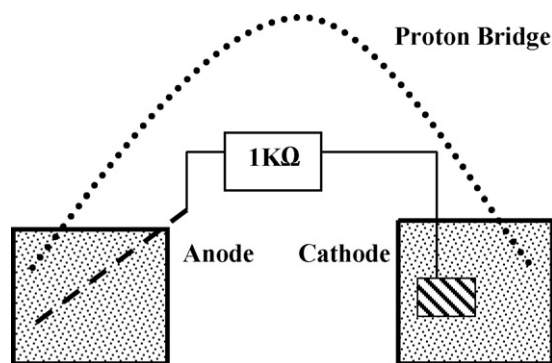


Fig. 1. Schematic drawing of the two-chambered fuel cell design with sealed anode and cathode chambers used in the MFC and SFC experiments.

electrons from the anode (site of microbial oxidation) to a cathode where the electrons are normally consumed in the reduction of oxygen to water [14]. However, we replaced the oxygen in the cathode chamber of this system with NO_3^- to determine if it could act as a terminal electron acceptor and thereby be reduced to N_2 gas and (or) NH_4^+ . Based on the positive results of this phase of the study, we employed a second power source, which was basically a double-cell MFC with a sacrificial anode (steel wool) in a sterile anode chamber. This steel fuel cell (SFC) provided a fairly constant source of electrons from the oxidation of the steel wool anode, which generated electron transfer to the cathode where nitrate was reduced.

2. Materials and methods

2.1. Microbial fuel cell nitrate reduction

Consider the field applicability of an MFC for enhanced remediation of nitrate in groundwater, a two-chambered MFC was constructed by modifying the design as described in Morris and Jin [15], using sealed glass jars (450 ml) with an agar-based proton bridge (45 cm long, 1.3 cm inner diameter vinyl tube, internal resistance $\sim 1\text{ K}$ to $1.5\text{ k}\Omega$), a stainless steel anode (6.4 g), a commercially available carbon-platinum cathode (16 cm^2), and a $1\text{ k}\Omega$ external resistor. To avoid galvanic reactions, the anode and cathode were connected to the resistor externally and these connection points were not submerged in the test solutions (Fig. 1).

The substrate source in the anode chamber was a 1:1 mixture of petroleum hydrocarbon-containing refinery wastewater and growth media [15] and the liquid in the cathode chamber was the same growth media used in the anode chamber except it was sterilized (boiled for 30 min). The anode chamber was sparged with N_2 gas to purge oxygen from the system and create an anoxic environment, which was verified with the redox indicator resazurin (0.13 mg/L) which turns a trace pink to clear color under anoxic conditions. The cathode chamber was sparged with ambient air to maintain dissolved oxygen (DO) concentrations of at least 6 mg/L so the MFC could produce electricity (the MFC could produce power (as high as $330\text{ }\mu\text{A}$ or $47\text{ }\mu\text{A}/\text{cm}^2$ cathode based on 328 mV produced through an external circuit with $1000\text{ }\Omega$ of resistance and a 16 cm^2 cathode). After 2 days of normal operation, oxygen was purged from the cathode chamber down to a concentration $\leq 0.73\text{ mg/L}$ and the chamber was sealed. The low redox state of the sealed cathode chamber was verified using resazurin (0.13 mg/L). After 5.5 days of anaerobic conditions in the cathode chamber and a concurrent decrease in power, a KNO_3 solution was injected into the cathode chamber to achieve a nominal concentration of $600\text{ mg NO}_3^-/\text{L}$. The electrical potential (mV) of the MFC was monitored using a data logger (ADC-16; Pico Technologies Limited, UK)

Table 1

Baseline characterization of groundwater used in the cathode chambers of the groundwater (GW) and sterile groundwater (SGW) treatments in the SFC experiment.

