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Elucidation of processes contributing to the biotransformation of monochlorobenzene in a pilot-scale constructed wetland

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Monochlorobenzene (MCB) is a frequently detected groundwater contaminant which is toxic and persistent under anoxic conditions. It may be a threat for drinking water abstraction from groundwater and thus, it is important to investigate its fate in the environment. Natural wetlands as transition zone between anoxic aquifers and oxic open water bodies may enhance the (bio)transformation potential for recalcitrant organic contaminants due to the unique geochemical conditions and gradients. Therefore, a pilot-scale horizontal subsurface-flow constructed wetland has been set-up directly at a contaminated field site in Bitterfeld, Germany, to investigate the processes contributing to the biotransformation of MCB. The system consisted of a gravel filter and an open water pond at the outflow and was fed with anoxic MCB-contaminated groundwater from the aquifer in Bitterfeld. We observed a decrease in the MCB concentration with concurrent ferric iron reduction along the flow path within the anoxic gravel bed of the wetland. In parallel, addition of ferric iron stimulated the mineralisation of MCB in laboratory microcosms with anoxic groundwater from the same source, indicating that the potential for anaerobic microbial degradation of MCB is present at the field site.

In order to elucidate the MCB biodegradation in the wetland, the potential contributing redox processes and the bacteria involved in the transformation process were investigated applying compound specific stable isotope analysis and carbon stable isotope probing concepts. The carbon stable isotope analysis of MCB did not show a significant enrichment of the heavier carbon isotope in the remaining MCB fraction along the flow path. *In situ* microcosms loaded with ^{13}C-labelled MCB and partly with ferric iron were installed in the upper (micro-) oxic and deeper anoxic zones of the gravel bed as well as in the pond for several weeks to enrich intrinsic microbial communities and analyse the MCB-derived carbon assimilation pattern. During the incubation, the isotope composition of CO_2 dissolved in groundwater was enriched in ^{13}C in the area of incubation by up to +20 ‰ compared to the background, indicating that the ^{13}C-labelled MCB was mineralised. Total lipid fatty acids (TLFA) derived from the indigenous microorganisms colonising the microcosms were extracted and differed qualitatively and quantitatively between microcosms incubated in the gravel bed and in the pond, demonstrating unique microbial communities in the different redox zones. Comparison of the TLFA pattern and its isotope labelling obtained from *in situ* microcosms with and without ferric iron amendment will allow determining the role of ferric iron in the microbial transformation of MCB.