Supporting Information for

Mass Transfer-Limited Biodegradation at Low Concentrations—Evidence from Reactive Transport Modeling of Isotope Profiles in a Bench-Scale Aquifer

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SUMMARY

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Chemicals

The following chemicals were used: 2,6-dichlorobenzamide (Sigma Aldrich, Germany), 2,6-dichlorobenzamide-3,4,5-d3, 98.4%-d3 (Alfa Chemistry, Ronkonkoma, NY).

The following chemicals were used for the medium preparation: disodium phosphate (6 g/L), monopotassium phosphate (≥99%), ammonium chloride (≥99%), magnesium sulfate heptahydrate (≥98%), calcium chloride dihydrate (≥99%), boric acid (≥99.5%), manganese sulfate monohydrate (≥99%), copper sulfate pentahydrate (≥98%), zinc chloride (≥98%), cobalt chloride hexahydrate (≥98%), sodium molybdate monohydrate (≥99%), glucose, and ferric chloride (≥98%). All the chemicals were from Sigma Aldrich, Germany.

Sample Preparation and Solid-phase Extraction (SPE)

Samples for carbon and nitrogen isotope analysis were filtered through 0.2 µM PES filter (Nalgene Thermo Scientific, Germany) and frozen at -20 °C immediately after each sampling event until enough sample volume (2 L) was collected. Samples for concentration measurements were filtered through 0.22 µM syringe filters (Merck KGaA, Germany), adjusted to pH 1.7 with HCl and spiked with internal standard 2,6-dichlorobenzamide-3,4,5-d3 before solid phase extraction (SPE). The SPE method was adapted from Torrentó et al.1 and Jensen et al.2. For the SPE of isotope samples, 0.2 g of hydrophobic polymer-based sorbent Bakerbond SDB-1 (J.T. Baker, USA) was self-packed into empty 6 mL PP SPE cartridges with PE frit (20 µm pore size; Sigma Aldrich, Germany). The cartridges were conditioned with 3 mL ethyl acetate, followed by 2 × 3 mL methanol and 2 × 3 mL Milli-Q water. 200 – 2000 ml samples were loaded to the SPE columns at a rate of 3 mL/min. After sample loading, the cartridges were washed twice with 3 mL of Milli-Q water and dried for 2 hours. BAM was eluted with 3 mL ethyl acetate, dried under a gentle stream of nitrogen at room temperature, and re-dissolved in 100–1000 µl ethyl acetate for CSIA. For the SPE method of concentration samples, 50 mg sorbent was packed in the 1 mL empty PP SPE cartridge with PE frit (20 µm pore size; Sigma Aldrich, Germany). The cartridges were conditioned with 1 mL ethyl acetate, followed by 2 × 1 mL methanol and 2 × 1 mL Milli-Q water. 1 ml samples were slowly loaded to the SPE columns. After sample loading, the cartridges were washed twice with 1 mL Milli-Q water and dried for 1 hour. Compounds were eluted with 1 mL acetonitrile, dried under a gentle stream of nitrogen at room temperature, and re-dissolved in 100-1000 µl 10% acetonitrile water solution for LC-MS/MS measurements.
**BAM and 2,6-DCBA Concentration Measurements on LC-MS/MS**

The method of the concentration measurement of BAM and 2,6-DCBA on LC-MS/MS was adapted from Jensen et al.² Briefly, liquid chromatography (LC) was performed on an Agilent 1100 HPLC system including a column compartment, an autosampler, a binary pump system, and a degasser (Agilent Technologies Inc, USA). Mass spectrometry (MS) was operated on a QTrap 4000 system using electrospray ionization (ESI) (Sciex, USA). Separation was carried out on a Kinetex® C18 column (2.6 µm, 10 nm, 100 × 2.1 mm i.d., Phenomenex, USA) at 40 °C. Mobile phase A was 5 mM of ammonium acetate with pH of 2.4 (adjusted by formic acid). Mobile phase B was acetonitrile. A gradient flow of 300 µL/min was used as follows: 0–5 min, 90% A; 5–9 min, 90%–10% A; 9–10 min, 10%–90% A; 10–15 min, 90% A. The injection volume was 10 µL. Each sample was analyzed twice in multiple reaction monitoring (MRM) mode with a temperature of 450 °C, a nebulizer gas at 50 psi, a heater gas at 40 psi, a curtain gas at 20 psi, and a collision gas at 11 psi. 2,6-Dichlorobenzamide and 2,6-dichlorobenzamide-3,4,5-d₃ (internal standard) were analyzed in positive mode with a capillary voltage at 4.5 kV. 2,6-Dichlorobenzoic acid was analyzed in negative mode with a capillary voltage at -4.5 kV. For each sample, two transitions were selected. The first transition was used for quantification and the second transition was used for qualification (shown in Table S1).

