

In-situ Protein-SIP in a constructed wetland model system reveals *para* ring hydroxylation as predominant initiative pathway

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Constructed wetlands are economical and efficient options for the treatment of wastewater. In these systems, organic pollutants are mainly degraded by microorganisms inhabiting the rhizosphere. Plants provide oxygen and organic exudates via their roots and thereby stimulate microbial degradation activity. While the effluents of well-operated CWs match the standard values of sewage treatment, the functional understanding of the microbial transformation processes taking place within these systems is still limited.

We investigated the process of microbial toluene removal in a controlled constructed wetland model system (Kappelmeyer, Wießner et al. 2002) spiked with ¹³C-toluene as sole external carbon source. Abiotic process parameters such as redox potential, oxygen concentration and pH were acquired continuously. By combining a pulsed *in situ* protein-SIP (Jehlich, Schmidt et al. 2010) experiment with metaproteomics and 16S rRNA gene sequencing, we identified bacterial key players of toluene degradation as well as the major catabolic pathway.

Molecular and proteomic analyses revealed that the rhizospheric bacterial community was complex and numerically dominated by *Rhizobiales* and *Burkholderiales*, which contributed each about 20% to total taxon abundances. Notably, metaproteome and protein-SIP data proved that *Burkholderiaceae* were main degraders of toluene in the wetland system. To a lesser extent, *Comamonadaceae* were also involved in toluene degradation. The initial degradation steps were apparently catalysed by *para* ring-hydroxylating monooxygenases which are known to be predominant under oxygen limiting conditions. Additionally, we found that the citric acid cycle and the polyhydroxyalkanoate metabolism were induced after the pulsed addition of ¹³C-toluene.

In conclusion, a deeper insight into the molecular processes of toluene degradation was achieved by combining functional and taxonomic information. Our study is a promising proof of concept for future investigations of rhizospheric ecosystems degrading emerging organic pollutants.

Jehlich, N., F. Schmidt, et al. (2010). "Protein-based stable isotope probing." *Nat. Protocols* 5(12): 1957-1966.

Kappelmeyer, U., A. Wießner, et al. (2002). "Operation of a Universal Test Unit for Planted Soil Filters - Planted Fixed Bed Reactor." *Engineering in Life Sciences* 2(10): 311-315.