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A Self-Sufficient Nitrate Groundwater Remediation System: *Geobacter Sulfurreducens* Microbial Fuel Cell Fed by Hydrogen from a Water Electrolyzer

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Nitrate contamination of groundwater is a major problem, especially in farming areas where nitrogen-based fertilizers are used. *Geobacter sulfurreducens* electrodes were electrochemically evaluated for their ability to reduce nitrate with implications for groundwater remediation. *G. sulfurreducens* were optimized for nitrate reduction by modifying growth media during subculture. The *Geobacter* were then cast on Toray carbon paper electrodes and immobilized with pectin. Cyclic voltammetry demonstrated that the electrodes bioelectrocatalytically reduce nitrate with an onset potential of −0.25 V vs. SCE. Amperometry was used to evaluate nitrate concentrations between 0.5 and 270 mM. The limit of detection is 8 mM with a linear range of 20 mM to 160 mM. Evaluation by a Michaelis Menten kinetic model yields a K_M of 110 ± 10 mM. The *Geobacter sulfurreducens* electrodes were incorporated into a nitrate reducing microbial fuel cell which was fed nitrate contaminated water by a peristaltic pump and hydrogen from a proton exchange membrane (PEM)-based water electrolysis cell and yielded a remediation rate of 6 mg/cm^2/day.

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Groundwater remediation is a major global problem. In particular, nitrate accumulation in ground water is a growing issue that needs to be addressed.[1,2] Nitrate pollution is especially problematic in rural areas due to farming. Fertilizer is required for increasing crop productivity and gives farmers economic viability. However, the use of nitrogen-based fertilizers results in excess nitrate runoff in the groundwater, which can cause significant health problems if consumed in large quantities. The cost to remove nitrates for drinking water can result in a 3–5 fold increase in operating costs for water treatment.[3]

Present technologies to remove nitrates include ion exchange, reverse osmosis, electrodialysis, distillation, electrocatalysis, and biological denitrification.[3–8] Ion exchange and reverse osmosis are high maintenance technologies, requiring the use of regeneration chemicals or cleaning to maintain the processes. Reverse osmosis and electrodialysis are particularly problematic where water conservation is important, as these processes can have a 50–90% reject rate.[4] Brine disposal is also a challenge, since it is often undesirable to place in a septic system or release into the environment. Additionally, the membranes tend to foul and need to be replaced periodically. Ion exchange technologies vary, but they also can use substantial amounts of water and chemicals to regenerate the resins. The resins can also become contaminated and need replacement. The presence of phosphates diminishes the effectiveness of resins, which is problematic for use in rural areas with high agriculture runoff. Distillation can have a very low reject rate and require minimal amounts of chemicals; however, energy usage can be very high and requires regular maintenance to prevent scaling of the equipment.

Electrochemical remediation of nitrates is an attractive option, because it is a potentially efficient process with low maintenance requirements and low water reject rates. Nearly a century of work has been conducted on electrochemical catalytic reduction of nitrate on solid-state catalysts.[6] However, the catalysts are non-specific for the complex reactions desired and wastewater contaminants deactivate the catalysts over time. In contrast, biological systems are often robust to wastewater components and can perform highly complex reactions. Microbial electrodes are renewable, recyclable, and regenerative. Bacterial biofilms grow and reproduce new microbes while consuming dead bacteria on the electrode surface, allowing microbial electrodes to be stable for 3–5 years.[9] To this end, an extensive amount of research has focused on using microbes to convert substrates found in groundwater as a method of removing contaminants.[7,8,10,11]

Microbial fuel cells were first invented in 1911 when researchers realized that microbe metabolism could be observed electrochemically at an electrode.[12] Until the early 2000s, the majority of microbial fuel cells in the literature required a small molecule redox mediator to transfer electrons between the electrode and microbes, but it has been found that some microbes can transfer their electrons directly to electrodes without the use of a small molecule redox mediator.[13] In recent years, microbial fuel cells have been proposed for wastewater treatment,[14] as well, because different types of bacteria are capable of remediating different water contaminants. For instance, *Shewanella* bacterial electrodes have been studied for oxidizing carbohydrate contaminants to carbon dioxide, *Enterobacter* bacterial electrodes have been for consuming hexavalent chromium contaminants, and *Gluconobacter* modified electrodes have been studied for consuming alcoholic contaminants (i.e. ethanol, butanol, glycerol, mannitol, ethylene glycol).[13,15,16] Over the past 20 years, microbial electrochemical systems have been studied for nitrate remediation.[7,8] Biological denitrification has been proven effective; however, many of the reactors use heterotrophic bacteria which require complex organic substances as energy sources, increasing operating costs and scalability. Extensive research has been conducted in the area of hydrogenotrophic denitrification.[17] Work in this area has shown that some biofilm electrodes are capable of nitrate remediation using hydrogen as the electron donor and carbon or carbonate as a carbon source for microbial regeneration. However, these technologies generally create hydrogen in situ at the electrode, causing a large decrease in the nitrate remediation rate as gradual scale formation on the surface suppresses hydrogen production.[17]

