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Bioavailability as bottleneck for biodegradation of organic micro-pollutants in groundwater? – Evidence from compound-specific isotope analysis in two-dimensional tank experiment

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In groundwater, organic pollutants persist at very low concentrations (sub-μg/L), although many of them are in principle biodegradable. Bioavailability limitation is one of the factors hypothesized to constitute bottlenecks of micro-pollutant biodegradation. Compound-specific isotope analysis (CSIA) has been brought forward as a promising approach to explore the onset of bioavailability limitation in biodegradation at low-level concentrations [1]. The role of membrane permeability as a barrier for atrazine biodegradation at low concentrations has been highlighted in a recent batch study by applying CSIA [2]. However whether bioavailability limitation – i.e. rate-limiting mass transfer towards and into the microbial cell – inhibits micropollutant biodegradation in environmental systems has not yet been verified by direct observation in experiments mimicking realistic situations in groundwater.

Here we present applications of CSIA to study biodegradation of micro-pollutants in a two-dimensional sediment tank experiment as a groundwater model system. A solution of 2,6-dichlorobenzamide (BAM) at its natural isotopic abundance was continuously injected into the middle of the tank, and a gradient of high to low concentrations of BAM was established by transverse dispersion. First, abiotic experiments were conducted to study the influence of hydrodynamic dispersion on observable isotope fractionation. Subsequently Aminobacter MSH1 was inoculated into the tank for “biotic” experiments. We took samples at different ports at the end of the tank to draw a picture of vertical distributions of concentration, biomass, isotope values etc.

Our results show that isotopic fractionation of BAM (13C/12C and 15N/14N) induced by hydrodynamic dispersion was negligible in the abiotic experiment. In biotic experiments, the highest isotope fractionation appeared in the fringes where O_2 and BAM were mixed. In the area near the top and bottom of the tank, in contrast, where BAM concentrations were lowest and where BAM degradation would, therefore, be expected to be most complete, observable isotope fractionation did not further increase, but started declining. We hypothesize that these results reflect the effect of bioavailability limitation during biodegradation. When concentrations of BAM were lower than a certain threshold concentration, mass transfer of BAM towards and into the cell became rate-limiting relative to the enzyme reaction inside the cell. Consequently, observed isotope fractionation decreased. Hence, the observed decrease of isotope fractionation at low-level concentrations in the gradient of a sediment tank may provide the first direct evidence of bioavailability limitation in a groundwater model system.

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[2] Ehrl, B., Gharasoo, M., Elsner, M. (2018). Isotope fractionation pinpoints membrane permeability as a barrier to atrazine biodegradation in gram-negative Polaromonas sp. Nea-C. Environ Sci Technol., p. acs.est. 7b06599.