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Using selectivity to evaluate aqueous- and resin-phase denitrification during biological ion exchange

Michael Edgar[a,b,*], Srivatsan Mohana Rangan[a,b,c,d], Anga G. Delgado[a,b,c] and Treavor H. Boyer[a]

[a] School of Sustainable Engineering and the Built Environment (SSEBE), Arizona State University, P.O. Box 873005, Tempe, AZ 85287-3005, USA
[b] Center for Bio-mediated and Bio-inspired Geotechnics (CBBG), Arizona State University, Tempe, AZ 85281, USA
[c] Biodesign Swette Center for Environmental Biotechnology, Arizona State University, Tempe, AZ 85287, USA
[d] Biodesign Center for Health Through Microbiomes, Arizona State University, Tempe, AZ 85287, USA
*Corresponding author. E-mail: michael.edgar.az@gmail.com

ME, 0000-0002-7060-6986

ABSTRACT

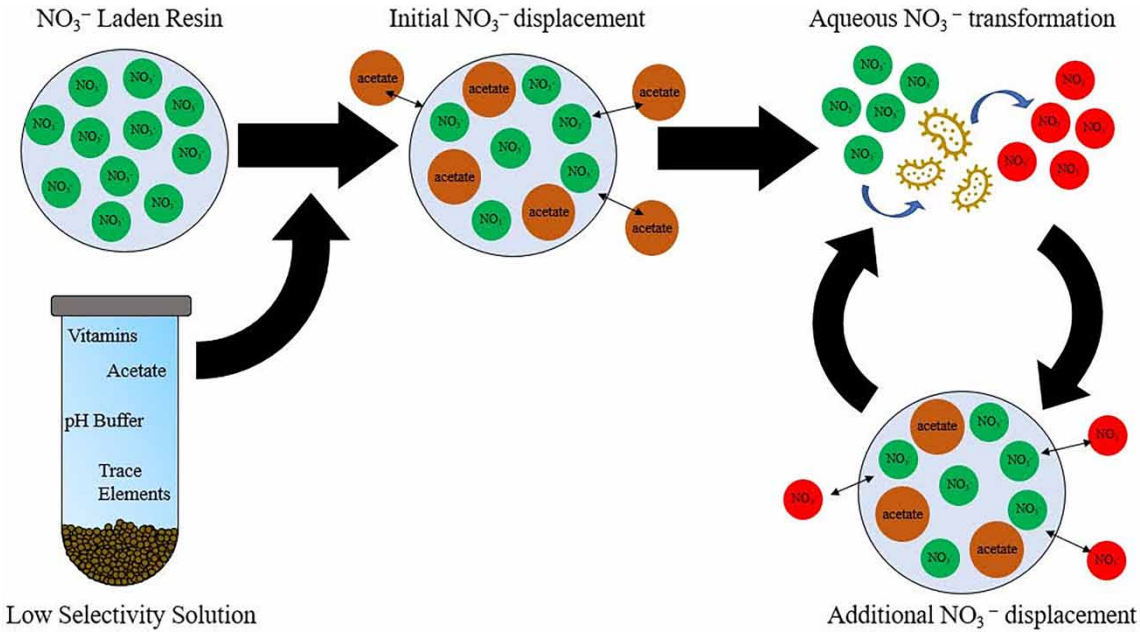
An increased fertilizer application for agricultural purposes has resulted in increased nitrate (NO_3^-) levels in surface water and groundwater around the globe, highlighting demand for a low-maintenance NO_3^- treatment technology that can be applied to nonpoint sources. Ion exchange (IEX) is an effective NO_3^- treatment technology and research has shown that bioregeneration of NO_3^- laden resins has the potential to minimize operational requirements and brine waste production that often prevents IEX application for decentralized treatment. In this work, batch denitrification experiments were conducted using solutions with low IEX selectivity capable of supporting the growth of denitrifying bacteria, while minimizing NO_3^- desorption from resins, encouraging resin-phase denitrification. Although only 15% of NO_3^- was desorbed by the low selectivity solution, this initial desorption started a cycle in which desorbed NO_3^- was biologically transformed to NO_2^-, which further desorbed NO_3^- that could be biotransformed. Denitrification experiments resulted in a 43% conversion rate of initially adsorbed NO_3^-, but biotransformations stopped at NO_2^- due to pH limitations. The balance between adsorption equilibria and biotransformation observed in this work was used to propose a continuous-flow reactor configuration where gradual NO_3^- desorption might allow for complete denitrification in the short retention times used for IEX systems.