| Compound                      | Precursor ion (m/z) | Product ion (m/z) | Declustering potential (V) | Entrance potential (V) | Collision energy (V) | Cell exit potential (V) |
|--------------------------------|---------------------|------------------|---------------------------|------------------------|----------------------|------------------------|
| 2,6-Dichlorobenzamide         | 190                 | 173              | 75                        | 10                     | 29                   | 7                      |
|                               | 190                 | 145.3            | 75                        | 10                     | 40                   | 7                      |
| 2,6-dichlorobenzamide-3,4,5-d₃| 193.1               | 176.1            | 70                        | 10                     | 27                   | 7                      |
|                               | 193.1               | 148.0            | 70                        | 10                     | 42                   | 7                      |
| 2,6-dichlorobenzoic acid      | 189                 | 144.9            | -26                       | -3                     | -13                  | -8                     |
|                               | 189                 | 35.2             | -26                       | -3                     | -33                  | -3                     |

Table S1. Parameters of target analytes on MS
Carbon and Nitrogen Isotope Measurements on GC-IRMS

This method was described by Sun et al. Briefly, a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific, Italy) and a Finnigan MAT 253 isotope ratio mass spectrometer (IRMS) were coupled through a Finnigan GC Combustion III interface (Thermo Fisher Scientific, Germany). A DB-5 analytical column (30 m, 0.25 mm i.d., 0.5 µm film, Agilent Technologies, Germany) was used to separate BAM in the gas chromatograph. Helium (grade 5.0) was the carrier gas. For the isotope measurement of the high BAM concentration samples, we used a Thermo injector in the split/split-less injection mode; for the isotope measurement of the low BAM concentration samples, we applied a programmable injector controlled by an Optic 3 system with liquid N₂-cryofocusing (ATAS GL, distributed by Axel Semrau, Germany) in on-column injection mode, in which a Rxi retention gap (fused silica, 3 m × 0.53 mm inner diameter) (RESTEK, Germany) was connected to a custom made on-column liner.

In the split/split-less injection mode, the GC method started at 80 °C. At a ramp rate of 15 °C/min, temperature increased to 280 °C and was held for 7 min. The flow rate was kept constant at 1.4 mL/min. In the on-column injection mode, the GC oven started at 35 °C and was held for 30 s. At a ramp rate of 5 °C/min, temperature increased to 80 °C. Then at a ramp rate of 15 °C/min, temperature increased from 80 °C to 280 °C. In the Optic 3, the method started at an initial temperature of 40 °C and was held for 300 s. Then temperature increased to 250 °C at a ramp rate of 2 °C/s. The initial flow rate was 0.3 mL/min and was held for 120 s. Then it was increased to 1.4 mL/min within 2 min. Thus, before the GC temperature program started a stable flow rate of 1.4 mL/min was established.

