

DEVELOPMENT OF NOVEL BIOELECTROCHEMICAL SYSTEMS
FOR IN SITU NITRATE REMOVAL FROM GROUNDWATER

by

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ABSTRACT
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Under the Supervision of Professor Zhen He

This study aims to develop new approaches for in situ nitrate removal from groundwater by using bioelectrochemical systems (BES). BESs employ bioelectricity generated from organic compounds to drive nitrate moving from groundwater into the electrode chamber and reduce nitrate to nitrogen gas by heterotrophic denitrification. In first phase experiments, nitrate ions were driven into the anode chamber of a de-coupled reactor, whose electrode chambers were separated, where heterotrophic denitrification took place with organic reduction. It was proved that by applying additional electrical potential at 0.8V, the reactor could achieve highest removal rate of $208.2 \pm 13.3 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$, when initial nitrate concentration in synthetic groundwater well is around 20 mg/L. Removal rate appeared a linear relationship with the initial nitrate concentration in groundwater. Electricity not only enhanced nitrate removal rate but also could inhibit ion exchange and prevent introducing other undesired ions into groundwater. In second phase experiments, the BES reactor was modified to single tubular. Nitrate ions transport across anion exchange membrane (AEM) into a mid-chamber between anode chamber and cathode, where they were concentrated and finally lead into anode chamber to be biological denitrificated. The

slower mid-chamber water flowing, the less cost would be for operation and the flow rate affect slightly to nitrate transport. It was found that nitrate concentration could reach equilibrium after about 17 hrs. Protons produced from cathode reaction were more likely travel across AEM into mid-chamber, which restricted nitrate ions' movement. The BES was also proved feasible when applying in real groundwater and tended to produce more coulomb of charge. Further development of this BES will need to address several key challenges

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Chapter 1 Introduction to groundwater remediation and bioelectrochemical systems

1 Groundwater contaminants

Mostly groundwater contamination is a result of poorly designed hazardous waste disposal facilities, leakage from underground storage tanks and mine tailings, accidental spills and application of fertilizers and pesticides in agriculture, resulting in a threat to groundwater supply and risk to human health [1].

Processes that remove pollution from groundwater are called groundwater remediation. It is required when concentrations of contaminants exceed or are expected to exceed the predetermined contamination levels for resource [2]. When a certain contaminant is released from resource; porous soil tend to transmit water along with the contaminant then move within aquifer in the same manner that groundwater moves[3]. Because of the generally slow movement of groundwater, contaminants remain concentrated in the form of a slowly moving plume [4]. To some degree it's possible to predict the movement of a plume hence the movement of pollutants.

2 Groundwater remediation strategies and technologies

Common groundwater remediation strategies for cleanup contamination problem include [4]: 1. complete source removal; 2. Source or plume containment; and 3. Mass reduction methods. Categorized by where it is taken place, remediation can be *ex-situ* or *in-situ*. A common means to contain or remediate *ex-situ* is to extract the water by

wells or drains, which is referred to as pump-and treat technology [5], [6]. In a pump-and-treat process, an extraction well is installed and contaminated groundwater is captured and pumped through this well to surface to be treated. In some cases injection wells are used for flushing towards extraction wells or drains for enhancement [6]. Commonly used in-situ techniques include:

1. Hydraulic containment. For relatively simple flow through aquifer, people can change the flow of plume in order to keep contaminants away from wells by pumping groundwater [5].
2. Air sparging. It is a cost-effective technique removing VOCs from unsaturated zone. The equilibrium between VOC phases is disturbed causing enhanced partitioning into vapor phase when air is purged in. Gas vapor then is treated using off-gas treatment systems [7].
3. *In-situ* oxidation. This technique introduces chemical oxidation transforming contaminant mass that stacked in soil or groundwater into less harmful chemical. Two most commonly used forms of injected oxidations are permanganate (MnO_4^-) and Fenton's (hydrogen peroxide [H_2O_2] and Ferrous iron [Fe^{+2}]) [8]. Primarily oxidation productions are carbon dioxide and water.
4. Permeable Reactive Barriers. It's an alternative to conventional pump-and-treat technique. Barriers are placed at the path of migrating contaminant plume. Proper reactive materials within the barrier are to promote reactions with pollutants [9].
5. Phytoremediation. Take advantages of some plant which can absorb metal elements, nutrients with their root system grounded in subsurface aquifer. When determine whether using a typical technique or combing several accesses together, people should take following factors into consideration such as hydrogeologic setting, contaminant characteristics, physical

properties (sink or float), chemical properties (solubility, sorption), subsurface access, land use, toxicity-risk, and cost [5].

3 Introduction to bioelectrochemical systems

The concept of converting chemical energy to electrical energy using living microorganisms or their enzyme systems was raised early [10] but undoubtedly is the working principle of a bioelectrochemical system (BES) in later researches. Although people presented treating domestic wastewater with microbial fuel cells (MFCs) in the early 1990s [11], due to limitation of power output only recently MFCs have developed providing possible opportunities for practical applications [12].

3.1 MFC working mechanisms

3.1.1 Anode

Generation of electricity in BES is basically due to bacteria metabolism [13]. Bacteria grow by catalyzing chemical reactions and storing the harnessed energy in the form of adenosine triphosphate (ATP). Some species of bacteria transfer electrons which are generated from reduced substance oxidation to respiratory enzymes then finally released to a terminal electron acceptor while protons flows back into the cell through enzyme ATPase creating ATP from adenosine diphosphate (ADP). Soluble substances such as nitrate sulfate or oxygen or some insoluble metal oxides such as Fe (III) and Mn (IV) can act as electron acceptors. Bacteria in bioelectrochemical systems do not directly transfer the electrons to their terminal electron acceptors;

instead, electrons are released to an electrode (the anode) [14]. This is how bioenergy turning into electricity, which links microbiology and electrochemistry [15]. Electron extraction from bacteria to electrodes has few many pathways [16]: by mediators (neutron red or anthraquinone-2, 6-disulfonate) or shuttles, by nanowires produced by bacteria, or by direct membrane associated electron transfer. Additional mediators or redox mediators produced by bacteria physically transport electron from intracellular compound, which becomes oxidized, to electrode surface [12]. Nanowires are electrically conductive pili that allow bacteria reach solid electron acceptor such as electrode. Even if bacteria are not in direct contact with electrode, they can release electrons via the pili [15]. Every pathway has its advantages and disadvantages. At current stage of knowledge, direct electron transfer allows high coulombic efficiencies but delivers low current and power output [16]. However there still exist difficulties in using mediators at a commercial scale [17].

So far pure strains such as *Shewanella putrefaciens*, *Escherichia coli*, *Geobacter sulfurreducens* and *Rhodospirillum rubrum* are found to be capable of transferring electrons from carbon source to electrode [12]. It has been proved that mixed communities typically produce higher current density and more power output than pure cultures [18]. Except for studying the process of electron transfer, mixed culture is more practical enhancing real application of BES.

3.1.2 Separator

Separator is one of the most important components in MFCs [19]. In most BES electron reaching cathode is accompanied with protons' transportation into anode chamber or other anion species' diffusion into anode chamber across separator in order to maintain charge balance [14]. Proton exchange membranes (PEMs) are the mostly used separator in membrane-based BESs [20] while cation exchange membranes (CEMs) are sometimes used as they are less expensive and structurally stronger [21]. Rozendal et.al [22] have found that other cations in anode medium are typically more responsible than protons transport across Nafion membranes, resulting in increased pHs at cathode and reduced voltage. Some research proved feasibility of substituting PEMs with anion exchange membranes (AEMs), ultrafiltration membranes, microporous filtration membranes, osmosis membranes, or even membrane less [18] [23] [24]. As to sediment MFC which extracts electric energy from organic-rich aquatic sediments, separator is sediment that embeds anode [25].

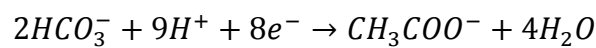
3.1.3 Cathode

Electrons extracted from cathode flowing via external circuit are to participate in cathode reduction. Cathode used for BESs are often carbon electrodes with or without coated catalyst. Pt is the most commonly used cathode catalyst but its high cost restrains commercial applications. Alternative applicable catalysts on cathode are reported such as transition metal porphyrines and phthalocyanines [26], activated carbon [27], etc. Catalysts on cathode are often required when oxygen is the electron

acceptor. Due to the poor kinetics of oxygen reduction in cathode chamber, more effective catholyte such as ferricyanide is considered to substantially increase power output [28]. No catalyst is necessary however ferricyanide must be replaced after converted to ferrocyanide while oxygen can be used continuously [26]. Meanwhile ferricyanide is not good for sustainable usage and may raise environmental risk if it is to be commercially applied. Disadvantages of conventional abiotic cathode can be overcome by biotic cathode to assist cathode reactions [29]. Besides reducing the cost of abiotic catalyst and increasing operation sustainability, some microorganism in biocathode may produce oxygen such as algae for cathode reduction, or remove other unwanted compounds e.g. Fe (III), Mn (IV), and nitrate compounds, etc [27].