Key words: adsorption, BIEX, biotransformation

HIGHLIGHTS

- Microorganisms preferentially transformed aqueous-phase NO_3^-, and no indicators of resin-phase NO_3^- transformation were observed.
- Aqueous- and resin-phase ion concentrations are driven toward an equilibrium state that is dependent on ion selectivities.
- The balance between adsorption equilibria and biotransformations is relevant to the implementation of a continuous-flow BIEX reactor.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Nitrate (NO_3^-) is a contaminant that contributes to eutrophication of surface water and groundwater and has adverse human health effects in drinking water (Ward *et al.* 2018). NO_3^- is effectively removed from water by ion exchange (IEX), but regeneration of these resins with concentrated salt brines requires onsite storage of concentrated chemicals and disposal of brine waste. This regeneration requirement is a significant barrier to the implementation of IEX for decentralized NO_3^- contamination that accounts for a majority of the NO_3^- loading to surface waters globally (Carpenter *et al.* 1998; Howarth *et al.* 2000; Howarth 2008). Biological ion exchange (BIEX) is a type of IEX where resins are regenerated biologically using a biofilm on the surface of the resins and has been suggested as an alternative to overcome barriers associated with chemical regeneration for application to decentralized treatment (Archna *et al.* 2012; Huno *et al.* 2018).

BIEX has been implemented in continuous-flow systems, where water flows into and out of the IEX reactor at a constant rate, for the removal of natural organic matter (NOM) from surface waters (Schulz *et al.* 2017; Amini *et al.* 2018; Winter *et al.* 2018; Edgar & Boyer 2021), resulting in a 100% reduction rate in brine waste and achieving as much as 60% continuous DOC removal. BIEX for NO_3^- removal, however, has been limited to batch configurations. For example, when NO_3^- laden brines (McAdam & Judd 2008) or resins (Meng *et al.* 2014; Ye *et al.* 2019) were contacted with microbial cultures for >4 h, 95–100% brine waste reduction and complete regeneration of resins were achieved. Batch NO_3^- BIEX experiments have relied on the desorption of NO_3^- adsorbed to the resin surface into the aqueous phase, followed by the microbial denitrification of aqueous-phase NO_3^- (Meng *et al.* 2014; Ye *et al.* 2019). Denitrification of adsorbed NO_3^- on the surface of IEX resins has not been experimentally evaluated. It is currently unknown if the denitrifying microorganisms can reduce the NO_3^- adsorbed to the IEX resin.

The desorption of adsorbed constituents from IEX resins by other constituents has been coined secondary ion exchange (SIEX) and is a key mechanism in both continuous and batch BIEX experiments (Schulz *et al.* 2017; Liu *et al.* 2020, 2022; Edgar & Boyer 2021, 2022). In a continuous-flow BIEX system, the desorption of NO_3^- by SIEX is undesirable because NO_3^- in the aqueous phase will flow out of the reactor before microbial denitrification can occur. This is because the timeframes of microbial denitrification are in the order of hours to days (Tiedje 1983; Archna *et al.* 2012; Huno *et al.* 2018), while most IEX resin columns have retention times of approximately 5 min. Previous work has identified the common SIEX constituents in different microbial cultures (wastewater sludge, lab-cultured microorganisms, and natural microorganisms from wood mulch) and quantified their affinities for IEX using separation factors (Edgar & Boyer 2022). The separation factors calculated in Edgar & Boyer (2022) show that buffer and salt solutions exist that could be used to culture microorganisms

while minimizing SIEX, promoting biological denitrification on the resin surface rather than the aqueous phase as would be desired in a continuous-flow system.

The goal of this study was to determine if resin-phase microbial denitrification is possible and identify potential configurations for continuous-flow BIEX implementation. The specific objectives of this work were to (1) identify a microbial mineral medium with low IEX selectivity to minimize secondary IEX with NO_3^-, (2) achieve aqueous-phase microbial denitrification in a low IEX selectivity microbial mineral medium, and (3) determine if microbial denitrification is possible using NO_3^- adsorbed to IEX resins (i.e., resin-phase denitrification). These objectives were accomplished using batch denitrification experiments in solutions with low IEX selectivity as identified in Edgar & Boyer (2022).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Mineral medium composition

A stock salt solution and trace element solutions (A and B) were prepared as described in Robles *et al.* (2021), where the tabulated composition of the stock solutions can be found (Robles *et al.* 2021). The stock salt solution was used to make a reduced anaerobic mineral medium and test media (modified the reduced anaerobic mineral medium with a low affinity for IEX). The reduced anaerobic mineral medium was prepared in a 2 L bulb flask filled with 1 L de-ionized (DI) water, 10 mL salt stock solution, 1 mL trace element solution A, and 1 mL trace element solution B. About 0.25 mL of 0.1% resazurin was added to ensure anoxic conditions in the mineral medium. The medium was prepared according to the Hungate method and was reduced with N_2 gas. The medium was buffered with 15 mM HEPES and pH was adjusted to 7.5 with 4 M NaOH. The mineral medium was reduced by adding 0.4 mM L-cysteine and 0.2 mM $Na_2S \times 9H_2O$. While sparging, 100 mL aliquots of growth media were transferred from the flask into 10 separate 120 mL serum bottles and then immediately capped with rubber stoppers and aluminum crimp caps. The bottles were then autoclaved and ready to be used for the growth of denitrifying bacteria.

