Supporting Information for

Towards improved bioremediation strategies: Response of BAM-degradation activity to concentration and flow changes in an inoculated bench-scale sediment tank

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26 SUMMARY

27 13 pages, 6 figures and 2 table

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54 **Chemicals**

55 The chemicals used in this study are: 2,6-dichlorobenzamide (Sigma Aldrich, Germany), 2,6-
56 dichlorobenzamide-3,4,5-d_3, 98.4%-d_3 (Alfa Chemistry, Ronkonkoma, NY), 2,6-
57 dichlorobenzoic acid (Sigma Aldrich, Germany), and metolachlor (Chemos GmbH &Co. KG,
58 Germany).

59 **Medium Preparation and Bacteria Cultivation**

60 The recipe of the growth medium for *Aminobacter* sp. strain MSH1 was adapted from the
61 optimized mineral salt medium $MSNC_{opt}$ developed by Schultz-Jensen et al.[1] and is described
62 in Sun et al.[2] Briefly, the medium was prepared in 990 mL MilliQ water, with Na_2HPO_4 (6
63 g/L), KH_2PO_4 (3 g/L), $MgSO_4 \times 7H_2O$ (0.2 g/L), $CaCl_2 \times 2H_2O$ (0.01 g/L). The pH of the
64 medium was adjusted to 7.0 with hydrochloric acid before autoclaving (121 ℃ for 20 min).
65 After autoclaving and cooling, 10 mL of trace element stock solution (with H_3BO_3 (39 mg/L),
66 $MnSO_4 \times H_2O$ (84.5 mg/L), $CuSO_4 \times 5H_2O$ (125 mg/L), $ZnCl_2$ (69 mg/L), $CoCl_2 \times 6H_2O$ (119.5
67 mg/L), and $Na_2MoO_4 \times H_2O$ (121 mg/L)) was filtered through 0.22µM syringe filters (Merck
68 KGaA, Germany) and added to the medium solution. To prepare the anoxic BAM solution
69 injected at the central inlet port of the tank, BAM (powder) was added to the medium and
70 stirred vigorously for 24 h to facilitate dissolution. The medium was flushed with N_2 gas for 4
71 h to remove the dissolved oxygen. The oxic medium solution was injected at the inlet ports
72 except for the central one, which was flushed with air for 2 h. All chemicals for the medium
73 preparation were from Sigma Aldrich, Germany.

74 The BAM degrader–*Aminobacter* sp. Strain MSH1 was obtained from the Department of
75 Geochemistry, the Geological Survey of Denmark and Greenland (GEUS), Denmark. The
76 bacteria cultivation steps are described in Sun et al.[2] Briefly, precultures were prepared in 200
77 mL medium solution in 1 L shaker flask with added glucose (400g/L, 2 mL, autoclaved) as
78 carbon source. A BAM solution (500 mg/L, 4 mL) was filtered through 0.22µM syringe filters
79 (Merck KGaA, Germany) and added to the preculture to ensure the BAM-degrading ability of
80 bacteria. The incubation was performed in an orbital shaker at 130 rpm at 20 °C. When the
81 optical density (OD) reached one, the preculture was centrifuged in four 50 mL centrifuge tubes
82 at 4000 rpm for 5 min. Then cells were resuspended and washed in 10 mL medium solution
83 (without glucose or BAM) three times. After resuspending the washed bacterial cell pellets in

84 2 L medium solution (without glucose or BAM), the culture was ready for inoculation to the
85 tank.