3.2 thermodynamics and electromotive force

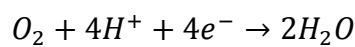
Suppose acetate is oxidized by bacteria in anode chamber. Reaction is written as consuming electrons according to IUPAC regulation [30]:



Deducted from Nernst equation and Gibbs free energy, theoretical anode potential is expressed as [14]:

$$E_{An} = E_{An}^0 - \frac{RT}{8F} \ln \left(\frac{[CH_3COO^-]}{[HCO_3^-]^2 [H^+]^9} \right)$$

When oxygen is the acceptor of electron in cathode, reaction is written as:



Similarly we have theoretical cathode potential as:

$$E_{cat} = E_{cat}^0 - \frac{RT}{4F} \ln \left(\frac{1}{pO_2[H^+]^4} \right)$$

Cell potential is calculated as: $E_{emf} = E_{cat} - E_{An}$. Theoretically it will not exceed 1.2V [14]. Factors that may cause potential loss in a BES come from ohmic polarization, activation losses during transport of electrons from or to compound reacting at electrode surface, bacterial metabolic losses and concentration losses which occur when the rate of mass transport of species from or to electrode surface limits current production [14] [31].

4 BESs configurations and applications

MFCs, which are the most recognized species of BESs, have many configurations. Traditional H-type two chamber MFCs are suitable for basic parameter research [14] for discovering anode and cathode mechanism, testing new materials that can enhance electricity production or for pure culture studying. As plenty of researches focus on increasing electrical production and organic reduction, some variations on basic designs have emerged such as flat plate MFCs [32], tubular MFCs [33], single chamber MFCs [34], and MFCs stacks [35].

If modified to add a saline solution chamber between anode and cathode chamber containing saline solution, MFC turns into a desalination device while simultaneously reducing organic matters [36]. When electricity generated from the reactor, electrical force will drive the movement of anions and cations into anode and cathode, respectively. They are referred to as microbial desalination cells (MDCs). Some

researchers place anode into aqueous sediment rich in organic matters making MFCs become sediment MFCs, which have potentials on powering marine devices or remote control sensors [37]. By applying additional potential to assist potential generating by bacteria in the anode, we can probably achieve hydrogen producing in cathode chamber [14]. These BESs are named microbial electrolysis cells (MEC). Since hydrogen production in cathode can happen only when overcoming energy barrier of the reaction, certain voltage is necessary. MECs play the role of converting electrical energy to H_2 , which is another form of energy. Other applications of BESs include biosensors [38], bioremediation [39], etc.

5 Groundwater remediation with BESs

Morris [40] reported a single-cell MFC that utilized petroleum contaminants as sole anode substrate for in situ bioremediation of petroleum hydrocarbons in the groundwater. Since that oxygen is only sufficient at or near ground surface, a depletion of oxygen will surely restrict cathode performance. Yuan [41] et.al proposed a tubular air-cathode MFCs submerged in simulated organic sediment to study its potential on bioremediation. It was proved the feasibility of treating sediment, and the reactor achieved relatively higher power output than previous chamber-less sediment MFCs. Nevertheless it was still limited by low oxygen availability

According to existing consumptions on BESs' applications in groundwater remediation, we can conclude that as a combination of biological and chemical

methods, BESs may perform more effective than just using biological or chemical technique themselves. The placement of BESs should be a problem since it is preferable to drill wells than just put them in unsaturated aquifers, which will raise costs on site construction. But fascinate of BESs makes it still worth trying in groundwater *in-situ* remediation.

Chapter 2 Nitrate removal from groundwater driven by electricity generation and heterotrophic denitrification in a bioelectrochemical system

1 Introduction

Human activity is extensively affecting global nitrogen circulation. Due to an increasing demand for more agricultural/industrial production and other resources because of a higher living standard and increased population, the discharge of nitrogen compounds such as inorganic nitrogen (e.g., ammonia/ammonium, nitrogen oxide, and nitrate) and organic nitrogen (e.g., urea, amines and protein) via water use also rises [42]. Excessive release of nitrogen and other nutrients into water bodies is responsible for eutrophication of aquatic systems, which deteriorates water quality[43]. High doses of nitrate, and nitrite, a reducing product of nitrate, also pose a potential risk to public health through human consumption of drinking water[44–47]. Because of its strong mobility, nitrate can move into groundwater with leachate or runoff. Groundwater plays a critical role in the supply of drinking water: in the United States, groundwater is the primary source of drinking water for 44% of the population[48]. Therefore, the United States government has established a strict standard for nitrate concentration in drinking water produced from groundwater, and groundwater contaminated with nitrate must be properly remediated before use[49].

The removal of nitrate can be accomplished by using physical, chemical, and biological methods[50]. For example, ion exchange is commonly used in producing drinking water from groundwater[51], in which the cations in groundwater are

exchanged with sodium ions and the anions are replaced by chloride ions when flowing through the packed ion exchange resin[52]. Reverse osmosis has not been particularly applied to remove nitrate, but its non-selective, semi-permeable membrane can stop many contaminants that may include nitrate. Biological nitrate removal is attractive because of its cost-effectiveness. Denitrification is commonly used to remove nitrate from sewage and natural water bodies including groundwater [53–55]and can be carried out by both heterotrophic and autotrophic bacteria[54, 56]. Nitrate removal from groundwater is conducted by either *ex situ* or *in situ* approaches, and *in situ* treatment is preferred because it avoids the use of the energy-intensive pump-and-treat approach[57].

Bioelectrochemical systems (BES) are a relatively new concept for removing organic matters (including some hazardous materials[58–60]) from water and wastewater, while simultaneously producing electricity[61]. Prior laboratory studies have demonstrated that BES can also be applied for nitrate removal, mainly from wastewater through autotrophic or heterotrophic denitrification; in such systems, electrons are produced from organic oxidation in the anode and transferred to a cathode electrode from which autotrophic denitrifying bacteria accept electrons for nitrate reduction.

A recent study applied the BES concept to nitrate removal from groundwater[62]. This submerged microbial desalination-denitrification cell takes advantage of the

working principles of both microbial desalination cells (MDCs) and bioelectrochemical denitrification in microbial fuel cells (MFCs): nitrate ions migrate into an anode compartment driven by an electric potential and then are transported into a cathode compartment for bioelectrochemical denitrification; an additional nitrifying bioreactor was added to the system to ensure that ammonium in the anode solution was converted to nitrate. The system achieved 90.5% removal of nitrate and exhibited a promising application of BES for *in situ* nitrate remediation in groundwater. However, the additional nitrifying bioreactor in this system could make the reactor construction and operation more complex and expensive; furthermore, since nitrate would first migrate into the anode compartment, it can be removed through heterotrophic denitrification, which could be more efficient than autotrophic denitrification in the cathode and may also avoid the limitation of using nitrate as a terminal electron acceptor of the cathode electrode.

In this study, we have advanced the above system through simplifying the reactor structure and operation, accomplished nitrate removal in the anode of a BES, and improved the nitrate removal rate by applying external electric potentials. Nitrate was attracted from groundwater into an anode compartment by an electric force, and then heterotrophic denitrification occurred in the presence of organic compounds, shown as Figure 1.

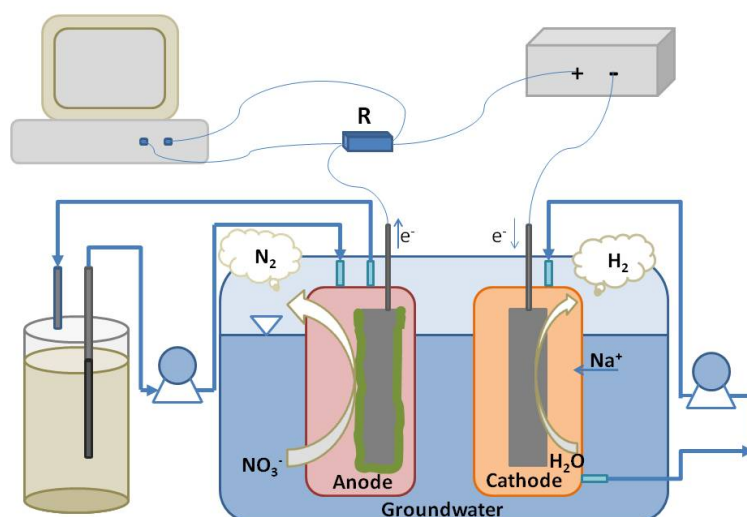


Figure 1: Experimental setup of a bioelectrochemical system (BES) for nitrate removal from groundwater. The BES is controlled by a power supply and the data are collected by a computer.

To examine its performance, we operated the present BES under several conditions with or without externally applied potential, and the effect of different applied potentials was studied. We investigated the role of ion exchange in nitrate removal and the influence of nitrate concentration on the BES performance. We also examined the nitrate removal in either a synthetic or an actual groundwater. The results are expected to provide a foundation for further development of a practical technology for *in situ* nitrate removal from groundwater.