Two liters of test media were prepared similarly to the reduced anaerobic mineral medium via the Hungate method with a few changes that minimized salt concentrations. No stock salt solution, resazurin, L-cysteine, or $Na_2S \times 9H_2O$ was added. Furthermore, the pH was adjusted to 6.7 (instead of 7.5) using 4 M NaOH. This modified reduced anaerobic mineral medium was then ready to be used as a low IEX selectivity microbial growth medium in the batch denitrification experiments.

2.1.2. IEX resin

The resin used in this experiment was A520E strong base anion exchange resin (Purolite). A520E is a gel, macroporous chloride-form resin with a polystyrene base. The resin has quaternary ammonium functional groups and a capacity of 0.9 eq/L, and was chosen for its high NO_3^- selectivity. The chloride-form resin was converted into the NO_3^- form by mixing 10 g of resin (dry weight) in a 150 mL concentrated KNO_3 solution (50× resin capacity) at 100 rpm for 24 h. The resin was then rinsed with DI water until the rinse water conductivity was <30 μS/cm to remove any remaining non-adsorbed KNO_3. The initial NO_3^- solution and the solution after 24 h of mixing with IEX resin were analyzed to determine the amount of adsorbed NO_3^-.

2.2. Experimental methods

2.2.1. Microbial inoculum

In an anaerobic glove chamber, two bottles of the reduced anaerobic mineral medium were spiked with 20 mM NO_3^-, 35 mM acetate, and 1 g/L yeast extract. Then, 600 μL anaerobic digester sludge from Mesa Northwest Water Reclamation Facility in Mesa, Arizona, was added as inoculum to each bottle. The inoculum collected was identified as rich in denitrifying bacteria by plant operators due to rapid denitrification observed in the anaerobic digester. The bottles were incubated at 32 °C and shaken on a shaker table at 100 rpm. A 1 mL sample was taken every 48 h and analyzed for NO_3^-. If NO_3^- was less than 5 mM, the culture was respiked with 20 mM NO_3^-. This process continued for 2 weeks.

After 2 weeks, six more bottles were spiked with 20 mM NO_3^-, 35 mM acetate, and 1 g/L yeast extract. The inoculum consisted of a 5 mL culture from the previously grown denitrifying culture cultivated for 2 weeks. These six bottles were incubated on a shaker table at 100 rpm and 32 °C and respiked with NO_3^-, acetate, and yeast extract identically to the previous bottles until the culture was sufficiently grown (visible flocs achieving complete NO_3^- reduction within 24 h of a spike).

Culture samples were collected from the microbial growth bottles and then concentrated and washed as follows. Bottles were transferred to an anaerobic chamber, and the contents were divided into 50 mL centrifuge tubes and centrifuged

using an Eppendorf microcentrifuge 5415R (Hauppage, NY) at 13,200 rpm for 15 min. The supernatant was discarded, and pellets from individual bottles were combined and resuspended into the mineral medium. The tubes were then centrifuged again, the supernatant was discarded, and the pellets were resuspended two additional times. This process ultimately resulted in a single 50 mL centrifuge tube containing the cultured bacteria that had been sufficiently rinsed for the batch denitrification experiment.

2.2.2. Batch denitrification experiment

The fresh reduced anaerobic mineral medium was prepared at the start of the batch denitrification experiments. The following six experimental conditions used are presented in Table 1: condition 1 provided a control for the test water; Condition 2 provided a control for microorganisms in test water without NO_3^-; Condition 3 was a control for microorganisms in test water with aqueous NO_3^-; Condition 4 was a control for the interactions between test water and resin; Condition 5 was the test condition containing test water, resin, and microorganisms; and Condition 6 was a control for resin in DI water. Each condition was tested in triplicate resulting in 18 total 120 mL glass serum bottles.

Ten milligrams of resin-adsorbed NO_3^- was added to each resin bottle, which is equivalent to 0.18 g of dry NO_3^- saturated resin (calculated during resin NO_3^- saturation). The appropriate medium was then added to each bottle, i.e., test water without NO_3^-, test water with NO_3^-, or DI water (Table 1). Next, the concentrated microbial culture was divided between the nine bottles for Conditions 2, 3, and 5 (5.5 mL concentrated culture). Test Condition 3 received 0.32 mL of 500 mM NO_3^- solution (equating to 10 mg of NO_3^-, identical to resin conditions). Conditions 1–5 received 0.28 mL of 1 M acetate solution, equivalent to adding 17.5 mg of acetate to each bottle. The bottles were incubated in a shaker table at 100 rpm and 32 °C. Samples were collected at $t = 0$ h, $t = 1$ h, 2 h, 1 day, 2 days, 3 days, 7 days, and 14 days. Filtered samples were analyzed for inorganic ions and acetate. A horizontally fixed frictionless syringe (borosilicate glass) was used to measure gas generation in bottles prior to collecting fluid samples. The syringe plunger was allowed to expand freely until stopping, at which point the gas volume was recorded and the plunger was fully depressed before the syringe was disconnected from the bottle. After 7 days, one bottle from each test condition had 2 mL of gas removed for gas chromatography analysis.