Parameter	Groundwater
pH	6.94
Conductivity ($\mu\text{S}/\text{cm}$)	713
Total bacteria (cells/ml)	65,593
Dissolved organic carbon	3.96 mg/L
Chloride	9.81 mg/L
Nitrate	9.68 mg/L
Sulfate	13.01 mg/L
Ammonia	<0.04 mg/L ^a
Calcium	45.2 mg/L
Iron	0.2 mg/L
Magnesium	7.3 mg/L
Potassium	2.6 mg/L
Sodium	9.6 mg/L
Aluminum	8.8 $\mu\text{g}/\text{L}$
Copper	0.7 $\mu\text{g}/\text{L}$
Nickel	1.2 $\mu\text{g}/\text{L}$
Zinc	3.8 $\mu\text{g}/\text{L}$

^a Below detection limit.

that recorded a measurement every 10 min and periodic samples were collected from the cathode chamber for NO_3^- analysis.

2.2. Steel fuel cell nitrate reduction

After analyzing the results of the preliminary MFC experiment, we designed an SFC that could produce an electrical current with no organic substrates or microbial inoculum in the anode chamber. These two-chambered SFC units were designed similarly to the MFC except we used 230 ml glass containers, stainless steel mesh cathodes (6.3 cm^2) with no platinum catalyst, and steel wool anodes (5.0 g). The anode chamber was filled with sterilized growth media and the cathode chamber was filled with 160 ml of groundwater (Table 1), sterilized groundwater, or reverse osmosis (RO) water. The cathode chambers also received 130 g of fine sand that was washed with 0.1 M hydrochloric acid (HCl) and 0.1 M nitric acid (HNO_3), rinsed with RO water, and dried. The purpose of adding this sand to the cells was to simulate the saturated matrix in the subsurface environment. The cathodes in each chamber were pushed into the sand and the dimensions of the proton bridges on each SFC were 30.5 cm long with an inner diameter of 1.3 cm.

The experiments were conducted in a static system. Four SFC treatments were established in triplicate under anaerobic conditions as follows: (1) a control treatment that contained N_2 -purged groundwater with no proton bridge or electrodes (no power production), (2) a groundwater (GW) treatment that contained N_2 -purged groundwater, (3) a sterilized groundwater treatment (SGW) that contained groundwater that was N_2 -purged and autoclaved for 2 h, and (4) a reverse osmosis water treatment (ROW) that contained N_2 -purged RO water. The control chamber and all cathode chambers received KNO_3 at a nominal target concentration of $1000\text{ mg NO}_3^-/\text{L}$. The pH was measured and samples were collected for nitrate and NH_4^+ analysis from the control and cathode chambers in an N_2 glove box to maintain anaerobic conditions. The voltage across all resistors was monitored periodically using a handheld multimeter throughout the 30 days experiment. Anaerobic conditions in the SFCs were verified by using resazurin as in the MFC experiment.

2.3. Analyses

All chemicals used for this study were reagent grade and purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise

indicated. The pH was measured with an Orion® Thermo model 720A+ pH meter equipped with an Orion® Ag/AgCl combination electrode. Major anions including chloride (Cl^-), bromide (Br^-), fluoride (F^-), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) were analyzed by ion chromatography on a DIONEX DX-100 ion chromatograph equipped with a 4×250 -mm IonPac AS14 anion exchange column (Sunnyvale, CA). Concentration of NH_4^+ was measured by the indophenol blue colorimetric method [18] on a Shimadzu UV-VIS spectrophotometer (Columbia, MD). Dissolved organic carbon (DOC) was analyzed on a Shimadzu total organic carbon analyzer (Columbia, MD). Cations including calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) were analyzed by graphite furnace atomic absorption spectrometry. Metals including aluminum (Al), copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). All anion samples were filtered to $0.2 \mu\text{m}$, stored at 4°C , and analyzed within 7 days of collection. Dissolved organic carbon samples were filtered to $0.45 \mu\text{m}$, acidified to a pH between 2 and 3 with 2 M HCl, stored in amber glass bottles at 4°C , and analyzed within 14 days of collection. Cation and metal samples were filtered to $0.45 \mu\text{m}$, acidified to a pH ≤ 2 with trace-metal grade HNO_3 , stored at 4°C , and analyzed within 30 days of collection. Bacteria were stained with acridine orange and enumerated using fluorescent microscopy [19].