2 Materials and methods

2.1 BES construction

The BES reactor consisted of two separated tubular compartments made of porous PVC tubes wrapped by an ion exchange membrane that was submerged in a 2-L beaker containing 1 L of either synthetic or actual groundwater (Figure 1). The anode

compartment was created by a tube of an anion exchange membrane, and the cathode compartment was contained in a tube of a cation exchange membrane (Membrane International Inc., Ringwood, NJ, USA). The anode compartment contained a carbon brush as an anode electrode, resulting in an anode liquid volume of 90 mL while the cathode compartment had carbon cloth as a cathode electrode coated with 0.5 mg Pt /cm² (prepared as previously described[63]), and a cathode liquid volume of 160 mL. The anode and the cathode electrodes were connected to an external circuit across a resistor of 10 Ω with or without being linked to a power supply.

2.2 BES operation

The BES was operated in a batch mode at a room temperature of $\sim 20^{\circ}$ C. The feeding solution to the anode compartment was prepared as: 0.5 g/L CH₃COONa; phosphate buffer solution (diluted from 1 M phosphate buffer solution containing 107 g/L K₂HPO₄ and 53 g/L KH₂PO₄); NH₄Cl, 75 mg/L; NaCl, 250 mg/L; MgSO₄, 7.5 mg/L; CaCl₂, 10 mg/L; NaHCO₃, 50 mg/L; trace mineral solution 0.1 mL/L[64]; and 1 g/L yeast extract. The conductivity of the fresh anode feeding solution was between 9-10 mS/cm and its pH was around 7. The anode feeding solution was purged with nitrogen gas before use. The catholyte was a 100 mM phosphorus buffer solution and was purged with nitrogen gas when the BES was operated in an MEC (microbial electrolysis cell) mode. The anode compartment was connected to a reservoir containing 400 mL of the anolyte. Both the anolyte and the catholyte were recirculated at 100 mL/min by using a peristaltic pump. The synthetic groundwater

was prepared by dissolving NaNO_3 in tap water. The actual groundwater was collected from No.BQ185 well, Wisconsin, USA. The anode was inoculated with digested sludge (South Shore Water Reclamation Facility, WI, USA) at a volume ratio of 5%. The anode feeding solution and the groundwater were replaced with fresh solutions every 24 hours.

The BES was operated under several different conditions: open circuit, the MFC mode (without externally applied potential), and the MEC mode (with an externally applied potential). In the MEC mode, a power supply (3645A, Circuit Specialist Inc, AZ, USA) was used to apply 0.5 V, 0.8 V or 1.0 V to the circuit, as previously described[63]. To maintain the conductivity and buffering capacity of the anolyte when using a low concentration of phosphate buffer solution (1 mM), 3.3 g of NaHCO_3 was added into the 400-mL of the anolyte.

2.3 Measurement and analysis

The BES voltage was recorded every 5 minutes by a digital multimeter (2700, Keithley Instruments, Inc., Cleveland, OH, USA). The pHs of the anolyte and the catholyte were measured by a benchtop pH meter (Oakton Instruments, Vernon Hills, IL, USA). The conductivity was measured by a benchtop conductivity meter (Mettler-Toledo, Columbus, OH, USA). The concentrations of chemical oxygen demand (COD), nitrate-nitrogen (NO_3^- -N), nitrite-nitrogen (NO_2^- -N), and total phosphate were measured using a colorimeter following the manufacturer's

instructions (Hach DR/890, Hach Company, Loveland, CO, USA). Nitrate removal rates were calculated based on the anode liquid volume. Two types of coulombic efficiencies, based on COD in the anode compartment or nitrate from groundwater, were calculated as the total coulomb output (integrating the production of time and current) divided by the total coulomb input (either the moles of COD removed assuming 4 mol of electrons/mol of COD, or the moles of nitrate removed from the system assuming 5 mol of electrons/mol of nitrate), according to the following equations:

$$CE_C = \frac{Q_{output}}{Q_{input}} = \frac{\sum It}{F \times COD_{removed} \times 4}$$

$$CE_N = \frac{Q_{output}}{Q_{input}} = \frac{\sum It}{F \times N_{removed} \times 5}$$

where CE_C is the Coulombic efficiency based on organic substrate, CE_N is the Coulombic efficiency based on nitrate, Q_{output} is the produced charge, Q_{input} is the total charge available in the substrate, F is the faraday constant (96485 C/ mol e^-), I is the electric current (A), and t is time (s). $COD_{removed}$ is the COD removed by the BES (mol) in the period of time t , and $N_{removed}$ is the amount of nitrate removed (mol) within time t .

3 Results and discussion

3.1 Effects of operating conditions

The BES was started in an MFC mode (without applying an external potential) for two weeks and then switched to an MEC mode that applied 0.8 V to the electrical

circuit. After a period of two months, the BES achieved an average current density of 28.2 A/m^3 at 0.8 V and a relatively stable removal of nitrate from the synthetic groundwater. The current generation exhibited a typical batch profile with a peak current of $35\text{-}40 \text{ A/m}^3$ upon replacement of the anode feeding solution, the cathode buffer and the synthetic groundwater, followed by a decrease to $24\text{-}25 \text{ A/m}^3$ (Figure 2A). With the use of a phosphate buffer solution, the pHs of the anolyte and the catholyte were maintained at 7.2 ± 0.1 and 7.4 ± 0.0 , respectively. The COD concentration in the anolyte was about 550 mg/L at the end of a batch, resulting in $66.9 \pm 7.7\%$ removal within one cycle; we

found that $15.7 \pm 1.6\%$ of COD was converted into electricity. Nitrate nitrogen in the synthetic groundwater was removed by 77.3% within 24 h (Figure 2B). The anode effluent contained a very low concentration of nitrite (below 0.1 mg/L) and almost no nitrate. The theoretical nitrate removal rate in the BES was $208.2 \pm 13.3 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$.

It was observed that the decrease in nitrate concentration in the synthetic groundwater

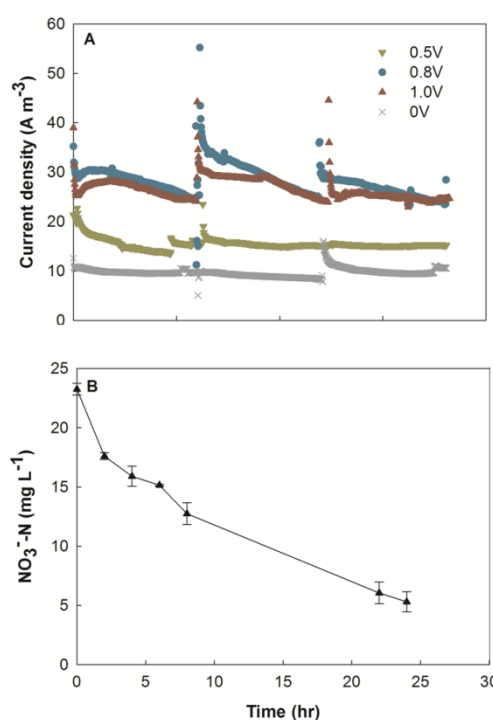


Figure 2: Current production in the BES under different operating conditions (A), and the nitrate concentration variation in the synthetic groundwater under an applied potential of 0.8 V (B).

was greater in the first 10 hours and then relatively slowed down, likely affected by current generation, which was related to organic supply. The consumption of organic

compounds in the anode compartment was mainly by electricity generation (electrochemically-active microbes), denitrification (denitrifying bacteria), and other microbial processes. With the nitrate removal from the synthetic groundwater, more and more nitrate ions migrated into the anode compartment and increased the competition of organic compounds among those microbial processes. As a result of this and other continuing consumption of organic compounds over time, the electric current became smaller and the electrical force for continuing nitrate removal became weaker. Consequently, the nitrate removal from the synthetic groundwater became slower. Those results indicate that, to ensure an efficient nitrate removal from groundwater, the organic supply should be sufficient, and/or the activity of the electrochemically active microbes should be further improved (e.g., to improve Coulombic efficiency).

To further understand the effect of the operating conditions, the BES was examined in the MEC mode with 0.5 or 1.0 V, and in an MFC mode without any additional potential. As shown in Figure 2A, the MEC mode with 0.8 or 1.0 V produced higher current densities than that with 0.5 V while the MFC mode ("0 V") generated the lowest current. Accordingly, the highest nitrate removal rate ($208.2 \pm 13.3 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$) was obtained with 0.8 V and the lowest one ($154.2 \pm 24.4 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$) was in the MFC mode. The MEC mode also significantly improved the Coulombic efficiencies based on either organics or nitrate. Better performance with 0.8 V than 0.5 and 0 V was because a higher energy input increases current generation,

as observed in other MEC studies[65, 66]. However, the 1.0-V condition did not lead to more current production than 0.8 V; the exact reason is not clear at this time. Surprisingly, there was a substantial nitrate removal with a nitrate removal rate of $158.5 \pm 4.2 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$ under an open circuit (no electricity generation), and while in the absence of the cathode chamber, the anode alone achieved a nitrate removal rate $135.0 \pm 3.9 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$, suggesting that other factors, likely ion exchange, played an important role in nitrate transport from the groundwater into the anode compartment. In addition, the Coulombic efficiency based on the nitrate consumption was less than 40%, indicating that the movement of some nitrate ions across the anion exchange membrane was not associated with electricity production.