2.2.3. Resin regeneration

After day 14, samples were collected, and the contents of all bottles were centrifuged, rinsed in DI water, re-centrifuged, and the resin and microorganisms were recovered. Sonication was performed for 5 min to detach microorganisms from the resin in Condition 5. The recovered resin and microorganisms were dried and weighed. Resins were regenerated by mixing in 100 mL of 3,500 mg/L Na_2SO_4 solution (SO_4^{2-} equivalent to 3× the capacity of each resin sample) for 24 h. Initial and final regenerant samples were collected and analyzed for inorganic ions and organic acids.

2.2.4. Adsorption equilibrium experiment

A batch adsorption equilibrium experiment was conducted to determine the final active site composition of resin and test water mixtures with various concentrations of NO_3^-, NO_2^-, and acetate. Four conditions were used for the experiment, each completed in triplicate 120 mL serum bottles as follows: (1) DI and resin control, (2) 100 mg/L NO_2^- (equivalent concentration to adsorbed NO_3^-), (3) 100 mg/L acetate (equivalent concentration to adsorbed NO_3^-), and (4) 20 mg/L NO_2^- + 175 mg/L acetate (identical to the final amount of NO_2^- and acetate present in Condition 5 from the denitrification experiment). About 10 mg of resin-adsorbed NO_3^- was added to each bottle and mixed on a shaker table for 24 h, then resins

Table 1 | Experimental conditions tested in triplicate in this study

Condition	Medium	Resin	Microbes	Acetate
1 (Test water only)	Test water	No	No	Yes
2 (Inoculum only)	Test water	No	Yes	Yes
3 (Aqueous NO_3^-)	Test water + aqueous NO_3^-	No	Yes	Yes
4 (Test water and resin)	Test water	Yes	No	Yes
5 (Resin and inoculum)	Test water	Yes	Yes	Yes
6 (Resin and DI)	DI water	Yes	No	No

The text in parentheses next to each test condition number represents how each condition is referenced hereafter.

were regenerated as in the denitrification experiment. Samples were collected before adding resin, after 24 h of mixing, and after regeneration. All samples were analyzed for acetate, NO_3^-, NO_2^-, and Cl^-.

2.3. Analytical methods

The pH and conductivity measurements were completed using an Orion Dual Star Multiparameter meter, an Orion 9156BNWP (Thermo Fisher Scientific, Waltham, MA, USA) combination pH probe, and an Orion Star A212 conductivity probe. Concentrations of PO_4^{3-}, NO_3^-, sulfate (SO_4^{2-}), nitrite (NO_2^-), and chloride (Cl^-) anions and sodium (Na^+), ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) cations in the aqueous phase were measured using ion chromatography (Dionex ICS 5000+, Sunnyvale, CA, USA). Anion chromatography was conducted using a Dionex AS-18 column with KOH eluent, and cation chromatography was conducted using a Dionex CS-15 column with methanesulfonic acid eluent. Both cation and anion chromatography utilized a 0.1–100 ppm calibration curve for all measured ions. Organic acids including acetate were measured using high-performance liquid chromatography (HPLC; Shimadzu LC-20AT) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) as detailed previously (Joshi *et al.* 2021). Gaseous N_2, dihydrogen (H_2), nitric oxide (NO), nitrous oxide (N_2O), and carbon dioxide (CO_2) were measured using a Shimadzu GC-2010 Pro gas chromatograph.

3. RESULTS AND DISCUSSION

3.1. Aqueous-phase denitrification

The first step toward understanding resin-phase denitrification is to compare aqueous NO_3^- concentrations in bottles with denitrifying bacteria present. Figure 1 presents the aqueous ion concentrations over time for the aqueous NO_3^- condition (Condition 3) and the resin and inoculum condition (Condition 5). The key difference between these conditions is that Condition 3 contains initially aqueous NO_3^-, while Condition 5 contains initially adsorbed NO_3^-. The aqueous NO_3^- control condition shows a decrease in aqueous NO_3^- at 3 days (the initial concentration of 90 mg/L) that continues until 14 days to an aqueous concentration of 1.4 mg/L. Increases in nitrite (NO_2^-) were observed in parallel to the NO_3^- decrease and stabilized at 59 mg/L after 14 days. The decrease in NO_3^- and increase in NO_2^- are the representative of the first step in microbial denitrification, and NO_2^- was not transformed further. Approximately 77% of the total N in the aqueous NO_3^- control condition is accounted for as NO_2^-, indicating that 23% of N was either assimilated as biomass or converted further into gas. The biological transformation observed in the aqueous NO_3^- condition establishes a baseline for the maximum potential NO_3^- conversion that may be observed in the test conditions with adsorbed NO_3^-.

