Supporting Information

Mass Transfer Limitation During Slow Anaerobic Biodegradation of 2-Methylnaphthalene

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Contains:

Page: 7

Figure: 0

Contents:

1. Introduction

1.1 Determination of enrichment factors by the Rayleigh equation:

Changes in isotope values are indicative of the extent of degradation and can be described by the Rayleigh equation (Eq. 1):

$$\frac{R_t}{R_0} = \frac{1 + \delta 13\mathrm{Ct}}{1 + \delta 13\mathrm{Co}} = f^{\varepsilon} \qquad (1)$$

where R_t and R_0 (or $\delta^{13}C_t$ and $\delta^{13}C_0$) describe the average isotope composition of the heavy isotope to the light isotope in a specific compound at a given time and at the beginning of the reaction, respectively (i.e., when nothing has been degraded so far). The remaining fraction *f* of the compound is given by the ratio C_t/C_0, where C_t is the concentration of this compound at a given time and C_0 at the beginning of the reaction.

2. Materials and Methods

2.1 Chemicals

Unless stated otherwise, all reagents and solvents were of analytical grade. 2-MN (98%) and naphthalene (99%) (used as internal standard for GC-MS measurements) were obtained from Aldrich Chemie, CAS: 91-57-6 and CAS: 91-20-3, respectively. n-Hexadecane (CAS: 544-76-3 (99%), used as solvent-carrier phase for 2-MN, was obtained from Acros Organics. Cyclohexane (CAS: 110-82-7), utilized as extractant for 2-

MN from the aqueous phase and solvent for measurements of 2-MN in the hexadecane carrier phase, was obtained from Fluka Analytical.

2.2 Cultivation conditions and degradation experiments

2-methylnaphthalene degrading pure culture NaphS2 were cultivated as described in Widdel and Bak[1] and Galushko et al. [2] Degradation experiments were carried out either in aqueous medium solution (one phase) or in two phase systems with hexadecane as an overlaying carrier phase. In the one phase system pure 2-MN crystals were added to 1 L bottles with 850 ml of anoxic artificial seawater medium [2]and 150 ml of CO_2/N_2 (20:80 v/v) headspace. Crystals were stirred until complete dissolution occurred. Final 2-MN concentration was 0.060 mM.

In the two-phase systems two different concentrations of 2-MN in hexadecane were used:

Ten milliliters of the carrier phase containing 2-MN concentrations of 80 mM and 10 mM were added to 220 ml bottles with 180 mL of anoxic artificial seawater medium and 10 ml of CO_2/N_2 (20:80 v/v) headspace, providing a final nominal concentration of 4 mM and

65 0.5 mM of 2-MN in the whole two-phase system (water phase + organic phase) in each
66 condition respectively. According to our abiotic experiments, the aqueous concentration
67 of 2-MN after establishment of equilibrium with the donor phase in the absence of
68 biodegradation was 4 and 0.5 µM, respectively (Table 1). The actual concentration in the
69 gas phase was not measured and was neglected because of the low hexadecane-water
70 partitioning coefficient ($10^{-5.8}$, Schwarzenbach Lehrbuch). Calculation of a substrate in
71 the water phase for two conditions of 10 and 80 mM was the same and was equal to 0.1
72 % (Table 1) because the partitioning coefficient was the same in all conditions and was
73 calculated to be in our systems approx. 20 000). The fraction of a substrate was calculated
74 according to the following equation

75 $f_{naphthalene,aq} = (V_{aq}/V_d)/(\text{partition coefficient})$

76 where V_{aq} is the volume of the aqueous phase and V_d the volume of the donor phase
77 in milliliters.

78 The reason why estimated partition coefficients where higher in our experiments than the
79 theoretical one is likely due to the elevated salt concentration of our medium (“salting out”
80 effect).

In order to decrease the accumulation of relevant toxic amounts of sulfide ^{3} by sulfate reduction we exchanged the aqueous medium by fresh medium, when concentrations of sulfide were around around 5-6 mM.

All cultivation bottles were sealed with Viton stoppers (Maag Technik, Dübendorf, Switzerland) and inoculated with 10% inoculum. All incubations were performed at 30 °C, in the dark, and with gentle shaking (52 rpm). This shaking speed was chosen as a compromise between homogeneous substrate distribution and minor disturbance of the culture. All experiments were conducted in triplicates surplus two controls. In the control experiments autoclaved culture solution was added.

2.3 Analytical Methods.

Concentration analyses. Analyses of the 2-MN degradation progress in the one phase system were done in duplicates for each sampling point. Thus for each replicate 0.8 mL of the aqueous solution were withdrawn. Extraction of 2-MN was done by vortexing the aqueous sample with cyclohexane in a v/v ratio of 2/1 (aqueous solution/cyclohexane) for 2 min in a 2-mL glass vial (Supelco, Bellefonte, PA) closed with a Teflon coated cap. After

30 min – time of separation of the aqueous and cyclohexane phase, 162 µL of the cyclohexane phase were transferred to a another 2-ml glass vial containing a 200 µL micro-insert (Carl Roth Chemicals, Karlsruhe) and 18 µL of the internal naphthalene standard stock solution (1 mmol/L in cyclohexane) were added. Extraction efficiency was never worse than 95 % (data not shown).

