Electrical stimulation for enhanced denitrification in woodchip bioreactors: Opportunities and challenges

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Abstract
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Keywords
Bio-electrochemical reactor, Woodchip bioreactors, Denitrification, Electrical stimulation, Nitrate, Drainage

Disciplines
Agriculture | Bioresource and Agricultural Engineering | Water Resource Management

Comments

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Research Paper

Electrical stimulation for enhanced denitrification in woodchip bioreactors: Opportunities and challenges

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ABSTRACT

Woodchip bioreactors are being implemented for the removal of nitrates in groundwater and tile water drainage. However, low nitrate removals in denitrifying woodchip bioreactors have been observed for short hydraulic retention time (HRT) and low water temperature (< 10 °C). One potential approach to improve woodchip bioreactor performance is to provide an alternative and readily available electron source to the denitrifying microorganisms through electrical stimulation. Previous work has demonstrated the capability of bio-electrochemical reactors (BER) to remove a variety of water contaminants, including nitrate, in the presence of a soluble carbon source. The objective of this study was to evaluate the denitrification efficiency of electrically augmented woodchip bioreactors and conduct a simple techno-economic analysis (TEA) to understand the possibilities and limitations for full-scale BER implementation for treatment of agricultural drainage. Up-flow column woodchip bioreactors were studied included two controls (non-energized, and without electrodes), two electrically enhanced bioreactors, each using a single 316 stainless steel anode coupled with graphite cathodes, and two electrically enhanced bioreactors, each with graphite for both anode and cathodes. Both pairs of electrically enhanced bioreactors demonstrated higher denitrification efficiencies than controls when 500 mA of current was applied. While this technology appeared promising, the techno-economic analysis showed that the normalized N removal cost ($/kg N) for BERs was 2–10 times higher than the base cost with no electrical stimulation. With our current reactor design, opportunities to make this technology cost effective require denitrification efficiency of 85% at 100 mA. This work informs the process and design of electrically stimulated woodchip bioreactors with optimized performance to achieve lower capital and maintenance costs, and thus lower N removal cost.

1. Introduction

The benefits of nitrogen fertilizer addition to increase agricultural yields are well recognized, but subsequent nitrogen losses from agricultural land have significant negative environmental impacts when nitrogen is conveyed to surface and ground waters (Robertson and Vitousek, 2009). While hypoxia is the most common problem, excessive nutrients in aquatic ecosystems also may result in acidification of these aquatic systems (Camargo and Alonso, 2006). In addition, nitrate poses risks to human and animal health when occurring in drinking water at concentrations exceeding 10 mg/L as N (Camargo and Alonso, 2006; USEPA, 2009), and such concentrations are regularly found in tile drainage of high-production agricultural landscapes (Hofmann et al., 2004; Ikenberry et al., 2014; Kalita et al., 2007; Lawlor et al., 2008).

The Hypoxia Task Force (2013), a collaboration of state and federal agencies led by the U.S. EPA, aims to reduce non-point source nitrogen export in Iowa by 41 percent through the implementation of multiple nutrient reductions strategies. The Iowa Nutrient Reduction Strategy (INRS) includes changes in land management practices, land-use practices, and edge-of-field practices to meet these goals (IDALS, 2013). Among edge-of-field practices, woodchip bioreactors are recognized as one of the promising technologies to remove nitrate from tile drainage (IDALS, 2013). A comparative study of field bioreactors at four separate locations in Iowa reported an average nitrate removal of 43 percent for treated drainage water (Christianson et al., 2012), demonstrating that such systems could achieve reductions close to those targeted by the Hypoxia Task Force. However, the performance of bioreactors is highly variable, with lower removal efficiencies occurring when temperatures...
are low, or flow is high (i.e., when hydraulic retention time (HRT) are low) (Hoover et al., 2015; Robertson et al., 2008). This is one of the motivations to improve bioreactor performance under such conditions, which typically occur in the early spring or high-flow season.

One potential approach in improving nitrate removal is to provide electrical power to an electrode system within the bioreactor, thus providing more readily available electrons as an energy source to the denitrifying microorganisms (Sakakibara and Kuroda, 1993). Such electrical stimulation of microbial metabolism to remove toxic pollutants has been practiced for over 50 years, and electrically-enhanced nitrate removal has previously been demonstrated (Thrash and Coates, 2008). Electrical stimulation is attractive because no chemical addition is necessary. Bio-electrochemical treatment potentially has the advantage of lower cost when treating a larger volume of wastewater as compared to addition of chemical amendments, which may have a higher cost of operation. Prosnansky et al. (2002) used electrical stimulation to remove nitrate in synthetic groundwater and estimated operating costs of 0.15–0.48 $/m³ of treated water with current densities set between 2.7 and 6 A/m³. If electrification can improve denitrification rate and thus volumetric removal, then it could facilitate smaller bioreactors which are even more attractive for edge-of-field treatment.