We used Vienna PeeDee Belemnite (V-PDB) and Air-N₂ to determine the carbon isotope values \( \delta^{13}C \) [%] and nitrogen isotope values \( \delta^{15}N \) [%] of the samples. We calculated the carbon and nitrogen isotope values \( \delta^{13}C \) and \( \delta^{15}N \) of the samples in relation to a lab reference gas (CO₂ and N₂, respectively. In the beginning and the end of each run, the reference gas was measured against V-PDB and air by using international reference materials (provided by IAEA), e.g., the CO₂ gases RM 8562, RM8563 for CO₂, and RM 8564 and NSVEC (N₂) for N₂.
Medium Preparation and Bacteria Cultivation

The medium for the growth of *Aminobacter* sp. Strain MSH1 in the biotic tank experiment was adapted from Schultz-Jensen et al. Briefly, medium solution was prepared with Na₂HPO₄ (6 g/L), KH₂PO₄ (3 g/L), NH₄Cl (1 g/L), MgSO₄ × 7H₂O (0.2 g/L), CaCl₂ × 2H₂O (0.01 g/L) and autoclaved at 120 °C. After autoclaving, 10 mL from the trace element stock solution was filtered through 0.22µM syringe filters (Merck KGaA, Germany) and added to 1 L medium solution. The trace elements stock solution was H₃BO₃ (39 mg/L), MnSO₄ × H₂O (84.5 mg/L), CuSO₄ × 5H₂O (125 mg/L), ZnCl₂ (69 mg/L), CoCl₂ × 6H₂O (119.5 mg/L), and Na₂MoO₄ × H₂O (121 mg/L).

*Aminobacter* sp. Strain MSH1 was from the Department of Geochemistry, the Geological Survey of Denmark and Greenland (GEUS), Denmark. The strain on the sterile plates was transferred to the medium solution with a sterile needle. Precultures were made in 1 L shaker flask containing 200 mL medium solution. 2 mL of autoclaved glucose were added to 200 mL medium solution as carbon source. To ensure that the culture maintain its BAM-degrading ability, BAM was added to the medium (10 mg/L). The preculture was incubated in an orbital shaker at 130 rpm at 20 °C until an optical density (OD) of 1 was reached. The preculture with OD = 1 was centrifuged in four 50 mL centrifuge tubes at 4000 rpm for 5 min. Cell pellets were resuspended and washed in 10 mL medium solution (without glucose or BAM) for three times to remove the remaining glucose or BAM in the preculture. Finally, the suspended bacterial cell pellets were resuspended in 2 L medium solution (without glucose or BAM); the experimental culture with OD value of 0.1 was ready for the inoculation to the tank.

Set-up of the Two-dimensional Flow-through Sediment Tank Experiment

The quasi-two-dimensional tank was made up of two glass plates, separated by a Teflon spacer, and all fitted into two aluminum rims at either side of the chamber which were screwed together. The tank respectively was fitted with sixteen, equally spaced (at 1.0 cm distance each), ports at the inlet and outlet to accurately inject different constituents at specified depths and to sample with a high depth resolution at the outlet. The inner dimensions of the domain were 95 cm × 18 cm × 1 cm (L × H × W), so that the tank represents a quasi-two-dimensional system. The tank was wet packed with sterilized sand (0.8–1.2 mm grain diameter). Stainless steel capillaries and tygon pump-tubes are used to connect to inflow and outflow peristaltic pumps (Ismatec, Germany). The pumping rate was maintained at 45 ± 2 µL/min per port. The
seepage velocity was 1.25 m/d. The tank was sterilized with 12 g/L NaOH solution and rinsed with autoclaved Milli-Q water before the experiment.

**Additional Equations for the Mass-Transfer limitation Scenario**

We assume that $k_{tr}$ [1/s], the mass transfer rate coefficient, primarily describes the transfer through the cell membrane and neglect the transfer resistance from the bulk solution to the cell. The mass transfer through the cell membrane between the bulk phase and the cytoplasm has different effects on the bulk-phase concentration than on the intracellular concentrations because of the disproportionate bulk solution vs. single cell volumes. Therefore, eq 1, in the main manuscript, and the following equations, which describe the degradation rates in the bulk solution, contain the ratio of the biomass concentration over the mass density of bacterial cells. While the concentration in the cell interior may be expressed in moles of substrate per volume of pore space, this would not be the concentration experienced by the enzymes. Thus, comparisons between studies using pure enzymes and those involving bacterial cells may not be valid.