For the determination of the concentrations of 2-MN in the two phase system, 18 µL of the hexadecane phase were taken and added to 144 µL of cyclohexane in 2-ml glass vial containing a 200 µL micro-insert. Further, 18 µL of the internal standard naphthalene stock solution (10 mmol/L in cyclohexane) were added to a final volume of 180 µL.

For all approaches, extracted samples were analyzed after preparation immediately. Determination of 2-MN concentration was carried out on a Agilent GC 7890A gas chromatograph hyphenated to a 5975C inertXL EI/CI MSD detector (Agilent Technologies, Waldbronn). Chromatographic separation was done on a fused silica HP-5MS column (30m x 0.250 mm, film thickness 0.25 µm) with the following temperature program: start at 50°C, 10°C/min heat up to 130°C, hold for 1 min, 5°C/min to 200°C, 30°C/min heat up to 280°C, hold for 3 min. The injection volume was 1 µL. The samples

were injected in splitless mode. Analyte detection was done in SIM (single ion mode) mode for the following masses: m/z = 132 and 131 for 2-MN and m/z = 129 for naphthalene. External standard series of 2-MN ranged from 20 µM to 100 mM.

Isotope analyses. For the C and H isotopic analyses of 2-MN during its degradation in the one-phase setup, aqueous samples of 12-37 mL (depending on the 2-MN concentration) were periodically taken from culture bottles, transferred into Supelco vials with Teflon coated caps, and stored immediately, at -20 °C prior to isotope analysis according to Elsner et al[4]. For the two-phase systems two replicates of 175 µL (C and H isotope analyses) of the hexadecane phase were taken and transferred to 2-mL vial with 200 µL micro-inserts and closed with Teflon coated caps and stored at -20°C until analyses.

Carbon and hydrogen compound specific isotope ratios of 2-MN were measured using a TRACE GC Ultra gas chromatograph (GC) (Thermo Fisher Scientific; Milan, Italy), coupled to a Finnigan TM MAT253 IRMS (Thermo Fisher Scientific; Bremen, Germany). The temperature of the combustion oven was 1050 °C for carbon isotope analysis. For hydrogen isotope analysis a pyrolytic interface was used running at 1430 °C. The GC was

129 equipped with a programmable temperature vaporizer (PTV) injector (Optic3, ATASGL
130 International B.V.; Veldhoven, Netherlands) with cryofocussing option by liquid N_2. A
131 purge and trap concentrator Tekmar VelocityXPTTM together with an autosampler
132 Tekmar AQUATek 70 (Tekmar-Dohrmann; Mason, Ohio, USA) were connected online to
133 the PTV injector of the GC-IRMS. Operation of the purge & trap system including
134 cryofocussing of analytes in the injector was accomplished according to Jochmann et al.[5]
135 The GC-oven program was identical to the GC-MS setup. The carrier gas was He with a
136 purity of 5.0. Carrier gas flow was 1.4 for carbon isotope analyses and 1.2 for hydrogen
137 isotope analyses. Injections were done in split mode with a split ratio ranging from 10 to
138 50.
139 For the carbon isotope analysis of 2-MN by GC-IRMS, a laboratory CO_2 standard was
140 used as calibration gas. This laboratory standard had been calibrated to V-PDB by
141 reference CO_2 standards (RM 8562, RM 8563, RM 8564). Hydrogen isotope analysis of
142 2-MN was performed using a laboratory H_2 monitoring gas, which had not been calibrated
143 against an international standard. For this reason, changes in hydrogen isotope ratios are
144 given as relative differences $\Delta\delta^2H = \delta^2H_t - \delta^2H_0$ where δ^2H_0 is the mean isotope value of

the control bottles at time point zero. Samples for C isotope analyses were measured in duplicate, for hydrogen isotope analyses in triplicate. Reproducibility for $\delta^{13}C$ and $\delta^{2}H$ was always better than 0.5‰ and 5‰.

References

1. Widdel, F.; Bak, F., Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd ed.; Balows, A.; Trüper, H. G.; Dworkin, M.; Harder, W.; Schleifer, K. H., Eds. Springer-Verlag: New York, N.Y., 1992; pp 3352-3378.
2. Galushko, A.; Minz, D.; Schink, B.; Widdel, F., Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. *Environ. Microbiol.* **1999,** *1*, (5), 415-420.
3. Reis, M.; Almeida, J.; Lemos, P.; Carrondo, M., Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnol. Bioeng.* **1992,** *40*, (5), 593-600.
4. Elsner, M.; Couloume, G. L.; Sherwood Lollar, B., Freezing to preserve groundwater samples and improve headspace quantification limits of water-soluble organic contaminants for carbon isotope analysis. *Anal. Chem.* **2006,** *78*, (21), 7528-7534.
5. Jochmann, M. A.; Blessing, M.; Haderlein, S. B.; Schmidt, T. C., A new approach to determine method detection limits for compound-specific isotope analysis of volatile organic compounds. *Rapid Commun. Mass. Spectrom.* **2006,** *20*, (24), 3639-3648.