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

87 The setup of the quasi-two-dimensional flow-through sediment tank was adapted from
88 Bauer et al.[3] and is described in Sun et al.[2, 4] Briefly, two glass plates made up the front and
89 back sides of the tank, with a Teflon spacer as the bottom and sidewalls. Two aluminum rims
90 at either side of the chamber held the glass plates and Teflon spacer together. The tank is a
91 quasi-two-dimensional system with inner dimensions of 95 cm × 18 cm × 1 cm (L × H × W).
92 Sixteen ports were equally spaced (with 1.0 cm distance) at the inlet (left side) and outlet (right
93 side) boundary of the tank. The tank was sterilized with 12 g/L NaOH solution and rinsed with
94 autoclaved ultra-pure MilliQ water. Pre-sorted uniform quartz sands (0.8–1.2 mm diameter)
95 was wet packed under water in thin layers in the tank. Peristaltic pumps (Ismatec, Germany)
96 were connected between the inlet ports of the tank and BAM/medium solution bottles, and
97 between the outlet ports of the tank and the sampling vials via stainless steel capillaries and
98 tygon pump-tubes.

99 **Sample Preparation and Solid-Phase Extraction (SPE)**

100 We conducted filtration and solid phase extraction to clean and separate the analytes before
101 concentration and isotope measurements. For carbon isotope measurements, water samples
102 were filtered through 0.2 µM PES filter (Nalgene Thermo Scientific, Germany) and
103 cumulatively collected every day until enough sample volume (1–2 L) was collected. For
104 concentration measurements, water samples (1 mL) were filtered through 0.22 µM syringe
105 filters (Merck KGaA, Germany) and the pH was adjusted to pH 1.7 with HCl; the internal
106 standard 2,6-dichlorbobenzamide-3,4,5-d_3 was spiked into the samples before SPE. We
107 adapted the SPE method from Torrentó et al.[5] and Jensen et al.[6] The SPE cartridges (PP SPE
108 cartridges with PE frit, 20 µm pore size, Sigma Aldrich, Germany) were self-packed with
109 hydrophobic polymer-based sorbent Bakerbond SDB-1 (J.T. Baker, USA). The SPE steps for
110 isotope samples and concentration samples are described in Sun et al.[2] and are briefly listed in
111 the table below.

112

113 Table S1 SPE steps

	Cartridges	Sorbents	Conditioning	Loading	Washing	Drying	Concentrating
Isotope samples	6 mL	0.2 g	3 mL EtOAc 2×3 mL MeOH 2×3 mL MilliQ	0.2–2 L	2×3 mL MilliQ	120 min	3 ml EtOAc
Concentration samples	1 mL	0.05g	1 mL EtOAc 2×1 mL MeOH 2×1 mL MilliQ	1 mL	2×1 mL MilliQ	60 min	1 ml MeCN

114 **Sediment Sampling and Pretreatment for Total Cell Counts Measurement**

115 For the total cell counts of attached bacteria, duplicate sediment samples (0.5 mL) were
116 collected after the disassembly of the tank at the end of the experiment on day 170. Before
117 disassembling the tank, both the inlet and outlet pumps were stopped. Then the remaining water
118 in the system was slowly drained downwards port by port along the outlets. After draining,
119 the tank was slowly laid down on a flat bench table and carefully disassembled by removing
120 the glass plate at the front of the tank. Once the front glass had been carefully removed and the
121 sediments were completely exposed, a front-cut 2 mL syringe was used to sample the sediments
122 at 1 cm intervals along the vertical cross-sections, 2 cm from the inlet boundary, in the middle,
123 and 2 cm from the outlet boundary of the tank, respectively.

124 The pretreatment of the sediment samples for bacterial cell counting has been described
125 elsewhere.[7,8] Briefly, 0.5 mL sediment sample aliquots were fixed with 1.5 mL glutaraldehyde
126 solution (with 2.5% final concentration) and stored at 4°C until further treatment. Subsequently,
127 glutaraldehyde solution was replaced by 1.5 mL of phosphate buffer solution (PBS). To release
128 the cells from the sediment, samples were swung at 20 Hz for 3 min on a swing mill (Retsch,
129 MM 200). Cells were further separated from sand particles via density gradient centrifugation.[9]
130 The layer with bacterial cells was collected for the cell counting using a flow cytometer.

