Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Electronic Supporting Information

Quantifying the Temperature Dependence of Nitrate Reduction in Woodchip Bioreactors: Experimental and Modeled Results with Applied Case-Study

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Experimental Apparatus

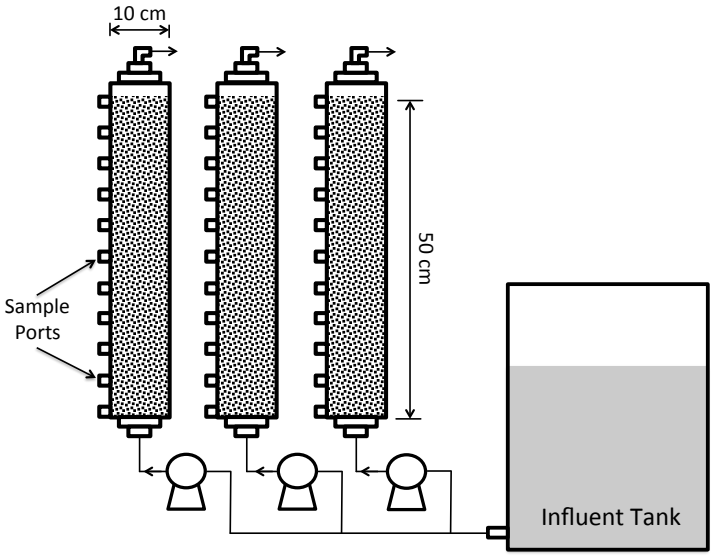

Figure S1. Schematic of up-flow woodchip reactor columns.

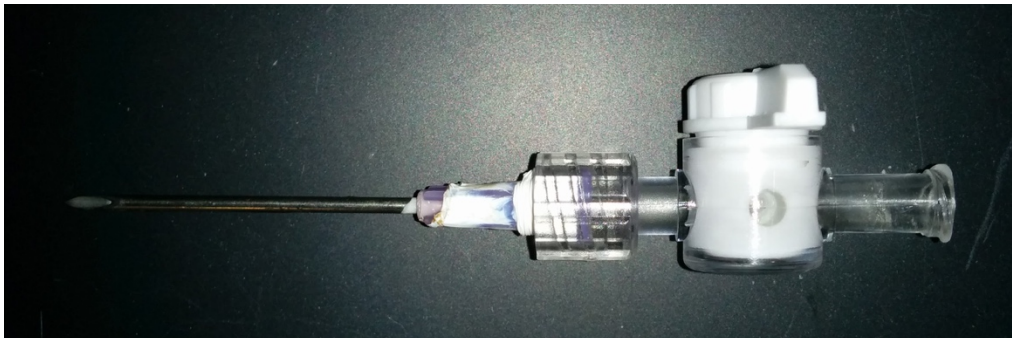

Figure S2. Image of sampling port mechanism. Luer-lock needles attached to ball valves were press-fit into holes along the side of the reactor columns to draw samples.

Tracer Experiments and Column Porewater Measurements

Potassium bromide was used as a conservative tracer. The columns were fed deionized water at 26 mL min^{-1} for 24 hours, then fed a 1mM KBr solution for 4 h (columns 2 and 3) or 5 h (column 1) (Figure S3). Bromide samples were collected from sample ports located 25 cm and 50 cm from the inlet every 15 minutes and measured with a Hanna Instruments HI4102 bromide combination electrode. Bromide concentration data were fit with a curve using[1] CXTFIT 2.0. Based on high tracer recovery, it was assumed that irreversible physical adsorption and chemical retardation of the tracer did not occur. Accordingly, the physical decay/irreversible adsorption coefficient, μ, and the retardation factor, R, were left at their default values (μ = 0, R = 1). Additionally, the curves were adjusted to account for 200mL of void space at the inlet of the columns. At 26 mL min^{-1}, the void space added 7.7 min to the tracer residence time, so 7.7 min were subtracted from sample times before fitting the curves. The porewater velocity and dispersion coefficient at 25 cm and 50cm were averaged for each column, and these values were used for subsequent calculations (Table S1). The average R^2 value for the tracer tests was 0.97 and a mass balance on the effluent bromide showed 99% recovery.

