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**Constructed wetland treatment of groundwater
contaminated by chlorinated volatile organic compounds**

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**Constructed wetland treatment of groundwater
contaminated by chlorinated volatile organic
compounds**

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Referat

In der vorliegenden Arbeit werden Untersuchungsergebnisse zur Behandlung von mit chlorierten flüchtigen organischen Verbindungen (VOC) verunreinigtem Grundwasser in *constructed wetlands* (Pflanzenklärsystemen) dargestellt. Monochlorbenzen, Dichlorbenzene und Chlorethene zählen weltweit zu den häufigsten Grundwasserkontaminanten. *Constructed wetlands* sind eine kostengünstige Technologie zur Vermeidung der Kontamination von Oberflächengewässern. Im Rahmen der Studie wurden die Eliminierungseffizienz und die Saisonalität der Eliminierung, die Relevanz verschiedener Eliminierungsprozesse, und der Einfluss der Bepflanzung bei der Behandlung von Chlorbenzen-kontaminiertem Grundwasser in horizontal unterhalb der Bodenoberfläche durchströmten *constructed wetlands* im Pilotmaßstab untersucht. Frachteliminierungseffizienzen von 59-65% für Chlorbenzen und 59-69% für 1,4-Dichlorbenzen wurden im Sommer erreicht. Die Entfernung der Chlorbenzene wurde durch die Bepflanzung (*Phragmites australis*) signifikant verbessert, was durch eine Modellierung der Eliminierungsprozesse bestätigt wurde. Der Einsatz von Stabile-Isotopen-Methoden und klassischen mikrobiologischen Methoden erbrachte den Nachweis für den aeroben und anaeroben mikrobiellen Chlorbenzen-Abbau. Emissionsmessungen zeigten, dass die Verflüchtigung von der Filteroberfläche ein untergeordneter Eliminierungsprozess war. Zusätzlich wird die Adaption der Pilotanlage an die simultane Behandlung von Chlorbenzen und Perchlorethen beschrieben. Die Bildung von Ethen und der Nachweis von *Dehalococcoides sp.* zeigten eine vollständige mikrobielle reduktive Dechlorierung von Perchlorethen. Damit eignet sich die Technologie zur Entgiftung von mit hoch- und niedrig chlorierten VOC verunreinigten Grundwässern. Die Optimierung der Behandlung durch Anpassung der Kohlenstofffracht wurde in Modell-Wurzelraumreaktoren untersucht. Eine hohe Eliminierungseffizienz konnte dadurch für Chlorbenzen (>99%) und Perchlorethen (79-87%) erreicht werden, wobei die Einstellung geeigneter Bedingungen für die mikrobielle reduktive Dechlorierung von Perchlorethen eine Herausforderung darstellte.

Abstract

Chlorinated volatile organic compounds (VOC) such as monochlorobenzene (MCB), dichlorobenzenes (DCBs) and perchloroethene (PCE) are among the most widespread groundwater pollutants worldwide. Chlorinated VOC contaminated groundwater causes environmental hazards particularly when it discharges into rivers and river floodplains. Constructed wetlands (CW) represent a promising cost-efficient technology to prevent the contamination of surface waters. In the presented study, the removal efficiency and the seasonality of removal, the relevance of various removal pathways and the influence of planting were determined for CW treatment of chlorinated benzenes contaminated groundwater in the temperate climate zone. In addition, the simultaneous treatment of microbially oxidizable and reducible chlorinated VOC (MCB and PCE) and the optimization of CW treatment by adaptation of operation conditions were investigated. Removal of MCB, DCBs and PCE from groundwater was studied in the field and laboratory, using pilot-scale horizontal sub-surface flow (HSSF) CW and rhizosphere model reactors.

A comprehensive pilot-scale study was carried out on the removal of MCB, 1,2-DCB and 1,4-DCB from groundwater in a HSSF CW. Treatment of MCB and 1,4-DCB was efficient, in particular in the upper filter layer, while 1,2-DCB was not considerably removed. Load removal efficiencies amounted to 59-65% ($2327\text{-}3179\ \mu\text{mol m}^{-2}\ \text{d}^{-1}$) for MCB and 59-69% ($27\text{-}35\ \mu\text{mol m}^{-2}\ \text{d}^{-1}$) for 1,4-DCB in the summer period. The removal of chlorobenzenes was significantly enhanced by the presence of plants (*Phragmites australis*). Modelling of removal processes demonstrated that plants play an important role in MCB removal due to root oxygen release, intensification of water table fluctuations and direct uptake. The application of stable isotope methods and conventional microbiological methods provided evidence for both aerobic and anaerobic microbial MCB degradation. Model calculations confirmed the high potential of oxidative microbial MCB degradation in the system. In contrast, emission measurements indicated that surface volatilization was a minor removal process.

Simultaneous treatment of MCB and PCE in the pilot-scale HSSF CW required a long adaptation period, as was evident from the development of PCE and metabolite concentration patterns, and was efficient 2.5 years after operation started. After

adaptation, complete removal of 10-15 μM PCE was achieved, and outflow concentrations of chlorinated metabolites were negligible. Ethene production and detection of *Dehalococcoides sp.* indicated complete PCE dechlorination. Up to 100% of the PCE inflow load ($1250 \pm 260 \mu\text{mol d}^{-1}$) were dechlorinated on a filter passage of 1 m, and a maximum of 30% was recovered as ethene. Thus, HSSF CW are a promising approach for the remediation of groundwater contaminated by mixtures of highly and lowly chlorinated VOC.

Technical optimization approaches to MCB and PCE treatment in CW by application of different treatment conditions were explored in macro-gradient free rhizosphere model reactors. MCB removal was very efficient ($>99\%$) under low carbon load (overall oxic) and moderate carbon load (overall reducing) conditions. Higher loads of easily degradable carbon resulted in lower removal efficiencies of 72-96%. Under low carbon load and high carbon load conditions, 79-87% of PCE loads were eliminated, but no microbial reductive dechlorination activity was detected. Thus, plant uptake and volatilization were considered as important removal processes for PCE. Under fluctuating moderate carbon load conditions, reductive dechlorination was initiated and minimum 10% of PCE inflow loads were dechlorinated. To conclude, moderate additional organic carbon loads enable both MCB and PCE removal processes, indicating a good optimization potential for CW treatment of mixed groundwater contaminations.

Zusammenfassung

Chlorierte flüchtige organische Verbindungen (VOC) wie Monochlorbenzen (MCB), Dichlorobenzene (DCBs) and Perchlorethen (PCE) zählen weltweit zu den häufigsten Grundwasserkontaminanten. Durch chlorierte VOC kontaminiertes Grundwasser verursacht vor allem dann eine Umweltgefährdung, wenn es in Flüsse und Flußauen eintritt. Angelegte Auenlandschaften (constructed wetlands, CW) stellen eine viel versprechende kostengünstige Technologie zur Vermeidung der Kontamination von Oberflächengewässern dar. In der vorliegenden Studie wurden die Eliminierungseffizienz und die Saisonalität der Eliminierung, die Relevanz verschiedener Eliminierungsprozesse, und der Einfluss der Bepflanzung bei der Behandlung von Chlorbenzen-kontaminiertem Grundwasser in CW in der gemäßigten Klimazone untersucht. Zusätzlich wurden die simultane Entfernung von mikrobiell oxidierbaren und reduzierbaren chlorierten VOC (MCB und PCE) sowie die Optimierung der Behandlung durch Anpassung der Betriebsbedingungen behandelt. Die Entfernung von MCB, DCBs und PCE aus Grundwässern wurde experimentell im Feld und im Labor untersucht, wobei horizontal unterhalb der Bodenoberfläche durchströmte (horizontal subsurface flow, HSSF) CW im Pilotmaßstab sowie Modell-Wurzelraumreaktoren zum Einsatz kamen.

Eine umfassende Pilotstudie wurde hinsichtlich der Entfernung von MCB, 1,2-DCB und 1,4-DCB aus Grundwasser in einem HSSF CW durchgeführt. Die Behandlung von MCB und 1,4-DCB war effizient, vor allem in der oberen Filterschicht, während 1,2-DCB nicht wesentlich eliminiert wurde. Frachteliminierungseffizienzen von 59-65% ($2327-3179 \mu\text{mol m}^{-2} \text{d}^{-1}$) für MCB und 59-69% ($27-35 \mu\text{mol m}^{-2} \text{d}^{-1}$) für 1,4-DCB wurden im Sommer erreicht. Die Entfernung der Chlorbenzene wurde durch die Bepflanzung (*Phragmites australis*) signifikant verbessert. Die Modellierung der Eliminierungsprozesse zeigte, dass die Bepflanzung durch den Sauerstoffeintrag, die Intensivierung der Wasserstandsschwankungen, und die direkte pflanzliche Aufnahme entscheidend zur MCB-Entfernung beiträgt. Der Einsatz von Stabile-Isotopen-Methoden und klassischen mikrobiologischen Methoden erbrachte den Nachweis sowohl für den aeroben als auch den anaeroben mikrobiellen MCB-Abbau. Modellberechnungen bestätigten das hohe Potenzial besonders des oxidativen

mikrobiellen MCB-Abbaus im HSSF CW. Emissionsmessungen zeigten, dass die Verflüchtigung von der Filteroberfläche dagegen ein untergeordneter Eliminierungsprozess war.

Die simultane Behandlung von MCB und PCE in dem HSSF CW benötigte eine lange Anpassungszeit, wie anhand der Entwicklung der Konzentrationsprofile von PCE und seinen Metaboliten beobachtet werden konnte, und war erst 2,5 Jahren nach Betriebsbeginn effizient. Nach erfolgter Anpassung wurde die vollständige Entfernung von 10-15 μM PCE erreicht, und die Ablaufkonzentrationen der chlorierten Metabolite waren vernachlässigbar. Die Bildung von Ethen und der Nachweis von *Dehalococcoides sp.* gaben Hinweise auf eine vollständige mikrobielle reduktive Dechlorierung von PCE. Bis zu 100% der PCE-Zulaufkraft ($1250 \pm 260 \mu\text{mol d}^{-1}$) wurden auf einer Filterpassage von 1 m dechloriert, wovon bis zu 30% als Ethen wiedergefunden wurden. Damit sind HSSF CW ein Erfolg versprechender Ansatz zur Behandlung von mit hoch- und niedrig chlorierten VOC kontaminierten Grundwässern.

Technische Optimierungsansätze für die Behandlung von MCB und PCE in CW durch Anwendung verschiedener Betriebsbedingungen wurden in makrogradientenfreien Wurzelraumreaktoren getestet. Die Eliminierung von MCB war sehr effizient (>99%) bei niedriger Kohlenstoffkraft (oxische Bedingungen) und moderater Kohlenstoffkraft (reduzierende Bedingungen). Höhere zusätzliche Frachten leicht abbaubarer Kohlenstoffverbindungen führten zu niedrigeren Eliminierungseffizienzen von 72-96%. Bei niedrigen und hohen zusätzlichen Kohlenstofffrachten wurde eine Eliminierung von 79-87% der zugeführten PCE-Frachten erreicht, jedoch wurde keine mikrobielle reduktive Dechlorierung festgestellt. Daher wurden die pflanzliche Aufnahme und die Volatilisierung als wichtige PCE-Eliminierungsprozesse betrachtet. Bei einer fluktuierenden mittleren Kohlenstoffkraft wurde die reduktive Dechlorierung initiiert und mindestens 10% der PCE-Zulaufkraft wurden dechloriert. Demnach ermöglichten moderate zusätzliche Kohlenstofffrachten sowohl die MCB- als auch PCE-Entfernung, was auf ein gutes Optimierungspotenzial für die Behandlung von mit verschiedenen VOC kontaminierten Grundwässern schließen lässt.