While there is great potential for the exploration of this technology, the bio-electrochemical reactor (BER) requires a higher capital cost than traditional woodchip bioreactors due to the material cost of electrodes and operating cost of power supply. Since the implementation of INRS, including bioreactor, is voluntary by land owners, the electrodes and operating cost of power supply. Since the implementa-tion of INRS, including bioreactor, is voluntary by land owners, the need for extensive modifications such as creating exclusively distinct oxidizing or reducing zones using baffles. To our knowledge, no previous studies have been conducted to evaluate the effect of electrical stimulation in woodchip bioreactors. By understanding the factors affecting the denitrification rate in this simple system, we hoped to provide insight on how woodchip-BER configurations can be optimized for nitrate removal. The objective of this study is to compare the nitrate removal in woodchip BERs with control woodchip (no electrical stimulation) bioreactors. To shed light on the mechanisms that might explain differences in performance between BERs and control reactors, parameters including pH, ORP and DO were monitored. In addition to the experimental work, a preliminary TEA was conducted to understand the possibilities and limitations for full-scale BER implementation for treatment of agricultural drainage.

1.1. Theory

Denitrification is a multi-step biological process accomplished by bacterial communities capable of enzymatic reduction of nitrate to nitrogen gas. These denitrifiers require an electron donor to reduce nitrate to nitrite, and eventually to nitrogen gas. Conventionally, hydrolysis products of woodchips are used as the sole electron donor in woodchip bioreactors. As is typical for biologically mediated reactions, decreasing temperatures result in lower reaction rates (Feyerisen et al., 2016; Hoover et al., 2015). For most bioreactor processes that are not mass-transfer limited, shorter HRTs are also associated with decreasing fractional nitrogen removal in these systems (Hoover et al., 2015). By stimulating the bioreactors with electricity, additional electrons can be readily produced to enhance the denitrification processes (Prosnansky et al., 2002; Thrash and Coates, 2008). As illustrated in Fig. 1, the electrons can be transferred to the denitrifiers from cathodes in three possible ways for biological denitrification: direct electron transfer, indirect electron transfer through electroactive substrates, and indirect electron transfer through hydrolysis of water (Thrash and Coates, 2008).

Direct electron transfer from a graphite cathode to microorganisms to reduce nitrate was demonstrated using pure cultures of Geobacter species (Gregory et al., 2004). Furthermore, mixed-culture denitrifying microbial communities enriched from wastewater sludge have been documented to have such capabilities (Park et al., 2005; Wrighton et al., 2010). This suggests the potential of woodchip bioreactors, which employ a diverse microbial consortium (Feyerisen et al., 2016), for the removal of nitrates through direct electron transfer.

Indirect electron transfer from cathode to microorganism via electroactive substrates is also known as electron shuttling (Thrash and Coates, 2008). Without being degraded, these substrates can accept
electrons from the cathode, and then donate to the microorganisms for biodegradation of water pollutants (Lovley et al., 1996; Lovley et al., 1999; Thrash et al., 2007). These substrates include quinones, phenazines, and humic substances (Thrash and Coates, 2008). In theory, humic substances present in woodchip bioreactors can act as electron shuttles, thus improving overall electron transfer efficiency. However, this mechanism has not been well studied and its significance is unclear.

Electrolysis of water is another indirect electron transfer mechanism, and different reactor configurations and operational parameters have been employed to leverage this mechanism (Gregory et al., 2004; Hao et al., 2013; Park et al., 2005; Prosnansky et al., 2005; Prosnansky et al., 2002; Sakakibara and Kuroda, 1993; Thrash and Coates, 2008; Wrighton et al., 2010). In this mechanism, H₂ produced from electrolysis of water can serve as an electron donor for the denitrifying microorganism. However, overproduction of H₂ may result in inhibitory effects (Flora et al., 1994). In some nitrate-removal BERs, ion exchange membrane or sponge was used to keep O₂, produced at the anode, from entering the cathode region (or nitrate reduction zone), while allowing a passage for proton and electron movement (Prosnansky et al., 2002; Prosnansky et al., 2005; Sakakibara and Kuroda, 1993; Wrighton et al., 2010). This electrolysis mechanism is probably likely to occur in a woodchip BER, although the impact of H₂ and O₂ is uncertain.

Lastly, electrochemical reduction is a non-biological nitrate removal mechanism that may occur in a BER (Li et al., 2009). This mechanism involves the change in oxidation state of nitrogen from nitrate to nitrite, nitric oxide, nitrous oxide, and eventually to nitrogen gas. However, this pathway is not certain, and may result in the formation of by-products that are more toxic (Katsounaros et al., 2012). In addition, it is difficult to achieve selective reduction of nitrate in tile drainage due to the presence of other ions. Nevertheless, it is important to recognize this potential reduction mechanism in a BER, and the need for the reactor configuration to be optimized to maximize the microbial reduction pathway.

2. Materials and methods

2.1. Overview

The study had two major phases: An experimental phase examining the performance of electrically-stimulated BERs compared to their non-electrically stimulated controls, and a technoeconomic phase where the results from the experimental phase were used to construct a simple spreadsheet-based cost model of full-scale BER.