Geobacter have been extensively studied for their extracellular direct electron transfer (DET) ability.[18–26] Proposed mechanisms explaining this ability include metallic-like conduction through pili, termed nanowires, with interfacial cytochromes[22,25,27] or a sequence of redox reactions between cytochromes that connect the cells to the electrode.[27,28] Regardless of the mechanism, the ability to directly

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communicate with the electrode has great implications in a variety of microbial fuel cell and other bioelectronics research as most other microbes require mediators to transfer electrons to electrodes.[18–22,29] *Geobacter* is a common bacteria used in microbial fuel cells for carbohydrate oxidation, but it contains a nitrate remediation pathway for consuming nitrate and forming nitrogen gas, which means that it does not have to be used entirely for microbial fuel cell anodes, but can also be used for biocathodes. *Geobacter* are autotrophic bacteria that can use hydrogen as an energy source. Several species of *Geobacter* have been shown to reduce nitrates.[30–32] *Geobacter sulfurreducens* has been electrochemically studied as a biofilm[18,19,33] as well as immobilized on carbon electrodes by pectin,[20] where DET still occurred when the *G. sulfurreducens* were immobilized on the electrode without forming a biofilm.

Here, a nitrate reducing *Geobacter sulfurreducens* fuel cell fed by hydrogen from a proton exchange membrane (PEM)-based water electrolysis cell is employed as a low cost alternative to current water treatment methods. *G. sulfurreducens* electrodes were electrochemically evaluated for their ability to reduce nitrate. *G. sulfurreducens* cultures were optimized for nitrate reduction by modifying growth media. The *Geobacter* were then cast on Toray carbon paper electrodes and immobilized with pectin. Cyclic voltammetry and amperometry were performed to evaluate the bioelectrocatalysis and kinetics of nitrate reduction by the *G. sulfurreducens* electrodes. A water treatment microbial fuel cell (MFC) was built with a *Geobacter* cathode and platinum/carbon anode. The MFC was fed hydrogen from a PEM-based water electrolysis cell. The concentrations of nitrate, nitrite, and ammonia were analyzed before and after one hour of constant potential operation of the fuel cell.

Methods and Materials

Medium and culture conditions.—All chemicals were purchased from Fisher Scientific unless otherwise stated. A *Geobacter sulfurreducens* (ATCC 51573) starter culture was grown in a standard fumarate rich media (pH 6.8) containing 1.5 g/L ammonium chloride (NH_4Cl), 0.6 g/L monosodium phosphate (NaH_2PO_4), 0.1 g/L potassium chloride (KCl), 2.5 g/L sodium bicarbonate ($NaHCO_3$), 0.82 g/L sodium acetate ($C_2H_3NaO_2$), 8.0 g/L sodium fumarate ($C_4H_2Na_2O_4$), and Wolfe's vitamin and mineral solutions from ATCC (2.0 mg/L biotin, 2.0 mg/L folic acid, 10 mg/L pyridine hydrochloride, 5.0 mg/L thiamine HCl, 5.0 mg/L riboflavin, 5.0 mg/L nicotinic acid, 5.0 mg/L calcium D-(+)-panthothenate, 0.1 mg/L vitamin B12, 5.0 mg/L p-aminobenzoic acid, 5.0 mg/L thioctic acid, 10.0 mg/L sodium selenite (Na_2SeO_3), 10.0 mg/L nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), 10 mg/L $Na_2WO_4 \cdot 2H_2O$) at 30°C. The solution was continuously sparged with 20% CO_2/80% N_2 gas (Airgas). Mid-exponential phase cells were harvested at half-maximal optical density (at 600 nm) of 0.28.

The *Geobacter* were optimized for nitrate reduction by subculturing in media where all components were the same, except 7.0 g/L of the sodium fumarate was replaced by sodium nitrate. Nitrate-grown cells were harvested in the mid-exponential phase at half-maximal optical density, then centrifuged at 5000 × g for 15 minutes in CO_2/N_2 flushed centrifuge tubes. The cells were rinsed with fresh media and centrifuged again, twice, to concentrate them. Cells were flash frozen with liquid nitrogen and stored in a −80°C freezer until needed.

