

*Water Resources Research*

Supporting Information for

**Development of a fully coupled biogeochemical reactive transport model to simulate microbial oxidation of organic carbon and pyrite under nitrate-reducing conditions**

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### Text S1 - Derivation of equation 19

It is assumed that the difference between the inflowing nitrate and nitrite concentrations, and the measured concentrations at the outflow between  $t_n$  (14 d) and  $t_m$  (60 d), which is after the tracer breakthrough but before sulfate increases due to pyrite oxidation, is entirely the result of heterotrophic denitrification.

The amount of nitrate which has been reduced can be calculated with:

$$n_{NO_3^-,red} = Q \cdot \int_{t_n}^{t_m} (c_{NO_3^-,in} - c_{NO_3^-}(t)) dt \quad S1-1$$

where  $c_{NO_3^-}(t)$  is the measured concentration at the outflow for nitrate at the time  $t$  [mol L<sup>-1</sup>],  $c_{NO_3^-,in}$  the inflowing concentration of nitrate [mol L<sup>-1</sup>], and  $Q$  the water volume flux [L d<sup>-1</sup>].

Using the stoichiometry of Eq. 9 the amount of nitrite reduced can be calculated with:

$$n_{NO_2^-,red} = Q \cdot \int_{t_n}^{t_m} ((c_{NO_3^-,in} - c_{NO_3^-}(t)) - c_{NO_2^-}(t)) dt \quad S1-1$$

where  $c_{NO_2^-}(t)$  is the measured concentration at the outflow for nitrite at the time  $t$  [mol L<sup>-1</sup>].

With both the stoichiometry of Eqs. 9 and 10, the amount of SOC oxidized during the reduction of nitrate and nitrite can be calculated with:

$$n_{SOC,ox} = \frac{1}{2} \cdot n_{NO_3^-,red} + \frac{3}{4} n_{NO_2^-,red} \quad S1-3$$

which becomes substituting for  $n_{NO_3^-,red}$  and  $n_{NO_2^-,red}$  from S1-1 and S1-2:

$$n_{SOC,ox} = \frac{1}{2} \cdot Q \cdot \int_{t_n}^{t_m} (c_{NO_3^-,in} - c_{NO_3^-}(t)) dt + \frac{3}{4} Q \cdot \int_{t_n}^{t_m} ((c_{NO_3^-,in} - c_{NO_3^-}(t)) - c_{NO_2^-}(t)) dt \quad S1-4$$

It follows that

$$n_{SOC,ox} = Q \cdot \left( \int_{t_n}^{t_m} \left[ \frac{5}{4} (c_{NO_3^-,in} - c_{NO_3^-}(t)) - \frac{3}{4} c_{NO_2^-}(t) \right] dt \right) \quad S1-5$$

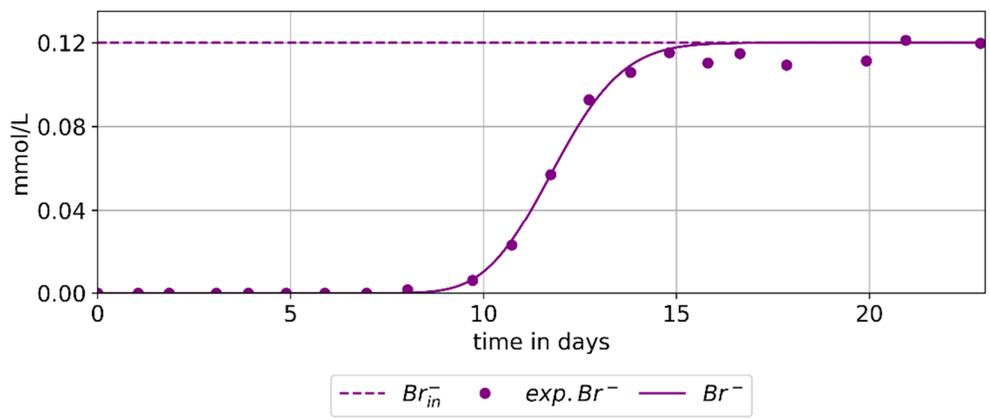
Assuming the SOC is distributed equally in the column the concentration with respect to solute volume, the SOC concentration can be calculated with:

$$c_{SOC} = \frac{n_{SOC,ox}}{V_w} = \frac{Q}{V_w} \cdot \left( \int_{t_n}^{t_m} \left[ \frac{5}{4} (c_{NO_3^-,in} - c_{NO_3^-}(t)) - \frac{3}{4} c_{NO_2^-}(t) \right] dt \right) \quad S1-6$$

where  $V_w$  is the water volume of the column [L].