131 **BAM and 2,6-DCBA Concentration Measurements on LC-MS/MS**

132 The method of the concentration measurements on LC-MS/MS was adapted from Jensen
133 et al.[6] Briefly, the LC-MS/MS system consisted of an Agilent 1100 HPLC system (Agilent
134 Technologies Inc, USA) coupled to a QTrap 4000 mass spectrometer (MS) equipped with
135 electrospray ionization (ESI) (Sciex, USA) interface. Chromatographic separation was

136 performed on a Kinetex® C18 column (2.6 µm, 10 nm, 100 × 2.1 mm i.d., Phenomenex, USA)
137 guarded by a precolumn. The column oven temperature was maintained at 40 ℃. The mobile
138 phase was composed of solvents A (water/ammonium acetate (5 mM), pH of 2.4 adjusted by
139 formic acid), and solvent B (acetonitrile). The separation was achieved by applying a gradient
140 flow of 300 µL/min as follows: 0–5 min, 90% A; 5–9 min, 90%–10% A; 9–10 min, 10%–90%
141 A; 10–15 min, 90% A. The injection volume was 10 µL. Detailed method description and
142 parameters of target analytes on the MS are as same as described by Sun et al.[2]

143 **Carbon Isotope Measurements on GC-IRMS**

144 Carbon isotope measurements were performed on TRACE GC Ultra gas chromatograph
145 (Thermo Fisher Scientific, Italy) coupled with a Finnigan MAT 253 isotope ratio mass
146 spectrometer (IRMS). A Finnigan GC Combustion III interface (Thermo Fisher Scientific,
147 Germany) was used to connect GC to IRMS. Compound separation was conducted on a DB-5
148 analytical column (30 m, 0.25 mm i.d., 0.5 µm film, Agilent Technologies, Germany) in the
149 GC. The carrier gas was Helium (1.4 mL/L, grade 5.0). For high concentration samples, a
150 Thermo injector in the split/split-less injection mode was used; for low concentration samples,
151 on-column injection mode was set up using a programmable Optic 3 injector (system) with
152 liquid N_2-cryofocusing (ATAS GL, distributed by Axel Semrau, Germany). The GC oven
153 temperature program in the split/split-less injection mode started at 80 ℃; temperature
154 increased to 280 °C at a ramp rate of 15 ℃/min, and held for 7 min. The GC oven temperature
155 program in the on-column injection mode started at 35 °C holding for 30 s; the temperature
156 increased to 80 ℃ at a ramp rate of 5 °C/min. Then the temperature increased from 80 °C to
157 280 °C at a ramp rate of 15 °C/min. International reference standards Vienna PeeDee Belemnite
158 (V-PDB) were used to determine the carbon isotope values δ^{13}C [‰] of the samples. The
159 carbon isotope values δ^{13}C of the samples were calculated in relation to a lab reference gas
160 (CO_2, RM8562, RM8563). Further analytical details are as same as described in Sun et al.[2, 4]

161 **T-RFLP Analysis**

162 T-RFLP analysis of bacterial 16S rRNA genes was performed according to the previous
163 protocol of Pilloni et al. 2011.[10] 16S rRNA genes were amplified using FAM-labeled primer
164 pairs Ba27f (5'FAM-aga gtt tga tcm tgg ctc ag-3') and 907r (5'-ccg tca att cct ttg agt tt-3').
165 The PCR thermal profile consisted of 25 cycles of denaturation step (30s, at 94 °C), an