2.2. Experimental phase – reactor overview

The experiment was designed to compare the nitrate removal efficiencies with and without electrical stimulation, and with different anode materials (316-stainless steel [SS] and graphite [C]). Graphite was used as cathodes for all columns. During the start-up period, all columns were flushed with nutrient solution for 31 days to remove excessive total organic carbon (TOC), and to inoculate denitrifying bacteria. Two pairs of BERs with 100 mA applied current and a pair of control reactors were then tested under 10 °C for 39 days, but no nitrate removal (data not shown) was observed in all BERs and control reactors. The same finding was reported by Feyereisen et al. (2016) in woodchip bioreactors. Since there was no observed improvement on denitrification using 100 mA electrical treatments at room temperature and 10 °C scenarios, the data for the 10 °C scenario were not discussed in the following sections. This is because we can expect the same explanation from the room temperature scenario to be applied to the 10 °C scenario, in addition to the expectation that minimal microbial activities are expected at low temperatures. Consequently, the operating temperature was increased to room temperature (22.5 °C), and re-inoculated with denitrifying bacteria during an 11-day transition period. The effect of current intensity on nitrate removal was evaluated by supplying 500 mA and 100 mA to the BERs in two consecutive periods. Differences in denitrification efficiency between BERs and control reactors during the room temperature study (Condition B and C) are reported here. The test matrix for the experiment is presented in Table 1.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Day</th>
<th>Number of</th>
<th>Current</th>
<th>Temp (°C)</th>
<th>HRT (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start-up period</td>
<td>0–31</td>
<td>31</td>
<td>0</td>
<td>10</td>
<td>5.9</td>
</tr>
<tr>
<td>A</td>
<td>32–70</td>
<td>39</td>
<td>100</td>
<td>10</td>
<td>5.9</td>
</tr>
<tr>
<td>Transition period</td>
<td>71–81</td>
<td>11</td>
<td>100</td>
<td>22.5</td>
<td>8.2</td>
</tr>
<tr>
<td>B</td>
<td>82–128</td>
<td>47</td>
<td>500</td>
<td>22.5</td>
<td>8.2</td>
</tr>
<tr>
<td>C</td>
<td>129–149</td>
<td>21</td>
<td>100</td>
<td>22.5</td>
<td>8.2</td>
</tr>
</tbody>
</table>

2.3. Reactor vessel and packing

The experiment was conducted with three pairs of duplicated upflow column woodchip bioreactors. Each column measured 15.2 cm (6 in.) in diameter and 50.8 cm (20 in.) in height. A pair of diffuser plates and a pair of flexible caps were fit onto each end of the column. One anode socket and two cathode sockets, which consisted of 2.5 cm (1 in) diameter electrode, 3.8 cm diameter (1.5 in.) slot, and 3.8 cm diameter (1.5 in.) flexible cap, were inserted into the sides of the column as shown in Fig. 2. The electrodes were 101.6 cm (40 in.) long. The column, sockets, and diffuser plates were made of polyvinyl chloride (PVC).

Each column, with total volume of 9.47 L, was packed with 2 kg of hardwood chips (Golden Valley Hardscapes, Story City, Iowa), resulting in a mean pore volume of 4.91 ± 0.1 L (mean ± SD). The average gravitational and internal porosity of the woodchip media were 0.52 ± 0.01 and 0.32 ± 0.03, respectively, yielding a total porosity of 0.84, which was comparable to 0.84 and 0.89 reported by Robertson (2010) and Hoover et al. (2015), respectively.

2.4. Electrical stimulation system

SS-C (anode-cathode) electrode combinations were employed in a pair of columns, while C-C electrode combinations were tested in the second pair of columns. The last pair of columns without electrodes (and power supply) served as controls.

The anode was placed in the center of the column, in between the two cathodes (Fig. 2). The anode was at a distance of 25.4 cm (10 in.) from both inlet and outlet. Each cathode was placed 12.7 cm (5 in.) from the anode, and from the inlet or outlet. All electrodes were connected to a power supply (Enduro™ E0303, Labnet, Edison, NJ).

The BERs received no electrical stimulation during the start-up period, and were supplied with 100 mA (7.52 A/m²) current during the 10 °C test period (Table 1). During the 11-day transition period for temperature adjustment, 100 mA of current was supplied to the BERs. Then, the BERs received current intensity at 500 mA (37.6 A/m²) for 47 days, and finally 100 mA for the last 21 days of operation.

2.5. Fluid handling system

Two 4-channel variable speed peristaltic pumps (Ismatec CP 78017-10, Cole-Parmer, Vernon Hills, IL) were used to supply nutrient solution to all columns. Flow rates were set to achieve average HRTs of 5.9 and 8.2 h (Table 1). The HRTs were estimated using measured pore volumes. Flow rates of the pumps were occasionally adjusted based on measured daily average flow rate, to compensate for flow variations.
due to tubing wear or other unforeseen factors such as clogging by humic substances. Tubing was replaced when flow rates decreased significantly. Synthetic nutrient solution containing 30 mg/L of NO$_3$-N, and other micronutrients (detailed in supplementary information) required for optimal bacterial growth (Nadelhoffe, 1990), was used to represent tile drain water (Hoover et al., 2015). The solution was prepared in a 170 L container as influent solution for all columns.

### 2.6. Thermal control

As mentioned above, the columns were initially placed in a temperature-controlled room at 10 °C. However, no nitrate removal was observed in our cold temperature study (data not shown), which was similarly reported by Feyereisen et al. (2016) for their 1.5 and 15.5 °C woodchip bioreactors experiments. Therefore, the temperature was increased and maintained at 22.5 °C for the remainder of the experiment. Due to the local heating effect from electrical stimulation, the water temperature was monitored at the inlet, In-Col 1, In-Col 2, In-Col 3 and outlet (Fig. 2) on a weekly basis.