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1 General introduction

1.1 Chlorinated volatile organic compounds as groundwater pollutants

Chlorinated volatile organic compounds (VOC) are among the priority groundwater contaminants worldwide (Plumb, 1991; US EPA, 1993; ATSDR, 2007). Chlorinated VOC of environmental relevance are mainly representatives of a group of chlorinated solvents and bulk chemicals ubiquitously used in the chemical industries: chlorinated methanes, chlorinated ethenes and ethanes, and mono- and dichlorinated benzenes. Main applications of chlorinated VOC are: solvents in chemical production and organic synthesis, building blocks for the production of pesticides, pharmaceuticals and dyes (especially chlorobenzenes), carriers for textile dyes and pesticides, degreasing agents in metal processing, and solvents in textile processing and treatment (chloroethenes) (van Agteren et al., 1998; WHO, 2004; WHO, 2006). For the purpose of this study, monochlorobenzene (MCB), dichlorobenzenes (DCBs) and perchloroethene (PCE) were chosen as model compounds. MCB is today mainly used in the production of nitrochlorobenzene and diphenyl oxide, while the major application of PCE worldwide is in dry-cleaning (53% of world demand in 1990) (IARC, 1995; WHO 2004). They are both very common groundwater contaminants in Europe and Northern America (McCarty, 1993; Kaschl et al., 2005; Field and Sierra-Alvarez, 2008), and were selected due to their exemplary characteristics. MCB is an aromatic compound with only one chlorine substituent, making it readily oxidizable by microorganisms under aerobic conditions (Field and Sierra-Alvarez, 2008). PCE is an aliphatic, fully chlorinated compound, thus being microbially degradable only via an initial reductive dechlorination step (McCarty, 1993, Maymó-Gatell et al., 1999). The selection of model contaminants allows tackling a demanding problem in biological remediation of chlorinated VOC contaminated waters, namely the divergent requirements of different members of the contaminant group regarding their microbial degradation.

1.1.1 Contaminant characteristics

The group of chlorinated VOC, though including quite diverse compounds, displays a range of characteristic physico-chemical properties. VOC are defined as compounds with a vapor pressure greater than 2.7 hPa at 25°C (NPI, 2007). A very useful parameter regarding environmental behaviour is the Henry's law constant H , describing the transfer of VOC between the water phase and atmosphere. It covers a range of 25-3080 Pa m³ mol⁻¹ (25°C) in the case of chlorinated VOC (Mackay et al., 2006). Most chlorinated VOC display a considerable water solubility, ranging between 30 mg L⁻¹ and 20 g L⁻¹ (25°C) (Mackay et al., 2006). Lipophilicity grows with the number of chlorine atoms (Henschler, 1994), and is most often described by the octanol water partition coefficient K_{OW} . Log K_{OW} values of chlorinated VOC are typically found in the range of 0.6-3.5 (25°C) (Mackay et al., 2006). The most important physical and chemical characteristics of the model compounds MCB and PCE are summarized in Table 1.

Table 1: Physico-chemical characteristics of the model compounds MCB and PCE in their standard state at 25°C. M : molecular mass; ρ : density; P_V : vapor pressure; S_W : water solubility; log K_{OW} : octanol water partition coefficient; H : Henry's law constant.

	M [g mol ⁻¹]	ρ [g cm ⁻³]	P_V [hPa]	S_W [mg L ⁻¹]	log K_{OW}	H [Pa m ³ mol ⁻¹]
MCB	112.6	1.11 ⁴	16.7 ²	484 ¹	2.98 ²	375 ²
PCE	165.8	1.62 ⁴	26.7 ^{3;6}	150 ⁵	2.53 ⁵	1510 ¹

¹Koch, 1995; ²van Agteren et al., 1998; ³RSC, 1999; ⁴Mackay et al., 2006; ⁵WHO, 2006; ⁶at 26.3°C

Chlorinated organics often exert a higher chemical reactivity and biological activity, and therefore toxicity, than comparable non-chlorinated hydrocarbons (Henschler, 1994; Naumann, 2003). This also leads to their extensive use as pharmaceutical drugs and plant protection agents, but also to great environmental and health risks. The chlorinated VOC form a group with – to a certain extent – common toxicological characteristics. Toxic impacts on higher animals and humans include: acute central anesthesia, usually increasing with the number of chlorine atoms, chronic toxicity affecting mainly the liver and kidney, and oftentimes severe genotoxicity (Henschler, 1994). Carcinogenic potential has been shown for chloromethanes, chloroethanes and chloroethenes, with vinyl chloride being a prominent example as a known human

carcinogen (Lee et al., 2002). Lower chlorinated benzenes display somewhat different toxicological characteristics, causing both central nervous depression and chronic liver and kidney toxicity, but showing no considerable mutagenic and carcinogenic properties.

Physico-chemical properties effectively govern environmental fate and behaviour of compounds in the environment. As evident from their Henry's law coefficients, chlorinated VOC have a tendency to be transferred from the aqueous to the gas phase easily, and thus show considerable potential to reach the atmosphere (US EPA, 1993). In water treatment applications, the transfer to the atmosphere can be an important removal pathway. Persistence in the atmosphere is moderate, due to reaction with reactive atmospheric species such as hydroxyl radicals (US EPA, 1994; 1995). For MCB, atmospheric half life is reported to be between 20 h and 9 d (US EPA, 1995) and for PCE around 96 d (US EPA, 1994). Carbon tetrachloride is also both ozone-depleting and an atmospheric green-house gas (Evans and Puckrin, 1996; Fraser, 1997). Their considerable water solubility causes the chlorinated VOC's hazardous potential for aqueous environments in general, and leads to a good mobility in soil and water (Koch, 1995). In aquifer environments, it gives rise to widespread groundwater contaminations and the formation of long groundwater plumes (Moran et al., 2007; Nijenhuis et al., 2007). Lipophilicity controls sorption to organic material and uptake into living organisms, bioavailability and biodegradability, and the persistence in soils and sediments. Uptake into higher plants potentially plays a significant role for compounds with a $\log K_{OW}$ between 0.5-3 (Trapp 1995; Trapp and Karlson, 2001). Sorption of chlorinated VOC to soils and sediments may lead to the formation of long-term contaminant sources (Ball and Roberts, 1991; US EPA, 1993). Sorption also diminishes bioavailability, and desorption resistant fractions may be formed, which are not or only slowly biodegraded (Lee et al., 2003). Nevertheless, the tendency of chlorinated VOC for bio- and geo-accumulation is, compared to larger and more highly chlorinated compounds (polycyclic aromatic hydrocarbons, polychlorinated dibenzodioxins and dibenzofurans), moderate (Koch, 1995).

1.1.2 Sources, dimensions and hazards of groundwater contamination

MCB, DCBs and PCE are among the chlorinated VOC produced in major amounts worldwide. In the 1980s, MCB production volume was reported to reach 102 000 t y⁻¹ in the US and 103 000 t y⁻¹ in Western Germany (Weissermel and Arpe, 1988), and US production of DCBs was estimated as around 50 000 t y⁻¹ (Koch, 1995). In 1985, world production of PCE was around 1 000 000 t y⁻¹ (Rossberg et al., 2002), but declined in recent years. In the mid-90s, ~ 160 000 t y⁻¹ were produced in the U.S. and 164 000 t y⁻¹ in the EU (WHO, 2006).

MCB is today mainly produced via direct chlorination of benzene in the liquid phase, in presence of lewis-acid catalysts like ferric chloride or anhydrous aluminium chloride. DCBs are formed as by-products in the process, with ratios depending on the reaction conditions (Gerhartz and Arpe, 1986). PCE is produced mainly by chlorinolysis of C₁-C₄ hydrocarbons or chlorinated hydrocarbon residues, a reaction series of radical crack and substitution reactions yielding PCE and carbon tetrachloride. Another important industrial process is the oxychlorination of ethylene or 1,2-dichloroethane with O₂ and Cl₂ in fluidized catalyst bed reactors leading to the production of PCE and trichloroethene (Gerhartz and Arpe, 1986) (Figure 1).

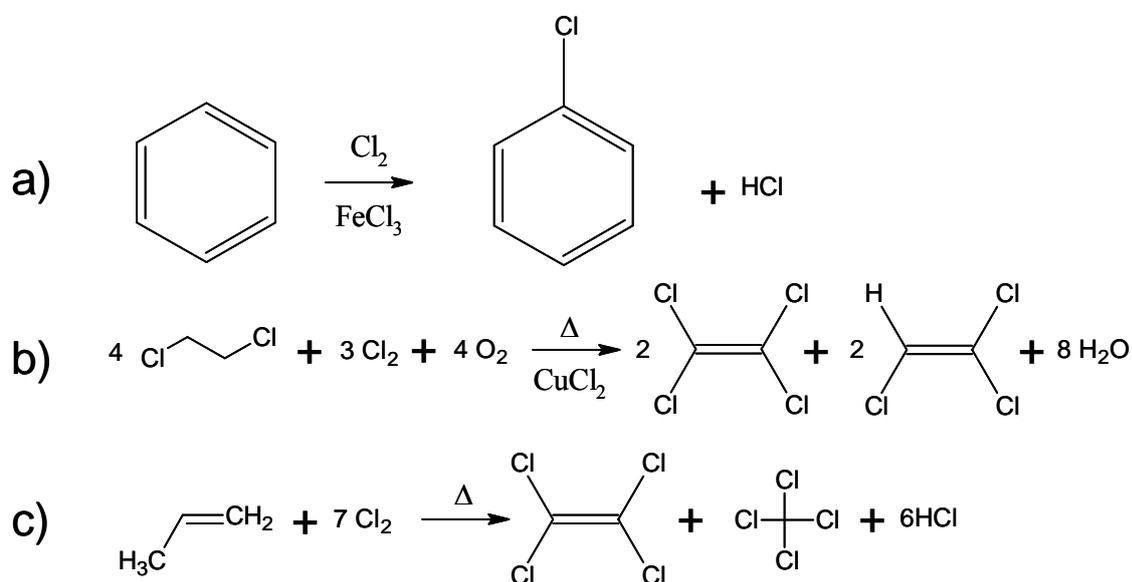


Figure 1: Processes for the industrial production of MCB via direct chlorination of benzene (a), and for the industrial production of PCE via oxychlorination of 1,2-dichloroethane (b) and via chlorinolysis of propylene (c).

Among the main environmental hazards emanating from chlorinated VOC in Europe and Northern America is the contamination of groundwater systems, due to their unique physico-chemical properties (Moran et al., 2007). Groundwater contaminations are in most cases associated to source areas such as industrial production facilities (e.g. Bitterfeld and Leuna, Germany), dry cleaners (Moran et al., 2007) and military sites (e.g. Aberdeen Proving Ground, MD, and other USEPA Superfund sites). Major sources of groundwater contaminations by chlorinated VOC are leakage of (underground) storage tanks, spills during production and application, and improper handling and disposal. Chlorinated VOC can also be formed in the groundwater *in situ*, via partial microbial degradation of higher chlorinated compounds (e.g. lindane) (Boyle et al., 1999; Moran et al., 2007; Nijenhuis et al., 2007). Total release of PCE into the environment in the US has been estimated as 1.81 Mio t between 1998 and 2001 (Moran et al., 2007). Worldwide emissions of chlorobenzenes are estimated to amount to 400 000 t y⁻¹ (Koch, 1995). Dimensions of groundwater contamination are extensive, considering the large amounts released and the tendency of chlorinated VOC to reach the groundwater and persist there. Total groundwater contamination at the Bitterfeld/Wolfen industrial megasite in Germany, where the field experiments for this study have been carried out, is estimated to affect an area of 25 km² and a volume of 200 million m³ (Weiß et al., 2001).

Severe hazards for human health and the environment are associated with groundwater contaminations by chlorinated VOC. On the one hand, groundwater is an important drinking water resource (Squillace et al., 2002). The dependency on clean groundwater as a resource might even increase in the future, due to increase of draughts as a result of climate change (IPPC, 2007), and growing population. In addition, contaminated groundwater is often not completely segregated from the above-ground environment. It may be in contact with, or discharge into, surface waters and river flood plains, endangering sensitive ecosystems (Lorah and Olsen, 1999; Bankston et al., 2002; Pardue, 2002; Heidrich et al., 2004; Kalbus et al., 2006; Tawney et al., 2008).