**Table S1.** Constant parameter values and initial calibration parameter values. \* indicates parameters which were set with manual trial-and-error. Rate constants for both processes were split to account for the partial reactions, the rate constants and relative growth yield for heterotrophic denitrification were further converted to the different SOC in this study ( $C_{org}$  compared to Ethanol in Rodríguez-Escales et al. [2016]).

Constant Parameters		Value	Reference
*SOC half saturation constant for step (a)	$k_{SOC,a}$ [mol L <sup>-1</sup> ]	$2.00 \cdot 10^{-5}$	Similar to <i>Clement et al.</i> [1997]
*SOC half saturation constant for step (b)	$k_{SOC,b}$ [mol L <sup>-1</sup> ]	$2.00 \cdot 10^{-3}$	Assumed higher than $k_{SOC,a}$ to match experimental nitrite accumulation
nitrate half saturation constant	$k_{NO_3^-}$ [mol L <sup>-1</sup> ]	$1.06 \cdot 10^{-5}$	<i>Clement et al.</i> [1997]
nitrite half saturation constant	$k_{NO_2^-}$ [mol L <sup>-1</sup> ]	$1.06 \cdot 10^{-5}$	assumed similar to $k_{NO_3}$
Attachment coefficient	$k_{att}$ [d <sup>-1</sup> ]	$1.98 \cdot 10^1$	Calculated with Eq. 5 from <i>Hornberger et al.</i> [1992]
*Decay coefficient	$d$ [d <sup>-1</sup> ]	$1.73 \cdot 10^{-1}$	<i>Rodríguez-Escales et al.</i> [2016]
Calibration Parameters		Initial Value	Reference
SOC oxidation rate constant for step (a)	$K_{m,SOC,a}$ [mol L <sup>-1</sup> d <sup>-1</sup> ]	$4.13 \cdot 10^{-2}$	Calculated from <i>Rodríguez-Escales et al.</i> [2016]
SOC oxidation rate constant for step (b)	$K_{m,SOC,b}$ [mol L <sup>-1</sup> d <sup>-1</sup> ]	$6.19 \cdot 10^{-2}$	Calculated from <i>Rodríguez-Escales et al.</i> [2016]
Heterotrophic relative growth yield	$Y_{SOC}$ [L mol <sup>-1</sup> ]	$2.76 \cdot 10^3$	Calculated from <i>Rodríguez-Escales et al.</i> [2016]
Initial concentration of SOC	$c_{SOC,0}$ [mol L <sup>-1</sup> ]	1.51	Calculated with Eq. (29)
Detachment coefficient	$k_{det}$ [d <sup>-1</sup> ]	$1.98 \cdot 10^{-1}$	Assumed two orders of magnitude lower than $k_{att}$ , since electron donors are solids.
Autolithotrophic relative growth yield	$Y_{pyr}$ [L mol <sup>-1</sup> ]	-	Assumed same as calibrated $Y_{SOC}$
SOC inhibition half saturation constant	$I_{SOC}$ [mol L <sup>-1</sup> ]	-	Assumed 1/4 of calibrated $c_{SOC,0}$
Pyrite oxidation rate constant for step (a)	$K_{m,pyr,a}$ [d <sup>-1</sup> ]	$1.65 \cdot 10^{-1}$	Calculated with $p_4$ from <i>Appelo et al.</i> [1998], see also Eq. (20)
Pyrite oxidation rate constant for step (b)	$K_{m,pyr,b}$ [d <sup>-1</sup> ]	$2.48 \cdot 10^{-1}$	Calculated with $p_4$ from <i>Appelo et al.</i> [1998], see also Eq. (21)



**Figure S1.** Comparison of measured (dots) and calculated (solid line) bromide concentrations in the effluent. Measurement error was below 0.6  $\mu\text{mol/L}$ . Inflowing concentration as dashed line.