### 2.7. Microbial inoculation

*Klebsiella* (DN2) and *Raoultella* sp. (DN3 and DN8A) bacteria cultures were obtained from Dr. Moorman’s laboratory. These bacteria used to inoculate the BERs were originally isolated from soil and they were confirmed to be denitrifying bacteria through their ability to produce N$_2$O from NO$_3$-N under O$_2$-free conditions in the presence of acetylene (Tiedje, 1994). They were inoculated into 25 mL of nutrient broth, and incubated at 30 °C on a rotary shaker for 4 days. They were then harvested by centrifuging at 5000 × g for 20 min. Cell pellets of each strain was re-suspended in 25 mL sterile phosphate buffer solution, respectively, and plated to determine cell concentrations before added together to form a 75 mL mixed culture. The first mixed culture containing over $10^{10}$ cells was added into a large influent container containing nutrient solution during the start-up period (Day 19), and fed continuously to each reactor for 24 h. During day 2 of the transition period (Day 72), the mixed culture was regrown and added into the influent tank.

### 2.8. Sample collection

Influent and effluent NO$_3$-N samples were collected every other day. 100 mL of influent NO$_3$-N sample was collected directly from the influent tank; 100 mL of 1-day (containing 3 or 4 pore volumes) composite sample of effluents were collected from respective effluent container. All NO$_3$-N samples were preserved with hydrochloric acid and stored at 4 °C until analysis. In addition, grab pH, ORP and DO samples of each reactor were collected weekly at five different locations: inlet, In-Col 1, In-Col 2, In-Col 3 and outlet (Fig. 2). These samples were analyzed immediately.

TOC samples were only collected after the color intensity of the effluent was reduced from dark to light tea color at Day-10. Daily samples were collected until Day-18, when average TOC concentration (3.6 ± 0.8 mg/L) was reduced to typical background concentration (< 5 mg/L DOC) observed in Iowa’s surface streams (Ruark et al., 2009). TOC samples were preserved with phosphoric acid and stored at 4 °C until analysis. At the end of experiment, the reactors were deconstructed and woodchip samples were collected from each reactor for microbial analysis. Woodchip samples were obtained from inlet, In-Col 1, In-Col 2, In-Col 3 and outlet. All microbial samples were frozen until DNA extraction and qPCR analysis.

### 2.9. Analytical methods

NO$_3$ - N + NO$_2$ - N concentrations were determined using Seal Analytical Method EPA-114A, rev. 7, which is equivalent to U.S. EPA method 353.2. Since there was no nitrate removal observed in all reactors during the 10 °C experimental period, the data was excluded and performance of each reactor was only evaluated under room temperature conditions. In addition, only data with daily influent concentration of 30 ± 4 mg/L was used for data analysis to exclude the effect of influent concentration on nitrate removal efficiency. Denitrification efficiency (DE, %) was calculated using the following formula:

$$DE = \left( \frac{C_{NO3-N,inf} - C_{NO3-N,eff}}{C_{NO3-N,inf}} \right) \times 100\%$$

where $C_{NO3-N,inf}$ and $C_{NO3-N,eff}$ are influent and effluent nitrate concentration (mg/L). Statistical analysis was conducted to compare the DE of each treatment and control, using ANOVA (normal distribution) and Wilcoxon test (non-normal distribution) in JMP software. All datasets were tested for normality using QQ-normal plot. P-value ≤ 0.05 was used to indicate statistically significant differences. The current intensity and type of treatment were considered as nominal data, while nitrate removal efficiency was treated as continuous data. In addition, current-denitrification efficiency ($\eta$, %) was calculated using the formula below (Prosnansky et al., 2002):

$$\eta = \left( \frac{Q(C_{NO3-N,inf} - C_{NO3-N,eff})}{I/nF} \right) \times 100\%$$

where Q is volumetric flow rate (cm$^3$/s), $C_{NO3-N,inf}$ and $C_{NO3-N,eff}$ are influent and effluent nitrate concentration (mol/cm$^3$), I is current intensity (A), apparent n is stoichiometric coefficient [n = 5, 

![Fig. 2. Exploded view of up-flow bio-electrochemical reactors. Blue arrow represents direction of water flow, which flows from inlet (bottom) to outlet (top) of the reactors. In-Col 1 and 3 are the locations of cathode; In-Col 2 is the location of anode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
representing the change in oxidation number of N from NO$_3^-$ N (+5) to N$_2$ (0)), and F is Faraday’s constant (C/mol).

For TOC analysis, persulfate-ultraviolet oxidation method was employed using Teledyne Tekmar Phoenix 8000 TOC analyzer. This is Method 5310C in Standard Methods for the Examination of Water and Wastewater, 22nd Ed.

The pH and ORP were measured using Thermo Scientific Orion Star A324, configured with pH (Orion™ ROSS Ultra pH/ATC Triode, Thermo Scientific, Waltham, MA) and ORP (Orion™ 9678BNWP ORP/Redox electrode, Thermo Scientific, Waltham, MA) probe, respectively. DO was measured using a DO meter (ProODO™, YSI, Yellow Springs, OH).

The microbial woodchip samples were thawed and chopped to approximately 0.5 cm wide and 1–2 cm long. Genomic DNA was isolated from woodchip samples using DNeasy PowerMax Soil Kit (QIAGEN, Inc., Germantown, MD) according to manufacturer’s protocol. DNeasy qPCR was targeted for nosZ denitrification genes (nitrous oxide reductase). In addition, 16S-rRNA genes were also quantified to obtain total gene number of Eubacteria so that relative abundance of nosZ gene can be determined. The detailed methods which were consistent with Kandeler et al. (2006) and Feyereisen et al. (2016) are provided in SI. The relative abundance of nosZ gene at the anode (In-Col 2 sampling location) was excluded from column average because of the oxidizing condition which may favor the growth of other microbes.