166 annealing step (30s, at 52 °C) and an extension step (60s, at 70 °C). The PCR reactions were
167 performed in a total volume of 50 µL, including 1× PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs,
168 1.25 U Taq polymerase (All from Fermentas, Germany), 0.2 µg/µL BSA (Roche, Switzerland),
169 0.5 µM of each primer (Biomers, Germany), and 1 µL of template DNA. Amplicons were then
170 purified with MinElute® PCR purification kit (Qiagen, Germany) according to the
171 manufacturer's instruction. Purified amplicons were then restricted using MspI (HpaII,
172 cleavage site 5'-ccgg-3') (Thermo Fisher Scientific, Germany) and resolved by capillary
173 electrophoresis on a 3730 DNA Analyzer (Applied Biosystems, USA). All samples were
174 performed in duplicates. Afterwards, electropherograms were analyzed by the software
175 GeneMapper 4.0 (Applied Biosystems, USA) as previously reported.[11] The data was then
176 further denoised and analyzed with T-REX software.[12] Software parameters were selected as
177 reported in Mueller et al.[13] The T-RFLP DNA fingerprint from the washed-out cells at different
178 outlet position in phase 2 is shown in Figure S4.

179 **Calculation of apparent isotope enrichment factor ε^* based on Thullner et al. 2008**

180

$$\varepsilon^* = 1 - \alpha^* = 1 - \alpha \cdot \frac{1 + \frac{1}{2}\left(\frac{a}{k_{\mathrm{tr}}} - \frac{c_{\mathrm{bulk}}}{K_{\mathrm{m}}} - 1\right) + \sqrt{\frac{a}{k_{\mathrm{tr}}} + \frac{1}{4} \cdot \left(\frac{a}{k_{\mathrm{tr}}} - \frac{c_{\mathrm{bulk}}}{K_m} - 1\right)^2}}{1 + \alpha_0 \cdot \left[\frac{1}{2}\left(\frac{a}{k_{\mathrm{tr}}} - \frac{c_{\mathrm{bulk}}}{K_{\mathrm{m}}} - 1\right) + \sqrt{\frac{a}{k_{\mathrm{tr}}} + \frac{1}{4} \cdot \left(\frac{a}{k_{\mathrm{tr}}} - \frac{c_{\mathrm{bulk}}}{K_m} - 1\right)^2}\right]} \tag{S1}$$

181

182 in which ε^* [-] is the apparent isotope enrichment factor, α^* [-] is the apparent isotope fractionation
183 factor, α [-] is the isotope fractionation factor with fixed value of 0.9992, a is specific affinity of the
184 bacteria promoting the enzymatic reaction given as $a = v_{\mathrm{max}}/K_{\mathrm{M}}$, v_{max} is the maximum hydrolysis rate
185 constant with fixed value of 67 µmol L^{-1} s^{-1}, K_{M} is the Monod coefficient of BAM with the fixed value
186 of 0.38 µmol L_{cell}^{-1}, k_{tr} is the mass transfer coefficient with the fixed value of 7.6 s^{-1}, c_{bulk} is the initial
187 BAM bulk concentration. The values of the fixed parameters are from Sun et al., 2021[14].

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Additional Supporting Tables and Figures

194 Table S2. Overall mass balance of the entire system calculated by the average values during the
195 sampling days over the three different operational phases defined by different feed concentrations
196 through the central inlet port. Data from the flow disturbance was excluded for the first 50 mg/L inlet
197 concentration experiment.

	$BAM_{\text{total}}^{\text{inlet}}$ [μmol/day]	$BAM_{\text{total}}^{\text{outlet}}$ [μmol/day]	$DCBA_{\text{total}}^{\text{outlet}}$ [μmol/day]	$DO_{\text{consumed}}^{\text{outlet}}$ [μmol/day]	$Carbon\ in\ Biomass$ [$\mu mol/day$]	*Biodegradation* Efficiency [%]	*Carbon-Assimilation Ratio* [%]
Phase 1	17.1	2.3±0.5	3.4±0.4	59.6±8.6	41±5	87±3	52 ± 8*
Phase 2	34.1	6.2±1.5	14.8±2.6	85.3±3.8	15±3	86±3	17 ± 10
Phase 3	17.1	0.04±0.05	5.0±1.2	72.4±5.1	6±1	99±2	7 ± 1

*The calculated carbon-assimilation ratio based on washed-out cell number in phase 1 may not represent the true value due to the adaptation of bacteria in phase 1. Uncertainties of $BAM_{\text{total}}^{\text{outlet}}$, $DCBA_{\text{total}}^{\text{outlet}}$, $DO_{\text{consumed}}^{\text{outlet}}$ are the standard deviation of the measurements, and the uncertainties of $Carbon\ in\ Biomass$, *Biodegradation Efficiency*, and *Carbon-Assimilation Ratio* are calculated based on Gauss' error propagation law.