The fitted value of the coefficient, $k_{tr}$ can be used to estimate the effective diffusion coefficient $D_{\text{eff}}$ [m² s⁻¹], and the apparent permeability of the cell membrane $P_{\text{app}}$ [m s⁻¹].⁵-⁷

$$k_{tr} = \frac{P_{\text{app}} \times A_{\text{cell}}}{V_{\text{cell}}} = \frac{D_{\text{eff}} \times K_{\text{lipw}} \times A_{\text{cell}}}{\sigma \times V_{\text{cell}}} \quad (\text{S1})$$

where $K_{\text{lipw}}$ [L_water L_membrane⁻¹] (value of 11) is the lipid-water distribution coefficient of BAM,⁸ $A_{\text{cell}}$ and $V_{\text{cell}}$ are the surface area and volume of a single cell, 6 µm² and 0.9 µm³, respectively,⁹,¹⁰ and we can assume that the diffusive distance, $\sigma$ [nm], from the substrates in the bulk solution to the location of the enzyme is the thickness of two lipid bilayer of the gram-negative bacterial strain *Aminobacter* sp. MSH1, $\sigma = 10$ nm.¹¹,¹²

**Governing Equations for the Scenario without Mass-Transfer Limitation**

In the simulation scenario without mass transfer limitation, the mass transfer process through the cell membrane was neglected, thus only the substrate concentrations in the bulk solution were simulated. The BAM degradation pathway can be simplified as,

$$BAM_{\text{bulk}} \xrightarrow{k_{\text{BAM bulk}}} D_{\text{CBA}}$$
in which BAM was irreversibly hydrolyzed to the main intermediate 2,6-DCBA via an amidase enzyme, then 2,6-DCBA is either degraded to CO₂ and ATP via aerobic respiration or utilized for the synthesis of biomass (C₅H₇O₂N).

The reactive transport of substrates in the bulk solution was coupled to microbial dynamics and was described by the following equations in two dimensions:

\[
\begin{align*}
\frac{\partial l, h c_{BAM, bulk}}{\partial t} &= -\mathbf{v} \cdot \nabla c_{BAM, bulk} + \nabla \cdot (D_{BAM} \cdot \nabla c_{BAM, bulk}) - r_{hydr}^{BAM} \\
\frac{\partial c_{O₂, bulk}}{\partial t} &= -\mathbf{v} \cdot \nabla c_{O₂, bulk} + \nabla \cdot (D_{O₂} \cdot \nabla c_{O₂, bulk}) - r_{deg}^{O₂} \\
\frac{\partial c_{DCBA, bulk}}{\partial t} &= -\mathbf{v} \cdot \nabla c_{DCBA, bulk} + \nabla \cdot (D_{DCBA} \cdot \nabla c_{DCBA, bulk}) + r_{hydr}^{BAM} + h r_{hydr}^{BAM} - r_{deg}^{DCBA} \\
\frac{\partial X_{im}}{\partial t} &= r_{growth}^{im} - r_{daughter} - r_{decay}^{im} \\
\frac{\partial X_{mob}}{\partial t} &= -\mathbf{v} \cdot \nabla X_{mob} + \nabla (D \cdot \nabla X_{mob}) + r_{daughter}
\end{align*}
\]

\[
\begin{align*}
l r_{hydr}^{BAM} &= r_{max}^{hydr} \cdot \frac{c_{BAM, total}^{BAM} + K_{BAM}^{BAM}}{c_{BAM, total}} \\
h r_{hydr}^{BAM} &= \alpha \cdot r_{max}^{hydr} \cdot \frac{c_{BAM, total}^{BAM} + K_{BAM}^{BAM}}{c_{BAM, total}} \\
r_{deg}^{DCBA} &= \mu_{max} \cdot \frac{\rho_{bio}}{Y} \cdot \frac{c_{DCBA, bulk}}{K_{m}^{DCBA} + c_{DCBA, total}^{DCBA}} \cdot \frac{c_{O₂, total}^{O₂}}{K_{m}^{O₂} + c_{O₂, total}^{O₂}} \\
r_{deg}^{O₂} &= p \cdot r_{deg}^{DCBA}
\end{align*}
\]

**Parameter Uncertainties and Sensitivities**

We fitted the log-parameter values (n = 10) using *lsqnonlin*, a MATLAB built-in nonlinear least squares data-fitting function, via minimization between model computed and measured concentration values. The delogarithmized fitted parameter values are presented alongside additional, fixed, physical parameters in
Table S2.