2.4. Statistical analysis

We conducted (1) statistical comparisons with ANOVA ($\alpha = 0.05$) followed by Tukey HSD post hoc pairwise comparisons and (2) simple linear regressions using Minitab™ Version 13.31 (Minitab Statistical Software, Minitab Inc.). We did not have to perform data transformations to satisfy the homogeneity of variance and normality assumptions of ANOVA. For the MFC test, we regressed all nitrate concentrations immediately after the nitrate spike and we regressed all electrical potential values starting 23 min after the nitrate spike (when the potential reached its highest value) without transforming the data.

3. Results

3.1. Microbial fuel cell nitrate reduction

The initial nitrate concentration in the cathode chamber of the MFC was below detection limit (0.31 mg/L). The voltage quickly decreased from $\sim 250 \text{ mV}$ after the chamber was purged with N_2 gas to $< 10 \text{ mV}$ over the course of 5.5 days as residual O_2 was depleted in the cathode chamber (Fig. 2a). After KNO_3 ($595 \text{ mg NO}_3^-/\text{L}$) was added to the cathode chamber, the voltage quickly increased and peaked at 100 mV (6.3 mW/m^2 cathode or 22.2 mW/m^3 of catholyte volume in the cathode compartment) approximately 23 min after KNO_3 addition. After the KNO_3 spike, the nitrate concentration decreased significantly from 595 to 344 mg/L at a rate of 11.4 mg/L/day over the course of 22 days. No nitrite was detected in the cathode chamber. The least squares linear regression for this 22-day period is: $\text{mg nitrate/L} = 637.3 - 11.9 \times \text{time}$ ($P < 0.001$, $R^2 = 0.87$, $n = 11$), where time is in days (Fig. 2b). Concurrently, the voltage decreased significantly from 100 to 54 mV over 22 days (it stabilized for the last 4.5 days of the experiment). The least squares linear regression for this 22-day period is: $\text{mV} = 95.6 - 1.7 \times \text{time}$ ($P < 0.001$, $R^2 = 0.85$, $n = 3151$), where time is also in days (Fig. 2b). The addition of resazurin 3.5-day into the experiment did not change the electrical potential of the MFC (Fig. 2a).

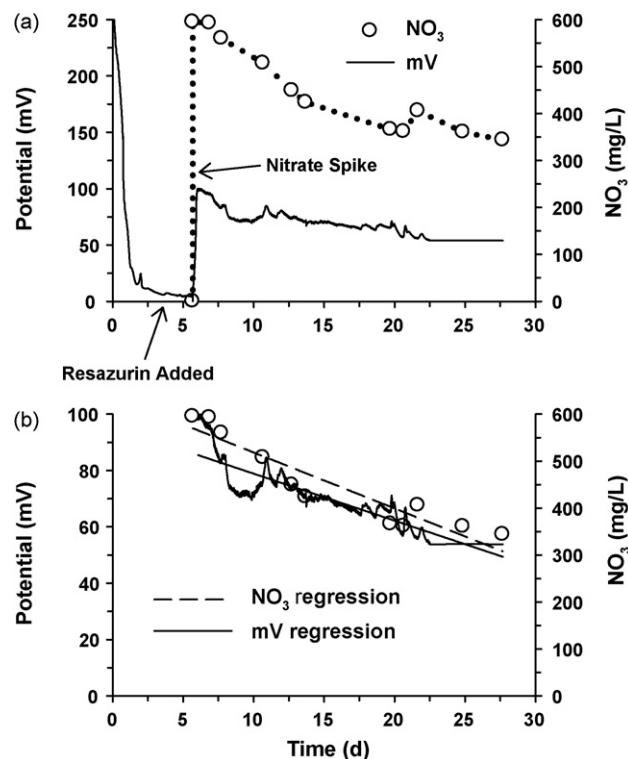


Fig. 2. Nitrate (NO_3^-) concentrations in the cathode chamber and corresponding electrical potential (voltage) (mV; $1 \text{ k}\Omega$) of the MFC (a). A redox indicating dye (resazurin) was added to the cathode chamber on day 3. The nitrate concentration and electrical potential (voltage) immediately increased following a KNO_3 spike, then decreased significantly ($P < 0.001$; see text for least squares regression equations) over the course of the final 22 days of the experiment (b).