1.1.3 Biodegradation of chlorinated VOC

Biological degradation is the main basis for any biological water treatment technology. Thus, it is of great importance to consider preconditions, requirements and efficiency of

chlorinated VOC metabolism. Chlorinated VOC form a group of compounds with similar physico-chemical properties and environmental behaviour, but have divergent biological degradation potentials, resulting in a high complexity of their biological treatment. In the biological metabolism of chlorinated compounds, chlorine atoms are enzymatically removed to form non-chlorinated intermediates, which can enter into metabolic pathways (Field and Sierra-Alvarez, 2004). Aerobic and anaerobic oxidation, anaerobic reductive dechlorination (dehalorespiration), and both oxidative and reductive cometabolism, are the most relevant microbial degradation processes for chlorinated VOC. In some cases, also plant metabolism leads to chlorinated VOC degradation. A problem complicating remediation of chlorinated VOC contaminated groundwater is the common occurrence of mixed contaminations (Moran et al., 2007). Oxidative and reductive microbial degradation steps are therefore often required to efficiently eliminate chlorinated VOC contaminations. Furthermore, detoxification of highly chlorinated compounds such as PCE requires a series of degradation steps, with divergent efficiencies according to the prevailing degradation conditions.

In microbial oxidation processes, microorganisms use chlorinated solvents as electron donor and carbon source to support growth, or they fortuitously (cometabolically) transform them while degrading other substrates, a process known as cooxidation (Field and Sierra-Alvarez, 2004). Oxygen is used as the most energetically favourable electron acceptor in aerobic oxidation, but also alternative electron acceptors like nitrate, ferric iron, sulphate and carbon dioxide can be used if oxygen is lacking (Schlegel, 1992).

The potential for oxidative microbial breakdown of chlorinated VOC decreases with increasing number of chlorine substituents. Highly chlorinated VOC like carbon tetrachloride, penta- and hexachloroethane, and PCE are not bio-oxidizable (Field and Sierra-Alvarez, 2004). Microorganisms from different genera have been shown to aerobically oxidize MCB and all DCB isomers as sole carbon and energy source (van Agteren et al., 1998). Prominent among them are several different *Pseudomonas* and *Burkholderia* strains (Field and Sierra-Alvarez, 2008), being ubiquitous in soil, water and groundwater environments. The dioxygenase-catalysed degradation pathway via chlorocatechol intermediates is generally accepted as the main microbial oxidation process (Reineke and Knackmuss, 1984; Schraa et al., 1986; Spain and Nishino, 1987; Haigler et al., 1988; Mars et al., 1997) (Figure 2). Evidence has been found for

anaerobic oxidation of chlorinated benzenes coupled to denitrification and ferric iron reduction, but could to date not be proven (Dermietzel and Vieth, 1999; Vogt et al., 2002; 2004). Oxidation of chlorinated methanes, ethanes and ethenes typically proceeds via monooxygenase catalysed insertion of one oxygen atom, often forming instable intermediates spontaneously converting to aldehydes or organic acids (Field and Sierra-Alvarez, 2004). Aerobic oxidation coupled to growth has been shown to occur for chloromethane (Hartmans et al., 1986), dichloromethane (Leisinger et al., 1994; Doronina et al., 1998), 1,2-dichloroethane (Stucki et al., 1992), cis-DCE (Coleman et al., 2002a) and vinyl chloride (Coleman et al., 2002b; Aulenta et al., 2003). Anaerobic oxidation of chloromethane and dichloromethane by anaerobic acetogens, methanogens and denitrifiers (only dichloromethane) was also observed (Traunecker et al., 1991; Magli et al., 1996; De Best et al., 2000). Cis-DCE and VC were oxidized in river and aquifer sediments under different terminal electron accepting conditions, including methanogenic, iron- and manganese reducing conditions (Bradley and Chapelle, 1997; Bradley et al., 1998).

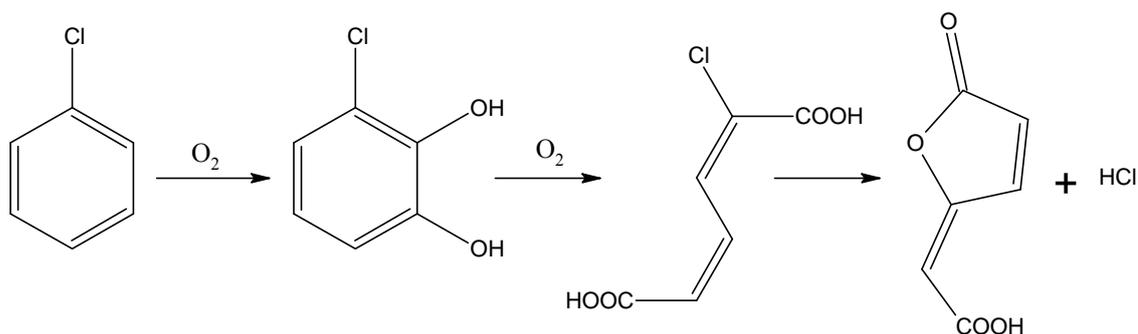


Figure 2: Dioxygenase catalysed aerobic microbial degradation of MCB via a chlorocatechol intermediate and ortho ring cleavage (modified from van Agteren et al., 1998).

Aerobic cooxidation is a widespread process with great importance for bioremediation. MCB can be cooxidized by methanotrophs (Halden, 1991; Jechorek, 2003), and *Pseudomonas* strains are able to cometabolize DCBs with benzene or MCB as a growth substrate (Ballschmitter and Scholz, 1981; Haigler et al., 1992; Brunsbach and Reineke, 1994). Chloromethane, dichloromethane, chloroform, chloroethane, and all di-, tri and tetrachloroethane isomers are cooxidized by monooxygenases of aerobic microorganisms, mainly with methane, butane or ammonia as primary substrates (Vanelli et al., 1990; Rasche et al., 1990; 1991; Alvarez-Cohen and McCarty, 1991; Bartnicki and Castro, 1994; Ely et al., 1997; Kim et al., 2000). Also TCE, DCEs and

VC are prone to monooxygenase (and some dioxygenase) catalysed cooxidation processes by a large number of microbial species (Janssens et al., 1988; Aziz et al., 1999; Alvarez-Cohen and Speitel Jr., 2001; Coleman et al., 2002a), usually involving a high degree of mineralization (Field and Sierra-Alvarez, 2004). Most common growth substrates are methane, ethane, ethene, propane, propene, ammonia, toluene and phenol. A group of methanotrophs cooxidizing TCE with methane monooxygenase, e.g. *Methylosinus trichosporium*, has been extensively studied (Field and Sierra-Alvarez, 2004). Only one study states the slow cooxidation of PCE by toluene-o-xylene monooxygenase (Ryoo et al., 2000).

In reductive microbial metabolism of chlorinated VOC, these serve as electron acceptors in the oxidation of organic substrates under anaerobic conditions, a process known as halorespiration (McCarty, 1993). It is of great importance in anaerobic groundwater environments, and leads to the degradation of chlorinated compounds that are not susceptible to oxidative transformation. Reductive hydrogenolysis is the most common mechanism, where one electron and one hydrogen atom are transferred onto the carbon atom and HCl is released (Holliger et al., 2003). The most important dehalorespirers are *Desulfitobacterium*, *Desulfuromonas*, *Dehalobacter* and *Dehalococcoides sp.*, with the former two using a range of organic substrates such as lactate, pyruvate or acetate as electron donors, and the latter two using only hydrogen (Field and Sierra-Alvarez, 2004). All dichlorobenzene isomers can be dechlorinated reductively (Bosma et al., 1988; Ramanand et al., 1993; Adrian and Görisch, 2002). Repeated evidence was found also for anaerobic conversion of MCB to benzene (Nowak et al., 1996, Kaschl et al., 2005), but it is to date unclear if the observed processes were metabolic or cometabolic, and conversion rates were very low (Adrian and Görisch, 2002). Different dehalorespirers have been shown to be capable of reducing chlorinated ethanes, namely 1,2-dichloroethane (*Dehalococcoides sp.*, *Desulfitobacterium sp.*), 1,1,1-trichloroethane (*Dehalobacter sp.*) and tetra-, penta- and hexachloroethane (PCE dechlorinating *Desulfitobacterium sp.*) (Maymo-Gatell et al., 1999; De Wildeman et al., 2001; Suyama et al., 2001; Sun et al., 2002). Only one reductive dehalogenase has been isolated that shows activity with lower chlorinated ethenes, which is produced only by *Dehalococcoides sp.* They can utilize all three DCE isomers and VC as sole electron acceptors, converting them to ethene (Maymo-Gatell et

al., 1999; Cupples et al., 2003; He et al., 2003) (Figure 3). In contrast, bacteria from several phylogenetic groups are capable to reduce PCE to TCE, and TCE to DCE with cis-DCE being the most commonly found daughter product (Neumann et al., 1995; Schumacher et al., 1997; Magnuson et al., 1998; Sung et al., 2003; Yoshida et al., 2007; Huang and Becker, 2009).

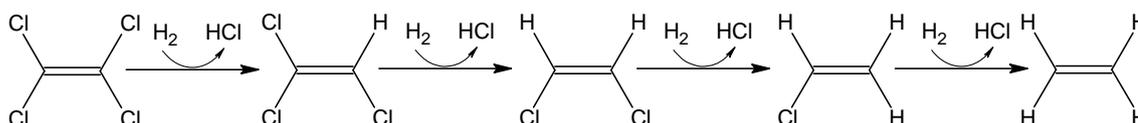


Figure 3: Complete reductive dechlorination of PCE to ethene.

Reductive cometabolism under anaerobic conditions has been found for chloroform, carbon tetrachloride and all chlorinated ethanes, mainly by methanogenic and acetogenic mixed cultures, and by sulphate and iron reducers (Egli et al., 1987; 1988; Picardal et al., 1993; Chen et al., 1996; van Eekert et al., 1999). Lower chlorinated ethenes can either be cometabolized during halorespiration (Distefano et al., 1999; Haston and McCarty, 1999) or during fortuitous reduction by other common anaerobes (Belay and Daniels, 1987; van Eekert et al., 2001). VC is cometabolically reduced by *Dehalococcoides ethenogenes* 195 during reduction of PCE (Maymo-Gatell et al., 2001). Slow conversion of PCE and TCE by methanogens and acetogenes was also observed, with methanol or H₂ as the electron donor (Egli et al., 1988; Fathepure and Boyd, 1988; Cabriol et al., 1998).

Also plants produce enzymes that degrade chlorinated VOC (Newman and Reynolds, 2004). The metabolism of TCE and carbon tetrachloride in poplar and cattail is well-known (Newman et al., 1997; 1999; Bankston et al., 2002; Wang et al., 2004), potentially contributing significantly to contaminant elimination in wetland environments (Shang et al., 2001). Degradation is usually oxidative, with intermediates such as trichloroethanol and trichloroacetic acid. As contaminants have to be taken up before they can be metabolized by plants, their lipophilicity plays a crucial role (Ryan et al., 1988).

