



Influence of substrate bioavailability on the apparent stable isotope fractionation

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Stable isotope fractionation analysis is an emerging technology for the qualitative and quantitative assessment of organic contaminant biodegradation in the environment. The fractionation processes are caused by the enzymatic discrimination of heavier isotopes during biotransformation processes, and lead to a relative enrichment of the heavier isotope in the residual substrate pool. To date, standard procedures determining fractionation factors are neglecting the influence of pollutant bioavailability on the degradation and on isotope fractionation. The bioavailability of the pollutant however can be an important factor for its degradation. In this case, the total degradation rate is influenced by the dynamics of the physico-chemical process controlling bioavailability rather than the rate of the microbially catalyzed biochemical reaction following Michaelis-Menten kinetics. As a consequence, the isotope fractionation factor observed in bioavailability affected systems might differ from values obtained for the non bioavailability-limited biochemical reaction only.

Here we present the theoretical background and the experimental evidence for the influence of bioavailability-limited pollutant degradation on apparent stable isotope fractionation. Simple laboratory setups were established to determine toluene isotope fractionation factors when exposing toluene-degrading *Pseudomonas putida* mt-2(pWWO) to different bioavailability constrains. Fractionation factors were found to vary significantly with mass transfer conditions, and consequently with substrate concentration during batch biodegradation. Our results confirm the hypothesized influence of substrate mass transfer limitation on apparent isotope fractionation and underline the importance of interactive physico-chemical processes on biodegradation.