Modeling enhanced denitrification in groundwater through electron competition among nitrogen species to identify N_2O emissions

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Background

Groundwater nitrate pollution represents a widespread threat to water quality, driven largely by inputs from septic systems and agricultural fertilizers. These sources contribute to eutrophication in surface waters via contaminated groundwater discharge. In Cape Cod (MA -USA), where both environmental and economic impacts of nitrogen are substantial, a coordinated regional effort is underway to reduce nitrogen levels in water bodies. Among the emerging strategies, permeable reactive barriers (PRBs) using emulsified vegetable oil (EVO) injections have shown strong potential for nutrient removal.

Demonstration tests of denitrifying PRBs highlight that dosing and longevity are crucial concepts to improve performance. This study utilizes column experiments and mathematical modeling to understand and describe the reactions that occur in the subsurface to transform nitrate into dinitrogen and other intermediate nitrogen oxides.

By coupling a process-based denitrification model with transport and emulsion emplacement we aspire to unravel the interplay between EVO degradation, nitrate reduction, and flow dynamics. This endeavor seeks to enhance our comprehension of the reactions driving denitrification in PRBs, providing insights for optimizing the design and operation of these systems for sustainable nitrogen removal. Questions on EVO dosing, flow rate influence and incomplete denitrification are better answered with the use of model simulations.

Materials and Methods

Two column experiments (15.2 cm length and 5.1 cm diameter) were packed with aquifer material from Orleans, MA – USA (medium sand, column porosity = 0.306) to simulate denitrification enhanced with EVO in PRB systems. The columns received 4.4 and 8.9 mg-EVO/g-dry soil (SRS-NR manufactured by Terra Systems) at 1 mL/min for 2.3 pore volumes followed by stream water with nitrate at 1 mL/min for the remainder of the experiment. Control columns were sectioned after emulsion delivery to determine initial conditions for emulsion emplacement and biomass distribution. Column effluent samples were analyzed for nitrate, nitrite, soluble substrate as COD and pH. Experimental data from two distinct columns were used for model calibration.

Reactions were defined based on a wastewater framework with a new feature that utilizes two biomass communities as illustrate in Figure 1. While EVO degradation transforms triglycerides into more available forms of carbon through hydrolytic bacteria, the denitrifying bacteria reduce nitrogen species independently, competing for a pool of electron mediators that can be in an oxidized or reduced form depending on the availability of acetate.

The calibrated model was utilized to generate multiple simulations that elucidate the role of flow, EVO dosage and influent nitrate in the system performance. The environmental and design parameters evaluated in the simulations are shown in Table 1. The model's ability to describe intermediate steps in denitrification enables the prediction of direct nitrous oxide emissions due to PRBs, an important secondary effect of the this treatment that was often neglected. Four performance metrics were evaluated in all simulations to identify trends and trade-offs in PRB design and operation: total N_2O emissions, N_2O emissions per influent nitrate, nitrate removal, and nitrate removal per influent nitrate.

Table 1. Environmental Conditions and Design Parameters for System Performance Simulations

	Darcy velocity (m/d)	EVO retained (mg-EVO/g-dry soil)	Influent nitrate (mg-N/L)
High flow	0.68	4.4	10
		8.9	30
		17.7	80
Low flow	0.03	4.4	10
		8.9	30
		17.7	80

Electron competition framework

This study introduces a novel dynamic electron competition model that links biomass growth and decay with electron mediator availability, improving simulation accuracy of denitrification in groundwater systems treated with emulsified vegetable oil (EVO). Unlike previous models assuming steady-state biomass, our approach accounts for temporal changes in denitrifier biomass, which influences the pool of reduced and oxidized electron mediators.

The model incorporates Monod kinetics for each denitrification step, mediated by specific reductases (e.g., *narG, nirS/nirK, norB, nosZ*), and simulates competition among nitrogen species under electron-limited conditions. EVO, a complex carbon source, undergoes hydrolysis via hydrolytic bacteria expressing the *fhs* gene, producing acetate and glycerol. Acetate fuels denitrifier growth and drives electron competition, while glycerol supports hydrolyzer biomass growth, modeled independently. Triglyceride hydrolysis is included as a potential rate-limiting step, linking carbon degradation to incomplete denitrification outcomes.