3.2 Effects of Ion exchange

The nitrate removal from the synthetic groundwater under an open circuit condition (without electricity generation) intrigued us to investigate the effect of an ion exchange on the nitrate transport. The anode compartment contained an anion exchange membrane that allows the movement of anions such as nitrate ions driven by a concentration gradient. In the present study, the anolyte contained 50-mM of a phosphate buffer solution that provided anions (e.g., phosphate ions) to exchange nitrate ions from the synthetic groundwater. We found that the phosphate concentration in the synthetic groundwater reached $36.0 \pm 3.1 \text{ mg/L}$ under the open circuit condition or $28.4 \pm 2.3 \text{ mg/L}$ in the absence of the cathode chamber (Figure 3).

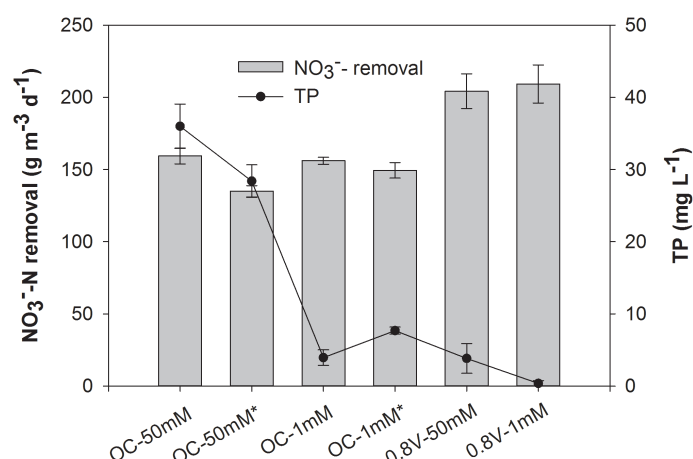


Figure 3: Nitrate removal rate in the BES and the phosphate concentration (TP) in the synthetic groundwater under open circuit or 0.8-V with different phosphate buffer concentration in the anolyte. “OC-50mM” means the open circuit condition with 50-mmol/L phosphate buffer solution. The “*” indicates that there was no cathode compartment in the groundwater during the test.

When we decreased the phosphate buffer concentration to 1 mM in the anolyte, the concentration of phosphate ions in the synthetic groundwater also notably decreased under the open circuit condition (Figure 3). At an applied potential of 0.8 V, electricity was generated and we observed a significantly lower concentration of phosphate ions (3.8 ± 2.1 mg/L) with an initial 50-mM phosphate buffer. The use of the 1-mM phosphate buffer in the anolyte resulted in a non-detectable concentration of phosphate ions in the synthetic groundwater under 0.8 V. Those results indicated that electricity generation inhibited the migration of phosphate ions from the anode compartment into the synthetic groundwater; however, without electricity generation, the activity of the ion exchange became much stronger.

According to those results, we propose different mechanisms of nitrate transport from

the synthetic groundwater into the anode compartment in the absence or presence of electricity generation. When there is no electricity production, the nitrate ions are exchanged by the anions (e.g., phosphate ions) in the anode compartment; thus, the anion movement across the anion exchange membrane is in dual directions to maintain a neutral ionic charge: the nitrate ions move into the anode and the anodic anions migrate into the synthetic groundwater. Phosphate ions could be a major anion for such exchange at a high initial concentration of the phosphate buffer (e.g., 50 mM). When the phosphate buffer is at a low initial concentration (e.g., 1 mM), other anions in the anolyte such as bicarbonate and chloride ions would act as exchange agents (unfortunately we did not monitor the concentrations of other anions). When electricity is produced, the anion movement across the anion exchange membrane is in a single direction: the nitrate ions move into the anode compartment. To keep electric neutrality in the synthetic groundwater, additional anions need to move in or cations in the groundwater will move out; in this case, we think cations move out into the cathode compartment driven by an electric potential. That matches the principle of electricity generation in an MDC, in which anions move into an anode while cations migrate into a cathode. Although the nitrate was removed in both conditions, we think the electricity-generating condition is more desirable, because it enhances the nitrate removal rate and prevents unfavorable anions from entering the synthetic groundwater (avoiding potential contamination).

3.3 Effect of nitrate concentrations

The concentration of nitrate in groundwater affects the conductivity and consequently the electricity generation and ion exchange in the present BES. The US federal NO_3^- -N threshold in groundwater is

10 mg/L while in some states this value can be as high as 45 mg/L; therefore, we examined a series of nitrate concentrations, including 6.4,

11.2, 23.4, and 32.0 mg/L, with 0.8 V applied. The current generation exhibited a batch profile (Figure 4A), and the four concentrations resulted in the produced coulombs of

176.2 ± 12.6 , 200.1 ± 8.8 , 177.0 ± 2.7 , and 205.3 ± 8.2 C, respectively. Accordingly, the Coulombic

efficiencies based on nitrate removal are $118.3 \pm 13.5\%$, $67.6 \pm 5.2\%$, $28.3 \pm 0.5\%$ and $24.8 \pm 1.4\%$. This suggests that as the C/N ratio decreases (with an increasing nitrate concentration), electricity generation played a weaker role in moving the nitrate out of the synthetic groundwater, likely due to a stronger activity of denitrification in the anode compartment that competed for electrons with the anode electrode.

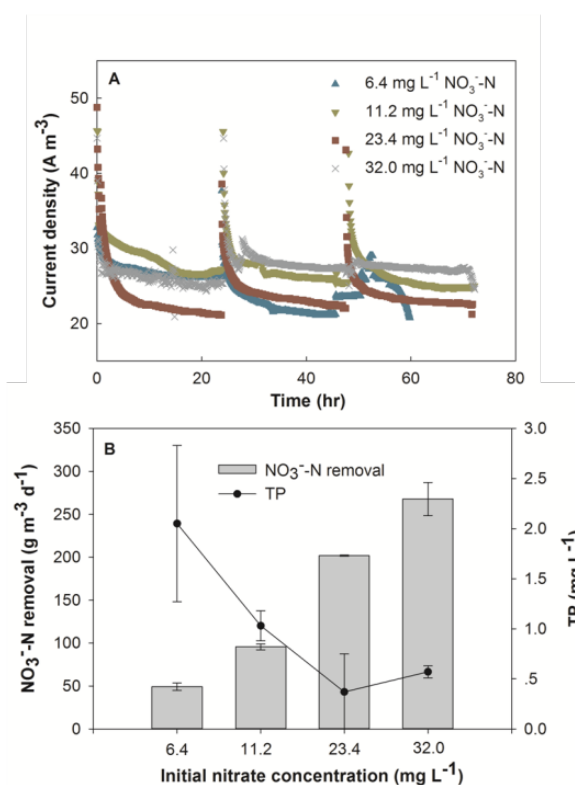


Figure 4: Current production in the BES (A), and the nitrate removal rates and the phosphate concentrations (TP) in the synthetic groundwater (B) at different initial nitrate concentrations. The applied potential was 0.8 V and the initial phosphate buffer in the anolyte was 1 mmol/L

We observed an unstable current generation with 6.4 mg NO_3^- -N/L, which was not shown with higher concentrations of nitrate. The possible reasons for this fluctuation may include a stronger ion exchange at a low-concentration of nitrate. For instance, we found that the change in the phosphate concentration in the synthetic groundwater increased with the decreasing nitrate concentration (Figure 4B). A higher initial nitrate concentration also resulted in a higher nitrate removal rate: the nitrate removal rate of $267.78 \pm 19.28 \text{ g NO}_3^-$ -N/ m^3 /d with 32.0 mg/L was more than five times the one ($49.25 \pm 4.21 \text{ g NO}_3^-$ -N/ m^3 /d) with 6.4 mg/L (Figure 4B). There was almost a linear relationship between the nitrate removal rate and the initial nitrate concentration ($Y=63.9X$, $R^2=0.9492$).

