Development of a LC-IRMS method for the compound-specific carbon stable isotope analysis of halo-benzoates

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Compound-specific stable isotope analysis (CSIA) has been developed and applied over the last decades for the investigation of *in situ* transformation as well as of reaction mechanisms of common environmental pollutants. Thus far, approaches are well developed and available for common GC-amenable groundwater contaminants such as the chlorinated ethenes or BTEX-substances, however, they are still challenging for more polar pollutants such as halogenated benzoic acids.
The occurrence of halogenated benzoic acids in the environment is related to their agricultural use, as for pure substances in herbicides or metabolites of other halogenated compounds as fungicides. Under anaerobic, denitrifying conditions halo-benzoates can be degraded by e.g. *Thauera chlorobenzoica* by using 3-chloro-, 3-bromo- as well as 2-fluoro- and 4-fluorobenzoic acids as their sole source of carbon and energy (Kuntze et al. 2011). The class I benzoyl-CoA-reductases (BCRs) were responsible for dehalogenation and the proposed mechanism for meta-substitution showed a primary dearomatization of the aromatic ring followed by a rearomatization under halogen-elimination. The mechanisms for ortho- and para-substitution is still unknown.

To further investigate the mechanism involved, we aimed to characterize the reductive dehalogenation by *T. chlorobenzoica* 3CB-1^T for degradation of various mono-halogenated benzoates using compound-specific stable isotope analysis (CSIA). The main challenge, regarding the carbon stable isotope analysis of polar compounds using liquid chromatography - isotope ratio mass spectrometry (LC-IRMS), is the requirement for a carbon-free liquid carrier phase as well as instability of commercially available LC-columns in pure aqueous systems.

Therefore, a LC method was established to separate halogenated and non-halogenated benzoic acids using solely inorganic buffer conditions. All tested halogenated benzoic acids could be eluted by using a potassium hydrogenphosphate buffer as carrier phase. Furthermore, series of pH-values and temperatures were tested. Various columns with modified C18 phase were tested which potentially were stable in pure aqueous phase.

Subsequently, the method will be tested for CSIA of pure halobenzoates via LC-IRMS. Futhermore, reference tests will be done using *T. chlorobenzoica* 3CB-1^T to evaluate carbon stable isotope analysis during microbial degradation of halobenzoates.

Reference: Kuntze, K., Kiefer, P., Baumann, S., Seifert, J., von Bergen, M., Vorholt J. A., Boll, M.; *"Enzymes involved in the anaerobic degradation of meta-substituted halobenzoates"*, Mol. Microbiol. (2011) **82**(3): 758-769.