Electrode fabrication.—50 μL of thawed, concentrated *Geobacter* was cast on 1 cm^2 Toray carbon paper (non-wet proof Toray-90, Fuel Cell Earth) electrodes and dried under a nitrogen atmosphere for two hours. Then, 50 μL of 4% pectin was cast on the electrodes, cross-linked with 20 μL of 25 mM magnesium chloride, and dried under a nitrogen atmosphere for two hours. After drying, electrodes were stored in 20% CO_2/80% N_2 sparged media until analysis. Generally, analysis was performed within 24 hours of electrode fabrication.

2.35 inch diameter circular Toray carbon paper electrodes for the fuel cell system were prepared in the same manner as described above, but with 800 μL aliquots of *Geobacter* and pectin and 200 μL aliquots of magnesium chloride. Electrodes were immersed in 20% CO_2/80% N_2 sparged media.

Scanning electron microscopy.—Electrodes of unmodified Toray paper, Toray paper modified only with pectin, Toray paper modified with the non-optimized *Geobacter* and pectin, and Toray paper modified with nitrate-optimized *Geobacter* and pectin were fixed with a 4% formaldehyde solution and imaged under low vacuum conditions (0.75 Torr) by scanning electron microscopy (SEM, FEI Quanta 600 FEG).

Electrochemistry.—Cyclic voltammetry (CH Instruments 660E) was performed from 0 V to −0.7 V vs. SCE (saturated calomel reference electrode) at 10 mV/s in a three electrode system where the counter electrode was platinum mesh. All solutions were sparged with nitrogen gas and a nitrogen blanket was maintained during analyses. Electrodes were equilibrated in solution for 20 minutes prior to analysis. The electrodes were allowed to rest for two hours between each CV. Solutions of sodium nitrate ($NaNO_3$), sodium acetate ($C_2H_3NaO_2$), and potassium chloride (KCl) were used for analysis. A solution of synthetic wastewater, pH 7, containing 50 mM each of sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl), monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), and potassium sulfate (K_2SO_4) was also used.[31] Amperometry was performed with the same three electrode system. The potential was held at −0.6 V vs SCE. After the current stabilized for a continuously stirred solution (1000 rpm) of 100 mM sodium acetate, injections of sodium nitrate were made to analyze a concentration range between 0.5 and 270 mM nitrate.

Nitrate remediation system.—A nitrate reducing microbial fuel cell was fed a 0.01 M nitrate solution by a peristaltic pump and fed hydrogen from a proton exchange membrane (PEM)-based water electrolysis cell. Figure 1 is a schematic of the system. The electrochemical cell consisted of Proton's 28 cm^2 production quality fuel cell hardware. A nitrate-optimized *G. sulfurreducens* electrode was placed on the cathode side of the fuel cell such that the microbes were separated from the Nafion PEM (2 mm thick) by a pectin layer. A platinum electrode (3 mg/cm^2 platinum black on carbon paper) was used on the anode side. Hydrogen was supplied to the anode side using a Proton OnSite lab unit (GC-600) which controlled the pressure to 50 psi. The hydrogen could permeate through the membrane (0.25 mL/min) to the cathode to feed the microbes and hydrogen could be sparged into the nitrate contaminated water directly. A peristaltic pump circulated disinfectant, process water, or rinse water depending on the operation mode. Temperature was controlled via a heating jacket surrounding an inlet tube to the stack. All experiments took place at 37°C (temperature conditioning involved equilibration for 20 minutes) and used 100 mL of fluid volume for circulation at 70 mL/min. Once the *Geobacter* electrode was placed in the fuel cell, it was incubated for 3 days in nitrate rich media before draining the system and flushing it with deionized water. Dissolved organic nitrogen concentration was monitored spectrophotometrically at 220 nm to confirm that the rinse procedure flushed a majority of the media components from the system.[34] This technique was also used to rapidly screen process water for nitrate.