1.1.4 Remediation options for groundwater contaminated by chlorinated VOC

In conventional pump-and-treat technologies, groundwater is pumped out of the aquifer and usually treated in above-ground reactors (Shevah and Waldman, 1995). These

methods are expensive and energy demanding, due to the pumping of large water volumes for long periods of time (Travis and Doty, 1990; Weiß et al., 2001). *In situ* methods include reactive barriers, *in situ* reactors (funnel & gate) and strategically placed injection and extraction wells, with the advantage of avoiding long-term pumping, but still involving high investment costs (Starr and Cherry, 1994; Shevah and Waldman, 1995; McGovern et al., 2002; Birke et al., 2003). A great number of physical and chemical methods are applicable for clean-up of chlorinated VOC contaminated groundwater. Prominent examples of physical treatment methods are adsorption on activated carbon (You and Chou, 2000) and air stripping of volatile contaminants (Gavaskar et al., 1995). Chemical contaminant break-up can be accomplished via chemical oxidation, using ozone or H₂O₂ and UV radiation (Bellamy et al., 1991; Gates and Siegrist, 1995; Weir et al., 1996) or O₂ and oxidation catalysts (Gavaskar et al., 1995), and via chemical reduction with zero-valent iron (reactive walls) (Phillips et al., 2010) or Pd metal catalysts (Kopinke et al., 2003; Hildebrand et al., 2009). Biological treatment methods make use of the degradation potential of microorganisms and plants. Biological degradation has been observed for most known xenobiotic compounds (Zhang and Bennett, 2005; Sinha et al., 2009). Advantages of biological treatment are mainly lower investment and maintenance costs and energy demands, compared to chemical and physical treatment methods (Sinha et al., 2009). Bioremediation of chlorinated VOC contaminated groundwater can be carried out *in situ* by biostimulation and bioaugmentation (Shevah and Waldman, 1995) as well as in bioreactors (Vogt et al., 2002) or constructed wetlands (CW), which will be described in detail in chapter 1.2.

At large-scale contaminated sites (industrial megasites, USEPA Superfund sites), a total remediation of contaminated groundwater is often technically and financially not feasible (Travis and Doty, 1990). Nevertheless, risk mitigation might in many cases be necessary, if valuable ecosystems or human health are endangered (Wycisk et al., 2003). Mere natural attenuation, relying on the self-cleanup potential of nature, is not always sufficient to avert dangers emanating from chlorinated VOC contaminated groundwater.

CW are a promising, nature orientated, energy and cost saving technology for biological treatment of chlorinated VOC contaminated groundwater. Indications for advantageous

application of CW for groundwater treatment are the presence of near surface groundwater, groundwater-surface water interfaces or seepage sites, such as natural discharge into river floodplains. In these cases, no groundwater pumping would be necessary, rendering the technology financially interesting. Realized in this way, CW qualify for a quasi *in situ* method of groundwater treatment. Another option is the use of parts of existing wetlands as engineered treatment zones to reduce impact of contaminated groundwater on the whole ecosystem.

1.2 CW treatment of chlorinated VOC

1.2.1 Technical and engineering aspects

CW mainly consist of helophyte vegetation growing either in a filter with a solid matrix, or in hydroponic setups without a matrix. Helophytes are wetland plant species adapted to growth in permanently waterlogged soils under reducing conditions. Reed (*Phragmites*), cattail (*Typha*), and rush (*Juncus*) species are mainly used in CW in the temperate climate zone (Kadlec and Wallace, 2008). Also trees which are adapted to waterlogged conditions are sometimes used in wetland applications, mainly poplar and willow species (Newman et al., 1999; Wang et al., 2004; Kuzovkina and Quigley, 2005). A feature of the helophyte vegetation with great importance for the water treatment process is the oxygen release into the rhizosphere, a process protecting the roots against reducing conditions and toxic compounds, and enabling root respiration (Brix, 1997; Stottmeister et al., 2003). Above-ground plant parts and roots are connected with an air channel system, the aerenchyma, allowing gas exchange between the atmosphere and rhizosphere. Gas flow is mainly diffusive and/or convective, driven by internal pressurization which is induced by thermoosmosis, humidity and by wind in the case of broken culms (Venturi effect) (Armstrong et al., 1996; Große et al., 1996; Große and Frick, 1999, Beckett et al., 2001). There is great variability found in the literature as to how much oxygen is actually released by helophytes, for example ranging between 0.02 and 12 g m⁻² day⁻¹ for *Phragmites* (Armstrong et al., 1990; Brix, 1990; 1997). As oxygen transported into the rhizosphere is not completely consumed by root respiration, a part is usually available for microbial processes (Sherwood et al., 1995). In addition, plants release carbon compounds into their rhizosphere (rhizodeposition), composed of root exudates and dead plant materials. Root exudates

are excreted by the plant to mobilize nutrients and to inhibit growth of competitors and pathogens (Brix, 1997), and consist mainly of organic acids, sugars and amino acids. Other impacts of plants on CW treatment include increasing the detention and contact time and soil hydraulic conductivity, providing a large surface area for microbial attachment and growth, and taking up nutrients (Brix, 1997). Plant uptake of chlorinated VOC, followed by metabolization or phytovolatilization (plant mediated release to the atmosphere), may play a role in certain CW applications. This is discussed in more detail in the Review article provided in Appendix A.1.

The major parameters for the choice of filter material for CW are the grain size distribution, pore volume and the hydraulic conductivity (k_f value) determining the flow characteristics in the filter (Kadlec and Wallace, 2008). Hydraulic conductivity should be chosen in a range where clogging is avoided as much as possible, and sufficient contact time between water and the biologically active parts of the system is guaranteed at the same time. A large surface area is beneficial to enable attachment and retention of microbial cells (Kadlec and Wallace, 2008). In modern CW engineering, mostly mixtures of sand and gravel with a grain size distribution of 0.06-10 mm are used, to achieve a k_f value greater than 10^{-5} m s^{-1} (Stottmeister et al., 2003). The filter material also affects sorption of contaminants. If mineral filter materials are used, sorption of chlorinated VOC increases contact time, but is reversible and not a permanent sink once a sorption equilibrium has developed. Sorption may have a greater impact if the filter material contains considerable amounts of organic carbon (Kassenga et al., 2003).

As another important precondition, climate has to be considered when planning CW, as it effects the seasonality of treatment performance. The major chlorinated VOC groundwater contaminations are found in the temperate climate zone (Europe and Northern America) and thus the present thesis focuses on temperate climate conditions.

There are several common CW types and operation modes, both with and without a filter body. Free water surface wetlands (FWS) possess areas of open water, such as hydroponics and ponds, with floating, emergent and/or submerged vegetation. These systems are fairly aerobic due to the direct contact between water and atmosphere (Kadlec and Wallace, 2008). They are expected to favour gaseous emissions in treatment of chlorinated VOC, which is an important disadvantage if immission

problems are likely to arise. The most commonly used CW types with a filter bed are horizontal subsurface flow (HSSF) and vertical flow (VF) systems. HSSF systems typically employ a gravel bed planted with helophytes, with water flowing horizontally below the surface from inlet to outlet (Figure 4). HSSF systems are technically simple and economically attractive due to their low maintenance costs. If a slope can be utilized, no pump is necessary, affecting a low energy demand, but filter volume utilization tends to be suboptimal, causing higher area demands (Bahlo et al., 1992). HSSF filters are oxygen limited and therefore not too well suited for nitrification, but have great advantages in applications where aerobic and anaerobic reaction zones are desired (Kadlec and Wallace, 2008; Faulwetter et al., 2009). Gaseous emissions are minimized by the vadose zone, but can still be considerable due to plant mediated phytovolatilization.

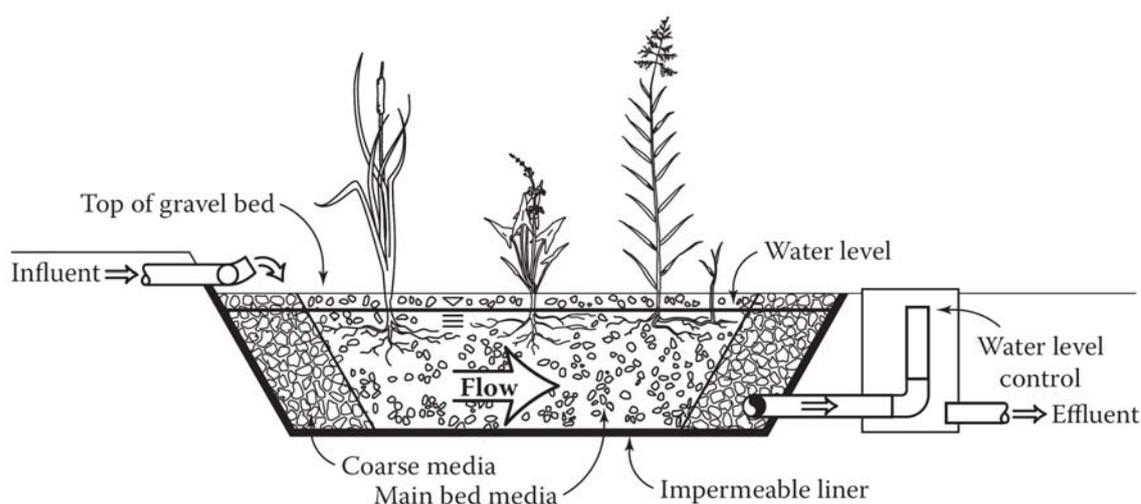


Figure 4: Typical operation scheme of a HSSF CW (from Kadlec and Wallace, 2008).

VF filter can be operated in downflow (percolating) or upflow mode, with the former technology being widespread in the treatment of domestic wastewater. The water is distributed across the surface of the filter bed either continuously or intermittently. These systems usually rely on pumping, but area demands are lower than for HSSF systems. VF filters are known to provide high oxygen input, and are efficient for nitrification and BOD removal, but not for denitrification (Bahlo et al., 1992; Cooper et al., 1996; Kadlec and Wallace, 2008). Upflow VF wetlands are interesting for groundwater treatment, in cases where natural infiltration or seepage at the bottom of a wetland can be utilized (Lorah and Olsen, 1999) and have been used to minimize

oxygen input and allow for reductive dechlorination of chlorinated solvents (Kassenga et al., 2004).

Operation parameters have to be considered according to the specific treatment task, which is mainly characterized by the contaminant or contaminant mixture and the actual groundwater concentrations and flow, as well as the target outflow concentrations (Kadlec and Wallace, 2008). The operation mode (type), area and volume of the CW, the hydraulic loading rate ($\text{L m}^{-2} \text{ day}^{-1}$) and retention time τ (days) have to be specified accordingly (Kadlec and Wallace, 2008). For CW treatment of domestic wastewater, which has been extensively studied since the 1950s (Vymazal, 2005), required areas for certain BOD loads are well known. Commonly suggested scaling factors are 5-8 g BOD $\text{m}^{-2} \text{ day}^{-1}$, to achieve outflow concentration of less than 30 mg BOD L^{-1} , or 5 m^2 per person equivalent of domestic wastewater (Kadlec and Wallace, 2008). This might be not directly applicable for CW treatment of chlorinated VOC, for which experimental data are still scarce. The divergent physical and chemical requirements for the microbial degradation of VOC (oxidative or reductive) have also not been in the focus of previous investigations.

1.2.2 Metabolic potential of CW and chlorinated VOC removal

Wetlands are very productive and biologically active ecosystems. The purpose of CW applications is to make use of the unique characteristics of the wetland root zone, as a biological reactor with an extremely high metabolic potential, for water clean-up. Many plants, and also helophytes, exert a so called rhizospheric effect on microorganisms living in their root zone. This effect includes a number of plant influences effectively enhancing growth, activity and diversity of microorganisms in the rhizosphere compared to non-rooted soils (Cunnigham et al., 1996; Banks et al., 2003a,b; McCutcheon and Schnoor, 2003). To name the most prominent effects, microbial growth and activity is stimulated by carbon exudation, providing growth and energy substrates (Jones et al., 2004), and also electron donors for reductive microbial contaminant degradation (Moormann et al., 2002; Stottmeister et al., 2003). Root exudation may also stimulate cometabolic transformation of contaminants (Donnelly and Fletcher, 1994; Horswell et al., 1997; Moormann et al., 2002). Aerobic microorganisms profit from root oxygen release. Not all the complex mutual

interactions in these plant-microbe systems have been fully understood so far. A HSSF CW system is dominated by complex spatial and temporal gradients, both at the macro (system) and at the micro (rhizosphere) scale. Dynamic oxic-anoxic interfaces are established throughout the system as a result of water table fluctuations, oxygen diffusion through the vadose and saturated zone, and active plant oxygen transport (Weishaar et al., 2009). Spatial and temporal variability of hydrological and geochemical conditions results in shifting redox potentials, and cyclic reduction and re-oxidation of alternative electron acceptors such as Fe^{3+} , NO_3^- and SO_4^{2-} . At the rhizosphere scale, gradients of oxygen, mineral nutrients, pH, redox potential, organic carbon from rhizodeposition, and microbial activities occur both in a radial and longitudinal axis along individual roots.