3.2. Steel fuel cell experiment

During the initial 5–7 days of the 30-day SFC experiments, the systems underwent a flux period where the voltage dropped considerably and NO_3^- concentrations fluctuated as the system stabilized. Therefore, although the NO_3^- values for the entire 30-day experiment are shown in Figs. 3 and 4, the starting values used for all calculations were measured on day 7.

3.2.1. SFC groundwater characterization

The groundwater used in the control and cathode chambers of the SFCs had a circum-neutral pH (6.94), concentration of NO_3^- was less than the MCL (9.7 mg/L), and NH_4^+ concentrations were below detection limit (0.04 mg/L ; Table 1).

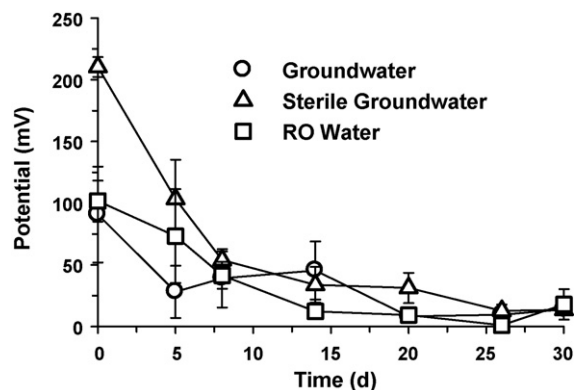


Fig. 3. Electrical potential (voltage) ($1 \text{ k}\Omega$) measured in SFCs. Error bars are standard error of the mean ($n = 3$).

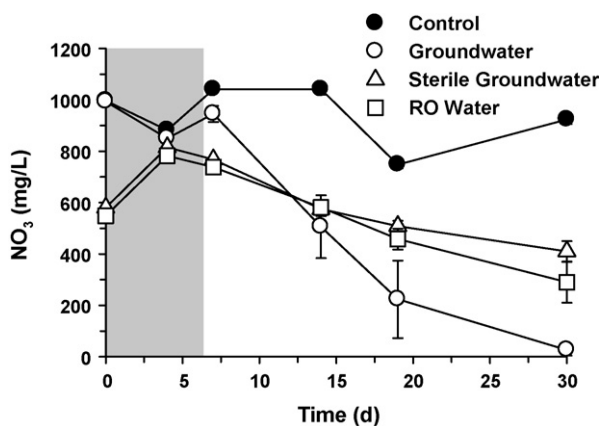


Fig. 4. Nitrate (NO_3^-) concentrations in the cathode chamber of each SFC treatment. The gray bar indicates an unstable period not considered in the nitrate reduction calculations. Error bars are standard error of the mean ($n = 3$).

3.2.2. SFC electrical potential

The GW, SGW, and ROW treatments all produced relatively low voltages throughout the experiment, starting from about 100 to 200 mV and then declining steadily to about 15 mV. However, after the initial decrease, as the system stabilized prior to day 7, the voltage in the GW treatment increased from 28 to 45 mV from days 5 to 15 and a similar increase was not observed in the SGW or ROW treatments (Fig. 3).

3.2.3. SFC nitrate reduction

Nitrate concentrations (mg/L) decreased slightly (11%) in the control treatments from 1040 ± 3 (standard error of the mean, $n = 3$) to 923 ± 18 ($5.1 \text{ mg NO}_3^-/\text{L/day}$) over a 23-day period. Nitrate concentrations decreased in the GW, SGW, and ROW treatments by 97, 47, and 61%, from 945 ± 32 to 26 ± 21 ($40.0 \text{ mg nitrate/L/day}$), 768 ± 4 to 410 ± 40 ($15.6 \text{ mg NO}_3^-/\text{L/day}$), and 738 ± 13 to 290 ± 79 ($19.5 \text{ mg NO}_3^-/\text{L/day}$), respectively (Fig. 4). At the end of the 30-day experiment, the percent of initial NO_3^- remaining in the cathode chambers of the GW treatment ($3\% \pm 2$) was significantly ($P < 0.001$) lower than in all other treatments and the percent of initial nitrate remaining in the SGW and ROW treatments was significantly ($P < 0.001$) lower than in the control (Fig. 5). The system of different letter cases was used in Fig. 5 to illustrate statistical results by following Nimick et al. [20]. The uppercase letters indicate significant differences in NO_3^- -N concentrations between different treatments (control, groundwater, sterile groundwater, RO water) the lowercase letters indicate significant differences in N_2 -N concentrations between different treatments and there are no letters for NH_4^- -N because there were no significant differences in NH_4^- -N between any of the treatments.