Cyclic voltammetry was used to verify that the electrodes were still able to perform nitrate bioelectrocatalysis before constant potential experiments were conducted. The system was tested with a solution of 10 mM nitrate and held at a constant potential of −0.144 V vs. SCE for one hour. Samples before and after the one hour were evaluated by Steams DHIA Laboratories for nitrate and nitrite concentrations with a standard procedure (SM 4500-NO3-F-97) in which nitrite is diazotized with sulfanilamide and coupled to N-(1-naphthyl)-ethylenediamine dihydrochloride to form an azo dye that can be colorimetrically measured. Nitrate is quantitatively converted to nitrite by cadmium reduction before measurement. Proton OnSite determined ammonia concentrations by the indophenol blue colorimetric method where, in the presence of an oxidizing agent and alkali, ammonia reacts with phenol to form indophenol. Nitroferricyanide acts as a

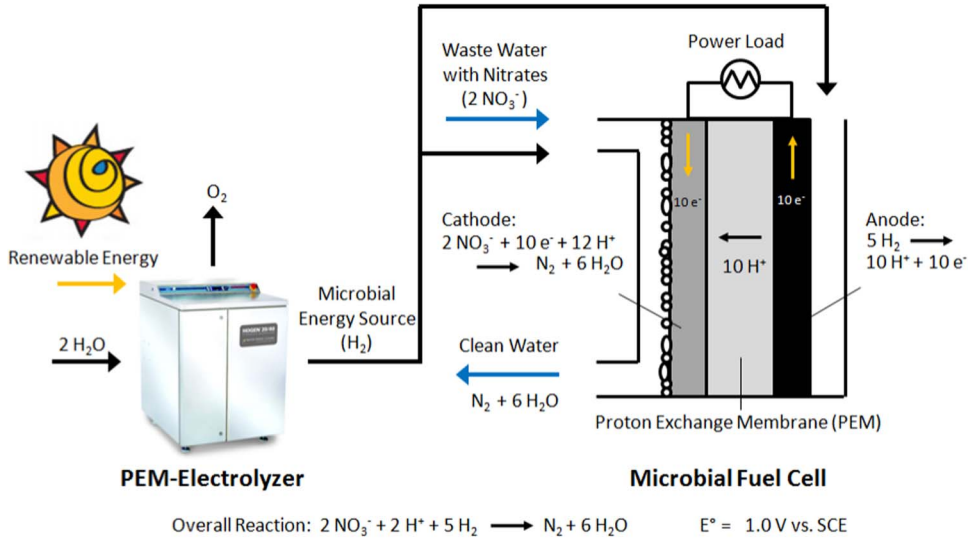

Figure 1. A schematic of the nitrate remediation system. A proton exchange membrane (PEM) based electrolyzer is integrated with a microbial fuel cell.

catalyst. A blue color develops in the samples and correlates with the concentration of ammonia present. The solutions were measured in a spectrophotometer (Radiometer Analytical XR400) at 630 nm.[35]

Results and Discussion

Scanning electron microscopy.—Figure 2 shows SEM micrographs of unmodified Toray carbon paper, Toray paper modified with pectin, Toray paper modified with pectin and non-optimized *G. sulfurreducens*, and Toray paper modified with pectin and nitrate-optimized *G. sulfurreducens*. Carbon fibers approximately 10 μm in diameter can be observed in all cases. When *Geobacter* are present, the bacteria can be observed as approximately 2 μm by 0.5 μm cylinders. The *Geobacter* appear to be intact after electrode fabrication. There is no major physical difference observed between the non-optimized *Geobacter* and the nitrate-optimized *Geobacter*. A biofilm has not been established given the spacing between microbes. The same amount of time elapsed between electrode fabrication and electrochemical characterization as electrode fabrication and SEM imaging, thus it can be assumed that no biofilm is formed at the time of the electrochemical assays.

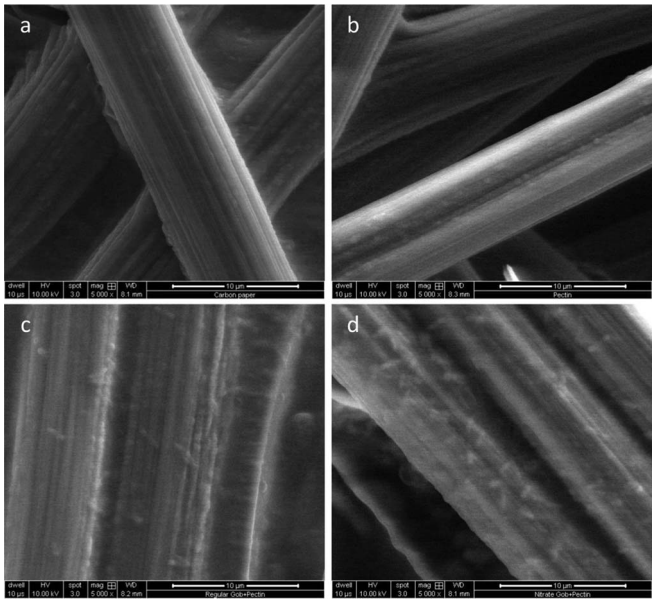

Figure 2. 5000× SEM images of formaldehyde fixed a) unmodified Toray paper, b) Toray paper modified with pectin, c) Toray paper modified with pectin and non-optimized *G. sulfurreducens*, and d) Toray paper modified with pectin and nitrate-optimized *G. sulfurreducens*. Scale bars are 10 μm.