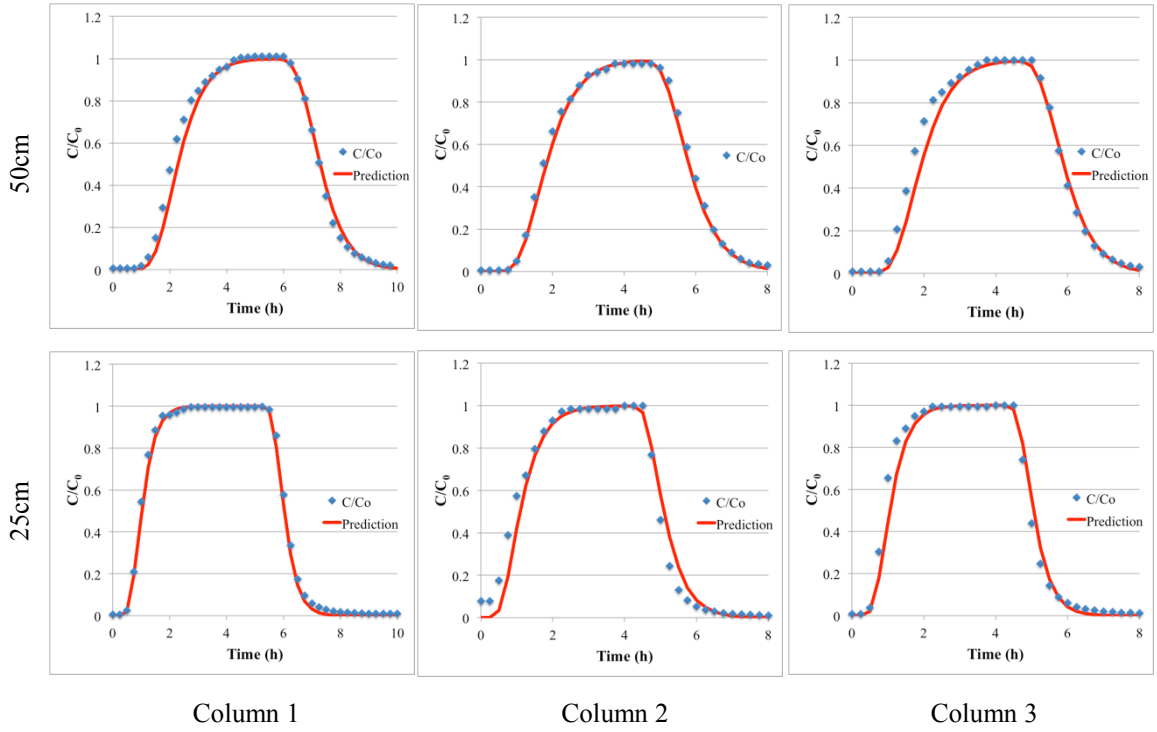

Figure S3. Bromide tracer test results for laboratory woodchip bioreactor column 1(left column), column 2 (middle column), and column 3 (right column). Bromide concentrations were measured at 25cm along reactor column (bottom row) and 50cm along reactor column (top row).

Table S1. Measured porewater velocity and dispersion coefficient for each column from tracer tests, and calculated porewater velocity and dispersion coefficient for column experiments.

Column	Tracer Tests (Q_1 = 26 mL min^{-1})			Column Experiments		
	v_1 (cm h^{-1})	D_1 (cm^2 h^{-1})	α_L (cm)	Q_2 (mL min^{-1})	v_2 (cm h^{-1})	D_2 (cm^2 h^{-1})
1	23.8	59.2	2.5	1.5	1.4	3.4
2	23.3	76.8	3.3	3.8	3.4	11.2
3	25.5	74.6	2.9	8.4	8.2	24.1

Porewater velocity for the column experiments was determined using the conversion $v_2 = v_1(Q_2/Q_1)$, where v_2 is porewater velocity for the column at the experimental flow rate (cm h^{-1}), Q_2 is the experimental flow rate (mL min^{-1}), v_1 is the porewater velocity from the tracer experiment (cm h^{-1}), and Q_1 is the tracer test flow rate (mL min^{-1}). The porewater velocities for the three columns during the experiment were 1.4 cm hr^{-1}, 3.4 cm hr^{-1}, and 8.2 cm hr^{-1}, respectively (Table S1). The dispersion coefficient for the column experiments was calculated as $D_2 = \alpha_L v_2$ where D_2 is the linear dispersion coefficient for the column at the experimental flow rate (cm^2 h^{-1}), and α_L is the linear dispersivity coefficient (cm). The linear dispersivity coefficient was calculated as $\alpha_L = D_1/v_1$, where D_1 is the dispersion coefficient from the tracer experiment (cm^2 h^{-1}).