3.4 Nitrate removal from actual groundwater

The actual groundwater contains a nitrate concentration of $\sim 18 \text{ mg NO}_3^-$ -N /L. The

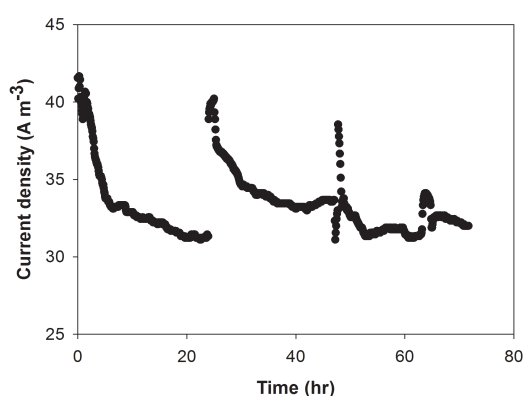


Figure 5: Current production in the BES with the actual groundwater.

current generation in the BES exhibited a similar profile as those from the synthetic groundwater (Figure 5). The nitrate removal rate was $130.0 \pm 12.2 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$, similar to that of the synthetic groundwater with a nitrate concentration between 11 and 24 mg/L, and also fit into the linear relationship developed with the synthetic groundwater. The average current density was 33.4 A/m^3 , higher than those with the synthetic groundwater (23.7 A/m^3 with 24 mg $\text{NO}_3^- \text{-N/L}$ and 27.3 A/m^3 with 32 mg $\text{NO}_3^- \text{-N /L}$); as a result, the total coulomb production was $244.7 \pm 9.1 \text{ C}$, much higher than those with the synthetic groundwater, likely due to other ionic species present in the actual groundwater that promoted ion transport across the ion exchange membrane and thus current generation.

3.5 Perspectives

The present BES provides a promising approach for *in situ* nitrate remove in groundwater remediation. It takes advantage of bioelectricity generation to attract nitrate ions out of groundwater and thus avoids the addition of other compounds for remediation (e.g., to stimulate microbial activities in groundwater bioremediation). The configuration of the separated anode and cathode improves the flexibility of the BES construction and operation and makes it possible to modify the components more conveniently (for example, modifying the anode/cathode or adding more units without affecting other units). Although the bioelectrochemical reactions in the anode and the cathode may affect the pH of adjacent groundwater via proton production/transport, this influence is minor due to a large volume of groundwater compared with much

smaller volumes of the anolyte and the catholyte.

To further develop the present BES for *in situ* nitrate remediation, we must address several challenges. First, a proper anode feeding solution should be selected: the use of purified organic compounds may increase the expense of remediation, while the low-cost substrates like wastewater must be used with caution to avoid introducing other contaminants into groundwater via the ion exchange processes. Second, the nitrate removal in the presence of other competing anions should be investigated: the competition could decrease the nitrate removal; on the other hand, the system may also remove other undesired ions (such as heavy metals into the cathode compartment) from groundwater. Third, the long-term performance of the BES with actual groundwater under a non-laboratory condition should be examined: the influence of environmental conditions (e.g., temperature and pH) on the BES performance is a key factor to its successful operation, and some issues such as fouling of ion exchange membrane due to ionic deposition require a long-term operation of the system. Fourth, the BES needs to be scaled up to an applicable size: the scaling up is always a challenge to the development of bioelectrochemical systems, and an advantage of the present BES for groundwater remediation is that it does not require a very large scale system like that for wastewater treatment, thereby making scaling up relatively feasible. Last but not least, we should properly evaluate this system from the aspects of capital investment, operating expense, and advantages/disadvantages compared with other *in situ/ex situ* nitrate remediation approaches once the BES is enlarged to a

certain scale.

4 Conclusions

This study has demonstrated the feasibility of using a bench-scale bioelectrochemical system to remove nitrate from groundwater. The BES effectively attracted nitrate out of groundwater driven by electricity generation and reduced it to nitrogen gas in the anode compartment via heterotrophic denitrification. Applying an external electric force improved the nitrate removal, and it was found that 0.8 V resulted in the highest nitrate removal rate. In addition to electricity generation, ion exchange was another major mechanism of nitrate migration into the BES anode, and there was competition between electricity-driven and ion exchange-driven nitrate movement, in which an electricity-driven process could inhibit the ion exchange process. The ion exchange process was also affected by the ionic concentration in the anolyte. The nitrate removal rate linearly increased with the increasing nitrate concentration in groundwater. The BES also achieved successful nitrate removal from actual groundwater sampled from a well in the state of Wisconsin. Further development of the BES for *in situ* nitrate removal from groundwater must consider the challenges such as the selection of anode substrates, the competition between nitrate and other anions, the long-term system performance, the reactor scaling up, and the economic evaluation of this approach.

Chapter 3 Development of a Bioelectrochemical System for *In Situ* Nitrate Removal from Groundwater

1 Introduction

Groundwater provides drinking water to more than one-half of the population in the United States and especially in many rural communities and large cities[67] . Hence safety of portable groundwater is of highly concern. Most groundwater pollution is related to structures and activities at or near the ground surface, which contribute bacteria and viruses, nitrate, and chemicals such as gasoline, pesticides, and industrial solvents to groundwater[68]. Major sources of nitrate are septic discharge, fertilization using natural (e.g., manure) or synthetic nitrogen sources, and concentrated animal feeding operations[69].

Process that is used to remove pollution from groundwater is named groundwater remediation. This involves two approaches of *ex-situ* and *in-situ*. In *ex-situ* approach, groundwater is extracted to surface where contaminants are removed, and then injected back, which referred to as pump-and-treat. *In-situ* method requires creating proper subsurface conditions that favor the degradation of contaminants however is not appropriate to contaminants that spread over large regions[70]. For point source contamination *in-situ* approach is preferred because it avoid using more energy-intensive apparatus[57].

Nitrate is thought to be recalcitrant pollutant and the success rate of clean up is poor. Techniques with physical, chemical, and biological methods are all taken into considered[49]. But for in-situ treatment most methods are based on chemical and/or

biological denitrification[40]. For biological denitrification, bacteria in soil and aquifer consume nitrate as nitrogen source in heterotrophic denitrification or autotrophic process under anaerobic condition. Organic carbon required for denitrification comes from either dissolved carbon or soil organic carbon[71]. The appealing thing for denitrification is that main product is nitrogen gas instead of other hazardous by-products remained in subsurface. However common problem of this method is that bio-film's building up in the aquifer may cause clogging in the subsurface[71]. Compared with denitrification technique, ion-exchange, reverse osmosis and electrodialysis can concentrate nitrate ions more effectively but hardly applied *in-situ* since the concentrated water need to be disposed[71].

Bioelectrochemical systems (BES) are new concepts that by integrating anaerobic reactor and membrane reactor together, achieving removal of organic matters from water and wastewater while simultaneously producing electricity[61]. Since storage of bacteria is easily achieved in the anode chamber of a BES, which is more suitable for well groundwater treatment. In a previous study a bench-scale BES demonstrated effectiveness of nitrate removal from a synthetic groundwater well. The BES's de-coupled electrode chambers were submerged in a synthetic groundwater well. Nitrate ions in groundwater migrate into anode chamber where they will be removed by heterotrophic denitrification process simultaneously with COD reduction in synthetic wastewater. Applied a proper electrical potential of 0.8V the system achieved the highest removal rate of $208 \pm 13.3 \text{ g NO}_3^- \text{-N/m}^3 \text{/d}$. Due to simply ion exchange effect when operated without present of current generation (open-circuit),

nitrate removal rate can still be maintained at $158.5 \pm 4.2 \text{ g NO}_3^- \text{-N/m}^3/\text{d}$ but indeed electricity production can prevent introducing other undesired ions into groundwater[39]. Basic working principle is proved in this phase's experiments, which exhibited potential of a new technique for in-situ nitrate remediation. Nevertheless there is still limitations hindering actual application such as anode wastewater places groundwater at high vulnerability since the porous PVC wrapped by anion exchange membrane is the only protective screen that separate waste to natural water resource.

Hence considering safer separation between groundwater well and the unwanted additional bacteria and organic contamination, an improved structure is designed in this study. Instead of de-coupled two-chamber structure, we use multi-chamber tubular shape shown as Figure 6 which is consisted of anode, cathode and mid-chamber. Nitrate ions are extracted from groundwater by electrical force through

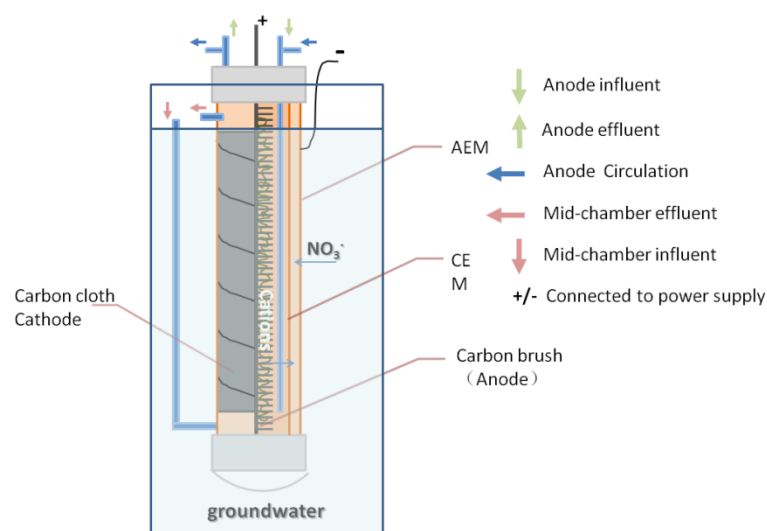


Figure 6: Schematic of phase 2 reactor

anion exchange membrane and concentrated in mid-chamber, which is filled with pure water while achieving organic matter reduction in anode and current production.