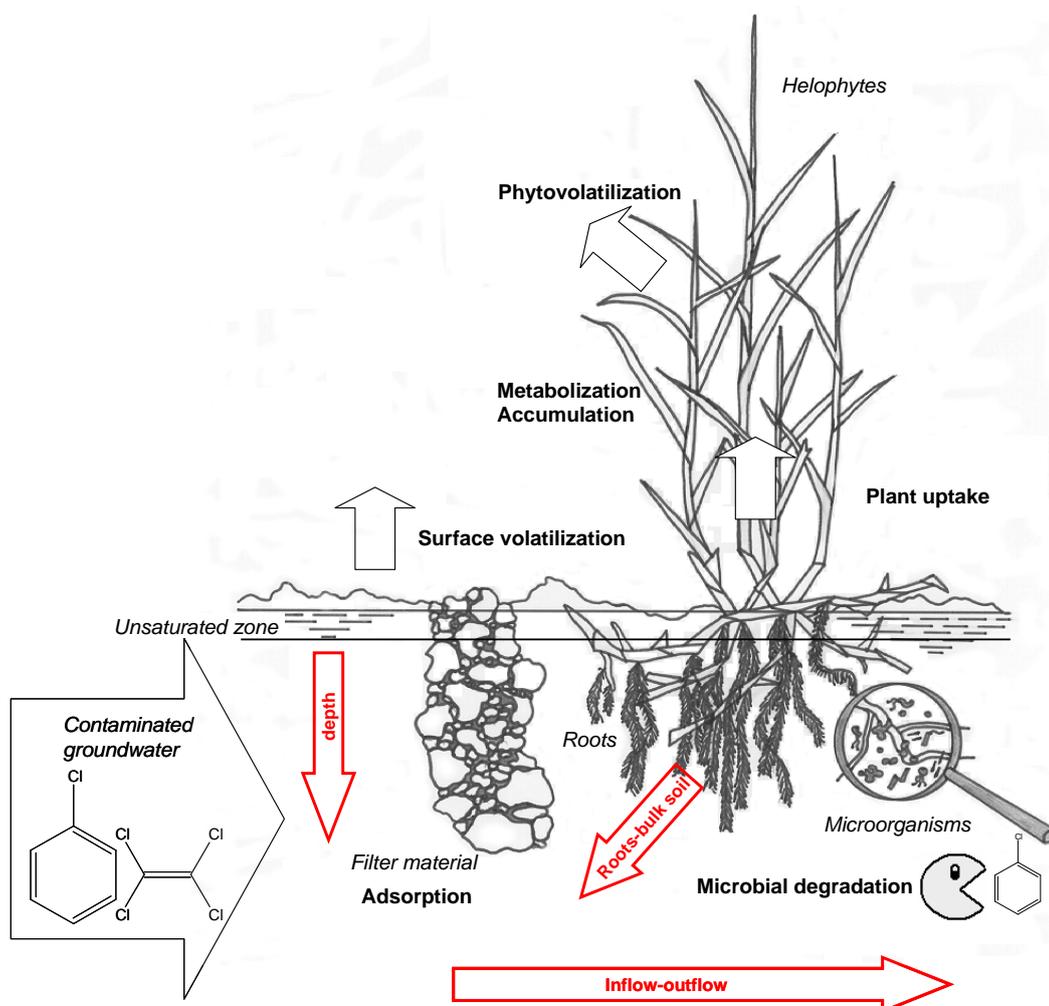


Figure 5: HSSF CW treatment of chlorinated VOC. CW components (*italics*); distribution and elimination processes (**bold**); main gradients (*red arrows*).

The simultaneous release of oxygen and organic carbon results in aerobic to anaerobic as well as redox gradients (Figure 5) and the development of a so called mosaic structure (Liesack et al., 2000; Wei et al., 2003).

Due to the critical role of electron donor and acceptor availability for contaminant degradation, these processes effectively govern organic contaminant turnover in CW systems. In this gradient-dominated system, many different microbial processes with divergent requirements, and carried out by different, often mutually interdependent microbial groups, take place simultaneously (Liesack et al., 2000; Wiessner et al., 2005). This also comprises important advantages for treatment of chlorinated VOC, particularly in the case of mixed contaminations of reductively and oxidatively degradable compounds (such as the model compounds MCB and PCE). In addition, some chlorinated VOC have to undergo a series of degradation steps for mineralization, where some steps are more efficient under aerobic and others under anaerobic conditions. For example, PCE and TCE are efficiently dechlorinated reductively, but for the lower chlorinated daughter compounds, aerobic transformation is generally faster and more efficient (Amon et al., 2007). Furthermore, reductive dechlorination of DCE and VC requires the presence and activity of *Dehalococcoides* species (Maymó-Gatell et al., 1999), which is not always given.

The fate of chlorinated VOC in CW systems is governed by both their physico-chemical and biological properties, and the inherent characteristics of the wetland system. To summarize, expected relevant removal processes for chlorinated VOC include oxidative and reductive microbial degradation, soil borne and plant mediated volatilization, and to a certain extent plant uptake with subsequent metabolization (see Appendix A.1) (Figure 5). Sorption and phytoaccumulation probably do not play an important role for the removal of chlorinated VOC. The specific system and operation conditions are anticipated to also determine the contribution of the specified processes to overall contaminant removal (Faulwetter et al., 2009). HSSF CW systems may provide a promising approach for the treatment of chlorinated VOC. Primarily, their characteristics as gradient dominated systems make HSSF filters more suitable for applications involving both oxidative and reductive processes than VF systems. FWS systems were expected to enhance volatilization, and therefore did not appear suitable for the treatment of volatiles. Furthermore, as a more simple and cost-efficient

alternative compared to VF systems, which usually require pumping, HSSF CW are more likely to be a realistic economical choice for groundwater remediation.

1.2.3 Current state of research

CW treatment of chlorinated VOC has attracted growing interest in recent years, mainly as a groundwater remediation technology. Removal of chlorinated VOC in natural wetlands and CW systems has been described in several studies, e.g. for carbon tetrachloride (Wang et al., 2004), chlorinated ethenes and ethanes (Lorah and Olsen, 1999; Mastin et al., 2001; Pardue, 2002; Lorah and Voytek, 2004; Tawney et al., 2008), and lower chlorinated benzenes (Jackson, 1999; Leppich, 1999; Lee et al., 2003). Researchers have pointed out the significance of microbial degradation (MacLeod, 1999; Kassenga et al., 2004; Amon et al., 2007) as well as the potential relevance of volatilization (MacLeod, 1999; Bankston et al., 2002; Ma and Burken, 2003; Keefe et al., 2004) of chlorinated VOC in CW applications. A detailed literature overview is given in the Review article provided in Appendix A.1, presenting experience in the field and laboratory, efficiency data and the state of knowledge concerning removal processes.

1.2.4 Challenges and engineering potentials

The general challenge associated with remediation of chlorinated VOC contaminated aquifers is to implement cheap and simple, but efficient treatment technologies. Only methods meeting these requirements stand a chance to be applied in real scale, due to political and economic constraints. Circumstances like the associated risk, availability of clean (drinking) water, useable areas for residential and commercial development, and the value ascribed to natural ecosystems, play a major role in the decision-making process concerning clean-up of contaminated groundwater (Wang and McTernan, 2002; Li et al., 2004). Comparing e.g. the situation in Germany to that in Denmark, groundwater remediation is tackled with much higher efforts in Denmark, probably due to the high value of areas for development, leading to cost effectiveness of groundwater clean-up.

Thus, before the technology can be implemented, detailed and established knowledge on removal efficiencies, ancillary conditions and associated advantages and

disadvantages has to be gathered, to ensure economic as well as ecological benefits. The treatment of chlorinated VOC contaminated waters in CW is not sufficiently studied to date, leaving a considerable gap of knowledge. Especially large scale applications are scarce to date. Findings are often not transferable due to the non-systematic application of various system types and operation conditions, or lack of insight into actual system conditions. The commonly applied black box approach to CW application does not always lead to satisfactory results, and reliable, transferable efficiency data, as a basis for design, are missing to date for chlorinated VOC removal. Potential removal pathways for chlorinated VOC in CW should be considered, and their relevance evaluated as far as possible. Like this, undesirable processes can be suppressed by suitable design decisions. Thus, additional knowledge and experience is needed concerning the role of different removal processes for the fate of chlorinated VOC.

On the basis of qualified knowledge, it is possible to attempt optimization of chlorinated VOC treatment by technological and operational adaptations. Potential possibilities for technical optimization of chlorinated VOC treatment in wetland systems should be economically justifiable, and benefits should outweigh additional costs. One potential option is the addition of subsidiary or stimulating compounds to the system (biostimulation), such as electron donors or acceptors, nutrients and carbon substrates, to create beneficial conditions for microbial degradation, and enable desired removal pathways. Biostimulation has been adopted for removal of organic contaminants from natural wetland systems (Mills et al., 2004; Garcia-Blanco et al., 2007), and for *in situ* groundwater remediation of chlorinated solvents (Lendvay et al., 2003; Hirschorn et al., 2007), but so far not to enhance chlorinated VOC removal in wetlands. If it is intended to stimulate aerobic processes, enhancement of oxygen input is an interesting approach (Faulwetter et al., 2009). In HSSF systems, this can be achieved by simulated tidal loading / fill-and-drain operation modes (Vymazal and Maza, 2003). Conventional aeration may harbour the risk of stripping out volatile compounds, leading to higher emissions to the atmosphere, and would also be connected to higher energy demand. Other potential optimization options include the adaptation of filter depth, water level, retention time, and other operation parameters. The introduction of suitable degrading microorganisms, the so called bioaugmentation, has been attempted mostly in *in situ*

groundwater remediation (Major et al., 2002, Lendvay et al., 2003), but there are also studies dealing with applications in phytoremediation (Barac et al., 2004).

Furthermore, the simultaneous treatment of highly and lowly chlorinated (oxidizable and reducible) VOC, though expected to be of great importance in real scale applications, has not been studied in detail, deterministically and target-oriented to date. Another crucial point for the implementation of CW treatment systems is the temporal evolution of system adaptation and the development of system performance, which will be a major focus of the present thesis. This is especially relevant for chlorinated VOC treatment, as these xenobiotic organic compounds are often not efficiently degraded by widespread environmental bacteria, and system adaptation may take much longer than for e.g. domestic or agricultural wastewaters.

The influence of climate conditions on CW treatment of chlorinated VOC, and the seasonality of removal efficiency are of great importance for applications in the temperate climate zone, where the majority of chlorinated VOC groundwater contaminations are found. They form the critical basis for assessment of true (yearly mean) system performance. A conclusive evaluation of whether and to what extent seasonality plays a role for CW treatment of this compound class has not been provided so far.

