Investigations on the biological transformation of chlorinated benzenes in a pilot scale constructed wetland

- Characterisation by means of physicochemical parameters and methods using the stable isotope ^{13}C -

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At the industrial mega site Bitterfeld in Saxony Anhalt a constructed wetland for the treatment of chlorobenzene contaminated groundwater was investigated. The goal of this investigation was the characterisation of horizontal and vertical zoning and seasonal variability of a planted soil filter and an unplanted reference system regarding chlorobenzene concentrations and environmental parameters. Conclusions should be drawn about microbial chlorobenzene degradation depending on the planting. A methodical main focus of the diploma work was the *in situ* and *ex situ* proof of microbial monochlorobenzene (MCB) degradation, the investigation of degradation pathways and involved microorganisms by means of different methods using the stable carbon isotope ^{13}C.

In the course of the soil filter passage an average chlorobenzene concentration decrease of up to 65% was observed. Processes probably contributing to the contaminant loss are sorption on brown coal particles, physiological transport and transformation processes of the reed plants, volatilisation from the soil surface and microbial degradation. The observed seasonal variability of chlorobenzene elimination with higher residual concentrations in the summer can partly be due to higher transpiration of the plants. The concentration decrease at an average of 20% more effective in the course of the planted soil filter in comparison to the unplanted system was lead back to the stimulation of microbial growth by the planting.

By evaluating gradients of environmental parameters insight on possible microbial degradation processes was gained. Oxygen concentrations of the pore water mostly ranged in the trace interval below 0.1 mg L^{-1}. In the upper zone of the soil filter and in proximity to the helophyte roots aerobic microbial degradation can be possible. The redox potential of the pore water samples - which are mixed samples due to the sampling method - lies in the range of iron(III) reduction with average values between 50 and 90 mV in both systems. The considerable iron(II) mobilisation of up to 40 mg L^{-1} in both soil filters also argues for a significant iron(III) reduction. Supporting this findings, in laboratory microcosm experiments MCB mineralisation was associated with iron(II) mobilisation. Furthermore, indications for microbial sulphate reduction were observed.

By means of *in situ* $^{12}C/^{13}C$ isotope fractionation a proof of microbial degradation in the planted as well as unplanted soil filter was provided on the basis of a change in isotope signature of 0.64-0.85‰. Through application of *in situ* microcosms (BACTRAPs) in both soil filters and in the free water ponds, which are situated at the outflow area of the soil filters, the microbial MCB degradation could also be demonstrated *in situ*. When supplied with ^{13}C labelled MCB as growth substrate microorganisms incorporated ^{13}C into their biomass. Hence, the application of BACTRAPs in a constructed wetland, which has not been described so far, has proved of value. By comparison of microbial fatty acid patterns in the different parts of the system it could be shown that slightly different microbial consortia are present in the water ponds and the soil filters.

A strong evidence for reductive dechlorination of MCB was adduced by detection of ^{13}C labelled benzene in soil BACTRAP samples. In anaerobic laboratory microcosm experiments the

mineralisation of MCB was shown by means of ^{13}C labelling of carbon dioxide. The possibility and mechanisms of anaerobic microbial MCB degradation is controversial to date.

For the clarification of the manifold processes occurring in the heterogeneous system of a constructed wetland further studies concerning the uptake and transformation of chlorobenzenes by wetland plants and the chlorobenzene emission are recommended. To evaluate the share of different potentially important concentration decrease processes, parameters have to be determined simultaneously as changing environmental conditions like the weather and season affect these processes.

The technology implemented at present for the operation of the pilot scale constructed wetland is suboptimal for biological degradation of persistent chlorobenzenes due to oxygen limitation. Particularly when higher chlorinated benzenes are present, more pronounced aerobic and anaerobic phases are necessary, for example by application of a vertical flow filter fed at intervals or operation with variable water levels. Thereby the degradation potential of aerobic as well as anaerobic microorganisms could be utilised to fuller capacity and higher removal efficiencies could be achieved.

A more profound investigation of anaerobic microbial MCB degradation in microcosm studies applying different degradation conditions is essential. The search for convenient electron acceptors for anaerobic MCB oxidation should be focused on the possible use of iron(III) and sulphate on the basis of present results. Moreover, further experiments regarding the identification of metabolites of anaerobic MCB degradation using stable isotope tracers are advisable. The application of bigger microcosms and of more parallel samples will allow the extraction of enough biomass and a better validation of the results.