After feasibility was proved, we examined restrictions to nitrate transport such as other ion species' competition and hydraulic retention time of mid-chamber flow. We led mid-chamber effluent into the most inside anode chamber where heterotrophic denitrification took place in order to achieve complete nitrate removal from the system. SEM images and EDX analysis were conducted to examine the effect of long time running to membrane morphology and element content.

2 Material and Methods

2.1 BES Construction

A multi-layer tubular reactor was constructed to prove the concept. From inside to outside are: porous PVC tube wrapped by a piece of cation exchange membrane (CEM, Membrane International, NJ, USA) as anode chamber and a piece of anion exchange membrane (AEM, Membrane International, NJ, USA) wrapped as outer wall of mid-chamber. A 20cm long carbon brush twined by a rubber spiral spacer was placed in anode chamber as anode. Purpose of placing the spiral spacer is to create adequate turbulence and have a better mixing of feeding solution in anode chamber. A piece of carbon cloth (Area) coated with 0.5 mg Pt /cm^2 was wrapped outside the AEM as cathode. Effective volume of anode chamber is $\sim 500\text{ml}$ and mid-chamber volume is $\sim 250\text{ml}$ (due to membrane deformation in water, mid-chamber volume may be little bit less than 250ml). The whole unit is submerged in a tubular tank as simulated groundwater well, which was filled with 2L simulated groundwater (deionized water with sodium nitrate). Use titanium wire to connect electrodes with a

1 Ω resistor and an additional power supply.

2.2 BES operation

Anode feeding solution was prepared as: 0.5 g/L CH_3COONa ; 10mM phosphate buffer solution (diluted from 1 M phosphate buffer solution containing 107 g/L K_2HPO_4 and 53 g/L KH_2PO_4); NH_4Cl , 75 mg/L; NaCl , 250 mg/L; MgSO_4 , 7.5 mg/L; CaCl_2 , 10 mg/l; NaHCO_3 , 50 mg/L; trace mineral solution 0.1 mL/L; and 0.1 g/L yeast extract. It was pumped into anode compartment continuously at a flow rate of 0.7mL/min and was recirculated at 100mL/min by two peristaltic pumps. Mid-chamber was fed with deionized water (with trace amount of nitrate ions that can be counted as zero) at various flow rates. Synthetic groundwater was prepared by dissolving NaNO_3 in deionized water and was replaced at the end of each cycle. Conductivity was maintained at about 200 $\mu\text{m/cm}$.

The BES was operated with or without 0.8V externally applied potential as previously described.

2.3 Measurement and analysis

The BES voltage was recorded every 5 minutes by a digital multimeter (2700, Keithley Instruments, Inc., Cleveland, OH, USA). The pHs of the anolyte and the catholyte were measured by a benchtop pH meter (Oakton Instruments, Vernon Hills, IL, USA). The conductivity was measured by a benchtop conductivity meter (Mettler-Toledo, Columbus, OH, USA). The concentrations of chemical oxygen

demand (COD), nitrate-nitrogen (NO₃--N), were measured using a colorimeter following the manufacturer's instructions (Hach DR/890, Hach Company, Loveland, CO, USA). Nitrate removal rates were calculated based on the nitrate from groundwater. Coulombic efficiencies were calculated as the total coulomb output (integrating the production of time and current) divided by the total coulomb input (moles of nitrate removed from groundwater well assuming 1 mol of charge/mol of nitrate ions), according to the following Equation 1:

$$CE_N = \frac{Q_{output}}{Q_{input}} = \frac{\Sigma It}{F \times M_{N\ removed} \times 1}$$

where CE_N is the Coulombic efficiency based on nitrate, Q_{output} is the produced charge, Q_{input} is the total charge available in the groundwater nitrate, F is the faraday constant (96485 C/ mol e⁻), I is the electric current (A), and t is time (s).

In order to estimate relationship between different components in the BES cathode and mid-chamber, the following Equation 2 describing electrochemical equilibrium over membrane is used[72]:

$$V^0 = N \frac{2\alpha_{av}RT}{zF} \ln \frac{a_c}{a_d}$$

Where V^0 is the overpotential loss on mass transfer and charge transfer in cathode electrode, α_{av} is average membrane permselectivity of an anion and cation exchange pair which is a fixed value to a certain sort of membrane, N is number of membrane pairs, R is the gas constant, T is absolute temperature (K), z is electrochemical valence, F is the Faraday constant, a_c is the activity (considered as concentration) of the concentrated salt solution (mol/L) and a_d is the activity of the diluted salt solution (mol/L). Theoretically in a MEC the applied voltage is divided in reversible and

irreversible energy loss[73]. Since no thermodynamical barrier of hydrogen production is in cathode, the reversible energy loss can be neglected. Total cell potential distributes to anode and cathode overpotential, pH gradient, transport loss, and ionic loss[74], which is represented by the above Equation 2.

3 Results and discussions

3.1 BES feasibility

We assumed that nitrate ions in synthetic groundwater would be driven by electric force migrating through AEM into mid-chamber, where nutrient ions accumulation happens. That should be how this reactor getting rid of excess nitrate ions in groundwater. First phase of this research is to prove the feasibility of this hypothesis. All the indicated tests were conducted in triplicate.

The BES was started up without additional voltage applied and connected to 1000ohm resistor for anode bacteria acclimation. Resistance was gradually decreased to 1ohm in two weeks after which 0.8V voltage was applied. Replacing synthetic groundwater was defined as beginning of each cycle. Since the remaining nitrate ion concentration would reach a balance and would not go lower after about 16 hrs, we defined that one cycle lasted for 17hrs. Mid-chamber was fed with deionized water and refilled cycle by cycle at start up stage and then switched to continuous mode at flow rate of 0.7mL/min. HRT of mid-chamber flow was about 6hrs. Input COD was ~450mg/L. When the BES performance got steady, after 12 hours' retention, it was achieved more than 70% organic removal in anode effluent. Average current density would

reach $\sim 28 \text{ A/m}^3$ and peak current density reached $\sim 40 \text{ A/m}^3$ upon replacement of the synthetic groundwater followed by a decrease to $\sim 20 \text{ A/m}^3$. Current generation exhibited a typical batch profile as Figure 7 displayed. It decreased drastically within

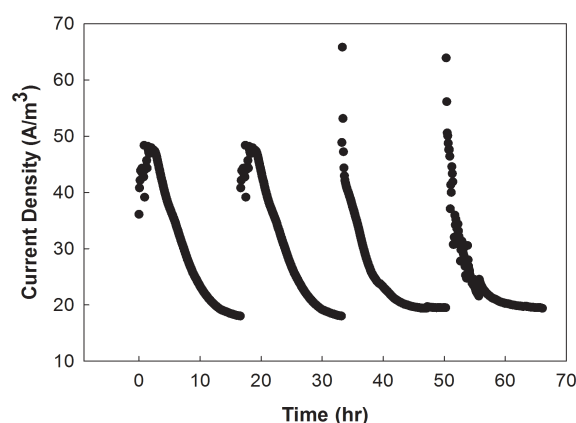


Figure 7: A typical profile of current density generation for initial NO_3^- -N concentration 20 mg/L

the former ten hours and gradually became flat within the rest time of a batch. In this phase, we used $21.5 \pm 0.8 \text{ mg/L}$ as initial nitrate concentration and it decreased to $10.0 \pm 0.4 \text{ mg/L}$ within 17 hrs. Meanwhile nitrate ions were cumulated in mid-chamber flow. Effluent nitrate concentration fluctuated within 20 to 30 mg/L. Basically nitrate decreasing in first 6 hours are more drastic than later hours, which is quite consistent with current profile. For mid-chamber, an increasing of nitrate concentration displayed at each sampling point. Value of each dot comes from the increasing of concentration at this time point compared with its former time point. Those values are positive at the first 8 hrs and then drop to negative or closing to zero. Since nitrate ions are more quickly driven into mid-chamber at the beginning, effluent nitrate will cumulate. However nitrate quantity is fixed in batch feeding, mid-chamber effluent nitrate will not increase when ions' migration rate slows down or close to zero. To

confirm the role of electrical power in driven nitrate ions migrating into mid-chamber, an open-circuit mode was conducted. Figure 8 presents nitrate decreasing and accumulation in both connect-with-power and open-circuit operation modes.

Averagely less than 2mg/L nitrate leave groundwater well within one batch time.