### 2.10. Technoeconomic analysis

A preliminary technoeconomic analysis (TEA) was conducted to provide a rough estimate of the cost (in US$, or USD) to remove a unit mass (kg) of NO$_x$N in full-scale reactor. A base case with no electrical stimulation and four BER scenarios were created (Table 2). The TEA includes three major costs associated with a BER: capital, operating, and maintenance costs. Capital costs were estimated using traditional woodchip bioreactor construction costs, which includes excavation, structure, and woodchips (Christianson et al., 2013). Cathode costs were also treated as capital costs because they are not expected to degrade and are therefore one-time costs. The BER operating costs were for electricity, which were based on scaling power per unit volume from the small to full-scale reactors, and on electricity rates assumed at $0.08/kWh. However, the BER was expected to operate with electrical stimulation under high-flow conditions only, which was assumed to be 10% annually (Ikenberry et al., 2014). The maintenance costs were for anode replacement, which were based upon anode degradation rates observed in the experimental reactors. It is important to note that this simple TEA did not account for the cost differences that can be caused by actual dimension (width: length: depth ratios) of the reactor, local availability of woodchips, distance of power line to bioreactors, wiring installation, engineering design fee and other detailed factors. Nevertheless, these estimated removal costs would serve as a preliminary work to determine the relative cost difference between electrical treatments and traditional woodchip bioreactors, and also to provide an insight on the strategy for cost reduction.

For the capital costs, we assumed a full-scale reactor excavation volume of 100 m$^3$. This in turn was used to estimate excavation, structural and woodchip costs (Christianson et al., 2013). The capital costs were amortized assuming 15 years operational life and 5% annual interest. No depreciation, salvage, or tax costs/benefits were assumed. The mass of cathodes required in full-scale treatment was determined based on ratio of cathode mass to reactor volume in the lab-scale experiments.

The anode material was considered as a maintenance cost due to the necessity for replacement over time. It was assumed to have the same anode and cathode loading factors (m$^3$/m$^3$) as our lab reactors (Table 2). The anode lifespan was projected based on the anode corrosion rate during the 149-day laboratory experiment. This yielded an estimated graphite anode lifespan of 6.4 years at 7.52 A/m$^2$ (or 100 mA in our lab reactor), and 1.3 years at 37.6 A/m$^2$ (or 500 mA) operating current. In contrast, the stainless steel anode was projected to have much longer lifespans at 349 and 69.9 years respectively. This effectively meant that the stainless steel anode was a one-time cost for our analysis. No salvage value was considered.

In this analysis, the nitrate removal efficiency of the full-scale BERs was expected to be equal to the results in our laboratory experiment. We also assumed treatment area of 22.2 ha with nitrate export rate at 31.4 kg NO$_3$N/ha-yr (Christianson et al., 2013; Ikenberry et al., 2014). We assumed 56% of the nitrate is exported during 10% of daily flow, and this drainage water was treated with electrical stimulation; the remaining 44% would be treated without electrical stimulation (Ikenberry et al., 2014). The nitrate removal efficiency (18.5%) of traditional treatment was assumed to be the same as our control reactors. The nitrate mass removal of each scenario was calculated based on nitrate export rate in tile drainage and nitrate removal efficiency of our lab reactors as presented in Table 2. Finally, the N removal cost was calculated by taking the ratio of total cost over nitrate mass removal.

A sensitivity coefficient analysis was performed on key parameters including bioreactor construction cost, cathode cost, incentive program, anode cost, anode lifespan, electricity cost, and nitrate mass removal. The change in N removal cost was determined after increasing 1% of the cost in the key parameters mentioned above.

### 3. Results and discussion

#### 3.1. Effect of electrical stimulation on denitrification efficiency

The effect of electrical stimulation on percent denitrification efficiency (DE) or percent nitrate removal efficiency was evaluated by supplying current at 100 mA (Day 82–128) and 500 mA (Day 129–149) to two pairs of BERs (SS-C and C-C), respectively, under room temperature conditions (Fig. 3). The DEs of electrically stimulated BERs

| Table 2: Input summary of techno-economic analysis. Capital costs were amortized for 15 years using annual interest rate of 5%. |
|---|---|---|---|---|---|
| Unit | Base Case | Scenario 1 | Scenario 2 | Scenario 3 | Scenario 4 |
| Cost | | | | | |
| Capital cost | $/yr | 627 | 1042 | 1042 | 1042 |
| Maintenance cost | $/yr | 0 | 402 | 1964 | 1753 |
| Operating Cost | $/yr | 0 | 441 | 441 | 7321 |
| Other input parameters | | | | | |
| Electrode pair | N/A | C-C | SS-C | C-C | SS-C |
| Anode: Reactor Volume | m$^3$/m$^3$ | N/A | 0.024 | 0.024 | 0.024 |
| Anode lifespan | yr | N/A | 6.4 | 15 | 1.3 |
| Cathode: Reactor Volume | m$^3$/m$^3$ | N/A | 0.047 | 0.047 | 0.047 |
| Current density | A/m$^2$ | N/A | 7.52 | 7.52 | 37.6 |
| Nitrate removal efficiency | % | 18.5% | 20.4% | 16.6% | 40.5% |
| Nitrate mass removal | kg NO$_3$N/yr | 129 | 136 | 122 | 215 |