The GW treatment resulted in the most favorable overall conversion of NO_3^- to desirable N_2 gas ($76.2 \pm 7.4\%$) and less desirable NH_4^+ ($21.2 \pm 9.9\%$; Fig. 5). Ammonium concentrations were below detection limit in the groundwater or RO water added to each treatment and the NH_4^+ concentrations in the C, GW, SGW, and ROW treatments increased to 13.2 ± 0.5 , 56.2 ± 21.0 , 70.1 ± 10.8 , and $31.2 \pm 15.0 \text{ mg/L}$ by day 30, respectively. Nitrite was not detected in any cathode chambers or the control chambers during this study.

The presence of ammonium indicates dissimilatory nitrate reduction was occurring. This was observed by Choi et al. [21] and Katsounaros and Kyriacou [22]. If oxygen is present in water the ammonium is converted to N_2 . Without oxygen and near the cathode, the ammonium is released as ammonia gas. Dissimilatory nitrate reduction is often followed by accumulation of nitrite from nitrification [23]; however, no nitrite was detected in the cathode solution, which indicates that conditions did not favor nitrification.

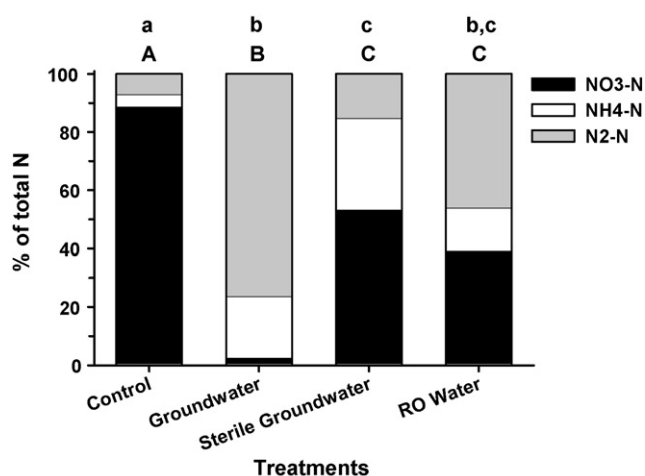


Fig. 5. Conversion of nitrate in the catholyte solution in each SFC treatment from day 7 to day 30 in terms of molar concentration of N calculated from final nitrate (NO_3^- -N), ammonium (NH_4^- -N), and nitrogen gas (N_2 -N) concentrations (initial N calculated using nitrate concentrations on day 7; N_2 calculated by difference). Different uppercase letters indicate significant ($P \leq 0.001$) differences in NO_3^- -N concentrations between different treatments and different lowercase letters indicate significant ($P \leq 0.001$) differences in N_2 -N concentrations between different treatments. There were no significant differences in NH_4^- -N between any treatments.

Therefore, it is reasonable to presume that nitrogen loss might have occurred in the testing system through ammonium to ammonia gas pathway. The formation of N_2 gas and N_2O may have occurred, but the formation of N_2O is presumed relatively insignificant when observable ammonium formation occurred. Katsounaros and Kyriacou [22] reported that N_2O consisted only 6% of the total by-products.

3.2.4. SFC microbial consortium

The total bacteria count in the cathode chambers of the control and GW treatments increased from approximately 6.6×10^4 cells/ml (baseline in groundwater collected from the field) to approximately $4.2 \times 10^5 \pm 1.4 \times 10^5$ and $4.7 \times 10^6 \pm 1.6 \times 10^6$ cells/ml, respectively; however, due to high variability, these concentrations were not significantly different. As expected, no bacteria were found in the cathode chambers of the SGW or ROW treatments.