Evaluations of microbial consortia on the basis of microbiology and molecular biology can provide knowledge about microorganisms responsible for MCB degradation and the prevailing degradation pathways in the different parts of the wetland system. The necessary biomass can advantageously be obtained by using the BACTRAP method. Through degradation experiments with indigenous microorganisms a fractionation factor for MCB degradation in the wetland system can be determined. This is crucial for the quantitative evaluation of biological degradation via *in situ* $^{12}C/^{13}C$ isotope fractionation.

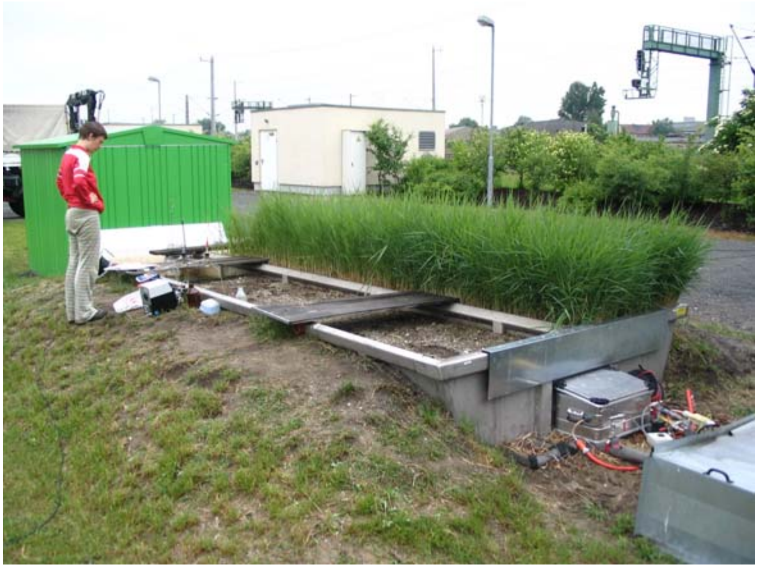

Figure 1: Constructed wetland for treatment of chlorobenzene contaminated groundwater in Bitterfeld

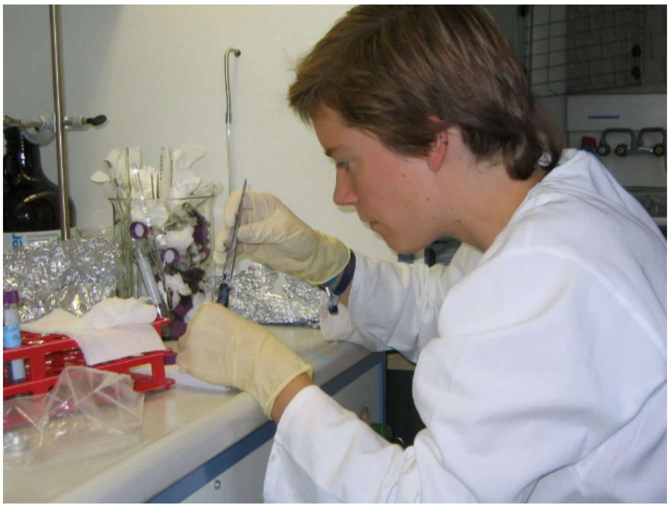

Figure 2: Extraction of fatty acids from BACTRAP samples

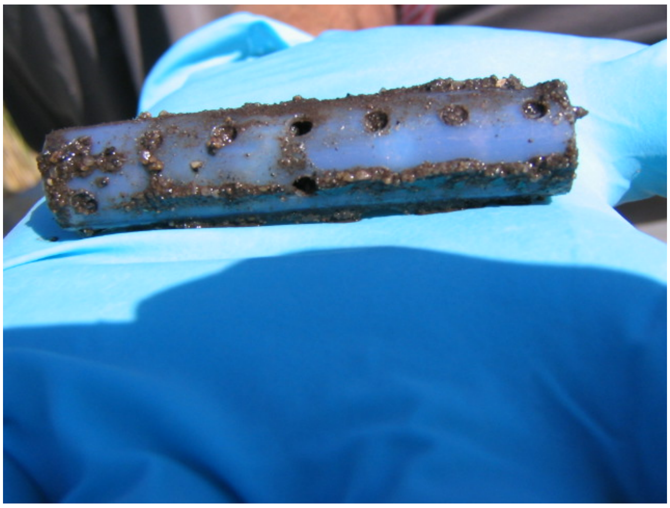

Figure 3: BACTRAP after exposition in the soil filter