Electrochemical assays of the Geobacter electrodes.—Cyclic voltammograms (CVs) in Figure 3 demonstrate bioelectrocatalytic nitrate reduction by the nitrate-optimized *G. sulfurreducens* electrode. A bioelectrocatalytic signal with onset potential near −0.25 V vs. SCE is observed for electrodes modified with *Geobacter* and pectin in the presence of nitrate. The CV morphology and onset potential is similar to that observed for *G. sulfurreducens* in the presence of fumarate and acetate[33] and similar to that observed for nitrate reduction by *Pseudomonas alcalipha*.[36] No significant signal is observed for unmodified electrodes nor for electrodes modified only with pectin in the presence of nitrate. The presence of the pectin film slightly increases capacitance, as expected for a biopolymer modified electrode. CVs are shown for solutions of 100 mM and 270 mM

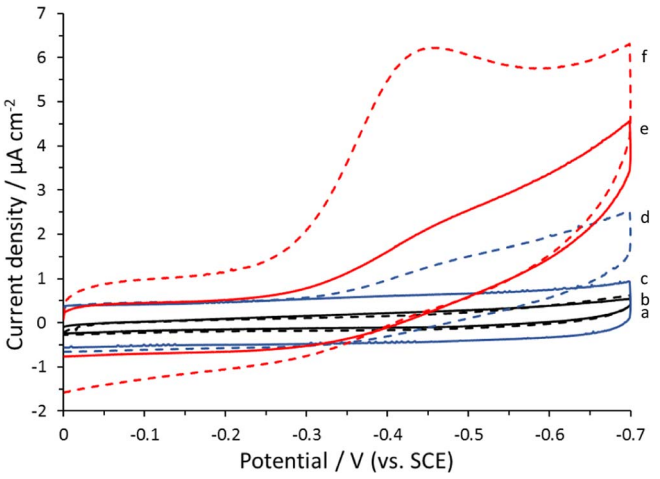

Figure 3. Overlaid 10 mV/s cyclic voltammograms for a) an unmodified Toray paper electrode in 270 mM sodium nitrate and 100 mM sodium acetate (black solid line), b) a Toray paper electrode modified with pectin in 270 mM sodium nitrate and 100 mM sodium acetate (black dashed line), and Toray paper electrodes modified with pectin and *G. sulfurreducens* in c) 100 mM potassium chloride (blue solid line), d) 100 mM sodium acetate (blue dashed line), e) 100 mM sodium nitrate and 100 mM sodium acetate (red solid line), and f) 270 mM sodium nitrate and 100 mM sodium acetate (red dashed line).

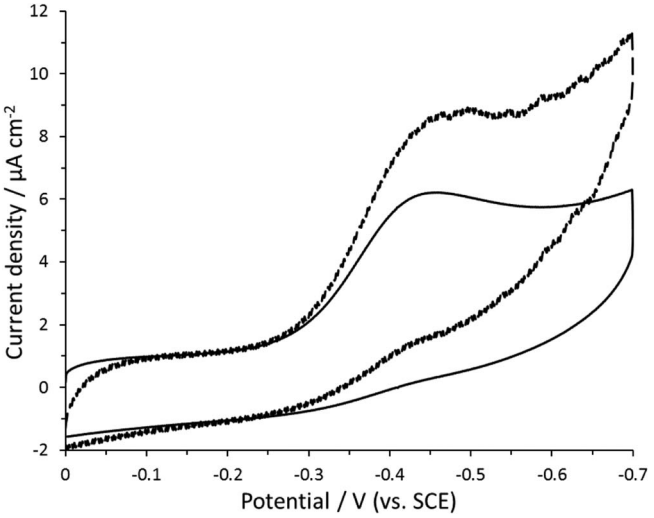

Figure 4. Overlaid 10 mV/s cyclic voltammograms for Toray paper electrodes modified with pectin and *G. sulfurreducens* in 270 mM sodium nitrate and 100 mM sodium acetate. Black solid line was measured in a stationary solution. Black dashed line was measured in a continuously stirred solution (1000 rpm).