were compared to control reactors, which were not electrically augmented. At 100 mA, the average DEs of SS-C and C-C treatments were 16.6 ± 4.8% (mean ± SD) and 20.4 ± 13.0%, respectively. Meanwhile, the control reactors showed an average DE of 18.5 ± 7.0%. Denitrification efficiency of SS-C (p = 0.53) and C-C (p = 0.61) treatments was not statistically different from the controls; suggesting that denitrification efficiency was not improved using electrical stimulation at 100 mA. Alternatively, SS-C and C-C treatments yield average DEs of 24.5 ± 11.4% and 41.1 ± 21.2%, respectively, when stimulated with current at 500 mA. The DE of the control reactors during this experimental period was 12.3 ± 4.2%, which was statistically lower than the DEs of SS-C (p < 0.01) and C-C (p < 0.01) treatments. This demonstrated the enhancement of denitrification efficiency using electrical stimulation at 500 mA. The lack of electrical influence on DE at 100 mA, and improvement on DE observed at 500 mA was because of our low current-denitrification efficiency, which will be detailed in the next section.

3.2. Effect of current intensity on denitrification efficiency and current-denitrification efficiency

DEs were higher at 500 mA than 100 mA in both treatments (p < 0.01). Alternatively, the estimated values of current-denitrification efficiency (η) decreased with higher current intensity. The η in this experiment was estimated by assuming all electrons uptaken by denitrifiers for denitrification in electrical columns were obtained from the cathodes, which were a more readily available electron source than hydrolysis products of wood chips. The η of SS-C and C-C treatments at 100 mA treatments were 28.7 and 35.2%, respectively. Our observed η were lower than Prosnansky et al. (2005)’s optimum η (61.5%), which was likely due to a smaller cathode surface area used in our reactors, with respect to the volume of our BERs. However, this comparison should only be used as a reference and direct comparison should not be made due to other differences such as reactor design and type of carbon source used. With small η and lower current intensity in our BERs, fewer electrons were provided to the denitrifiers at 100 mA. Accordingly, DEs were improved when the BERs received five times more electrons when the current was supplied at 500 mA. This observation suggested that DE can be improved by supplying sufficient electrons using higher current intensity, although it is important to note that the η (SS-C: 9.4%; C-C: 14.2%) at 500 mA was further reduced. Even though more electrons were delivered to denitrifiers for denitrification, η was not reduced proportionally. One possible reason was that a larger fraction of electrons was lost due to excessive production of H2 gas, which was not captured efficiently by the denitrifiers (Fig. 1). This trend was consistent with the work by Prosnansky et al. (2005) where a decrease in η was observed but not proportionally with increasing current intensity. Increasing the required current intensity or current density for the BERs may make them less economically feasible.

The maximum denitrification potential was not achieved in our reactors. The DE and η can likely be improved by increasing the cathode surface area, while maintaining low current density. Higher DE and η reported by Prosnansky et al. (2005) was likely due to their larger cathode surface area per unit pore volume (m2/m3). Prosnansky et al. (2005) had a reactor which used 123 m2 of graphite cathodes per cubic meters pore volume, while our reactor’s graphite cathode loading factor was only 15 m2/m3. Since cathode surface area plays an important role in electron transfer efficiency, appropriate current density (current intensity/cathode surface area) should be used to select the suitable current intensity when rescaling the BER for full-scale practices. Other reactor configurations, such as placement of electrodes and use of baffles, also can be modified for better DE and η, which will be discussed in the last section of this paper.

3.3. Effect of anode material on denitrification efficiency

No significant difference in DE was found between SS-C and C-C treatments at 100 mA (p = 0.33). However, there was a significant difference in DE between the two treatments at 500 mA (p < 0.01). The C-C treatment (41.1 ± 21.2%) demonstrated the highest average DE, followed by the SS-C treatment (24.5 ± 11.4%) and control reactors (12.3 ± 4.2%).

The higher removal efficiency in C-C treatment was likely due to the oxidation of graphite anode into CO2, which provided a buffering capacity for the system (Thrash and Coates, 2008). Despite its higher DE, corrosion of the graphite anode was also significant. An average mass loss of the graphite anode was 65.1 ± 16.9% after receiving 100 mA current for 71 days, and 500 mA current for 47 days. In contrast, O2 was produced at the anode of SS-C treatment, causing the DO level at locations above the anode (In-Col 2, In-Col 3 and outlet) to elevate. Higher DO level in SS-C treatment likely impacted the DE, as observed in this experiment. Only 1.22 ± 0.3% of the stainless-steel anode in the SS-C treatment was degraded throughout the experimental period.

3.4. Factors affecting pH, ORP, and DO and their effect on denitrification efficiency

Despite the improved DE observed in BERs, it is important to recognize the high variability in DE of the BERs at 500 mA, which was likely due to the inconsistent pH and ORP profile within the reactors. As presented in Fig. 4, the pH and ORP values at each sampling location of each BER varied greatly (error bars) even though the current intensity
and water flow rate were kept constant during the treatment periods.