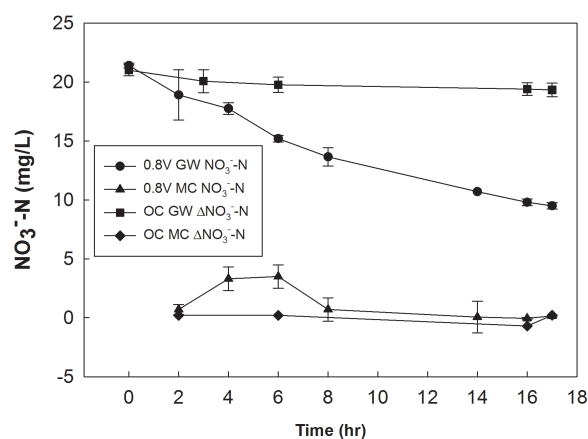


Figure 8: NO_3^- -N variation with time in groundwater well with 0.8 V applied or open circuit and ΔNO_3^- -N variation with time in mid-chamber with or without voltage applied. GW is for groundwater well, MC is for mid-chamber and OC is for open circuit

Most of the time nitrate accumulation in mid-chamber is negative, indicating that only more pure water is pumped in, instead of nitrate ions, which lowers effluent concentration. Since anions can move freely through AEM, nitrate ions rarely stay and cumulate in mid-chamber when device operated in open-circuit mode. When connected to power, the reactor relies much less on ion exchange itself. More nitrate ions are likely to be driven by electric force moving to one direction.

3.2 Limitation for the reaction process

We have found that once nitrate concentration in synthetic groundwater reaches a certain level (around 10 mg/L) after about 17 hrs, it seems that no more nitrate ions will be driven into mid-chamber. To an extent it achieved balance between nitrate ions in groundwater and mid-chamber. We then used 50 mg/L as initial nitrate

concentration in synthetic groundwater. Figure 9A is profile of current. Current density is $29.6 \pm 3.4 \text{ A/m}^3$. This value does

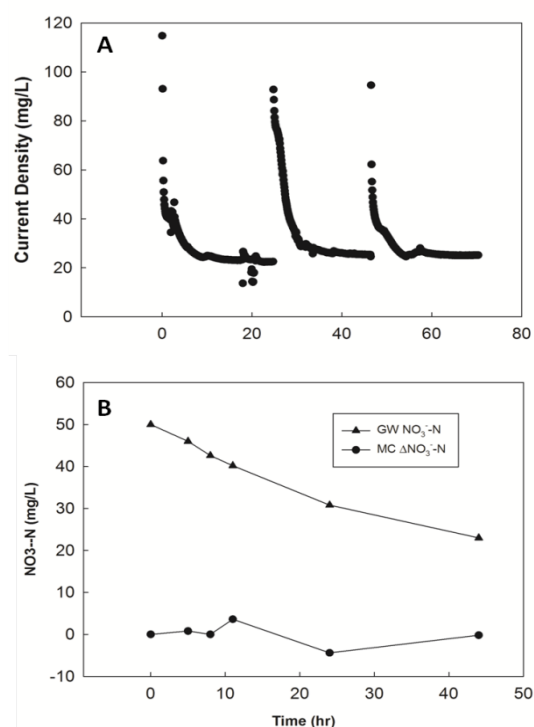


Figure 9: A) Current density profile when initial NO3--N is 50 mg/L. B) NO3--N in GW and ΔNO3--N in MC variation with time. GW is abbreviation for groundwater well and MC is abbreviation for mid-chamber

not exceed too much to what 20 mg/L group produced. However it takes longer time reaching balance, which is displayed in Figure 9B. After 44 hrs nitrate concentration remaining in synthetic groundwater is around 20 mg/L (data not shown), mid-chamber effluent concentration fluctuates at 60 mg/L, the fluctuation can be seen on Figure 9B. It is indicated that equilibrium is determined by initial ion concentration and electric force applied. On using Equation 1 in former section, coulombic efficiency based on nitrate removal (20 mg/L initial nitrate) is $414.6 \pm 95.5\%$. Total output charge is much greater than the charge which is generated from nitrate ion migration. There must be other anion species moving through AEM. Cathode reaction will consume H^+ in synthetic groundwater thus OH^- will be produced. Since other

anion species in DI water (solvent of synthetic groundwater) are of trace amount, OH^- is the anion species that also contributes current producing. In order to verify the assumption, mid-chamber liquid was switched to internal circulation from continuous flow.. All the migrated NO_3^- -N from groundwater well is $18.1 \pm 0.3 \text{ mg}$ ($1.3 \times 10^{-3} \pm 2.1 \times 10^{-5} \text{ mol}$). At the mean time $18.9 \pm 0.6 \text{ mg}$ NO_3^- -N ($1.4 \times 10^{-3} \pm 4.3 \times 10^{-5} \text{ mol}$) was cumulated in mid-chamber (calculation based on mid-chamber side data, 250 mL mid-chamber volume). Considering AEM and CEM deformation, these two values are equal, which indicates the conservation in ion migration. Due to cathode reaction, H^+ was consumed in groundwater well raising the pH of synthetic groundwater from 6.6 ± 0.5 to 10.7 ± 0.1 . Inner side of mid-chamber is CEM that facing the anode chamber. To compensate electron shortage in anode chamber, H^+ ions move into mid-chamber through the CEM. Phosphorous buffer solution keeps anode feeding pH between 7 and 8. But finally pH in mid-chamber is 12.0 ± 0.2 . Much more OH^- ions from synthetic groundwater get into mid-chamber than H^+ from anode chamber. The increased amount of OH^- in mid-chamber is about $2.5 \times 10^{-3} \text{ mol}$ which are greater than increased NO_3^- -N. We can conclude that NO_3^- , OH^- and H^+ have all contributed to current generation, among which OH^- contribute the most. Inner structure of ion exchange membrane contains anion or cation exchange groups that are able to transport anions or cations[75]. When contact with electrolyte, ions with same charge as exchange group will be excluded, only counter ions move through membrane. As to the strong base anion exchange membrane used in this study, it is quaternary ammonium fixed as positive-charged functional group. Fixed charge density is

defined as number of exchange group per volume of water in membrane backbone, which determines the counter ions transport through membrane[61]. Then the mobility of ions within membrane matrix affects electrical resistance. As mentioned before, other components of the total potential loss are more likely related to the configuration of electrode chambers, theoretical potential of electrode reactions and other anode operation conditions which are relatively fixed variables. According to Equation 2, equilibrium nitrate concentrations in bulk solutions at two sides of the anion exchange membrane are determined by potential loss on cathode nitrate ion transportation. However bulk solution volumes at two sides are different, Equation 2 cannot be used for calculation. This can be one of the reasons that nitration concentration will not decreasing after reaching certain equilibrium value. Once applied 1V voltage, average current density in each cycle could reach $37.3 \pm 6.1 \text{ A/m}^3$. Equilibrium nitrate concentration would remain around 10 mg/L causing $23.2 \pm 1.1 \text{ mg NO}_3^- \text{-N}$ was extracted from groundwater well and $22.6 \pm 1.7 \text{ mg NO}_3^- \text{-N}$ increasing in mid-chamber. Table 1 is a comparison of value changes to some key parameters at both the beginning and end of a batch applied by both 0.8V and 1V potential. Except

Table 1 Nitrate concentrations', conductivities' and pHs' variations at the beginning and the end of each operational cycle, in both synthetic groundwater well and mid-chamber

		0.8V		1.0 V	
		initial	End	initial	End
Nitrate mg/L	Groundwater	21.6 ± 0.6	12.5 ± 0.5	21.9 ± 0.2	10.4 ± 0.4
	Mid-chamber	1.6 ± 0.3	77.5 ± 2.4	0.9 ± 0.6	92.3 ± 6.8
pH	Groundwater	6.6 ± 0.5	10.7 ± 0.1	6.2 ± 0.1	10.3 ± 0.1
	Mid-chamber	9.2 ± 0.7	12.0 ± 0.2	8.9 ± 0.1	11.9 ± 0.1
Conductivity $\mu\text{S/cm}$	Groundwater	196.3 ± 15.3	276.0 ± 4.2	181.2 ± 2.2	219.8 ± 29.0
	Mid-chamber	40.5 ± 1.9	3786.7 ± 262.7	47.3 ± 22.2	3860 ± 658.8

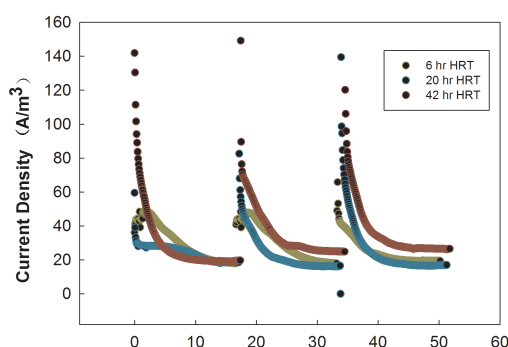
for nitrate concentration, we cannot see distinct variation on other parameters when applying these two different voltages. What we can confirm from the

comparison is that when applying higher potential it is more possible that nitrate ions' contribution to current generation increases and according to Equation 2, the concentration variation across the membrane will increase when higher V is achieved, which can partially explain lower equilibrium NO_3^- -N concentration when using 1V.

Ions transport through exchange membrane by occupying charged groups with counter ion species. It was found that Nafion 117 membranes have slightly higher affinity for Na^+ than H^+ thus H^+ ions were less responsible for the transport of positive charge through the Nafion membrane[22, 76]. Cooperband et.al [77] found potential interference from other anions (NO_3^- and SO_4^{2-}) on phosphorou extraction with anion exchange membrane. Although there is no explicit explanation to the interference of certain anion species transporting through AEM, we can assume that compared to NO_3^- , OH^- ions are more likely bonded with charge groups than NO_3^- , decreasing the contribution of NO_3^- .