Results of a local sensitivity analysis, performed by perturbing each parameter value by 10% and comparing the model outcome to that of the optimal case, are presented for all relevant model output in Figure 1. The sensitivities presented in Figure 1 are calculated by comparing the model outcome to measurements at each depth-location. Thus, Figure 1 shows the spatial dependence of each parameter’s sensitivity, that is, where along the depth profile does a parameter most influence the model outcome. A linearized uncertainty quantification was performed on the log-parameter values considering the sum of squared residuals (obtained from lsqnonlin) and parameter sensitivities obtained from the local sensitivity analysis. The relative parameter uncertainties are presented, for each parameter, along the legend of Figure 1. Thus, the relative uncertainty range for each parameter value is given by the multiple and quotient (× / ÷) of the fitted value and its relative uncertainty. That is, the closer the relative uncertainty is equal to 1, the more accurate the estimated parameter.

The results from our uncertainty quantification suggest that most parameters are well constrained, in particular, the parameters, \( k_{\text{BAM}}^{\text{tr}} \), \( r_{\text{max}}^{\text{hydro}} \), \( k_{\text{BAM}}^{\text{m}} \), \( Y \) and \( X_{\text{max}} \) exhibited a narrow uncertainty range (low relative uncertainty) and these were also the parameters that the model outcome was most sensitive to. The relative uncertainty for \( k_{\text{att}} \) was not reported, because a 10% perturbation of the fitted parameter value did not yield a quantifiable change in the model output, and thus the absolute value of \( k_{\text{att}} \) was poorly constrained.
Table S2. Flow and transport parameters and reaction rate coefficients, for the reactive transport model, used as either fixed values obtained from the literature or fitted to measured data.