## Text S2 – RMSE

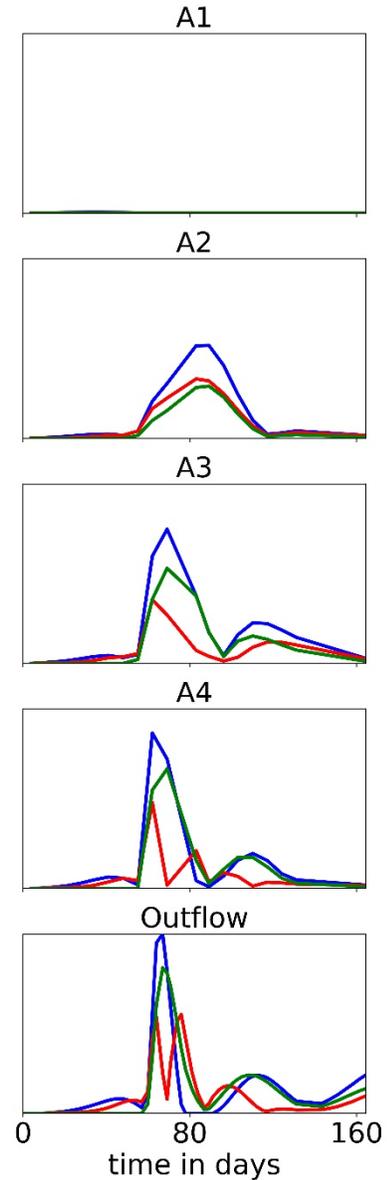
The calculated RMSE (root-mean square error) (Table S2) can be used to compare the fit between the measurement locations and measured species. The RMSE shows that the model prediction accuracy is lowest for nitrate and highest for nitrite. The prediction accuracy is higher for the discharge than for measurements collected along the column, except port A1. Especially the mismatch of sulfate and nitrate concentration at port A3 results in higher RMSE values.

**Table S2.** RMSE for each species and location, as well as totals of location and species normalized by the RMSE of the entire calibration dataset.

RMSE/RMSE <sub>total</sub>	A1	A2	A3	A4	Outflow	Total
Nitrate	0.63	2.25	3.28	0.92	0.50	1.16
Nitrite	0.02	0.60	0.96	0.70	0.86	0.71
Sulfate	0.51	1.16	1.59	1.38	1.10	1.13
Total	0.39	1.34	1.94	1.00	0.82	1.00

### Text S3 – Observation Sensitivity

PEST calculates the observation sensitivity of measured observation data (Figure S2). Between day 60 and 80 observation points are highly sensitive, and thus sensitivity rises with the beginning of the autolithotrophic denitrification. Observation sensitivities between ports A3, A4 and the discharge are in the same range, while they decrease for port A2 and are reduced to almost zero at port A1. This low sensitivity is coupled with the limited denitrification at port A1.



**Figure S2.** Observation sensitivities for nitrate (blue), nitrite (red) and sulfate (green) for all measurement locations (A1 to A4) and the discharge normalized by the highest value.

#### **Text S4 – Enrichment factor $\epsilon$ calculation**

The calculation of  $\epsilon$  was carried out according to the following equation (Knöller et al., 2011, Mariotti et al., 1981), taking into account the initial nitrate concentration and isotope ratio of the synthetic groundwater and the measured nitrate concentrations and isotope ratios (sampling during the experiment).

$$\epsilon = \frac{10^3 \ln \frac{10^{-3} \delta(NO_3^-)_{measured} + 1}{10^{-3} \delta(NO_3^-)_{initial} + 1}}{\ln \frac{c(NO_3^-)_{measured}}{c(NO_3^-)_{initial}}}$$

Where  $\delta(NO_3^-)$  is the delta  $^{15}N$ - $NO_3^-$  value [‰],  $c(NO_3^-)$  is the concentration of nitrate [mol/L]. For the calculation of  $\epsilon$  the initial concentration and isotope ratio was assumed as constant over the time of the experiment, with a nitrate concentration of 100 mg/l and a delta value of 0.1‰.

Since the processes of heterotrophic and autotrophic denitrification are overlapping, determining the initial values (concentration and delta value) for each process is difficult. To determine the enrichment factors for each process (heterotrophic and autolithotrophic denitrification and denitrification with nitrate or nitrite) isotopic fractionation would be needed to be added to the model, and more isotopic measurements would be necessary for a reliable result.