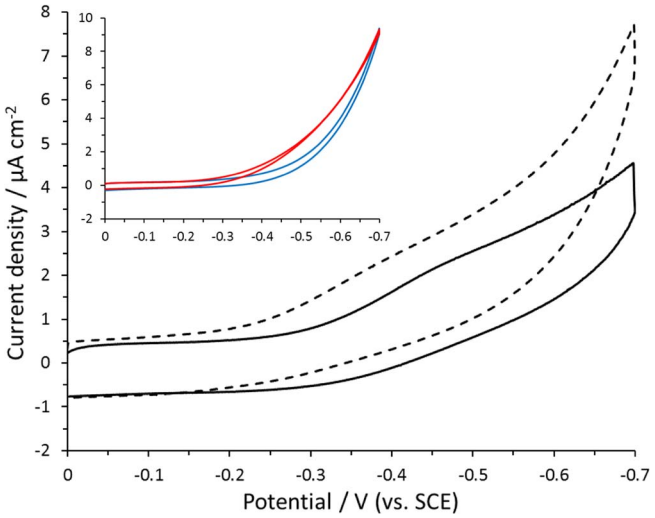

Figure 5. Overlaid 10 mV/s cyclic voltammograms for Toray paper electrodes modified with pectin and *G. sulfurreducens* in 100 mM sodium nitrate and 100 mM sodium acetate (black solid line), and synthetic wastewater consisting of 50 mM each of sodium nitrate, ammonium chloride, monosodium phosphate, disodium phosphate, and potassium sulfate (black dashed line). Inset is overlaid 10 mV/s cyclic voltammograms for Toray paper electrodes modified with pectin and *G. sulfurreducens* in 100 mM ammonium chloride (blue solid line), and 100 mM monosodium phosphate (red solid line).

sodium nitrate with 100 mM sodium acetate. Only very slight signals are observed for electrodes modified with *Geobacter* and pectin in the absence of nitrate (in the presence of a 100 mM potassium chloride solution or 100 mM sodium acetate solution), although the presence of *Geobacter* on the electrode increases capacitance slightly. Acetate was included in the solutions as an electron donor for the *Geobacter* given that the cells were immobilized on the Toray paper, rather than established as a biofilm that would directly transfer a large number of electrons between the cells and electrode to use the electrode as the sole electron donor. When CVs were repeated without rest time, there was a 40 ± 10% decrease in current density. Trials with various rest times showed that two hours were needed to recover the same current density as the initial run. Slightly higher capacitance is observed in the 270 mM nitrate CVs, likely due to the change in relative electrolyte concentration. Taking into account the higher capacitance of the baseline, the peak current density for 270 mM nitrate of 6.5 ± 0.9 µA cm^{-2} is still higher than the peak current density for 100 mM nitrate of 2.2 ± 0.3 µA cm^{-2} (taken at −0.45 V vs. SCE). CVs of Toray paper modified with non-optimized *Geobacter* and pectin do not have a clear bioelectrocatalytic signal and the current density at −0.45 V vs. SCE for 100 mM nitrate/100 mM acetate is 0.5 ± 0.1 µA cm^{-2}.

Figure 4 shows a 10 mV/s CV for a continuously stirred (1000 rpm) solution of 270 mM sodium nitrate and 100 mM sodium acetate overlaid with a CV taken without stirring. The onset potential stays the same while the peak current increases from 6.5 ± 0.9 µA cm^{-2} to 8.9 ± 1.2 µA cm^{-2} due to enhanced mass transport. The same amount of capacitance can be observed in the stirred 270 mM nitrate CVs. Quiescent CVs taken before and after stirring are consistent with each other, indicating that no delamination or loss of electron contact was caused by stirring. Therefore, we predict that the flow system in the microbial fuel cell (described in Methods and materials section) will not negatively affect the performance and may increase the performance.