1.3 Resulting tasks and outline of the thesis

In the present thesis, CW treatment of model chlorinated VOC (MCB and PCE) has been studied under conditions of the temperate climate zone, both in HSSF systems and in rhizosphere model reactors. HSSF systems have been chosen for several reasons: their characteristics as gradient dominated systems make HSSF filters suitable for supporting both oxidative and reductive processes, and they are considered to involve only a low volatilization potential. Resulting from the gaps of knowledge described in the previous chapter, the main aims of the thesis were to:

- 1.) investigate the seasonality of chlorinated VOC removal in HSSF CW under the conditions of the temperate climate zone;
- 2.) consider and elucidate the role and relevance of removal pathways, namely microbial degradation and gaseous emissions;

- 3.) determine potentials and constraints of simultaneous treatment of oxidizable and reducible chlorinated VOC in CW;
- 4.) investigate the potential of technical and engineering optimizations, especially biostimulation options (optimization of oxygen, carbon source and electron donor availability for different applications);
- 5.) provide reliable and transferable removal efficiency data, based on specific treatment conditions; and finally
- 6.) study the qualitative and temporal aspects of system adaptation to chlorinated VOC treatment.

The knowledge obtained is expected to contribute to evaluation of the emerging technology of CW treatment of chlorinated VOC, and provide necessary insight into contaminant removal processes, as a basis for optimization of the technology for different specific applications.

Following the theoretical background given in **Chapter 1**, and preceding the summary and concluding remarks (**Chapter 5**), this thesis on CW treatment of chlorinated VOC is divided into three chapters. **Chapter 2** describes and discusses results of a comprehensive pilot-scale field study on the treatment of MCB and DCBs contaminated groundwater in HSSF CW, carried out at the Bitterfeld field site (for pictures of the system see **Appendix A.2**). In **Chapter 2.1**, European summer time removal of MCB, 1,2-DCB and 1,4-DCB is presented, and spatial concentration dynamics of pollutants, oxygen and ferrous iron, redox potential, and the influence of vegetation are discussed to evaluate the processes involved. **Chapter 2.2** deals with the seasonality and long-term efficiency of MCB and DCBs treatment, and the contribution of different processes to MCB removal performance in a theoretical modelling approach. The role of plants for oxygen input and contaminant uptake, and the role of VOC emissions are in the focus of the study. The assessment of microbial degradation of MCB is in the focus of **Chapter 2.3**, presenting a combined approach to tackle *in situ* biodegradation of the contaminant. Natural attenuation and main processes controlling the wetland system were evaluated, based on a detailed chemical characterization explored with the help of multivariate statistics, stable isotope composition analysis, and stable isotope

tracer experiments. In **Chapter 3**, simultaneous treatment of MCB and PCE in the pilot-scale is presented. System adaptation was investigated over the course of 2.5 years, and removal efficiency for MCB in the presence of PCE was determined. Particular focus was laid on the development of PCE degradation and metabolite patterns, the potential to achieve complete dechlorination to the non-toxic end product ethene, and the time period necessary to obtain steady state system performance. Finally, **Chapter 4** explores technical optimization approaches to MCB and PCE treatment in CW, carried out in rhizosphere model reactors (for an operation scheme and picture of the reactors, see **Appendix A.3**). The impact of different treatment conditions (oxygen surplus, sulphate surplus, and different organic carbon loads) on removal efficiency was investigated, with the aim of providing a range of removal performance data transferable to real scale applications.

The review article provided in **Appendix A.1** gives a broad overview of recent progress in organic chemicals treatment in CW, embedding the thesis into the current state of the art of the emerging research field. An exhaustive literature survey is presented on treatment of chlorinated VOC and other important contaminant groups. The main degradation pathways are summarized, and key characteristics of CW controlling the removal of organic chemicals are examined. Furthermore, approaches and techniques to follow up removal processes are addressed.

2 Pilot-scale study on the treatment of chlorobenzenes in constructed wetlands

2.1 Treatment of chlorobenzene contaminated groundwater in a pilot-scale constructed wetland

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Abstract

At industrial megasites worldwide, the natural seepage of contaminated groundwater from surface-near layers into rivers and river floodplains may give rise to serious environmental hazards. To prevent adverse effects on the environment, affordable and energy-efficient treatment methods for these sites are required. Large-scale constructed wetlands (CW) may represent a promising approach to protect river catchment areas. A pilot-scale horizontal subsurface flow CW planted with *Phragmites australis* and an unplanted reference plot were investigated for the removal of monochlorobenzene (MCB), 1,4-dichlorobenzene (1,4-DCB) and 1,2-dichlorobenzene (1,2-DCB) from polluted groundwater in Bitterfeld (Germany). At inflow concentrations of 20 mg L⁻¹ MCB, 0.19 mg L⁻¹ 1,2-DCB, and 0.25 mg L⁻¹ 1,4-DCB, the planted system showed high removals of MCB and 1,4-DCB already after 2 m of the flow path with averages of 48% MCB and 38% 1,4-DCB over all depths (71% MCB and 62% 1,4-DCB in the upper layer of the wetland). The unplanted wetland showed its highest mean removals only after 4 m of the flow path with 35% MCB and 32% 1,4-DCB. 1,2-DCB was not removed in either system. Oxygen limitation prevailed in both systems but concentrations were higher in the planted wetland with redox potentials of 65 to 90 mV (0 to 65 mV in the unplanted wetland). The presence and activity of aerobic MCB degraders in the wetland was shown via most probable number (MPN) determination and aerobic microcosm experiments. Since plants have a significant effect on

chlorobenzene removal, CW provide a high potential for the treatment of chlorobenzene-contaminated groundwater.

Keywords: Constructed wetlands, Groundwater remediation, *Phragmites australis*, monochlorobenzene, dichlorobenzene

Introduction

Groundwater contaminations caused by chlorinated hydrocarbons are a global hazard particularly at industrial megasites. The Bitterfeld area in the middle-eastern part of Germany was characterized by extensive activities of the chemical industry and open-cast lignite mining for more than one hundred years. This led to changes in the groundwater system and groundwater contamination affecting an area of 25 km² and a volume of about 200 million m³ with the main contaminants being chlorinated aliphatic and aromatic hydrocarbons (Weiß et al., 2001). During recent years, groundwater flow conditions changed and the groundwater table rose due to the abandonment of mining water management. This development is expected to lead to the mobilization of contaminant plumes and in the mid-term to the infiltration of contaminated groundwater from surface-near layers into the floodplains of the nearby river Mulde, a tributary of the river Elbe. The situation is exemplary as industrial megasites worldwide are often located in the vicinity of rivers and surface-near groundwater from contaminated aquifers may thus enter the river floodplains. Large-scale constructed wetlands (CW) represent a promising approach to protect river catchment areas against the impact of contaminated groundwater and to reduce environmental damage.

There are strong indications from several investigations that the interface between anaerobic groundwater and surface water can play an important role in the natural attenuation of organic contaminants (Bradley and Chapelle, 1998; Lendvay et al., 1998; Witt et al., 2002). Steep gradients of oxygen concentrations and other geochemical parameters greatly enhance the intrinsic biodegradation potential for the breakdown of many contaminants such as monochlorobenzene (MCB), dichloroethenes (DCEs) and vinyl chloride (VC). These substances are persistent or only slowly degraded under the anaerobic conditions usually found in aquifers (Lorah and Voytek, 2004) and are therefore widespread in the anaerobic aquifer at the large-scale contaminated site in Bitterfeld, Germany (Heidrich et al., 2004). They are dead-end products of the

anaerobic dechlorination of highly chlorinated benzenes and ethenes in anaerobic environments. A promising way of taking advantage of interface phenomena is to utilize surface-near anaerobic/aerobic mixing zones in CW. CW can be constructed on a large scale, integrated into the landscape, and may lead to an efficient removal of organic contaminants (Haberl et al., 2003; Kassenga et al., 2003 and 2004; Lorah et al., 2001; Lorah and Voytek, 2004).

During recent years, the phytoremediation of waters and soils contaminated by chlorinated hydrocarbons has moved into the focus of research, with most of the investigations being targeted on the removal of chlorinated volatile organic compounds (VOC), particularly ethenes and ethanes. Highly chlorinated compounds, e.g. perchloroethylene, require initial anaerobic microbial degradation steps, and thus the high removal efficiencies of more than 90% were found mainly under anaerobic soil conditions (Lorah and Voytek, 2004; Strand et al., 2005). However, under these conditions low-chlorinated compounds may accumulate if the process is not properly managed. Other authors showed the effectiveness of coupled anaerobic and aerobic systems (Kassenga et al., 2004; Richard et al., 2002) and the enhancing impact of plants on the removal of chlorinated VOC (Cho et al., 2005; Bankston et al., 2002; Wiltse et al., 1998).

In contrast to chlorinated ethanes and ethenes, only little is known about the relevant removal processes for chlorinated benzenes and the impact of the plants on removal efficiency in CW. Volatilization was found to be a dominant elimination process for monochlorobenzene (MCB) in laboratory microcosms planted with reed (MacLeod et al., 1999). However, MCB mineralization was enhanced by the presence of the plants and accounted for 27% of the added MCB in comparison to 16% in unplanted microcosms. Non-reversible sorption to wetland soils was shown to limit bioavailability and mineralization particularly of dichlorobenzenes (Lee et al., 2003). The removal efficiency of a large-scale CW has so far only been evaluated for 1,4-dichlorobenzene with values ranging from 63% to 87% (Keefe et al., 2004). In general, most of the investigations on phytoremediation of waters and soils contaminated by chlorinated hydrocarbons have been carried out under laboratory conditions using micro- or meso-scale systems. Field investigations in pilot or full-scale treatment wetlands are lacking. Furthermore, potential impacts of undesirable processes such as volatilization have not

been addressed adequately. Volatilization of toxic chlorinated hydrocarbons may be increased by technological problems such as clogging and subsequent flooding, and may lead to serious air pollution.

A pilot-scale horizontal subsurface flow CW was installed and operated at the Bitterfeld site for field investigations on the removal of chlorobenzenes as the main groundwater contaminants. In order to evaluate the processes involved, spatial concentration dynamics of monochlorobenzene (MCB), 1,2-dichlorobenzene (1,2-DCB), 1,4-dichlorobenzene (1,4-DCB), oxygen and ferrous iron as well as the redox potential were investigated in relation to the distance from the inflow and the depth of the wetland bed. To provide further evidence for the hypothesis that plants display an enhancing effect on chlorobenzenes removal efficiency, investigations were carried out in parallel in a planted wetland and an unplanted reference plot.

Materials and Methods

The SAFIRA (remediation research in regionally contaminated aquifers) groundwater research site is located in the south-east of the city of Bitterfeld in Saxony-Anhalt, Germany. Monochlorobenzene (MCB) is the predominant groundwater contaminant at the site. Averages and standard deviations of the main pollutant concentrations and geochemical characteristics as observed in June 2005 are given in Table 1. The groundwater passing through the wetlands was pumped from 22 m depth from the SAFIRA well no. 5.

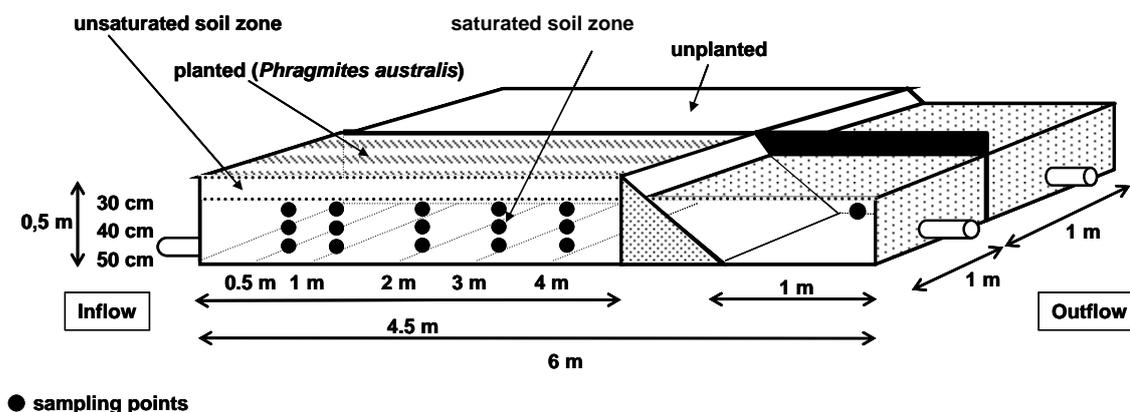


Figure 1: Process scheme and sampling points (●) of the pilot-scale CW for groundwater treatment.