Table S2. Measured hydraulic properties of the experimental woodchip reactor columns.[27]

Column	Drainable Por. (-)	Specific Ret. (-)	Total Por. (-)	Q (mL min^{-1})	v (cm h^{-1})	D (cm^2 h^{-1})	$\bar{t}$ (h)
1	0.50	0.34	0.84	1.5	1.4	3.4	35.7
2	0.56	0.31	0.87	3.8	3.4	11.2	14.7
3	0.57	0.33	0.90	8.4	8.2	24.1	6.1

Q=flowrate, v=effective porewater velocity, D=dispersion coefficient, $\bar{t}$=actual mean hydraulic retention time

Table S3. Parameter values used in the denitrification model with DO inhibition.

Parameter	Est. Value	Units
K_O	0.1	mg-O_2 L^{-1}
K_N	0.05	mg-N L^{-1}
K_I	0.1	mg-O_2 L^{-1}
$V_{O,21°C}$	16.54	mg-O_2 L^{-1} h^{-1}
$V_{N,21°C}$	0.15	mg-N L^{-1} h^{-1}
θ_O	1.20*	-
θ_N	1.15*	-

Parameters values with "*" were estimated using experimental data from this study. The remaining parameters were from Halaburka et al[28] "Model 3" (nitrate and DO inhibition model), and were determined through training the model with some data sets and validating with others. Inclusion of confidence intervals for non-linear models is known to be problematic and misleading[37] and are thus not included; indeed, single value parameter reporting is standard for denitrification modeling.[38,39]

Principal Component Analysis Numerical Data.

The principal component analysis (Figure 2 of the manuscript body) is a projection of the data using the two largest Eigenvectors. The orthogonal plane formed from the first and second largest Eigenvectors of the data generates the first and second principal components, respectively, of Figure 2. The data are then projected onto the plane formed. For these data, the first principal component explains 46% of the variance in the data. The second and third components explain 19% and 17% of the variance, respectively. Although the outstanding variance demonstrates that the PCA visualization is not complete (i.e., greater explanation of variance in the first two principal components yields the most robust results), PCA is still helpful in the context of the visualization of the factor analysis. For example, the results in Figure 2 demonstrate that DOC concentrations and nitrate concentrations are negatively correlated on PCA1 and DO concentration and porewater velocity are negatively correlated on PCA2; these results make physical sense and the PCA is merely a visualization aid for the data.

Table S.4. The Eigenvalues of the numerical data measured in the column experiments (six variables described in Table S.4).

	Eigenvalues of Experimental Column Data			
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.761443	46.02405	2.761443	46.0241
2	1.120720	18.67866	3.882163	64.7027
3	1.004526	16.74211	4.886689	81.4448
4	0.707326	11.78877	5.594015	93.2336
5	0.301854	5.03090	5.895869	98.2645
6	0.104131	1.73551	6.000000	100.0000

Table S.5: Numerical values correlation matrix of the factor coordinates plotted in the principal component analysis (PCA) of Figure 2 in the manuscript body. Significant values (bold) have an absolute value >0.5. Factors 4, 5, and 6 contain no significant values.

	Factor coordinates of the variables					
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Temp	**0.717321**	-0.186882	**0.619860**	0.098904	0.157770	-0.177838
PWV	-0.372395	**-0.755578**	0.297932	-0.439852	0.005189	0.090355
Distance on Col	0.473610	-0.458502	**-0.724360**	-0.129633	0.084645	-0.129627
DO Conc	**-0.726497**	0.426726	-0.006773	-0.452576	0.270943	-0.108748
Nitrate Conc.	**-0.904723**	-0.173318	0.048868	0.139405	-0.308276	-0.185961
DOC Conc	**0.733204**	0.304202	0.066373	-0.512846	-0.318319	-0.033607