At 100 mA, the pH at sampling location In-Col 1 (cathode) in both SS-C and C-C treatments increased due to production of OH\(^{-}\) ions at the cathode. The pH was then decreased at In-Col 2 (anode) as H\(^{+}\) ions were produced at the anode. Unsurprisingly, the pH at In-Col 3 in C-C treatment was increased. However, pH at In-Col 3 in SS-C treatment remained at approximately 6.3 (Table S2). It was suspected that CO\(_2\) produced at the anode of C-C treatment act as a pH buffer for the upper half of the column. At 500 mA, pH profile in all BERs shared the same trend: increased at In-Col 1, then decreased along the reactor, and finally leveled off around 5.73 at In-Col 3. The pH pattern at In-Col 1 and In-Col 2 followed the same explanation for 100 mA scenario. Interestingly, the pH at In-Col 3 did not increase even in the SS-C treatment. This was likely due to better mixing of H\(^{+}\) and OH\(^{-}\) ions in the upper half of the reactor resulting from greater production of gas bubbles at higher current intensity. Nevertheless, the greater swing of pH in SS-C treatment possibly contributed to its lower nitrate removal efficiency as compared to the C-C treatment.

Lower ORP values were observed in electrical treatments than in controls (Fig. 4), which indicated a more conducive reducing condition for denitrification. Recall that reducing zone is formed around the cathode, while oxidizing zone is created around the anode. As expected, the ORP values at 100 mA scenario decreased after the influent entered the BERs at In-Col 1 (cathode), and then increased at In-Col 2 (anode). Finally, the ORP decreased again as water passed through In-Col 3 (cathode). At 500 mA, even lower ORP values were observed in SS-C treatment but the values remained relatively the same in C-C treatment, as compared to 100 mA scenario. This suggested that a better reducing condition can be created with SS-C treatment, despite the pH (discussed in previous paragraph) and DO (discussed in next paragraph) issues in this up-flow column design. Note that the ORP profile at 500 mA did not follow the same and obvious trend as observed in the 100 mA scenario, which was also likely due to greater mixing at upper column by gas bubbles produced at bottom part of the column reactor.

Meanwhile, the average DO of the influent was 7.9 ± 0.3 mg/L, but immediately reduced to an average of 1.6 ± 0.5 mg/L after entering the reactors at In-Col 1 (Fig. 4). This suggested microbial activity took place immediately by consuming oxygen. In addition, In-Col 1 was located below the anode (In-Col 2), thus leaving it unaffected from O\(_2\) or CO\(_2\) produced at the anode. In both 100 and 500 mA scenarios, the DO levels in SS-C treatment increased at the anode (In-Col 2) and above the anode (In-Col 3 and outlet). However, DO levels at all sampling locations in C-C treatment remained below 2 mg/L. This was because O\(_2\) was produced at anode of SS-C treatment, while CO\(_2\) was likely produced at the anode of C-C treatment. Consequently, the higher DO level in the SS-C treatment may explain the lower DE when compared to the C-C treatment, although an equal amount of external energy source (electron) was provided.

3.5. Denitrifying bacterial communities and their role in denitrification

The abundance of denitrification genes ranged from 2.02 × 10\(^{11}\) to 2.96 × 10\(^{12}\) copies of nosZ gene per gram of dry substrate in SS-C treatment; 1.35 × 10\(^{10}\) to 1.56 × 10\(^{11}\) 10\(^{11}\) nosZ gene copy/g dry substrate in C-C treatment; and 3.05 × 10\(^{11}\) to 3.11 × 10\(^{12}\) nosZ gene copy/g dry substrate in control reactors (Table S3). Meanwhile, the abundance of 16S-rRNA genes ranged from 4.15 × 10\(^{12}\) to 1.70 × 10\(^{14}\) 16S-rRNA gene copy/g dry substrate in SS-C treatment; and 3.10 × 10\(^{12}\)–2.24 × 10\(^{13}\) 10\(^{12}\) 16S-rRNA gene copy/g dry substrate in C-C treatment; and 3.70 × 10\(^{10}\) to 1.14 × 10\(^{11}\) 16S-rRNA gene copy/g dry substrate in control. The gene abundances in C-C treatment were lower than the SS-C treatment and control, but all values were
comparable to other studies where active denitrifying genes were quantified (Feyereisen et al., 2016; Ilhan et al., 2011; Kandel et al., 2006; Warnke et al., 2011). This suggested that microbial denitrification occurs in electrical treatments and the control, with possible electrochemical reduction of nitrate in electrical treatments. However, microbial reduction was likely to be the dominant nitrate removal mechanism because if electrochemical reduction was the dominant mechanism, then the change in current intensity from 500 to 100 mA is expected to yield a much lower nitrate removal efficiency (~ 4-5 times lower) than what was observed. The notable effect of DO on DEs between SS-C and C-C treatments at 500 mA further suggests that microbial denitrification was the dominant mechanism; although, the electron transfer pathway (direct vs indirect) cannot be determined from our experiments.

The average relative abundances of nosZ gene (nosZ to 16S-rRNA) from two replicated SS-C columns were 1.3% and 0.9%, respectively. In the duplicated C-C columns, the average relative abundances were 0.4% and 0.6%, respectively. Meanwhile, the control reactors had 1.2% and 1.3% relative abundance of nosZ gene, respectively. The lower gene abundance and relative abundance in C-C treatment suggested that electrical stimulation may alter the total population and density of microbial communities in the BERs. This may be caused by differences in pH, ORP and DO levels, as well as growth capabilities of denitrifiers and other microbes by utilizing electrons from electrical stimulation. Therefore, it is important to recognize the presence of other microbes, which may outcompete denitrifiers if the environmental conditions become favorable.