| Symbol  | Parameter                          | Values   | Unit          | References            |
|---------|------------------------------------|----------|---------------|-----------------------|
|         | Transport parameters               |          |               |                       |
| $d_{\text{grain}}$ | grain size                        | 0.001    | [m]           | experimental          |
| $\phi$  | porosity                           | 0.45     | [-]           | experimental          |
| $\alpha_1$ | longitudinal dispersivity          | $6 \times 10^{-4}$ | [m] | fitted |
| $\alpha_t$ | transverse dispersivity           | $1.9 \times 10^{-4}$ | [m] | fitted |
| $D_{\text{BAM}}^{\text{bio}}$ | bacteria diffusion coefficient   | $1.5 \times 10^{-11}$ | [m$^2$s$^{-1}$] | Kathryn, et al. 13 |
| $D_{\text{O}_2}^{\text{bio}}$ | diffusion coefficient            | $4.3 \times 10^{-10}$ | [m$^2$s$^{-1}$] | Jorgensen, et al. 14 |
|         | Biokinetic parameters              |          |               |                       |
| $k_{\text{BAM}}^{\text{tr}}$ | mass transfer coefficient of BAM  | 7.6      | [s$^{-1}$]    | fitted                |
| $k_{\text{DCBA}}^{\text{tr}}$ | mass transfer coefficient of DCBA | 3.9      | [s$^{-1}$]    | fitted                |
| $k_{\text{O}_2}^{\text{tr}}$ | mass transfer coefficient of O$_2$| $3 \times 10^6$ | [s$^{-1}$] | fitted |
| $K_{\text{BAM}}^{\text{m}}$ | Michaelis Menten coefficient of BAM for the hydrolysis to form 2,6-DCBA | 0.38    | [µmol L$_{\text{int}}^{-1}$] | fitted |
| $K_{\text{DCBA}}^{\text{m}}$ | Monod coefficient of 2,6-DCBA for further degradation | 10.8    | [µmol L$_{\text{int}}^{-1}$] | fitted |
| $K_{\text{O}_2}^{\text{m}}$ | Monod coefficient of O$_2$ for further degradation of DCBA | 3.9    | [µmol L$_{\text{int}}^{-1}$] | fitted |
| $r_{\text{hydro}}^{\text{max}}$ | maximum hydrolysis rate constant from BAM to 2,6-DCBA | 67.3    | [µmol L$_{\text{int}}^{-1}$s$^{-1}$] | fitted |
| $k_{\text{att}}$ | bacterial attachment rate constant | $6.2 \times 10^{-6}$ | [s$^{-1}$] | fitted |
| $\mu_{\text{max}}$ | maximum specific growth rate constant | $1.5 \times 10^{-3}$ | [s$^{-1}$] | fitted |
| $X_{\text{max}}$ | maximum carrying capacity for biomass growth | 97   | [µmol$_{\text{biomass}}$ L$^{-1}$] | fitted |
| $Y$     | yield coefficient                  | 0.24     | [µmol$_{\text{biomass}}$ µmol$^{-1}$] | fitted |
| $V_{\text{cell}}$ | single cell volume                | 0.9      | [µm$^3$]      | Elleegaard, et al. 10 |
| $M_{\text{cell}}$ | dry weight per cell volume        | $3 \times 10^{-7}$ | [µg$_{\text{biomass}}$] | Schultz-Jensen, et al. 4 |
| $\rho_{\text{bio}}$ | biomass density                   | $3 \times 10^6$ | [µmol$_{\text{biomass}}$ L$_{\text{int}}^{-1}$] | $\rho_{\text{bio}} = M_{\text{cell}} / V_{\text{cell}}$ |
|         | Isotope parameters                |          |               |                       |
| $\varepsilon_{\text{C}}$ | C isotope enrichment factor       | -8       | [%o]          | Reinnicke, et al. 16  |
| $\varepsilon_{\text{N}}$ | N isotope enrichment factor       | -13.7    | [%o]          | Reinnicke, et al. 16  |
|         | Inflow concentrations             |          |               |                       |
| $c_{\text{BAM}}^{\text{in}}$ | BAM inlet concentration           | 100      | [µmol L$^{-1}$] | experimental          |
| $c_{\text{O}_2}^{\text{in}}$ | O$_2$ inlet concentration         | 244      | [µmol L$^{-1}$] | experimental          |
| $X_{\text{bio}}^{\text{in}}$ | Biomass inlet concentration       | 32.6     | [µmol L$^{-1}$] | fitted                |

The fitted parameters in the model were obtained via the automated model calibration. Other parameters were determined either by laboratory measurements or from literature.
Figure S1. Parameter sensitivities plotted at each measurement location along the column depth-profile, where $C_i$ denotes the $i$-th model outcome and $p_j$ the $j$-th parameter ($n = 10$). Relative parameter errors (that is, $\times / \div$ the fitted parameter value) are presented alongside each parameter in the figure legend. Parameters with a relative error close to 1 are well constrained. The uncertainty for $k_{att}$ is not reported, because a 10% perturbation of the parameter did not yield a change in the model output.
Figure S1 plotted in different x-scales.
Derivation of the Apparent Enrichment Factor (Equation 18)

The theoretical background of the derivation of eq 18 is based Thullner et al.17 Governing equations without consideration of isotopologues:

\[ \frac{\partial c_{\text{bulk}}}{\partial t} = \frac{X}{\rho_{\text{bio}}} \cdot k_{\text{tr}} \cdot (c_{\text{int}} - c_{\text{bulk}}) \quad (S11) \]

\[ \frac{\partial c_{\text{int}}}{\partial t} = k_{\text{tr}} \cdot (c_{\text{bulk}} - c_{\text{int}}) - r_{\text{max}} \cdot \frac{c_{\text{int}}}{c_{\text{int}} + K_m} \quad (S12) \]