198

199 The calculation of carbon assimilation ratio (CAR) is based on the equations below:

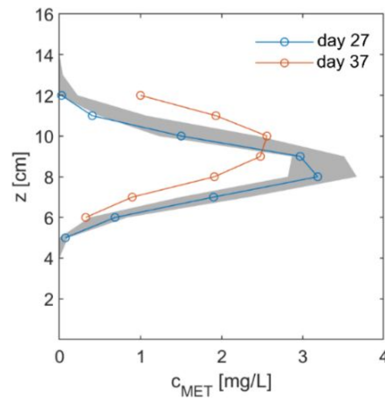
$$CAR = \frac{Carbon\ of\ washed\ out\ biomass}{Carbon\ of\ consumed\ BAM\ or\ DCBA\ to\ CO_2\ or\ biomass} \quad \text{(S2)}$$

$$Carbon\ of\ washed\ out\ biomass\ \ (\text{mol/L}) = \text{Washed out cell number (mol)} \times \frac{130\ fg\ per\ cell}{12\ g/mol} \quad \text{(S3)}$$

$$Carbon\ of\ BAM_{consumed}\ or\ DCBA_{consumed}\ (\text{mol/L}) = (BAM_{inlet} - BAM_{remained} - DCBA_{remained})\text{m} \times \frac{12\ g/mol\ \times 7}{191\ g/mol} \quad \text{(S4)}$$

200 with molar mass of 2,6-DCBA of 191 g/mol, and biomass of 130 fg per cell[15].

201

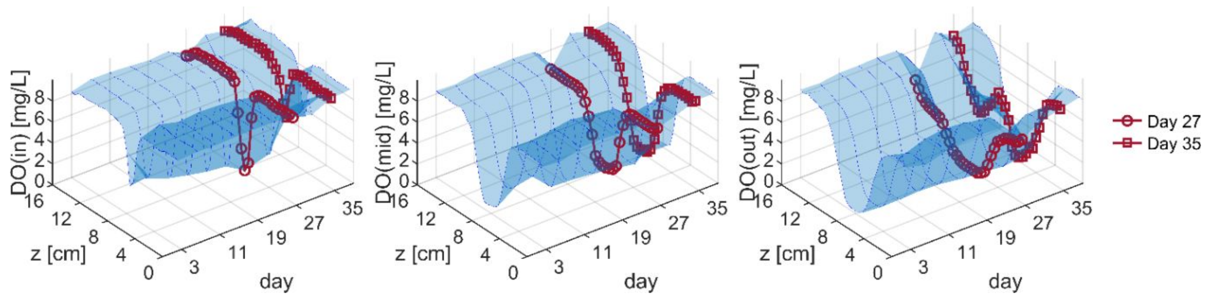


202

203 Figure S1. Metolachlor (conservative tracer) concentration profile changing with time under the first
204 50 mg/L BAM inlet concentration condition (phase 1). Grey shade represents the concentration range
205 in the quasi-steady state period. Blue and orange data points represent the metolachlor concentration
206 profiles on the flow fluctuation days.

207

208



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210 Figure S2. Dissolved oxygen (DO) profiles at the inlet, in the middle, and at the outlet of the tank,
211 changing with time under the first 50 mg/L BAM inlet concentration condition (phase 1). Red data
212 points represent the DO profiles on the flow fluctuation days.