Experimental setup. At the SAFIRA site a meso-scale pilot plant was built in order to investigate the remediation of chlorinated contaminants in a wetland system. The CW was designed as a horizontal flow subsurface system and consisted of a 6 m×2 m×0.7 m stainless steel container divided into two subsystems. Both parts were filled with the local aquifer material to a height of 0.5 m and a length of 5 m. The last metre of each part was left as an open water (FW) compartment (Fig. 1). The aquifer material used as filter material for the wetland consisted of 36% gravel, 58% sand and 6% clay (Vogt et al., 2002). According to the Hazen formula, k_f was determined as $2.1 \times 10^{-3} \text{ m s}^{-1}$. Due to residues of the local lignite seam, TOC varied between 1.5 and 2.0% by weight. The iron content of the sediment was in the range of 0.8 to 1.3% by weight.

Table 1: Analysis of the groundwater inflow of the CW.

		Groundwater inflow	
		Average ^a	Standard deviation
MCB	[mg L ⁻¹]	19.7	±2.3
1,2-DCB	[mg L ⁻¹]	0.19	±0.01
1,4-DCB	[mg L ⁻¹]	0.25	±0.03
Fe _{ges}	[mg L ⁻¹]	1.00	±0.59
Ca ²⁺	[mg L ⁻¹]	394.2	±10.4
Mg ²⁺	[mg L ⁻¹]	48.3	±0.5
Mn ²⁺	[mg L ⁻¹]	0.14	±0
Fe ²⁺	[mg L ⁻¹]	0.12	±0.15
NH ₄ ⁺	[mg L ⁻¹]	3.38	±0.53
Na ⁺	[mg L ⁻¹]	134.7	±13.7
K ⁺	[mg L ⁻¹]	180.8	±6.8
PO ₄ ³⁻	[mg L ⁻¹]	4.83	±0.17
SO ₄ ²⁻	[mg L ⁻¹]	957.1	±85.1
Cl ⁻	[mg L ⁻¹]	271.4	±39.1
S ²⁻	[μg L ⁻¹]	0.03	±0
O ₂	[mg L ⁻¹]	0.037	±0.010
σ	[mS cm ⁻¹]	2.35	±0.20
Eh	[mV]	15	±24
pH		6.72	±0.25

^a Average values of three sampling campaigns carried out in June 2005

One of the wetlands was planted with common reed (*Phragmites australis*); the other one was kept unplanted as a reference plot. Both systems were continuously supplied

with anaerobic, contaminated groundwater at a flow rate of 4.7 L h^{-1} . The water level was adjusted to 0.4 m by float valves in the FW compartments and volumes pumped off were controlled by flow meters. Based on the flow rate of 4.7 L h^{-1} and a porosity of 0.28, the theoretical hydraulic retention time in the wetlands was calculated and found to be 119 h. The system was installed in March 2003 and was operated for more than two years to ensure steady state conditions before the current investigations were carried out in June 2005.

Sampling. Water samples of approximately 60 mL for the evaluation of chlorobenzene concentrations and the redox-sensitive parameters were taken from the middle of the wetlands at 0.5, 1, 2, 3 and 4 m distance from the inflow at depths of 0.3, 0.4 and 0.5 m. The FW compartments were sampled at 0.4 m depth only. Water sampling at the various depths was carried out using stainless steel lancets (3.5 mm inner diameter) and peristaltic pumps with a flow rate of 78 mL min^{-1} . All water samples for chemical analysis were stored without headspace at 4°C until analysis. For sediment sampling a Pürckhauer soil corer was used.

Analysis. Chlorobenzenes were analysed in duplicates by means of headspace GC-FID (HP 6890 with HP 7694 autosampler, Agilent Technologies, Palo Alto, USA) using a HP-1 column ($30 \text{ m} \times 0.32 \text{ mm} \times 5 \mu\text{m}$). The following temperature program was applied: 45°C (1 min), 20 K min^{-1} to 200°C (2.5 min), 65 K min^{-1} to 250°C (1 min). The detector temperature was 280°C . Helium served as carrier gas with a constant flow of 1.7 mL min^{-1} . For equilibrating, the samples were shaken for 1 h at a temperature of 60°C directly before injection. 1 mL of the headspace was injected with a split 1:5 (injector temperature: 250°C). Detection limits (dl) were $1.3 \mu\text{g L}^{-1}$ for MCB, $2.4 \mu\text{g L}^{-1}$ for 1,4-DCB and $1.2 \mu\text{g L}^{-1}$ for 1,2-DCB. Aqueous concentrations of chlorobenzenes were determined by applying an external calibration (equilibrium headspace gas chromatography).

The photometric detection of ferrous iron was carried out as described previously (Lovley and Phillips, 1986). NH_4^+ concentrations were determined according to DIN 38406-E5 (1983) (dl: 0.01 mg L^{-1}) and PO_4^{3-} concentrations according to DIN EN ISO 6878-D11 (2004) (dl: 0.07 mg L^{-1}). Photometric measurements were performed using a Cadas 100 photometer (Hach Lange, Düsseldorf, Germany).

Sulphide concentrations (dl: $0.3 \mu\text{g L}^{-1}$) and electric conductivity were measured in batch using ion-sensitive Ag/S 500/800 and conductivity electrodes (WTW, Weilheim, Germany). Oxygen concentration and redox potential were measured in a flow-through unit using an optical oxygen trace sensor system (sensor FTC-TOS7 and instrument FIBOX-3-trace, PreSens, Regensburg, Germany) (dl: $1 \mu\text{g L}^{-1}$) and redox electrodes (Pt/Ag⁺/AgCl/Cl⁻ Typ Sentix ORP, WTW, Weilheim, Germany).

Analysis of Cl⁻ and SO₄²⁻ (dl: 2.5 mg L^{-1}) concentrations was performed by means of ion chromatography (DX 500) with conductivity detection (CD 20) and an IonPacAG11 column (4x250 mm) (Dionex Corporation, Sunnyvale, USA). The flow rate was 2 mL min^{-1} , the mobile phase was A = 5 mM NaOH, B = water and D = 100 mM NaOH. The following gradient was applied: 10% A and 90% B (3 min), linear from 10 to 100% A in 4 min, linear from 0 to 35% D in 7 min, return to 10% A and 90% B in 3 min (5min). Total Fe (dl: 0.03 mg L^{-1}), Mn²⁺ (dl: 0.02 mg L^{-1}), K⁺ (dl: 0.2 mg L^{-1}), Na⁺ (dl: 0.4 mg L^{-1}), Mg²⁺ (dl: 0.01 mg L^{-1}) and Ca²⁺ (dl: 0.03 mg L^{-1}) concentrations were analysed by means of atomic emission spectrometry with inductively coupled plasma excitation (ICP-AES, Spectro Ciros Vision CCD, Spectro Analytical Instruments, Kleve, Germany).

To test the statistical significance of observed differences in removal efficiency, non-parametric Mann-Whitney-Wilcoxon tests were applied using the SPSS 14.0 software.

Microbial studies. For the investigation of microbial MCB degradation, water samples were collected from a depth of 0.1 m at 1 m distance from the wetland inflow. The samples were incubated at 16°C in aerobic microcosms (25 mL in 118 mL serum bottles) on a shaker and were repeatedly spiked with 0.5 mg MCB after initial disappearance of the contaminant. The oxygen amount initially introduced into the microcosms with the headspace air volume (circa $870 \mu\text{mol O}_2$) represented an excess reservoir of oxygen throughout the period of the experiments. Sodium azide (1.2 g L^{-1}) was added to negative controls. For one week, MCB concentrations in the aqueous phase were analysed by GC-FID as described above.

Most probable numbers (MPN) were determined for liquid samples from 0.1 m depth at 1 m, 3 m and 4.5 m distance from the wetland inflow. They were incubated in microtiter plates in a closed vessel of 3 L volume as described elsewhere (Vogt et al., 2002). For

the liquid samples, a dilution sequence to 10^{-8} was prepared in mineral medium ($2.44 \text{ g L}^{-1} \text{ Na}_2\text{HPO}_4$, $1.52 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$, $0.5 \text{ g L}^{-1} (\text{NH}_4)_2\text{SO}_4$, $0.5 \text{ g L}^{-1} \text{ NaCl}$, $0.2 \text{ g L}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.05 \text{ g L}^{-1} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 mL L^{-1} of trace solution SL-10 (Trueper and Pfennig, 1992) and 5 mL L^{-1} of vitamin solution (Pfennig, 1965) in distilled water, pH 6.9) before being transferred to microtiter plates. A filter with $400 \mu\text{l}$ MCB as the sole carbon source was added before air-tight closure of the vessel. MPN counts were determined after an incubation period of one month at room temperature.

Results and Discussion

In order to investigate the pollutant removal and the influence of plants in the pilot-scale CW, the concentrations of MCB, 1,2-DCB and 1,4-DCB from the contaminated groundwater were measured at various distances from the inflow and depths of the wetlands. The mean concentrations of MCB and 1,4-DCB decreased with distance from the inlet in both planted and the unplanted system but the removal was more effective in the planted wetland, indicating the enhancement of contaminant removal by the plants. Within the first 3 m of the flow path, significant differences in mean removal efficiency (average of the investigated depth) were observed between the planted and unplanted wetland ($p < 0.05$). The planted system showed mean removals of 43% for MCB and 39% for 1,4-DCB whereas the unplanted system removed only 10% and 16%, respectively. At the outflow of the planted wetland (4 m distance from inflow), the mean removal was 43% for MCB and 43% for 1,4-DCB. The efficiency of the unplanted system was lower with 35% for MCB and 31% for 1,4-DCB (Fig 2). However, differences in removal efficiency were no longer significant after 4 m of the flow path, possibly suggesting that the advantage of the planted over the unplanted system is reduced or levelled out if the wetland passage is long enough. Also the increasingly impaired development of the plants towards the outflow of the wetland passage may diminish removal efficiency, as will be further discussed later on.

The removal efficiency showed high variability over the depths of the wetland beds. In the planted wetland, removal at 0.3 m (upper layer with the rhizosphere) tends to be 15 to 20% higher in comparison to those at 0.5 m for both MCB and 1,4-DCB. For example, removal of MCB at 2 m distance from the inflow amounted to 71% at 0.3 m depth but only 25% at 0.5 m depth. Similar ranges were found for 1,4-DCB. The depth

effects were much less distinct in the unplanted wetland. These findings may indicate considerable effects of oxygen particularly in the upper layer of the planted wetland. However, statistical significance of differences in removal efficiency between depths could not be tested due to the small data volume.

It is notable that the concentration eliminated was much lower for 1,4-DCB than for MCB ($0.11 \pm 0.02 \text{ mg L}^{-1}$ and $8.7 \pm 2.7 \text{ mg L}^{-1}$ average removal after 4 m of the flow path in the planted wetland, respectively). This indicates either a lower microbial degradability of 1,4-DCB or a limitation of microbial 1,4-DCB degradation by concentration thresholds, such as was already observed for other chlorinated aromatic compounds (Boethling et al., 1979). For 1,2-DCB, only insignificant concentration changes within a range of 6% of the inflow concentration could be observed in both wetlands. No clear concentration decrease between inflow and 4 m of the flow path could be detected. However, most of the residual contaminants were removed from the adjacent FW compartments with open water surface in both planted and unplanted wetlands, presumably due to volatilization (overall removals of 89 and 95% MCB, 78 and 87% 1,4-DCB and 59 and 100% 1,2-DCB after the FW compartments of the planted and unplanted wetland, respectively).