Assume quasi-steady state in the bacterial cell interior:

\[ k_{\text{tr}} \cdot (c_{\text{bulk}} - c_{\text{int}}) - r_{\text{max}} \cdot \frac{c_{\text{int}}}{c_{\text{int}} + K_m} = 0 \quad (S13) \]

\[ \Rightarrow (c_{\text{bulk}} - c_{\text{int}}) \cdot (c_{\text{int}} + K_m) - r_{\text{max}} \cdot k_{\text{tr}} \cdot c_{\text{int}} = 0 \quad (S14) \]

\[ \Rightarrow - c_{\text{int}}^2 + \left( c_{\text{bulk}} - K_m - \frac{r_{\text{max}}}{k_{\text{tr}}} \right) \cdot c_{\text{int}} + c_{\text{bulk}} \cdot K_m = 0 \quad (S15) \]

yields the quasi steady-state interior concentration:

\[ c_{\text{int}} = \frac{c_{\text{bulk}} - K_m - \frac{r_{\text{max}}}{k_{\text{tr}}} \cdot c_{\text{int}} + c_{\text{bulk}} \cdot K_m}{2} \quad (S16) \]

Now we consider the light and heavy isotopologues:

\[ \frac{\partial c_{\text{light}}}{\partial t} = \frac{X}{\rho_{\text{bio}}} \cdot k_{\text{tr}} \cdot (c_{\text{light}} - c_{\text{light}}) \quad (S17) \]

\[ \frac{\partial c_{\text{light}}}{\partial t} = k_{\text{tr}} \cdot (c_{\text{light}} - c_{\text{light}}) - r_{\text{max}} \cdot \frac{c_{\text{light}}}{c_{\text{light}} + K_m} \quad (S18) \]

\[ \frac{\partial c_{\text{heavy}}}{\partial t} = \frac{X}{\rho_{\text{bio}}} \cdot k_{\text{tr}} \cdot (c_{\text{heavy}} - c_{\text{heavy}}) \quad (S19) \]

\[ \frac{\partial c_{\text{heavy}}}{\partial t} = k_{\text{tr}} \cdot (c_{\text{heavy}} - c_{\text{heavy}}) - \alpha \cdot r_{\text{max}} \cdot \frac{c_{\text{heavy}}}{c_{\text{heavy}} + K_m} \quad (S20) \]

With \( c_{\text{int}} \) as derived above, quasi-steady state in the interior implies:
\[ \begin{align*}
 k_{tr} \cdot (c_{\text{light bulk}} - c_{\text{light int}}) - r_{\text{hydro max}} \cdot \frac{c_{\text{light int}}}{c_{\text{int}} + K_m} &= 0 \quad (S21) \\
 k_{tr} \cdot (c_{\text{heavy bulk}} - c_{\text{heavy int}}) - \alpha \cdot r_{\text{hydro max}} \cdot \frac{c_{\text{heavy int}}}{c_{\text{int}} + K_m} &= 0 \quad (S22) \\
 \Rightarrow c_{\text{light int}} &= \frac{c_{\text{light bulk}}}{1 + \frac{r_{\text{hydro max}}}{k_{tr} \cdot (c_{\text{int}} + K_m)}} \quad (S23) \\
 \Rightarrow c_{\text{heavy int}} &= \frac{c_{\text{heavy bulk}}}{1 + \frac{\alpha \cdot r_{\text{hydro max}}}{k_{tr} \cdot (c_{\text{int}} + K_m)}} \quad (S24)
\end{align*} \]