DOC and DO Profile Plots

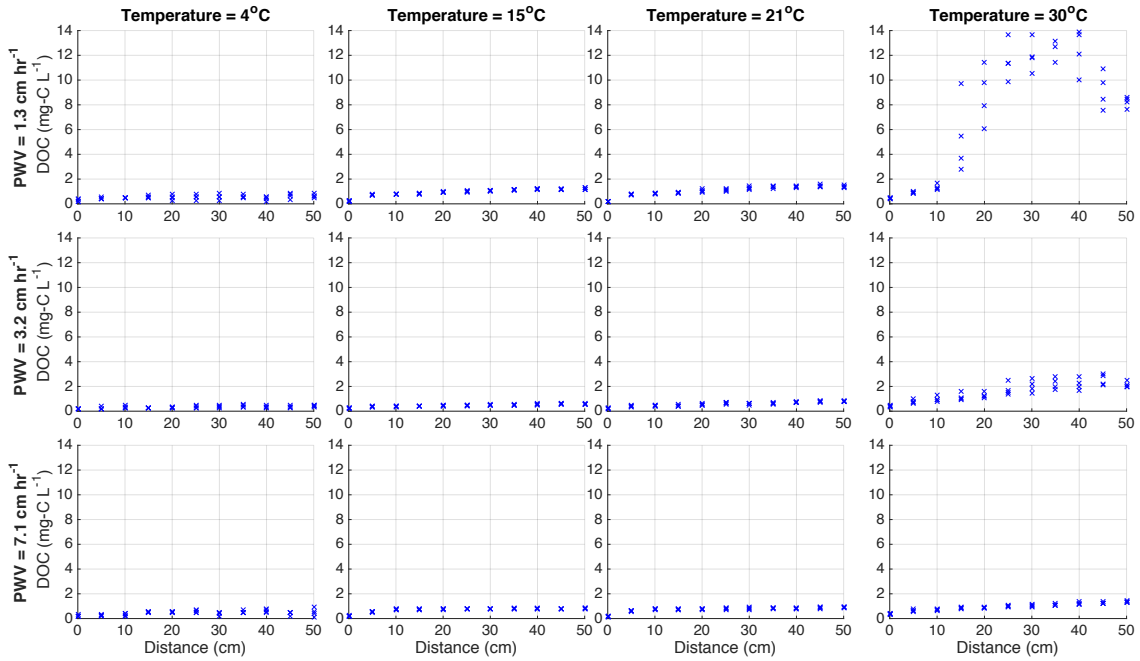

Figure S4. DOC concentration profile data (blue x-symbols) for porewater velocities of 1.4 cm h^{-1} (row 1), 3.4 cm h^{-1} (row 2), and 8.2 cm h^{-1} (row 3) and temperatures of 4 °C (column 1), 15 °C (column 2), 21 °C (column 3), and 30 °C (column 4).