After using the aqueous NO_3^- control condition to determine a baseline for biological transformation, aqueous ion concentrations are observed in the test conditions to determine if the experimental conditions successfully minimized the influences of SIEX. The resin and inoculum condition showed constant low concentrations of chloride, phosphate, and sulfate. Because the concentrations of these inorganic ions remained low and unchanged throughout the experiment, SIEX involving these ions is believed to have little to no influence. There was some initial NO_3^- desorption over the first 2 days due to acetate

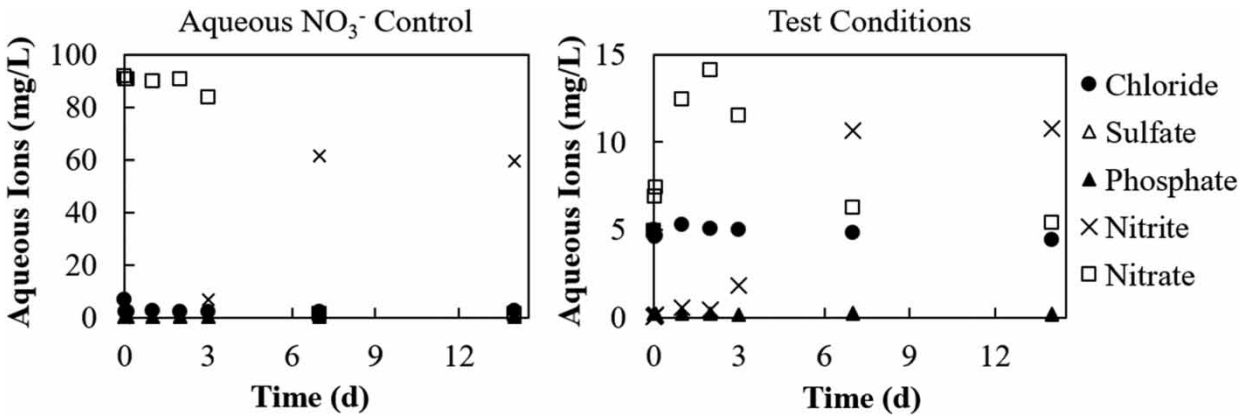


Figure 1 | Aqueous ion concentrations versus time for the aqueous NO_3^- control bottle and the test bottle containing resin and inoculum. Low aqueous ion concentrations verify that secondary IEX was minimized. Increasing NO_2^- concentrations signifies biological activity.

adsorption, resulting in an aqueous NO_3^- concentration of 14 mg/L that began decreasing after 3 days and dropped to 5.3 mg/L after 14 days. As in the aqueous NO_3^- condition, the disappearance of aqueous NO_3^- and the generation of NO_2^- occur concurrently and signify biological transformation. At the end of the experiment, the amount of aqueous NO_2^- and NO_3^- accounted for approximately 15 and 6%, respectively, of the total N initially added to the system as adsorbed NO_3^-. Therefore, approximately 21% of the total N was in the aqueous phase at the end of the experiment.

Because N_2 gas production is an indicator of complete biological denitrification, gas generation was monitored for all conditions. Gas production versus time for all bottles except the resin and DI control (Condition 6) is shown in Figure 2. The aqueous NO_3^- control condition generated the highest gas volume of 5.1 mL, while the resin and inoculum condition and the inoculum-only condition (Condition 2) generated 2.0 and 2.6 mL, respectively. The low volume of gas generated in the resin and inoculum condition indicates that microbial denitrification is not proceeding past NO_2^- and producing gas, since there was less gas generation than the inoculum-only condition, and the only gases detected during gas chromatography (GC) measurement for the resin and inoculum condition were H_2, N_2 (both present in the anaerobic chamber), and CO_2 (a byproduct of the NO_2^- production). The gas generation observed in the aqueous NO_3^- condition had some amounts of NO and N_2O but was mostly gaseous CO_2 produced during the conversion of NO_3^- to NO_2^-. The low volume of gas generated in other control conditions is likely due to the change in temperature from the anaerobic chamber where the experimental bottles were set up (25 °C) and the incubator where the bottles were incubated during the experiment (32 °C). Both gas production data and aqueous inorganic ion data indicate that denitrification is stopping at NO_2^-, but this limitation is not due to available NO_3^- being bound to the resin because it also occurred in control bottles. It was not possible to quantify N_2 gas generation, as the atmosphere of the anaerobic chamber used was over 98% N_2.

The aqueous- and gas-phase analyses presented in this section help to identify the biological transformations taking place and confirm that the experimental conditions used were appropriate for both the stimulation of biological transformation and the inhibition of SIEX. Both aqueous and gas data confirm biological activity in the expected bottles, but the data are also indicative of incomplete denitrification that stops at NO_2^-. Since this observation holds true for the aqueous NO_3^- control bottles, it is apparent that the low selectivity solution used is the likely cause of incomplete denitrification rather than the lack of availability of adsorbed N species. The aqueous ion trends observed in test conditions were indicative of limited SIEX by inorganic ions, but as much as 21% of total N may have been desorbed by acetate.