Cyclic voltammetry was also performed in synthetic wastewater (pH 7) containing 50 mM each of sodium nitrate, ammonium chloride, monosodium phosphate, disodium phosphate, and potassium sulfate. A signal is still observed, although the current is higher than would be expected for only nitrate reduction as shown by the overlay of 10 mV/s CVs for the synthetic wastewater (containing 50 mM sodium nitrate) and for the 100 mM sodium nitrate/100 mM sodium acetate in Figure 5. The current density taken at −0.45 V vs. SCE is 2.2 ± 0.3 µA cm^{-2} for the 100 mM sodium nitrate/100 mM sodium acetate solution and 3.1 ± 0.5 µA cm^{-2} for the synthetic wastewater solution. The higher current observed for wastewater may be due to the *Geobacter* interacting with other compounds in addition to nitrate. Ammonium chloride and monosodium phosphate are compounds also used in the *Geobacter* growth media. CVs of the *Geobacter* and pectin electrodes with 100 mM ammonium chloride only and with 100 mM monosodium phosphate only are shown in the Figure 5 inset. A non-catalytic current increase starting at −0.55 V reminiscent of the hydrogen evolution reaction is observed. The contribution of these currents in the synthetic wastewater CVs likely explains the higher overall current.

Figure 6 shows a current versus nitrate concentration plot obtained by amperometry for a concentration range between 0.5 and 270 mM

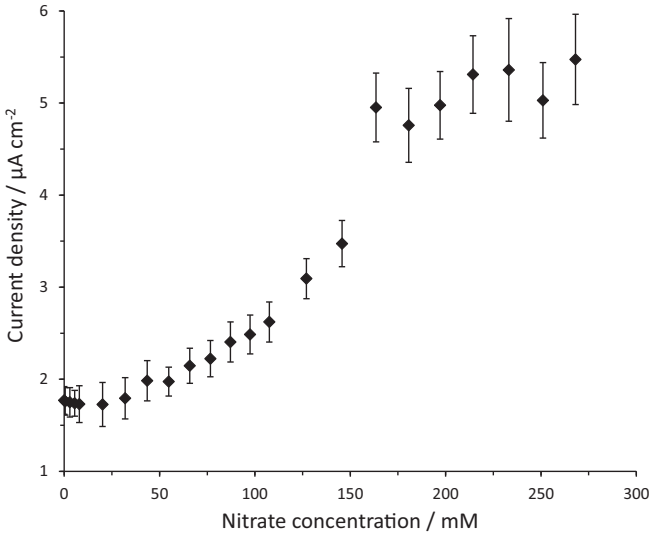

Figure 6. Current density (µA cm^{-2}) versus concentration of sodium nitrate (mM) for amperometric analysis of Toray paper electrodes modified with pectin and *G. sulfurreducens*. Injections of 1.0 M sodium nitrate and 100 mM sodium acetate were made to a continuously stirred (1000 rpm) solution of 100 mM sodium acetate.

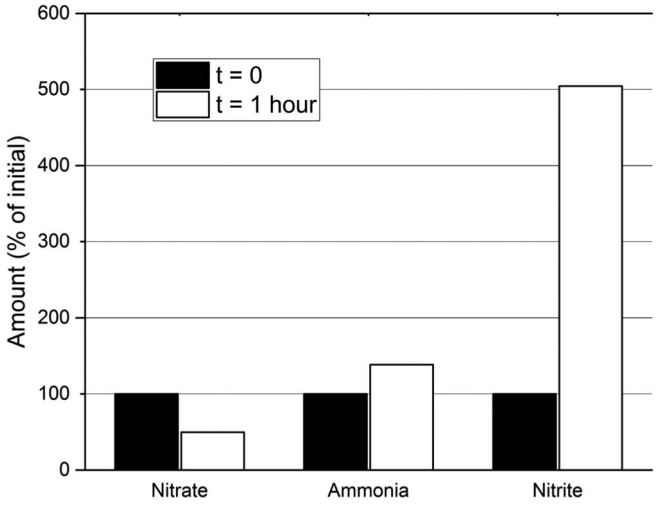

Figure 7. Water composition (in % of initial concentration) of nitrate, ammonia, and nitrite before and after one hour of constant potential operation at −0.144 V vs. SCE in the microbial fuel cell. Black bars are before operation and white bars are after one hour of operation.

nitrate. The limit of detection is 8 mM. with a linear range of 20 mM to 160 mM. Evaluation by a Michaelis Menten kinetic model yields a K_M of 110 ± 10 mM.

Nitrate remediation system.—During initial tests, with no media circulation prior to operation, a constant potential of −0.644 V vs. SCE (400 mV overall cell potential) was applied for six hours. The spectrophotometric technique did not detect major changes in composition of the process water over the six hours. After circulating nitrate-rich media on the cathode side for three days, the collected media was turbid upon drainage, indicating bacterial growth occurred. After operating a cell with 10 mM nitrate at a constant potential of −0.144 V vs. SCE for one hour, the spectrophotometric technique detected major changes in the composition of the process water. Figure 7 shows the concentrations of nitrate, nitrite, and ammonia determined by the azo dye colorimetric method and the indophenol blue colorimetric. There was a significant decrease in nitrate of about 50% (from 139 ppm to 69 ppm) corresponding with an increase in ammonia of 138% (from 7.22 to 9.99 ppm) and an increase in nitrite of 500% (from 0.22 to 1.11 ppm).