3.3 Effects of mid-chamber flow rate

In former tests, mid-chamber DI water flow rate was 0.7 mL/min. However, compared with nitrate driven from synthetic groundwater, DI water consumption is huge. It is calculated that if 1mg NO_3^- -N is extracted into mid-chamber, averagely 30 mL DI water is consumed, which will definitely never be an economical way if this technique is to be applied in reality.



We will probably find if lowering the mid-chamber DI water feeding rate could have any effect on the BES performance. In this phase, two lower feeding rates: 0.1 mL/min and 0.2 mL/min (accordingly HRTs are: ~42 hr and ~20 hr) together with 0.7 mL/min were applied. Figure 10 shows current profile under these three distinct operation conditions. Highest coulomb produced in each cycle is 1047.4 ± 105.2 C, came from 42 hr HRT group. 6hr group produced 854.5 ± 75.5 C while 20 hr group produced 752.2 ± 50.1 C. Nitrate removal in each group corresponds with electricity production. Shown in Figure 11, 22.9 ± 2.2 mg NO_3^- -N was extracted within one cycle in 42 hr group. 17.7 ± 2.2 mg NO_3^- -N and 19.9 ± 1.5 mg NO_3^- -N is for 20 hr and 42 hrs, respectively. Here the corresponding relation between current generation and nitrate transport is proved again. The more nitrate ions migrating into mid-chamber within a certain time, the higher current the BES will have. Compared with others, 42 hr group is obviously more electrical active and has better nitrate removal performance. Lowering the mid-chamber flow rate mightily is an effective way optimizing performance of this BES. 20 hr group does

not present better than 6 hr group, but statistically saying, the former one could not be described a worse performance because there is no statistical significant difference between these two groups. The

reason could be that 20 hr HRT is not long enough to cause more significant difference

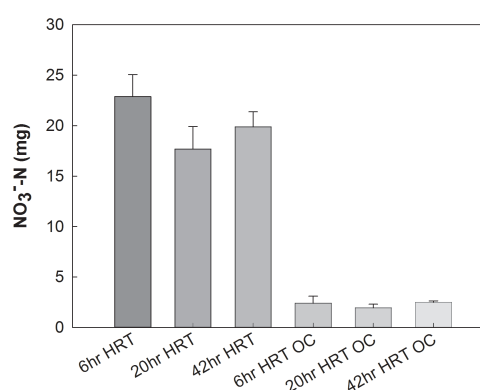


Figure 11: NO_3^- -N removed from groundwater well with or without potential applied, under 3 different mid-chamber retention time conditions

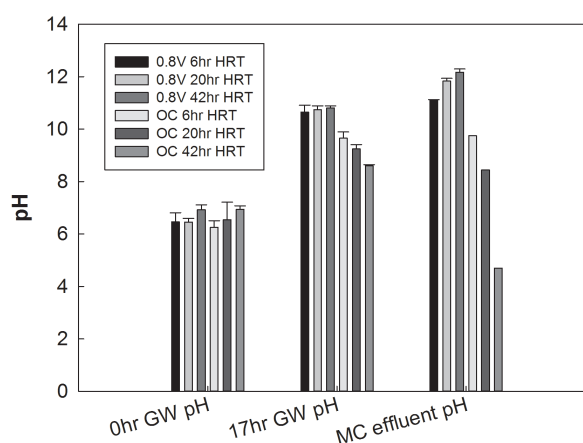
with 6 hr group or other uncontrollable factors during routine operation.

We confirm that excess DI water consumption can be drastically cut by lowering mid-chamber flow rate. Getting 1 mg NO_3^- -N extracted will consume 11.7 ± 1.6 mL and 5.2 ± 0.39 mL DI water in 24 hr and 48 hr group, respectively. However feeding rate decreasing does not show much effect on synthetic groundwater pH after one cycle, according to Figure 12, but it does on mid-chamber effluent pHs. With the decreasing of mid-chamber flow rate, effluent pH will increase indicating more OH^- cumulating in mid-chamber. But we cannot assume that more OH^- ions competing with nitrate ions are moving in because the renewing of low pH DI water slows down meanwhile.

Open-circuit tests in this phase also conducted. Seen from Figure 11, we can hardly see any distinct among those operation conditions. Basically rare nitrate ions will cumulate in mid-chamber, changing feeding rate will have no significant effect. PH is likely to drop according to Figure

12. This is because experiments are conducted in chronological order.

In open-circuit mode less and less OH^- will stay steady either in mid-chamber and they will



transport back to water well in order to maintain concentration

Figure 12: pH variations with or without potential applied under 3 different mid-chamber retention times. GW is for groundwater well, MC is for mid-chamber and OC is for open-circuit

equilibrium (literature). Since more DI water is feeding in as time goes, high pH

mid-chamber water is also diluted.

3.4 System improvement

In former phase experiments we achieved nitrate being concentrated in mid-chamber, which guaranteed a protection for groundwater from anode feeding wastewater. Nonetheless, nitrate wasn't thoroughly got rid of from the system. In order to remove concentrated nitrate ions by biological conversion, we led mid-chamber effluent to anode feeding solution at flow rate of 0.1 mL/min and the mixed solution was fed at 0.7 mL/min. Due to nitrate accumulation in previous tests, mid-chamber effluent nitrate concentration was 102.0 ± 2.8 mg/L. Sobieszuk et.al[78] suggest that critical C/N ratio for microbial denitrification is almost equal to 7.6 g O_2 /g N. Initially 4L anode feed solution with 578 mg/L COD was prepared. Compared with nitrate supplement, carbon source was more than just enough for denitrification. Averagely 10.4 ± 0.3 mg of nitrate flew into anode feeding tank in one cycle (17 hr) accompanied with 271 ± 43.8 mg COD consumed. Anode feeding solution could be diluted as mid-chamber effluent flowing in, but this would rarely affect electricity producing since current density maintained at a level of 33.6 ± 0.1 A/m³. Since this phase was conducted after 3 month's running the reactor[79], anion migration was hindered thus only 17.7 ± 1.7 mg was extracted within 17 hrs. Test result of anode effluent showed 0 mg/L nitrate remaining after passing through the anode chamber. NO_2^- concentration was less than 1mg/L. This indicated that cumulated nitrate could be completely removed from the system. For practical usage, mid-chamber flow should be

furthermore slowed down in order to reduce cost. Besides it is proved in former phase tests that decreasing the flow rate will not have too much influence on extracting nitrate ions from synthetic groundwater.

4 Perspectives

The BESs presents a more feasible and safety approach for in-situ nitrate enriching and biological denitrification, compared with previous study. In this study the configuration of reactors was modified to multi-layer tubular. Single tubular configuration is not only space-saving but also makes it more possible to bury it directly in an unsaturated aquifer, soil or sediment. Separation of anode chamber with surroundings can be more guaranteed that organic or microorganism mass will not easily permeate into aquifer or groundwater wells.

There are still plenty issues to be discussed in order to further develop this system. First, nitrate ions' migration into mid-chamber is accompanied with protons, which finally raise pH of surrounding water. USEPA suggests the upper limit of groundwater pH (for drinking) to 10, however in this study after one cycle synthetic groundwater will always reach 11. In real application groundwater body will surely have much larger volume and buffer capacity, but proton migration should be considered since meanwhile proton ions are strong competitors with nitrate ions. By slightly increase additionally applied voltage, nitrate ions are less affected by protons. We can assume a voltage level that more protons can be restrained and at the same time bacteria is guaranteed safe, still, managing a reasonable cost. Second, strength of

outer AEM should be considered if this reactor is to run for long time. Since separation between natural water and concentrated and high-pH water is only a piece of AEM, it cannot be too fragile. Only four months' safe running will not predict a longer safe time. Long operation time and even strength and deformation tests should be conducted if necessary. Third, we should evaluate potential cost if this system is to be set by addressing issues such as operation cost, whether to put this system under shallow aquifer or drilling extraction well and compare it with other groundwater remediation techniques in order to find optimal combination of conventional and novel methods.

5 Conclusions

This study proposed a development of BES for simultaneously organic reduction, nitrate concentration and biological denitrification. It was proved the feasibility of the assumption that nitrate ions in synthetic groundwater can be driven by electrical force across anion exchange membrane and reach equilibrium in a mid-chamber for concentration. It was found that nitrate concentration across two sides of the AEM would reach a certain level at which it will not be decreased in synthetic groundwater within 17 hrs. Protons are more likely to move into mid-chamber hence they are main competitors to nitrate ions on getting through AEM. By decreasing mid-chamber flow, we achieved a more economic way to operate the system with rarely negative influence on nitrate migration. Outlet of mid-chamber was lead to anode chamber together with anode feeding solution. 0mg/l nitrate or nitrite was detected in anode

effluent, indicating concentrated nitrate ions could be completely removed from this system.

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