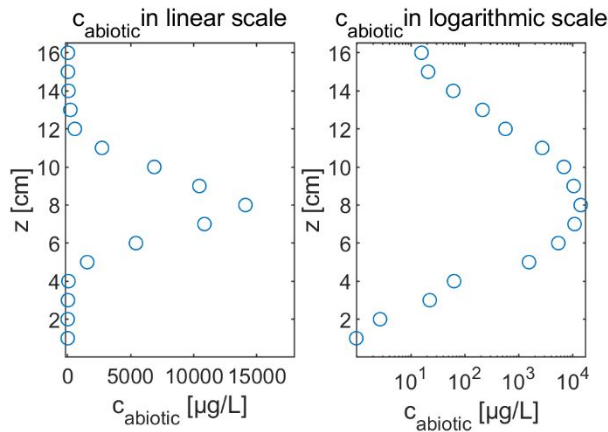


Figure S3. Abiotic BAM concentration profile under the 50 mg/L BAM inlet concentration condition.

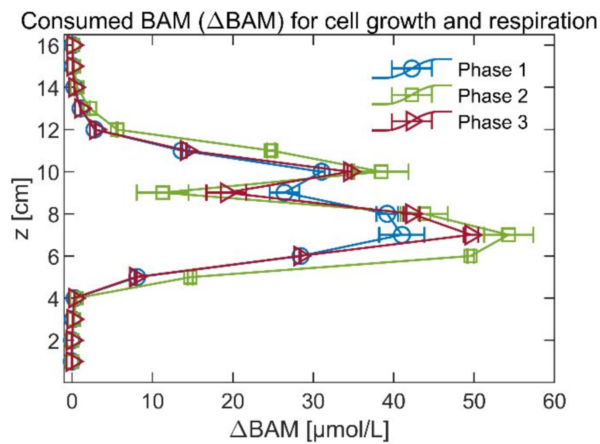
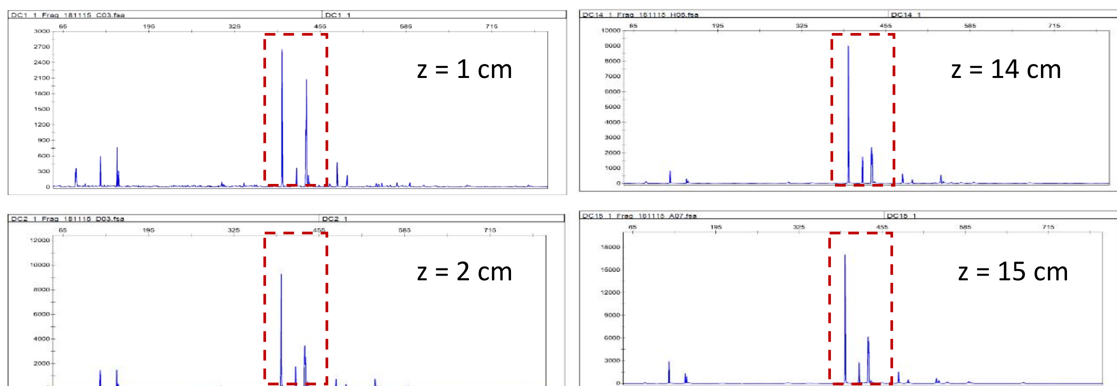
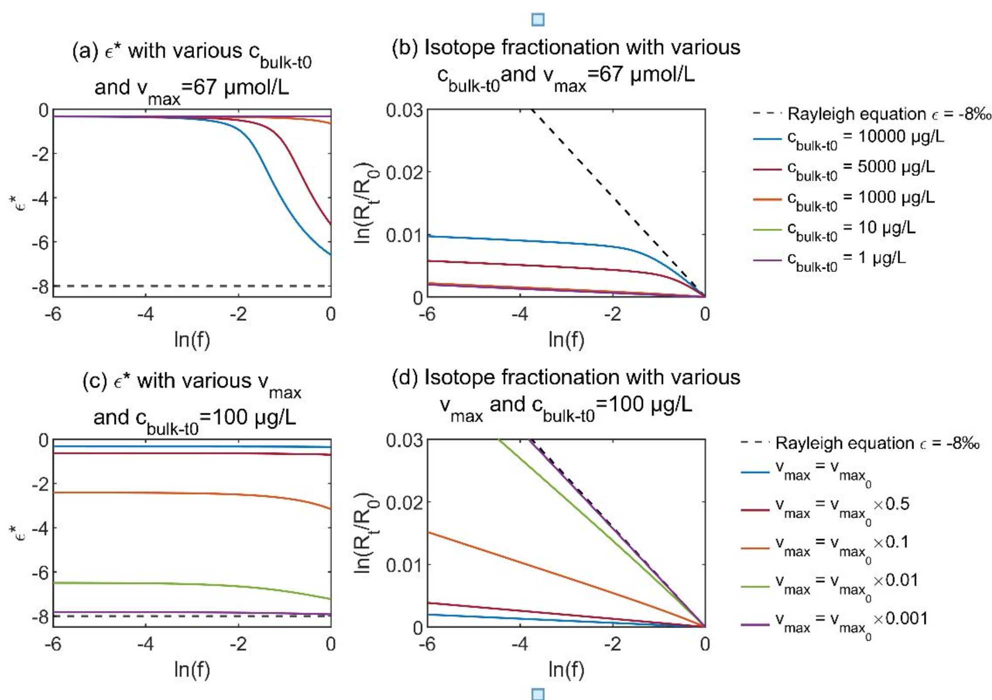