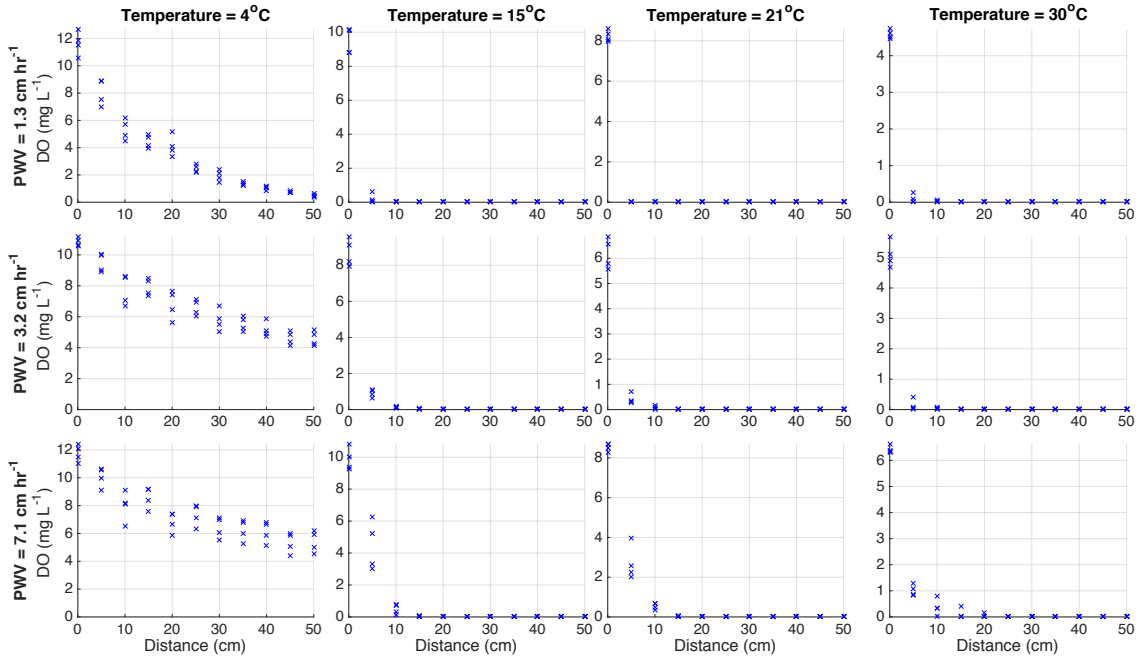

Figure S5. DO concentration profile data (blue x-symbols) for porewater velocities of 1.4 cm h^{-1} (row 1), 3.4 cm h^{-1} (row 2), and 8.2 cm h^{-1} (row 3) and temperatures of 4 °C (column 1), 15 °C (column 2), 21 °C (column 3), and 30 °C (column 4).