The resulting remediation rate from this preliminary data is 6 mg nitrate/cm^2/day, which surpasses the estimated rate of 4 mg nitrate/day/cm^2 needed to make the technology economically feasible compared to competing technologies. This system compares favorably to similar competing technologies. Biofilm electrode reactors (BERs) consist of immobilized bacteria on an electrode, with reduction stimulated electrochemically. Typically, the hydrogen for this reaction is co-evolved at the electrode. This technique is cited for being simple and easy to operate, and selective for reduction of nitrate to nitrogen gas. However, because the hydrogen is produced on the same electrode as the bacteria, complications arise achieving adequate hydrogen formation and distribution as the biofilm matures and scaling occurs. Reported BER removal rates range between 0.2–1 mg nitrate/cm^2/day.[7] Membrane biofilm reactors (MBRs) address the hydrogen distribution issue by feeding the microbes hydrogen gas through a membrane (typically hollow-fiber membranes), but there is no electrochemically driven process. This technique has seen high removal rates, likely because of the improved hydrogen distribution. Reported MBR removal rates range between 0.1–6 mg nitrate/cm^2/day.[17] Here, a hybrid of these two approaches has been created, which combines the benefit of electrochemically supplying the electrons and driving the reactions with efficient supply of hydrogen to the bacteria. It is encouraging to see that preliminary work yields a remediation rate similar to the best rates recorded for MBRs.

Thermodynamic analysis based on the cathode reaction in Equation 1 and the anode reaction in Equation 2 yields a theoretical cell potential of 1.0 V.

$$2NO_3^- + 10e + 12H^+ \rightarrow N_2 + 6H_2O \quad E^0 = 0.76\ V\ vs.\ SCE \quad [1]$$

$$5H_2 \rightarrow 10H^+ + 10e \quad E^0 = -0.24\ V\ vs.\ SCE \quad [2]$$

$$2NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O \quad E^0_{cell} = 1.0\ V \quad [3]$$

The biocathode open circuit potential (OCP) was measured 0.1 V vs. SCE in the three electrode setup with 100 mM nitrate and there is negligible overpotential for platinum black anodes, resulting in a calculated cell potential of 0.34 V. The microbial fuel cell had an average current of 0.1 mA for the operation at 100 mV overall cell potential, producing 10 μW or 36 mJ in one hour. Assuming all electrons from the 0.1 mA were related to the reaction in Equation 3, 0.002 mmoles of H_2 were reduced at the electrode over one hour. From the moles of nitrate reduced, Equation 3 can be used to calculate that 0.3 mmoles of H_2 were consumed by the *Geobacter* in the one hour period. The electrolyzer cycled on and off at 1.8 A cm^{-2} and 2 V (100 W) to deliver a constant 50 psi of H_2 to the anode. 0.25 mL/min H_2 (0.7 mmoles/hour) crossed the PEM to the cathode. Assuming 100% faradaic efficiency for the electrolyzer, 0.7 mmoles of H_2 were delivered to the MFC over the one hour period and 140 C were used. The electrolyzer was on for 3 seconds in the hour and expended 300 J. For an assumed heat loss of 2 K/hour, the system lost 800 J in heat over the hour. Given that this system has not been optimized, preliminary data are promising and it is expected that a greater proportion of the nitrate reduction will happen electrochemically as the electrode integration with a real world system is tuned.

Conclusions

G. sulfurreducens electrodes bioelectrocatalytically reduce nitrate. This makes them a good candidate for groundwater remediation devices. Results from the fuel cell/electrolyzer system have great implications toward a cost-effective, low-maintenance, and green remediation technology for nitrate contamination. By combining the microbial fuel cell with an electrolyzer, a low-maintenance system emerges which does not require the addition of chemicals; the water electrolyzer provides hydrogen gas to feed the microbes. This system could be easily integrated with a renewable energy source. The achieved nitrate remediation rate of 6 mg nitrate/cm^2/day makes the technology cost-effective in comparison with other technologies. Thus, this system could be ideal for use in isolated, economically poor, rural areas.

Acknowledgments

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