**Differentiation of aerobic and anaerobic PAH biodegradation employing hydrogen isotope fractionation**

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Polycyclic aromatic hydrocarbons (PAHs) are widely distributed pollutants of great concern due to their potential toxicity, mutagenicity and carcinogenicity. Caused by their hydrophobic nature, PAHs basically accumulate in sediments from where they are slowly released into the groundwater. Microbial degradation is thought to be one of the major mechanisms leading to a sustainable restoration of PAH contaminated sites. *In situ* biodegradation of the poorly water-soluble PAHs is difficult to monitor due to the generally limited bioavailability and associated slow reactions kinetics of PAHs. For monitoring and evaluating environmental PAH biodegradation processes, knowledge about responsible organisms and biochemical pathways is crucial.

We applied compound-specific stable isotope analyses (2D-CSIA) to determine characteristic carbon and hydrogen isotope fractionation patterns of aerobic and anaerobic PAH degradation. Naphthalene and 2-methylnaphtalene were used as model compounds to characterize the stable isotope effect of the first reaction step initiating both degradation pathways. Dihydroxylation of naphthalene and 2-methylnaphthalene by *Pseudomonas putida* NCIB 9816-4 and *Pseudomonas fluorescens* ATCC 17483 using a naphthalene dioxygenase was associated with small but significant carbon isotope fractionation (εC = -0.8 ± 0.1 ‰ to -1.6 ± 0.2 ‰). In contrast, anaerobic activation of naphthalene by a carboxylation-like mechanism by the enrichment culture NaphS6 was linked to negligible carbon isotope fractionation (εC = -0.2 ± 0.2 ‰ to -0.3 ± 0.6 ‰). Notably, anaerobic activation of naphthalene by strain NaphS6 exhibited a normal hydrogen isotope fractionation (εH = -11 ± 2 ‰ to -46 ± 14 ‰) whereas an inverse hydrogen isotope fractionation was observed for the strains containing a naphthalene dioxygenase (εH = +15 ± 2 ‰ to +71 ± 6 ‰). Additionally, isotope fractionation of strain NaphS6 was determined in an overlaying non-polar carrier phase, resulting in more reliable enrichment factors compared to immobilizing the PAHs on the bottle walls without carrier phase. The observed differences in carbon and hydrogen fractionation might be used to differentiate between aerobic and anaerobic naphthalene and 2-methylnaphthalene biodegradation pathways at PAH-contaminated field sites.