Figure S4. Consumed BAM (ΔBAM) for cell growth and respiration in experimental phase 1, 2, and 3, which was the total consumed BAM ($c_{abiotic}^{BAM} - c_{biotic}^{BAM}$) minus the remaining 2,6-DCBA (c_{biotic}^{DCBA}), i.e., $BAM = c_{abiotic}^{BAM} - c_{biotic}^{BAM} - c_{biotic}^{DCBA}$ [µmol/L]. Uncertainties represent the standard deviation of the measurements.



223 Figure S5. T-RFLP DNA fingerprint from the washed-out cells at different outlet positions in phase 2
224 (with BAM inlet concentration of 100 mg/L). The DNA fingerprint of strain *Aminobacter* sp. MSH1
225 (accession number DQ401867.1) was framed in the dashed-line rectangle.

226



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228 Figure S6 (a-b) Calculated apparent enrichment factor ε* and isotope values based on eq S1 with fixed
229 isotope fractionation factor $\alpha = 0.992$, maximum hydrolysis rate constant v_{max0}= 67 μmol L^{-1} s^{-1}, mass
230 transfer coefficient k_{tr} = 7.6 s^{-1}, Monod coefficient of BAM K_M = 0.38 μmol L_{cell}^{-1} and various initial
231 substrate bulk concentration $c_{bulk\text{-}t0}$. (c-d) Calculated apparent enrichment factor ε^* and isotope values
232 with fixed $c_{bulk\text{-}t0}$ = 100 μg L^{-1} and various v_{max}. The calculated ε* values are the slopes of the isotope
233 value plot at a given location.

234 When biotransformation is only subject to mass-transfer limitation but without physiological
235 adaptation (i.e., v_{max} is constant), isotope fractionation is tremendously masked by the mass-
236 transfer limitation, with apparent isotope enrichment factor, ε*, and isotope fractionation close
237 to zero at low concentrations (e.g., at c_{bulk_t0} = 1μg/L, 100μg/L). In contrast, with decreasing
238 v_{max} due to physiological adaptation, isotope fractionation becomes more and more observable
239 and closer to the Rayleigh equation, and the apparent isotope enrichment factor, ε*, is closer to
240 the true isotope enrichment factor, ε.

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