3.2. Resin-phase denitrification

To complete a mass balance on N species, inorganic ions, and acetate and to investigate whether biological transformations occurred on the resin surface or exclusively in the aqueous phase, it is necessary to determine the composition of constituents adsorbed to IEX resins. Adsorbed species were analyzed by regenerating resins and measuring aqueous ion concentrations in the regenerant solution. The active site composition for all resin-containing conditions is presented in Figure 3. The resin and DI condition contained 88% NO_3^- and 9% Cl^-, indicating that some Cl^- was not initially desorbed by NO_3^- during resin

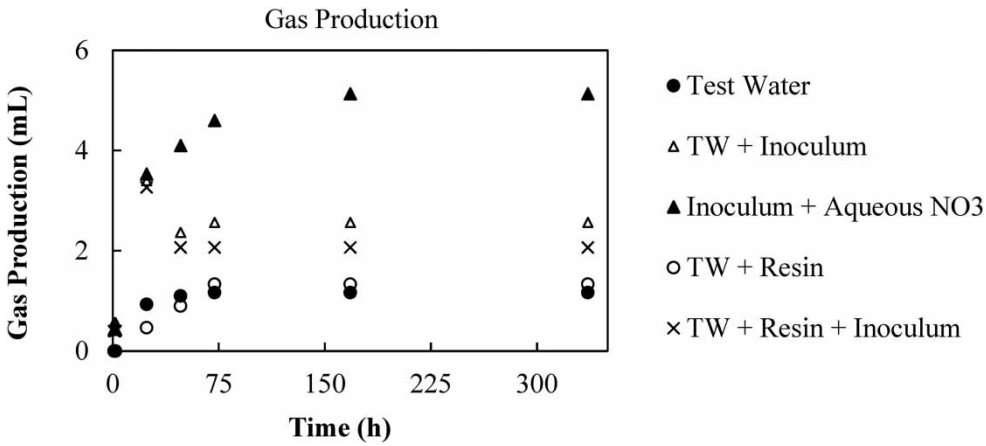


Figure 2 | Gas production versus time for all conditions. The aqueous NO_3^- condition had the highest gas generation, while the test conditions achieved slightly less gas generation than inoculum-only conditions.

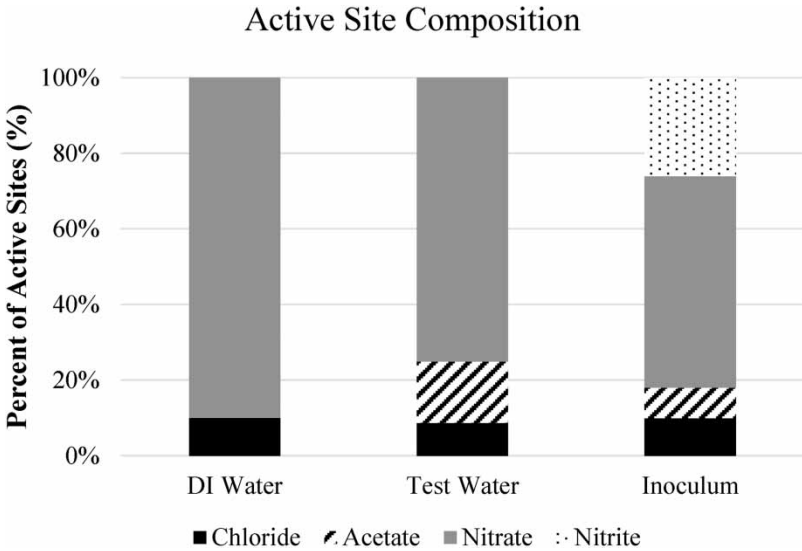


Figure 3 | The active site composition of resins as determined by resin regeneration. Resins contained almost entirely NO_3^- and Cl^-, with NO_2^- detected only in the inoculum + test water + resin bottles.

saturation, which may have been caused by insufficient contact time during resin saturation, insufficient KNO_3 concentrations during resin saturation, or an equilibrium balance between the amount of aqueous and adsorbed Cl^- during resin saturation. The test water-only condition performed similarly and verified that only acetate was displacing NO_3^-, as acetate accounted for 15% of active sites in this condition. This is consistent with 21% of aqueous TN species observed to have been desorbed in resin and inoculum conditions, as discussed in the previous section. From the perspective of a charge balance on the test water and resin conditions (no inoculum), approximately 0.025 meq of acetate was adsorbed to the resin at the end of the experiment, and approximately 0.023 meq of NO_3^- was observed in the aqueous phase. This confirms that the increase in aqueous NO_3^- concentrations discussed in the previous section is due to the adsorption of acetate.

The resin and inoculum condition showed less adsorbed acetate (7.7% of active sites) and much less adsorbed NO_3^- (53% of active sites) at the end of the experiment compared to the resin and test water condition without inoculum. Most notably, 25% of active sites were occupied by NO_2^-. This resin-phase composition highlights the possibility of resin-phase biotransformation of NO_3^-, since a large fraction of NO_3^- has been converted to NO_2^-, while only a small fraction was observed to have been desorbed by acetate. Additionally, the amount of adsorbed acetate highlights the possibility that adsorbed acetate was also used by microorganisms. Because the initial NO_3^- desorption in resin and inoculum conditions was nearly identical to resin and test water conditions (approximately 0.023 meq desorbed NO_3^-), one would have expected to find similar amounts of adsorbed acetate in the resin and inoculum conditions, but the observed amount of adsorbed acetate was much less (7.7% in resin and inoculum conditions versus 15% in resin and test water conditions). The aqueous- and resin-phase ion compositions observed in the resin and inoculum condition are strong indicators for resin-phase biological denitrification.