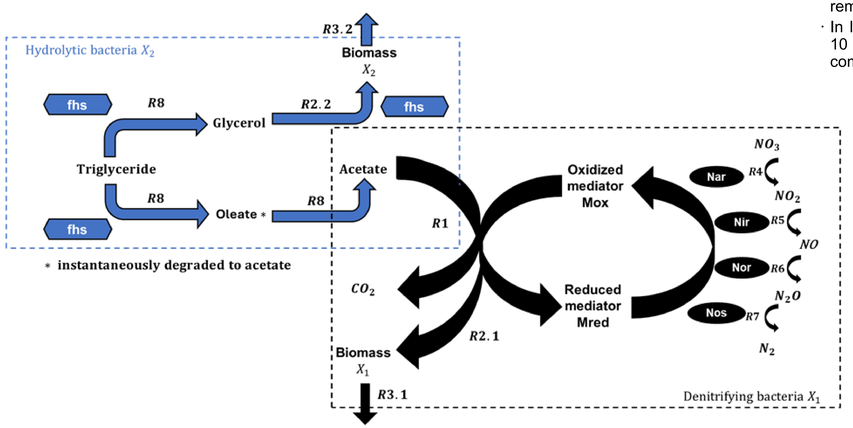

Figure 1. Oxi-reduction of Carbon and Nitrogen mediated by electron carriers and the respective target genes for each bioreduction/ bio-oxidation step. Adapted from Pan et al., 2013.

Results and Discussion

Across all simulations, effluent nitrate levels initially declined during biostimulation, followed by a sustained treatment phase with no detectable nitrate. As the carbon source was depleted, nitrate concentrations rebounded to influent levels, signaling treatment exhaustion.

Eighteen simulation scenarios at low (0.03 m/d) and high (0.68 m/d) Darcy velocities (Figure 2) revealed that total nitrate removal improves with higher EVO or nitrate inputs. Simulations show that low-flow conditions led to ~50% less nitrate removal and significantly longer treatment durations (5–14×) due to lower nitrate flux and extended residence time.

Overall, high-flow systems were more influenced by advection, while low-flow systems were dominated by biochemical processes—highlighting the need to identify the critical flow regime where this shift occurs.

N_2O total emissions are higher as total nitrate removed is higher. N_2O emissions normalized by influent nitrate varied with the degree of nitrate reduction, with two unexpected trends emerging:

- In high-flow, 10 mg-N/L nitrate scenarios, N_2O emissions increased with higher EVO dosages, suggesting enhanced duration of electron competition despite improved nitrate removal. This is a result of limited denitrifying biomass growth.
- In low-flow simulations, treatment duration remained unchanged across EVO dosages at 10 mg-N/L nitrate, with a surprising 35% shorter duration at the highest EVO dose compared to higher nitrate concentrations.

Figure 2. Nitrous oxide emissions and nitrate removed (left); nitrous oxide emissions per influent nitrate and nitrate removed per influent nitrate (right) in simulations with high flow and low flow under multiple nitrate and dosage conditions

How N_2O emissions compare with other N control technologies

To contextualize nitrous oxide emissions from permeable reactive barriers, we compared them with other nitrate removal technologies, ranging from passive (e.g., natural attenuation, constructed wetlands) to active (e.g., sequencing batch reactors, SBRs). PRBs demonstrated high nitrate removal efficiencies (82–100%) and notably lower N_2O emissions factor (0.02–0.30%), which is defined as the fraction of nitrous oxide formed per nitrate inflow in the system. **Even though PRBs are a less controlled technique when compared to conventional wastewater treatment, the model indicates that PRBs will not results in significantly higher N_2O emissions.**

PRBs also maintained performance across a wide range of nitrate loads (10–80 mg-N/L) and hydraulic conditions. Their vertical design and small footprint make them well-suited for space-limited sites. Enhanced flow designs offer additional optimization potential while keeping emissions low. Future work should measure nitrous oxide emissions directly to confirm emissions factors.