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Iron oxides stimulate microbial monochlorobenzene in situ transformation in constructed wetlands and laboratory systems

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HIGHLIGHTS

- MCB removal in anoxic gravel bed of a planted and an unplanted constructed wetland was accompanied by iron(II) mobilisation.
- Higher MCB removal related to *Phragmatis* plants and summer season
- MCB mineralisation was stimulated with nitrate and iron(III) as electron acceptor in anoxic laboratory microcosm.
- MCB removal appears mainly linked to iron reduction; benzene removal may be linked to both sulphate and iron reduction in situ.

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ABSTRACT

Natural wetlands are transition zones between anoxic ground and oxic surface water which may enhance the (bio)transformation potential for recalcitrant chloro-organic contaminants due to the unique geochemical conditions and gradients. Monochlorobenzene (MCB) is a frequently detected groundwater contaminant which is toxic and was thought to be persistent under anoxic conditions. Furthermore, to date, no degradation pathways for anoxic MCB removal have been proven in the field. Hence, it is important to investigate MCB biodegradation in the environment, as groundwater is an important drinking water source in many European countries. Therefore, two pilot-scale horizontal subsurface-flow constructed wetlands, planted and unplanted, were used to investigate the processes in situ contributing to the biotransformation of MCB in these gradient systems. The wetlands were fed with anoxic MCB-contaminated groundwater from a nearby aquifer in Bitterfeld, Germany. An overall MCB removal was observed in both wetlands, whereas just 10% of the original MCB inflow concentration was detected in the ponds. In particular in the gravel bed of the planted wetland, MCB removal was highest in summer season with 73 ± 9% compared to the unplanted one with 40 ± 5%. Whereas the MCB concentrations rapidly decreased in the transition zone of unplanted gravel to the pond, a significant MCB removal was already determined in the anoxic gravel bed of the planted system. The investigation of hydro-geochemical parameters revealed that iron and sulphate reduction were relevant redox processes in both wetlands. In parallel, the addition of ferric iron or nitrate 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.

1. Introduction

Wetlands, river and lake sediments are ecosystems which are characterized by steep gradients of numerous physicochemical parameters like redox potential and oxygen concentrations. They provide highly reactive environments creating hot spots for microbial activity. The interaction of these chemical, physical and (micro)-biological processes is crucial for biochemical gradients and element cycling of e.g. iron and sulphur (Borch et al., 2010) but also for turn-over of organic substances including organic contaminants (Bauer et al., 2008; Imfeld et al., 2009).

In wetlands, these microbial metabolic hot spots include e.g. the rhizosphere or the interface between the groundwater and surface water providing a microbial habitat, high turnover rates of organic carbon (Stern et al., 2007) and redox processes of relevant elements (O_2, N, P, Fe, Mn and S) (Borch et al., 2010), which are affected by annual variation depending on the season and vegetation dynamics (Kadlec

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et al., 2012). Several natural processes e.g. microbial degradation, oxidation, volatilisation, and/or plant uptake contribute to the organic carbon turnover in wetlands (Imfeld et al., 2009; Kadlec et al., 2012).

Contaminant removal in natural and constructed wetlands or related water treatment systems (horizontal and vertical sediment columns) has been reported for compounds including ammonium (Bauer et al., 2008), pesticides e.g. hexachlorocyclohexanes (HCH) (Bhatt et al., 2009; Brunke and Gonser, 1997), pharmaceuticals such as ibuprofen and clofibric acid (Matamoros et al., 2009) as well as metalloids e.g. arsenic (Bauer et al., 2008) or for typically encountered groundwater contaminants such as the chloroethenes (Imfeld et al., 2008; Kassenga and Pardue, 2002; Lorah and Voytek, 2004; Novak et al., 2002), benzene (Rakoczy et al., 2011) and monochlorobenzene (Braeckevelt et al., 2007a,b).

Monochlorobenzene (MCB), a recalcitrant and ubiquitous groundwater contaminant (EPA, 2009) accumulates under anoxic conditions as for example found in the contaminated aquifer (Bitterfeld/Wolfen region, Germany) of a former production site of lindane (γ-HCH) as result from microbial conversion of HCHs. The aerobic microbial degradation of MCB is well characterised (Haigler et al., 1992; Müller et al., 1996; van Agteren et al., 1998). Moreover, MCB biodegradation under hypoxic conditions was proven for laboratory cultures of *Acidovorax* sp. and *Pseudomonas* sp. (Balcke et al., 2008). However, to date only few reports are available for anaerobic MCB biodegradation. Evidences for the in situ biodegradation were obtained using compound specific stable isotope analysis in combination with stable isotope tracer experiments (Braeckevelt et al., 2007a; Kaschl et al., 2005; Nijenhuis et al., 2007; Stelzer et al., 2009). The mineralisation of ^{13}C-labelled MCB and incorporation of MCB-derived carbon into biomass was proven in laboratory microcosms (Nijenhuis et al., 2007; Stelzer et al., 2009). Martinez-Lavanchy et al. (2011) suggested the involvement of *Proteobacteria*, *Fibrobacteres* and microbial members of the candidate division OD1 in the anaerobic MCB degradation, though; a single MCB degrading strain was not identified. Additionally to a complete mineralisation, the dechlorination of dichlorobenzene (DCB) and MCB to benzene by *Dehalobacter* sp. was reported (Fung et al., 2009; Liang et al., 2011).