4. Discussion

The correlation between the changes (initial increase and subsequent decrease) in the NO_3^- concentrations and the electrical potential in the MFC and SFC experiments suggests that NO_3^- was being utilized as a terminal electron acceptor in the cathode chamber. Our preliminary MFC experiment demonstrated that nitrate reduction can occur in the presence of a low electrical current under anaerobic conditions. Clauwaert et al. [24] reported that a tubular MFC can successfully enhance microbial denitrification at a maximum rate of about $645.5 \text{ mg NO}_3^-/\text{L/day}$ when acetate was used as the anode substrate; however, our study is the first experiment to our knowledge that demonstrates concurrent degradation of two groups of listed contaminants in aqueous phase in a MFC setup (petroleum hydrocarbons (electron donor) and NO_3^- (electron acceptor)) in an MFC system. Due to the un-optimized system configurations and the resultant high internal resistance, the denitrifying rates in our MFC ($11.4 \text{ mg NO}_3^-/\text{L/day}$) and SFC ($40.0 \text{ mg NO}_3^-/\text{L/day}$) systems were substantially lower than the rate of $645.5 \text{ mg NO}_3^-/\text{L/day}$ as reported by Clauwaert et al. [24]. We attribute the high denitrifying rates in the tubular MFC used by Clauwaert et al. to its higher system efficiency. For example, the

cathode chamber of their system was packed with carbon granular, which might have provided larger surface areas for both microbial growth and electron transfer from the electrode to nitrate. The population difference in the cathode chamber may also contribute to different denitrifying rates as observed. Further investigations are needed to decipher the involved mechanisms and improve the system performance including denitrifying rates and capacity of electron generation and transfer.

Although our results and the work of others demonstrates that an MFC design could potentially be employed in the field to enhance nitrate reduction in anoxic groundwater, the drawback is that organic material must be fed to the bacteria in the anode chamber and optimal chemical and physical conditions for microbial activity must be maintained. This could be problematic because maintaining favorable operating conditions in the field is sometimes impossible. Therefore, we identified the SFC as a reliable, low maintenance, low voltage, abiotic power source that could potentially achieve the same level of denitrification in a field system. This apparatus utilizes a similar electron flow mechanism as in an MFC without having to maintain optimal biological conditions in the anode chamber. The design of the SFC relies on iron oxidation of a steel wool anode to generate electron flow and eliminates the need for an expensive catalyst such as platinum on the cathode. Just like an MFC, electrons in an SFC pass from the anode to the cathode, but this electrical current originates from the abiotic oxidation of steel wool.

Similar to the MFC results, the SFC experiments indicate the flow of electrons from the anode enhances nitrate reduction in the cathode chamber; however, the rate of nitrate reduction in the SFC is much higher (40.0 mg NO₃⁻/L/day). Additionally, the population of microorganisms in the cathode chamber increases with electron flow and the presence of these microbes correlate to a significantly larger reduction in nitrate than in sterile systems. Interestingly, the voltage in these SFCs is influenced by the NO₃⁻ concentration in the cathode chamber but not by the presence of microbes in the cathode chamber. It appears that electrons flowing to the cathode are capable of directly reducing NO₃⁻ and stimulating microbial activity and growth, thereby, also indirectly enhancing microbial denitrification. This observation also agreed with the recent results on biocathodes by other researchers [25,26]. Dash et al. [27] has reported that 70–97% direct reduction of nitrate using aluminum, iron, and titanium electrodes; but no observable nitrate reduction was achieved when graphite electrodes were used. Based on preliminary screening, we selected stainless steel for cathode in this SFC setup. We did not determine the mechanism behind this enhanced microbial growth; however, it has been shown that electrodes can serve as direct electron donors for anaerobic microbes involved in nitrate reduction [28] and this process might have enhanced the metabolism and growth of denitrifying bacteria in our system. The relatively low concentration of DOC (3.96 mg/L) in the groundwater was apparently insufficient to sustain denitrification in the control treatments, which supports our conclusion that microbes in the GW treatment were utilizing electrons generated from steel wool oxidation as an electron source.