Rate of change of concentration in the bulk phase:
\[ \begin{align*}
 \frac{\partial c_{\text{light bulk}}}{\partial t} &= \frac{X_{\text{im}}}{\rho_{\text{bio}}} \cdot k_{tr} \cdot (c_{\text{light int}} - c_{\text{light bulk}}) = \frac{X_{\text{im}}}{\rho_{\text{bio}}} \cdot k_{tr} \cdot c_{\text{light bulk}} \cdot \left( \frac{1}{1 + \frac{r_{\text{hydro max}}}{k_{tr} \cdot (c_{\text{int}} + K_m)}} \right) \quad \text{(S25)} \\
 \frac{\partial c_{\text{heavy bulk}}}{\partial t} &= \frac{X_{\text{im}}}{\rho_{\text{bio}}} \cdot k_{tr} \cdot (c_{\text{heavy int}} - c_{\text{heavy bulk}}) = \frac{X_{\text{im}}}{\rho_{\text{bio}}} \cdot k_{tr} \cdot c_{\text{heavy bulk}} \cdot \left( \frac{1}{1 + \frac{\alpha \cdot r_{\text{hydro max}}}{k_{tr} \cdot (c_{\text{int}} + K_m)}} \right) \quad \text{(S26)}
\end{align*} \]

Apparent fractionation factor:
\[ \alpha^* = \frac{\frac{dc_{\text{heavy bulk}}}{dt} \cdot c_{\text{light bulk}}}{\frac{dc_{\text{light bulk}}}{dt} \cdot c_{\text{heavy bulk}}} = \frac{1}{1 + \frac{\alpha \cdot r_{\text{hydro max}}}{k_{tr} \cdot (c_{\text{int}} + K_m)}} - 1 \]
\[ = \frac{1}{1 + \frac{r_{\text{hydro max}}}{k_{tr} \cdot (c_{\text{int}} + K_m)}} - 1 \]

\[ S14 \]
\[
\frac{c_{\text{int}} + K_m}{c_{\text{int}} + K_m + \frac{\alpha \cdot r_{\text{hydro}}}{k_{tr}}} - 1 = \frac{c_{\text{int}} + K_m + \frac{\alpha \cdot r_{\text{hydro}}}{k_{tr}}}{c_{\text{int}} + K_m + \frac{r_{\text{hydro}}}{k_{tr}}} - 1
\]
\[
= \frac{\alpha}{c_{\text{int}} + K_m + \frac{\alpha \cdot r_{\text{hydro}}}{k_{tr}}}
\]

Substitute eq S16 into eq S27, we will get the final equation:
\[
\alpha^* = \frac{c_{\text{bulk}} + K_m + \frac{r_{\text{hydro}}}{k_{tr}}}{c_{\text{bulk}} + K_m + \frac{r_{\text{hydro}}}{k_{tr}}} + \left(\frac{c_{\text{bulk}} - K_m - \frac{r_{\text{hydro}}}{k_{tr}}}{k_{tr}}\right)^2 + 4c_{\text{bulk}} \cdot K_m
\]

The calculation based on eq 18 and the one based on Thullner et al (eq S29) yielded same estimations (Figure 4).
\[
\alpha^* = \alpha \cdot \frac{1 + \frac{1}{2} \left(\frac{a}{k_{tr}} - \frac{c_{\text{bulk}}}{K_m} - 1\right) + \sqrt{\frac{a}{k_{tr}} + \frac{1}{4} \cdot \left(\frac{a}{k_{tr}} - \frac{c_{\text{bulk}}}{K_m} - 1\right)^2}}{1 + \alpha_0 \cdot \left[\frac{1}{2} \left(\frac{a}{k_{tr}} - \frac{c_{\text{bulk}}}{K_m} - 1\right) + \sqrt{\frac{a}{k_{tr}} + \frac{1}{4} \cdot \left(\frac{a}{k_{tr}} - \frac{c_{\text{bulk}}}{K_m} - 1\right)^2}\right]}
\]

(S29)
**Additional Supporting Figures**

Figure S2. Simulated transient development of isotope values $\Delta \delta^{13} C$, BAM concentration $c_{\text{BAM}}^{\text{bulk}}$, total washed-out cell number $TCC$, and oxygen concentration $c_{\text{bulk}}^{O_2}$ at outflow. System reached to steady state on day 17.

Figure S3. Concentration difference between the bulk solution $c_{\text{BAM}}^{\text{bulk}}$ and the intracellular solution $c_{\text{int}}^{\text{BAM}}$ along the vertical outlet profile.
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