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Monitoring BTEX degradation by CSIA – chances and challenges

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Monitoring is crucial for evaluating the success of any geobiotechnological applications. Compound- specific stable isotope analysis (CSIA) has emerged as a key method for monitoring biogeochemical transformation processes. Isotope compositions of residual reactants may change during the first rate-limiting step in (bio)chemical reactions; measurement of these changes are the basis for CSIA. Caused by differences in the activation energy, light isotopologues often react slightly faster than heavy isotopologues, resulting in enrichment of heavy isotopes at the reactive site in the substrate or of light isotopes in the product. This is termed isotope fractionation. Upon multi-dimensional CSIA (2D-CSIA, 3D-CSIA), the isotope fractionation of two or more different elements within a molecule is determined, allowing highly resolved analyses of degradation processes as masking effects typically occurring in one-dimensional CSIA are cancelled. In the last years, 2D-CSIA making use of the ratio of stable carbon to hydrogen isotopes (13C/12C, 2H/1H), turned out to be an important tool for elucidating the environmental biodegradation pattern of BTEX compounds which are global notorious contaminants. This presentation aims to summarize the current knowledge on 2D-CSIA of BTEX, to point out the prospects and to indicate future perspectives upon monitoring in the field.

Degradation experiments for determining carbon and hydrogen isotope fractionation factors were carried out using several pure and mixed cultures performing different BTEX-activating reactions. Various anaerobic key reactions showed pronounced hydrogen isotope fractionation: (i) fumarate addition to the methyl moiety of toluene, xylene isomers and probably ethylbenzene catalyzed by benzylsuccinate synthases, (ii) anaerobic hydroxylation of the ethyl side chain of ethylbenzene catalyzed by ethylbenzene dehydrogenase, and (iii) anaerobic activation of benzene by yet unknown biochemical mechanisms. Due to the high hydrogen isotope fractionation, the ratios of hydrogen vs. carbon isotope fractionation in two-dimensional plots (lambda values, Λ) were generally higher than 10 (in extreme cases > 100). Upon aerobic activation reactions at the aromatic ring catalyzed by mono- or dioxygenases, usually Λ values smaller than 10 were observed due to small, absent or inverse hydrogen isotope fractionation. An exception is the aerobic monooxygenation of methyl or methylene moieties which is linked to large hydrogen and carbon isotope fractionation. Since Λ values are highly indicative for specific transformation reactions, 2D-CSIA has a great potential for evaluating biodegradation processes of BTEX in the environment. Moreover, reactions catalyzed by benzylsuccinate synthases showed partially variable Λ values, indicating slightly different reaction mechanisms of isoenzymes, probably permitting the detection of specific isoenzymes by 2D-CSIA in field applications. In contrast, ethylbenzene dehydrogenase of three tested organisms showed similar, very characteristic isotope fractionation pattern even under different redox conditions.

The major goal of future investigations is to use 2D-CSIA at contaminated field sites for elucidating specific degradation pathways. Single data for benzene are promising, demonstrating e.g., anaerobic benzene degradation by 2D-CSIA at a highly contaminated site. Nevertheless, 2D-CSIA field data for BTEX are yet lacking and need to be surveyed for a proper evaluation of the 2D-CSIA concept for BTEX.