It appears that two possible mechanisms of nitrate reduction may have caused the decrease in NO₃⁻ concentrations in the SFCs. First, the decrease of NO₃⁻ in the treatments with sterile cathode chambers (SGW and ROW) but not in the disconnected control suggests that electrons are passed directly to NO₃⁻ with no microbial intermediary. Second, the significantly larger decrease in NO₃⁻ in the treatment with a robust microbial population in the cathode chamber (GW) compared to all other treatments indicates that the presence of bacteria enhances nitrate reduction over sterile conditions in the presence of an electrical current and that this flow of electrons to a non-sterile system stimulates bacterial growth. This enhanced nitrate reduction could be due to direct electron

utilization by nitrate reducing bacteria on the cathode, thereby stimulating microbial denitrification or a combination of electron transfer directly to NO₃⁻ and electron transfer to nitrate reducing bacteria, which may serve as a type of bio-catalyst on the cathode for nitrate reduction. Further characterization work on this cathode-bound bio-catalyst is needed.

Our SFC design for nitrate reduction may be a significant improvement over other NO₃⁻ treatment systems using similar mechanisms. For instance, although zero-valent iron has been used to enhance denitrification in both laboratory and field studies [29–33], the risk of iron contamination and surface masking by iron oxides is high when used for in situ remediation of contaminated groundwater. The SFC design eliminates this risk by keeping the iron electron source isolated from the NO₃⁻ through indirect oxidation (i.e., the iron and NO₃⁻ would be only connected through an electrical circuit and a proton bridge). Till et al. [34] designed an iron oxidation/nitrate reduction system that also separated the steel wool from the nitrate-contaminated water, to avoid exposing microorganisms to potentially toxic iron corrosion products. Their system required the production and transfer of H₂ from a separate chamber to the chamber containing NO₃⁻. This system enhanced complete removal of 25 mg NO₃⁻/L within 4 days (6.3 mg NO₃⁻/L/day) with 93% denitrification. Interestingly, our SFC system had a 6-fold higher nitrate reduction rate (40.0 mg NO₃⁻/L/day) with up to 76% denitrification and did not require inputs of H₂.

Although some ammonium was detected (as much as 70 mg/L) during the SFC electron-mediated denitrification (Fig. 5), this represents a small overall percentage of the initial NO₃⁻ concentration (15–21%) and should not present a significant contamination risk in the groundwater. The formation of ammonium was attributed to reduction of nitrate at lower redox potential. Ammonium has been determined to be a preferential nitrogen source to heterotrophic bacteria [35,36], and therefore we expect this residual NH₄⁺ to be readily assimilated during microbial metabolism and growth. In addition, a number of indigenous bacteria (e.g., anammox bacteria) can oxidize NH₄⁺ under anaerobic conditions and produce N₂ gas [37].

5. Conclusions

Results from this study indicate that electron transfer in an MFC equivalent design of SFC may facilitate direct nitrate reduction in the cathode chamber. The simple design of our SFC, separation of the electron donor (anode) from NO₃⁻ in groundwater, may offer a new technique for nitrate reduction as well as a potential alternative for remediation of other contaminants capable of accepting electrons, such as perchlorate, perchloroethene, and trichloroethene. Additionally, the SFC system has the potential to be an effective, low cost, low maintenance NO₃⁻ remediation strategy due to the use of indigenous microbes in the groundwater, waste material such as scrap iron as an electron donor, and a cathode that does not require expensive catalysts such as platinum. The lifespan of SFC can be easily extended by increasing the amount of steel wool, if needed for specific field applications, in which steel wool would be kept in a container at the surface that is connected to the groundwater through proton bridges for easy accessibility when maintenance and material exchange are deemed necessary.

Acknowledgments

We thank Joel Mason and Felipe Burns (Western Research Institute) for assistance with this project. Partial financial support was provided by the United States Dept of Energy (DoE) through Western Research Institute's Cooperative Agreement No.

DE-FC26-98FT40322 with DoE. Any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not reflect the view of the DoE.

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