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Monitoring biodegradation of hydrocarbons by stable isotope fractionation

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In the last decade, several studies have demonstrated that stable isotope tools are highly applicable for monitoring anaerobic biodegradation processes. An important methodological approach is to characterize distinct degradation pathways with respect to the specific mechanism of C-H-bond cleavage and to quantify the extent of biodegradation by compound specific isotope analysis (CSIA). Here, enrichment factors (bulk) needed for a CSIA field site approach must be determined in laboratory reference experiments. Recent research results from different laboratories have shown that single bulk values for similar degradation pathways can be highly variable; thus, the use of two-dimensional compound specific isotope analysis (2D-CSIA) has been encouraged for characterizing biodegradation pathways more precisely. 2D-CSIA for hydrocarbons can be expressed by the slope of the linear regression for hydrogen versus carbon discrimination known as lambda $\approx$ Hbulk/Cbulk.

We determined the carbon and hydrogen isotope fractionation for the biodegradation of benzene, toluene and xylenes by various reference cultures. Specific enzymatic reactions initiating different biodegradation pathways could be distinguished by 2D-CSIA. For the aerobic di- and monohydroxylation of the benzene ring, lambda values always lower than 9 were observed. Enrichment cultures degrading benzene anaerobically produced significant different values: lambda values between 8-19 were oberved for nitrate-reducing consortia, whereas sulfate-reducing and methanogenic consortia showed always lambda values greater than 20 [1,2]. The observed variations suggest that (i) aerobic benzene biodegradation can be distinguished from anaerobic biodegradation, and (ii) that more than a single mechanism seems to exist for the activation of benzene under anoxic conditions. lambda values for anaerobic toluene degradation initiated by the enzyme benzylsuccinate synthase (BSS) ranged from 4 to 41, tested with strains using nitrate, sulfate or ferric iron as electron acceptor or using light as energy source [3,4,5]. Significantly different lambda values were also observed for the anaerobic degradation of xylenes initiated by the BSS [5]. The different lambda values obtained for the anaerobic degradation of toluene and xylenes might be caused by slightly different reaction mechanisms of BSS isoenzymes. In comparison, lambda and/or bulk values for the methyl monohydroxylation of toluene with oxygen as co-substrate were significantly different for two tested strains each containing a different toluene attacking enzyme, indicating that specific enzymes for aerobic methyl group oxidation reactions can be detected by CSIA and 2D-CSIA.
Our results show that the combined carbon and hydrogen isotope fractionation approach has great potential to elucidate biodegradation pathways of monoaromatic hydrocarbons in microcosm and field studies. Current work focus on (i) 2D-CSIA of aromatic and aliphatic hydrocarbons in degradation experiments using whole cells, and (ii) 2D-CSIA of aromatic hydrocarbons in in vitro experiments using cell extracts.

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