To date, it is not clear how and via which pathways MCB is removed from contaminated anoxic aquifers. MCB may undergo different degradation reactions in situ (Fig. 1). From laboratory experiments it is known that under strictly anoxic conditions MCB can be i) reductively dechlorinated to benzene (Fung et al., 2009) which can be subject to mineralisation, shown for nitrate, iron and sulphate reducing as well as methanogenic conditions (Burland and Edwards, 1999; Liang et al., 2013; Lovley, 2000; Vogt et al., 2007). Alternatively, ii) we postulate that MCB can be directly mineralised with nitrate, iron, sulphate or CO_2 as terminal electron acceptor. Therefore we propose that these two main pathways, in which MCB functions as either i) electron acceptor or ii) electron donor, may take place sequentially or simultaneously in the environment leading to MCB mass reduction.

To investigate the MCB removal under anoxic conditions and at anoxic/oxic interfaces and to elucidate the contributing pathways in situ, a model subsurface flow constructed wetland, representing a highly reactive gradient system, was used. Previously, MCB in situ biodegradation was demonstrated in the model wetland and appeared to correlate with ferrous iron mobilisation. However, the use of aquifer material with high organic carbon content (brown coal) restricted the analysis of processes in the previous study (Braeckevelt et al., 2007a,b).

Therefore, the aquifer material was replaced by characterised gravel as sediment to provide a more defined system for the investigations. Additionally, reference experiments with anoxic groundwater from the field site were conducted using laboratory microcosms.

In our study we aimed at understanding the processes contributing to anaerobic MCB degradation with the following questions: 1) is MCB degraded in the model system, 2) which potential for microbial MCB degradation provides the contaminated groundwater and 3) which conditions support the anaerobic microbial MCB degradation in situ?

2. Material and methods

2.1. System description

Two horizontal subsurface flow wetlands, planted and unplanted, respectively, were set up at the SAFIRA project area in Bitterfeld/Wolfen, Germany (Wycisk et al., 2003) and consisted of a stainless steel basin with the dimension of 6 m (length) × 1 m (width) × 0.6 m (depth), each (Fig. 2/Supporting Information (SI) Fig. S1). Both wetlands contained a gravel bed (5 m × 1 m × 0.5 m) and an open water pond (1 m × 1 m × 0.4 m) at the outflow side. The gravel with a grain size of 0.6 to 6 mm (commercially obtained from the "Kiesgrube Wallendorf", quaternary gravel of the "Saale"-terrace, Germany) had a porosity of 41% and contained 252 ± 67 mg kg^{-1} iron, based on extraction with 0.5 N HCl (analysis with ICP-AES, SI, Table S1). To assess the vegetation effect on MCB removal and wetland geochemistry, one wetland segment was planted with common reed (*Phragmites australis*

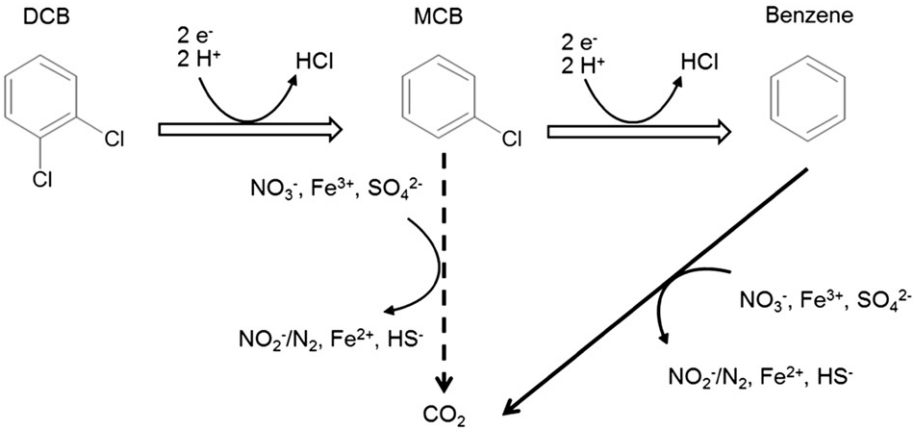

Fig. 1. Proposed anaerobic degradation pathways, suggested (dashed line) and published (solid line), for the degradation of dichlorobenzene (DCB), monochlorobenzene (MCB) and benzene. Reductive dechlorination of DCB via MCB to benzene (open arrows) reported by Fung et al. (2009) with hydrogen as electron donor. Anaerobic oxidation of benzene was reported under nitrate (Burland and Edwards, 1999), iron (Lovley, 2000) and sulphate reducing conditions (Vogt et al., 2007). Direct mineralisation of MCB with NO_3^-, Fe^{3+} or SO_4^{2-} as electron acceptor was suggested as metabolic pathway at corresponding reducing conditions (this study).