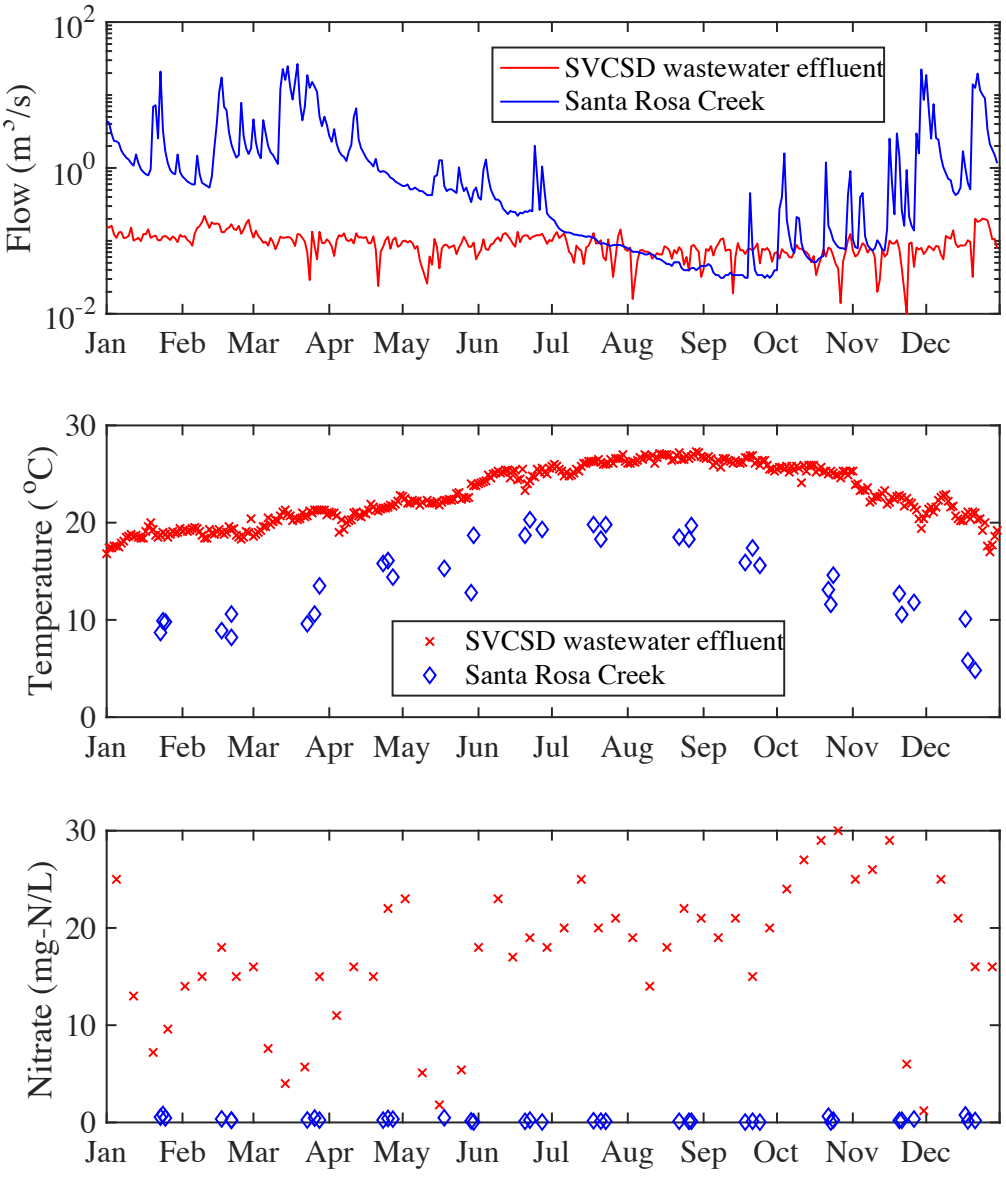

Figure S6. Flow, temperature, and nitrate concentrations plotted by calendar day for both SVCSD wastewater effluent and Santa Rosa Creek.

Model of Woodchip Bioreactor (*Abbreviated description; developed in full previously in Halaburka et al. Environ. Sci. Technol. 2017, 51, 5156−5164*)

The generalized model for each constituent takes the form:

$$\frac{\partial C_i}{\partial t} = D\frac{\partial^2 C_i}{\partial x^2} - v\frac{\partial C_i}{\partial x} - R_i \quad (1)$$

where C_i is the concentration of the ith species (mg L^{-1}), t is time (h), D is the dispersion coefficient (cm^2 h^{-1}), v is the effective porewater velocity (cm h^{-1}), x is distance along the column (cm), and R_i is the biological reaction rate term for the ith species (mg L^{-1} h^{-1}).
The aerobic reaction rate can be expressed in the form:

$$R_O = X_O V_O \left(\frac{C}{Kc + C}\right)\left(\frac{O}{Ko + O}\right) \quad (2)$$

where R_O is the rate of oxygen uptake (mg-O_2 L^{-1} h^{-1}), X_O is the concentration of aerobic heterotrophs (mg-biomass L^{-1}), V_O is the maximum uptake rate of DO (mg-O_2 mg-biomass^{-1} h^{-1}), C is the concentration of DOC (mg-C L^{-1}), Kc is the half-saturation constant for DOC (mg-C L^{-1}), O is the concentration of DO (mg-O_2 L^{-1}), and Ko is the half-saturation constant for DO (mg-O_2 L^{-1}).

Denitrification can similarly be expressed as a coupled Michaelis-Menten reaction, with the addition of a non-competitive inhibition term representing the inhibiting effect of DO on denitrification. This reaction rate takes the form:

$$R_N = X_N V_N \left(\frac{C}{Kc + C}\right)\left(\frac{N}{K_N + N}\right)\left(\frac{K_I}{K_I + O}\right) \quad (3)$$

where R_N is the rate of denitrification (mg-N L^{-1} h^{-1}), X_N is the concentration of heterotrophic denitrifiers (mg-biomass L^{-1}), V_N is the maximum rate of denitrification (mg-N mg-biomass^{-1} h^{-1}), N is the concentration of nitrate (mg-N L^{-1}), K_N is the half-saturation constant for nitrate (mg-N L^{-1}), and K_I is the inhibition constant of DO (mg-O_2 L^{-1}).

DOC is consumed through both aerobic respiration and denitrification, and the DOC reaction rate is modeled as a combination of the Michealis-Menten reaction equations for the two processes. The DOC reaction term takes the form

$$R_C = \beta_O X_O V_O \left(\frac{C}{Kc + C}\right)\left(\frac{O}{Ko + O}\right) + \beta_N X_N V_N \left(\frac{C}{Kc + C}\right)\left(\frac{N}{K_N + N}\right)\left(\frac{K_I}{K_I + O}\right) \quad (4)$$

where R_C is the rate of DOC uptake (mg-C L^{-1} h^{-1}), β_O is the uptake coefficient for DO (mg-C mg-O_2^{-1}), β_N is the uptake coefficient for nitrate (mg-C mg-N^{-1}). The uptake coefficients for DO and nitrate are the ratios of the mass of DOC consumed per mass of DO or nitrate consumed, respectively. Thus the three partial differential equations to model DO, nitrate, and DOC mass transport are:

Dissolved Oxygen: ***(Model 1)***

$$0 = D\frac{\partial^2 O}{\partial x^2} - v\frac{\partial O}{\partial x} - V_O\left(\frac{C}{Kc + C}\right)\left(\frac{O}{Ko + O}\right)$$

where D is the dispersion coefficient (cm^2 h^{-1}), v is the effective porewater velocity (cm h^{-1}), and x is distance along the column (cm). V_O is the maximum uptake rate of DO (mg-O_2 mg-biomass^{-1} h^{-1}), C is the concentration of DOC (mg-C L^{-1}), Kc is the half-saturation constant for DOC (mg-C L^{-1}), O is the concentration of DO (mg-O_2 L^{-1}), and Ko is the half-saturation constant for DO (mg-O_2 L^{-1}).

Nitrate:

$$0 = D\frac{\partial^2 N}{\partial x^2} - v\frac{\partial N}{\partial x} - V_N\left(\frac{C}{Kc + C}\right)\left(\frac{N}{K_N + N}\right)\left(\frac{K_I}{K_I + O}\right)$$

where V_N is the maximum rate of denitrification (mg-N mg-biomass^{-1} h^{-1}), N is the concentration of nitrate (mg-N L^{-1}), K_N is the half-saturation constant for nitrate (mg-N L^{-1}), and K_I is the inhibition constant of DO (mg-O_2 L^{-1}).

Dissolved Organic Carbon:

$$0 = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \beta_O V_O\left(\frac{C}{Kc + C}\right)\left(\frac{O}{Ko + O}\right) - \beta_N V_N\left(\frac{C}{Kc + C}\right)\left(\frac{N}{K_N + N}\right)\left(\frac{K_I}{K_I + O}\right) + V_{h1}\left(\frac{O}{K_O + O}\right) + V_{h2}\left(\frac{K_I}{K_I + O}\right)$$

where β_O is the uptake coefficient for DO (mg-C mg-O_2^{-1}), β_N is the uptake coefficient for nitrate (mg-C mg-N^{-1}). The uptake coefficients for DO and nitrate are the ratios of the mass of DOC consumed per mass of DO or nitrate consumed, respectively.

The second model (Model 2) simplifies Model 1 by assuming DO does not significantly impact the overall denitrification rate. The DO terms are removed from the system of equations, and the model takes the form

Nitrate: ***(Model 2)***

$$0 = D\frac{\partial^2 N}{\partial x^2} - v\frac{\partial N}{\partial x} - V_N\left(\frac{C}{Kc + C}\right)\left(\frac{N}{K_N + N}\right)$$

Dissolved Organic Carbon:

$$0 = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \beta_N V_N\left(\frac{C}{Kc + C}\right)\left(\frac{N}{K_N + N}\right) + V_{h2}$$

The third model (Model 3) is an alternate simplification of Model 1 by assuming denitrification is not dependent on DOC concentrations. Model 3 takes the form:

Dissolved Oxygen: ***(Model 3)***

$$0 = D\frac{\partial^2 O}{\partial x^2} - \nu\frac{\partial O}{\partial x} - V_O\left(\frac{O}{Ko + O}\right)$$

Nitrate:

$$0 = D\frac{\partial^2 N}{\partial x^2} - \nu\frac{\partial N}{\partial x} - V_N\left(\frac{N}{K_N + N}\right)\left(\frac{K_I}{K_I + O}\right)$$

The fourth model (Model 4) assumes both DO inhibition and DOC concentrations have little impact on the denitrification rate, thus both the DO and the DOC equations are removed from Model 1 and Model 4 takes the form:

Nitrate: ***(Model 4)***

$$0 = D\frac{\partial^2 N}{\partial x^2} - \nu\frac{\partial N}{\partial x} - V_N\left(\frac{N}{K_N + N}\right)$$

The last model evaluated (Model 5) is a zero-order reaction rate equation commonly used to describe nitrate reduction rates in WBRs:

Nitrate: ***(Model 5)***

$$N = N_0 - V_N(x/\nu)$$

where N is nitrate concentration, N_O is influent nitrate concentration, V_N is the zero-order denitrification rate, v is porewater velocity, and x is distance along the column. Model 5 was constrained such that $N \geq 0$ in order to provide a more accurate comparison between models.