To emphasize the importance of the resin-phase compositions in the resin and inoculum conditions, a complete N balance can be conducted for the resin, gas, and aqueous phases. Figure 4 presents the fate of nitrogen in all bottles containing NO_3^-, which is presented as a percentage of total nitrogen in the adsorbed and aqueous phases. The aqueous NO_3^- and inoculum condition presented on the left side of Figure 4 provides a baseline for biological transformation and shows that nearly all (>85%) of the initial aqueous NO_3^- in the system is converted to NO_2^-, biomass, and gas (approximately 13% associated with gas generation or biomass). The test water and resin condition quantifies SIEX, with NO_3^- desorption occurring due to acetate adsorption. The key result from this figure is the resin and inoculum condition, as it shows that there is an observed transformation of NO_3^- to NO_2^- both in the aqueous and resin phases.

The results presented in Figures 2–4 clearly show biological activity under the provided conditions, and that SIEX only occurred with <15–18% of adsorbed NO_3^- due to desorption by acetate. These results provide a positive outlook for denitrifying BIEX implementation in a continuous-flow system due to the clear stepwise transformation observed for adsorbed NO_3^-. The potential utilization of adsorbed acetate by microorganisms is an added benefit, as well as the adsorption of NO_2^-, which

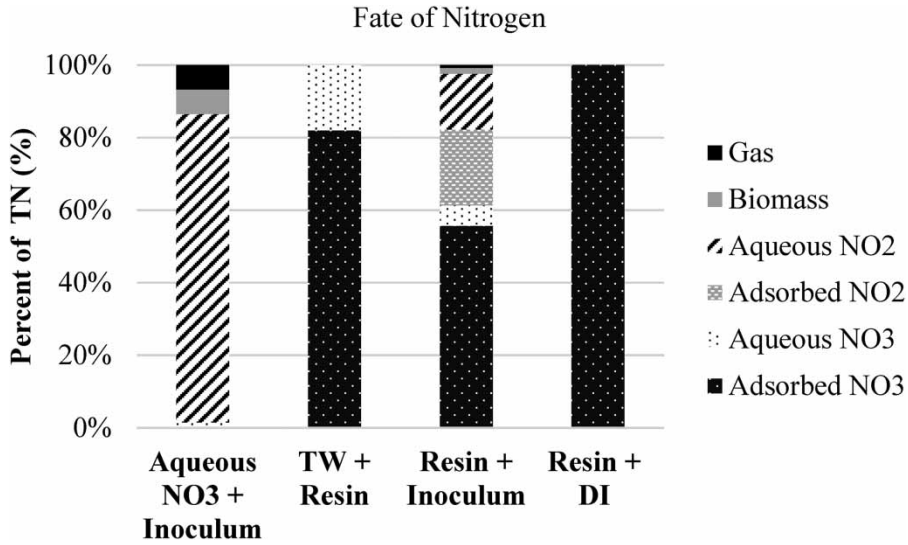


Figure 4 | The fate of N was determined after resin regeneration using a mass balance on adsorbed and aqueous ionic species.

will need to be further transformed for complete denitrification. Although these conditions are favorable for implementation in a continuous-flow system, further investigation is still required to determine if biological transformations actually occur on the resin surface or if the ultimate resin-phase composition observed is due to continuous adsorption and desorption of acetate, NO_3^-, and NO_2^- as the aqueous- and resin-phase concentrations are driven toward an equilibrium state that is continuously fluctuating due to biological transformations. To further address this theory, an adsorption equilibrium experiment was completed.

3.3. Adsorption equilibrium

The adsorption affinity of an ion is its likelihood of adsorbing to an IEX resin and is often quantified using the aqueous- and resin-phase concentrations of that ion, as well as the aqueous- and resin-phase concentrations of the ion that it will need to desorb in order to adsorb to the IEX resin (Edgar & Boyer 2021). Because of the constantly changing adsorbed and aqueous concentrations in the denitrification experiments, it is possible that there was continuous adsorption and desorption of NO_3^-, NO_2^-, and acetate as biological transformations occurred and the adsorption equilibrium changed. A batch adsorption equilibrium experiment was conducted to evaluate whether the final active site composition observed in the denitrification experiments could be attributed to a repetitive cycle of aqueous-phase biotransformations followed by additional NO_3^- and acetate desorption by NO_2^- generated from the biotransformations. The results of the batch adsorption experiment are presented in Figure 5.