3.6. Technoeconomic analysis

As presented in Fig. 5, the electrical treatment did not appear to be an attractive approach from the perspective of additional costs for the benefit of improved denitrification efficiency. The base case, which resembles the traditional woodchip bioreactor had nitrate removal cost at $4.86/kg NO$_3$-N. Our estimated value was almost four times greater than the estimation ($1.07/kg NO_3$-N) by Christianson et al. (2013). The divergent of our base case as compared to Christianson et al. was due to the differences in several input parameters, which includes lifespan of woodchip bioreactors (15 vs 40 years), interest rate (5 vs 4%), and denitrification efficiency (18.5 vs 37.5%). Consequently, our BER scenarios were only compared to our base case.

Scenarios 1 and 2, which corresponded to the low operating current (7.52 A/m$^2$) had much higher N removal costs compared to the base case. Nevertheless, the N removal cost of Scenario 1 can be reduced to base case level with 85% nitrate removal efficiency (data not shown), which can be potentially achieved with a better-designed reactor. The high-current scenarios were even less cost effective. We explored how the cost per unit N removed would change if Scenarios 3 and 4 achieved 100% N removal (data not shown) – but those scenarios were still not economically competitive with the base case.

As shown in Fig. 6, a few of the primary reasons that contributed to the high cost of BER include cathode installation cost, anode maintenance cost and electricity cost. BER typically requires a large cathode surface area, which yield the additional cost with respect to traditional woodchip bioreactors. In Scenario 1, the high degradation rate of graphite anode resulted in frequent need for replacement every 6.4 years, thus contributed to a large portion of the total cost. Although stainless steel anode (Scenario 2) had a much lower degradation rate and does not require replacement, it had a significantly higher material cost than graphite. The sensitivity coefficient analysis for Scenario 1 found that 1% increment in bioreactor’s construction cost, cathode cost, anode cost and electricity cost will increase N removal cost by 0.51%, 0.23%, 0.22% and 0.25%, respectively (Table S4); while the sensitivity coefficient analysis for Scenario 2 showed that 1% increment in bioreactor’s construction cost, cathode cost, anode cost and electricity cost will increase N removal cost by 0.45%, 0.20%, 0.32% and 0.22%, respectively. This suggested that improved denitrification efficiency (thus lower HRT, smaller reactor size), smaller anode, and lower current intensity can be the key to reducing the N removal cost of BER. A better denitrification efficiency can be attained in horizontal-flow reactors as described in Prosnansky et al. (2005). Since SS anode undergo little degradation over a long period, its size can be reduced significantly. Finally, lower current intensity can be used to achieve the same or higher denitrification efficiency by improving the $\eta$ in both scenarios. This can be achieved by using cathode shape that would yield a larger surface area given the same mass.

4. Implication and future of work

Here, we found that up-flow woodchip bio-electrochemical reactors were difficult to operate, and did not achieve the expected denitrification potential which was reported in other studies. This is because it was difficult to optimize the three denitrification parameters (pH, ORP, DO) simultaneously in an up-flow reactor without the use of a pH buffer. An ideal zone for denitrification includes neutral pH, low ORP and low DO. Initially, we aimed to offset the pH difference at anode and cathodes by placing the anode in between the two cathodes. However, inconsistent and extreme pH values were still observed in some locations adjacent to the electrodes. Due to the center location of the anode, distinct oxidizing and reducing zones were not created. The reducing zone, where denitrification takes place, needed to be larger and separated from the oxidizing zone for improved denitrification. In addition, the DO level at the top half of SS-C BERs was significantly higher than C-C BERs and control reactors in this experiment. The increment in DO was because of the O$_2$ gas produced at the stainless steel anode.

Prosnansky et al. (2005) recommended that extreme pH can be avoided by placing the anode at upstream of a horizontal flow reactor while operating at a current density below 12 A/m$^2$. With horizontal flow and upstream-anode design, the ORP and DO concerns also can be overcome by separating the anode and cathode zones with baffles. Larger reducing zone, or low ORP zone, can be created using cathodes with a larger surface area, and therefore plate-shaped instead of the rod-shaped cathode is also recommended.

We mentioned in Section 2.2 that no improvement in DE was observed using 100 mA during the 10 °C treatment period, and because of this finding, the DE at 500 mA current intensity and 10 °C was not evaluated. Future work is recommended to test the performance of a well-designed BER under low temperature conditions to determine if DE can be increased in times such as early spring flow conditions. The
improved DE using electrical stimulation at room temperature could result in greater NO$_3$-N removal during high flows in summer storm events.

5. Conclusion

This study demonstrated improvement in nitrate removal efficiency of woodchip bioreactors using electrical stimulation. The primary nitrate removal mechanism of these electrically modified reactors was suspected to be microbial denitrification. Higher denitrification efficiencies using SS-C (24.0 ± 11.0%) and C-C (40.5 ± 19.5%) BERs were obtained with a current intensity of 500 mA, as compared to control woodchip bioreactors (14.0 ± 6.5%). However, the enhanced denitrification efficiency is associated with additional costs of electrode material cost and electricity cost. In a well-designed BER, the additional costs may be offset with greater denitrification efficiency.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2017.10.002.

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