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Isotope Fractionation Reveals Limitations and Microbial Regulation of Pollutant Biodegradation at Low Concentrations

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Compound-specific isotope fractionation analysis (CSIA) of chemical trace contaminants (“micropollutants”) could reveal bottlenecks of degradation at low, relevant (mg/L) concentrations. When enzyme-associated isotope effects were observable, this provided evidence that molecules diffused freely into and out of bacterial cells demonstrating that mass transfer was not limiting. In contrast, when isotope fractionation was pronounced at high concentrations, but isotope effects were masked at trace levels, this provided evidence that mass transfer into and out of the cell became limiting for biodegradation specifically at low concentrations [1]. An onset of masking was observed for atrazine when degraded by *Arthrobacter aurescens* TC1 at concentrations below 60 mg/L in chemostat [1, 2] with complete rate control at 10 mg/L in retentostat [3]. Proteomics revealed that such mass transfer limitation served as trigger for physiological adaptation, where catabolic enzymes remained highly expressed, whereas other cellular functions were downregulated.

CSIA also demonstrated mass transfer limitations in a quasi-two dimensional sediment tank system mimicking realistic conditions of natural aquifers. High, unmasked isotope fractionation in the center of the plume indicated that 2,6-dichlorobenzamide degradation by *Aminobacter* sp. MSH1 was not limited by substrate availability, but rather by oxygen supply. In contrast masked isotope fractionation pinpointed rate-limiting mass transfer during cellular uptake towards the lower end of the concentration profile [4]. For bioremediation approaches of low-level concentrations, the direct observation of limitations offers an enabling tool to identify relevant bottlenecks and observe bacterial regulation / adaptation over time [5]. For Isotope Biogeochemistry these findings have, moreover, significant implications for the interpretation of isotope profiles at low concentrations: they imply that, based on isotopic evidence, turnover of substances at low concentrations may be widely underestimated.

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