Condition 2, which started with equivalent (meq:meq) concentrations of aqueous NO_2^- and adsorbed NO_3^-, resulted in an average of 39% NO_3^- desorption. Condition 3, which started with equivalent concentrations of aqueous acetate and adsorbed NO_3^-, resulted in 11.3% of desorbed NO_3^-. This is consistent with literature that has suggested IEX selectivity follows the order of $NO_3^- > NO_2^-$ > acetate (Marina *et al.* 1997; Pohl *et al.* 1997; Li & Yang 2015; Edgar & Boyer 2022). Condition 4, which mimicked the test conditions in the denitrification experiment, resulted in active site compositions of 17.1% of NO_2^- and 8.8% of acetate, which is very similar to the ultimate active site composition observed in the denitrification experiment, which was 24.9% of NO_2^- and 7.7% of acetate. The similar active site composition in both experiments indicates that adsorption equilibria are the major driving force for NO_3^- desorption in both experiments. However, the higher amount of NO_3^- desorbed in the denitrification experiments indicates that the presence of denitrifying bacteria encourages further NO_3^- desorption by decreasing aqueous NO_3^- concentrations and increasing aqueous NO_2^- concentrations.

Though these results indicate that resin-phase biotransformation was unlikely, this gradual NO_3^- desorption still has positive implications for a continuous-flow denitrifying BIEX system, since the small amounts of NO_3^- released from the resin over time could potentially be denitrified in the short retention times used in IEX treatment systems, particularly if desorbed NO_3^- could stay present in the extracellular matrix of the biofilm during diffusion to and from active sites through the biofilm (de Beer &

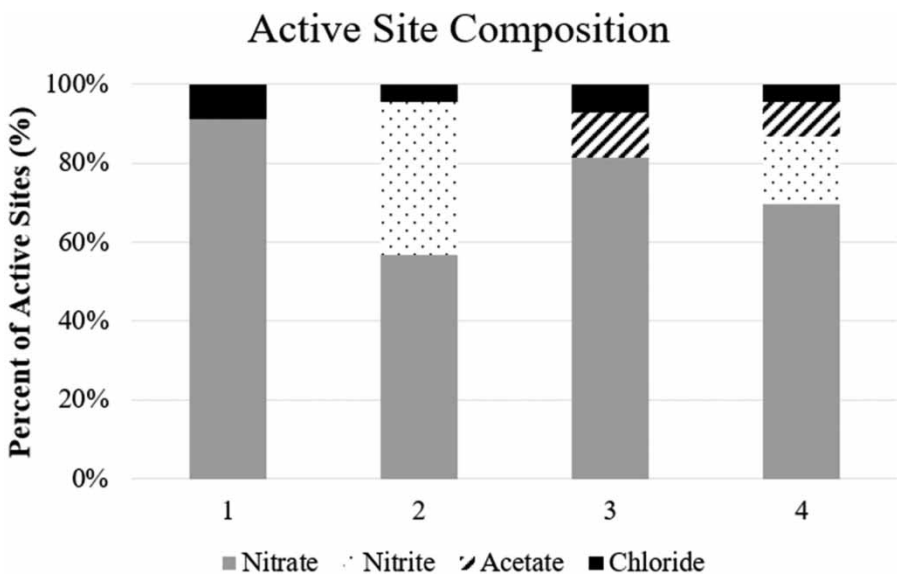


Figure 5 | Active site composition in adsorption equilibrium experiments containing either (1) DI water only, (2) equivalent concentrations NO_2^- and NO_3^-, (3) equivalent concentrations acetate and NO_3^-, or (4) concentrations identical to Condition 5 from the denitrification experiment (20 mg/L NO_2^- and 175 mg/L acetate).

Stoodley 1995; Horn & Morgenroth 2006). NO_2^- accumulation is a common barrier to achieving denitrification under a range of conditions (Glass & Silverstein 1998; Cao *et al.* 2013; Yuan *et al.* 2013; Rocher *et al.* 2015), and the NO_2^- accumulation observed in this work may be due to pH limitations, inappropriate C/N ratio, or a lack of available nutrients, vitamins, or other trace elements. pH is a likely contributor as all bottles had a pH value from 6.3 to 6.5 at the end of the denitrification experiment. The next step toward a continuous-flow denitrifying BIEX system should be flow-through experiments that determine if NO_3^- washout occurs at a variety of flow rates, water compositions, resin types, and at various stages of biofilm growth.

4. CONCLUSIONS

This work has identified a low selectivity solution, containing constituents with low IEX affinity, and IEX resin pair that are amenable to achieving biotransformation of NO_3^- in a batch solution with approximately 47% of NO_3^- desorption, showing promise for BIEX implementation in a continuous-flow denitrification system. Denitrifying bacteria were shown to transform desorbed NO_3^- to NO_2^-, driving adsorption equilibria toward additional NO_3^- desorption. The gradual NO_3^- desorption facilitated by this biotransformation presents an opportunity for a continuous-flow denitrifying BIEX configuration where desorbed NO_3^- can be denitrified in the short retention times used for IEX systems. The success of a continuous-flow configuration will still be dependent on overcoming the barrier of NO_2^- accumulation, which means maintaining the appropriate pH conditions, vitamins, minerals, and trace elements required for denitrifying bacteria while still minimizing SIEX. The successful biotransformation achieved under these test conditions has enhanced the understanding of BIEX and specifically indicated the remaining gaps in knowledge that must be addressed for its implementation in a continuous-flow configuration.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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