The Henry's Law constants of 1,2-DCB ($0.0019 \text{ atm. m}^3 \text{ mol}^{-1}$) and 1,4-DCB ($0.0016 \text{ atm. m}^3 \text{ mol}^{-1}$) differ only slightly (van Agteren et al., 1998). Therefore, the fact that 1,2-DCB was not efficiently eliminated in the wetlands but was eliminated to a high extent in the FW compartments clearly indicates that volatilization was not the dominant removal process in the wetlands. In contrast to MCB and 1,4-DCB, 1,2-DCB can not be easily degraded by the indigenous microbial consortium from the Bitterfeld aquifer (Dermietzel et al., 1999). This is also consistent with the hypothesis that microbial degradation processes are responsible for the MCB and 1,4-DCB removal in the wetlands.

Most probable number (MPN) determinations of MCB-degrading bacteria were carried out using pore water from the pilot-scale CW. 10^2 to 10^5 MPN mL^{-1} of aerobic MCB degraders were determined, with the highest value found at 1 m distance from the inflow and the lowest value at 4.5 m. Apparently, MCB-degrading bacteria are most

abundant in the inflow area of the wetland where chlorobenzene concentrations are highest.

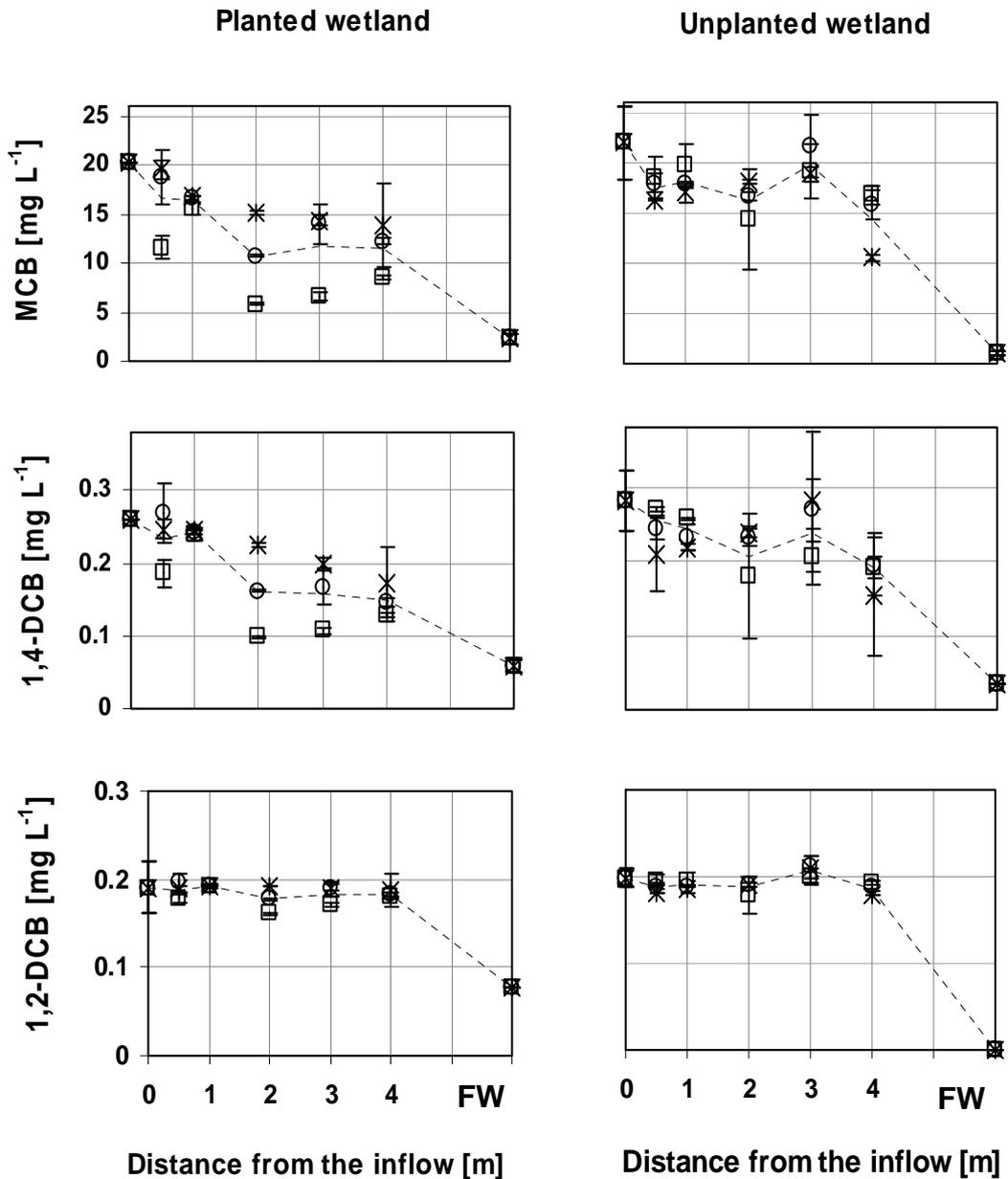


Figure 2: Monochlorobenzene (MCB), 1,4-dichlorobenzene (1,4-DCB), and 1,2-dichlorobenzene (1,2-DCB) concentrations of the pore water depending on the depth of the wetland and the distance from the inflow; (\square) 0.3 m; (\circ) 0.4 m; (\times) 0.5 m; (----) mean; FW free water compartment.

In the aerobic microcosm experiments carried out with liquid field samples, MCB was degraded by microorganisms within 3 days and subsequently every spike of MCB thereafter disappeared within 24 h. This definitely shows the presence of aerobic MCB degraders in 0.1 m depth of the wetland sediment where aerobic conditions are expected

to prevail. At least some of these MCB-degrading organisms are cultivatable under the conditions applied in the aerobic microcosms.

The decrease of chlorobenzene concentrations in the wetlands depended on the flow distance, the depth of the wetland and the presence or absence of vegetation. The concentrations diminished with increasing distance from the inflow and decreasing depth of the wetlands (Fig. 2). This effect was most pronounced in the planted system. The highest removal efficiency was already observed on the first half of the planted wetland (first 2 m of the flow path) with 71% for MCB and 62% for 1,4-DCB determined in 0.3 m depth. Surprisingly, on the second half of the flow path a slight increase of concentrations was observed, which could be attributed to water losses due to the transpiration of the plants. Chloride was used as an indicator, because the change in chloride concentration was much higher than that which would be expected from chlorobenzene degradation. Microbial degradation of 10 mg L^{-1} MCB would result in a release of only $3.2 \text{ mg L}^{-1} \text{ Cl}^-$. However, chloride concentrations were actually found to increase from 245 mg L^{-1} at 2 m distance from the inflow up to 298 mg L^{-1} in the FW compartment, resulting in an additional enrichment of at least 17%. Therefore, mean chlorobenzene removals corrected for the water loss will lead to much higher actual values in the planted CW. Similar concentration effects were also observed in other experiments (Wand et al., 2006). Previous publications reporting the evaluation of a full-scale wetland supplied with water containing $0.74 \text{ } \mu\text{g L}^{-1}$ 1,4-DCB (Keefe et al., 2004) showed an overall decrease of 1,4-DCB concentrations between 63 and 87%. These eliminations exceed our mean removals but are in a similar range to the values obtained for the upper aerobic zone in our experiment.

In theory, the overall decrease of chlorobenzene concentrations can be caused by microbial degradation, sorption onto solid materials and volatilization; in the planted system it can also be caused by plant uptake followed by transport, transformation and phytovolatilization. Due to the long-term operation of the wetlands preceding the present investigations, leading to an established adsorption/desorption balance, a removal by sorption onto soil materials can be assumed to be negligibly small. Biodegradation is expected to be a dominant process of chlorobenzene removal. However, the extent of degradation cannot be estimated without data concerning the volatilization and plant uptake. The analysis of MCB emissions from the soil surface of

the investigated planted CW showed that emissions only account for less than 10% of the total contaminant mass decrease (Reiche, 2005). Other authors found in laboratory experiments that 31% of the added [UL-¹⁴C] MCB is volatilised and 16% mineralized in unplanted systems (MacLeod et al., 1999). In planted systems, the extent of volatilization was found to be in the same range as in unplanted systems, but mineralization was much more effective, accounting for the removal of 27% of the added compound. This result shows the enhancement of microbial degradation by the plants. However, not much is yet known about phytosorption, plant uptake and phytovolatilization of chlorobenzenes by wetland plants. Compounds displaying a logarithm of the octanol-water partition coefficient ($\log K_{OW}$) between 1 and 3.5 are considered to be transferred through the root epidermis and can thus undergo transport, transformation or accumulation in the plant tissue (Paterson et al., 1990). Taking into consideration the chlorobenzenes' $\log K_{OW}$ values (MCB: 2.98; DCBs: 3.38 (van Agteren et al., 1998)), transfer into the plants may be an important factor for fate assessment.

The considerably higher decrease of chlorobenzenes concentration in the surface-near layer of the wetland (Fig. 2) indicates aerobic microbial degradation supported by oxygen transfer via the soil surface and by oxygen release from helophyte roots (Wießner et al., 2005). Previous studies have shown that MCB is completely degraded by microorganisms from Bitterfeld groundwater if enough oxygen is provided, and that the inhibition of microbial MCB mineralization under oxygen limited conditions is due to accumulation of the toxic intermediate 3-chlorocatechol (Vogt et al., 2004a, 2004b). Higher removal efficiencies in the upper layer of horizontal flow CW have been observed previously, e.g. in an experimental wetland treating water contaminated with diesel oil (Omari et al., 2003), and have been attributed to higher oxygen availability. The pore water oxygen concentration was determined at all investigated sampling points as an indicator for the redox conditions and possible aerobic microbial activity (Fig. 3).

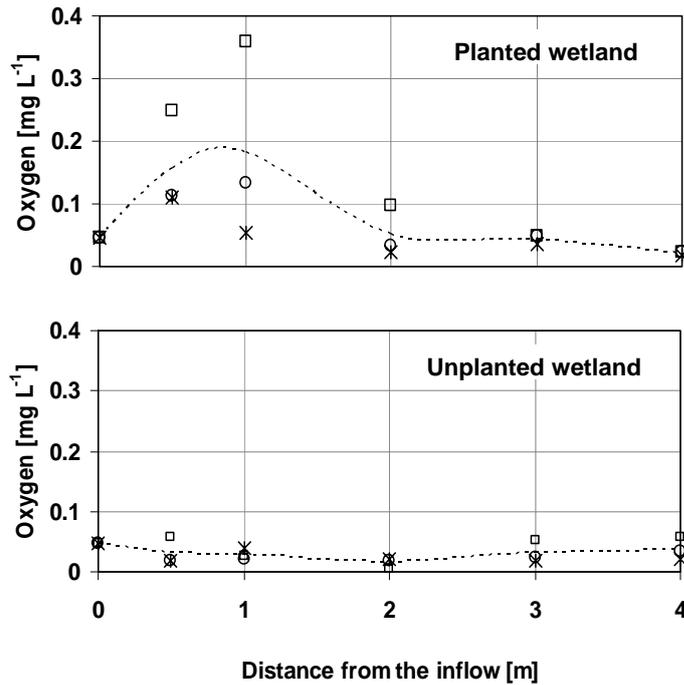


Figure 3: Oxygen concentrations of the pore water depending on the depth of the wetland and the distance from the inflow; (\square) 0.3 m; (\circ) 0.4 m; (\times) 0.5 m; (----) mean.

Oxygen concentrations amounted to 0.37 mg L^{-1} in the top layer of the first half of the planted wetland, indicating an enhanced physiological activity of the plants to release oxygen into the rhizosphere. Corresponding to oxygen concentrations below 0.05 mg L^{-1} a steady state of the chlorobenzene concentrations (Fig. 2) was observed in the planted wetland after 2 m of the flow path. In the unplanted reference plot, oxygen concentrations only reached up to 0.05 mg L^{-1} throughout the sampling transect and no concentration dynamics were observed (Fig. 3). Therefore, it can be concluded that most of the oxygen input was induced by the plants. Oxygen diffusion via the soil surface only has, if at all, an impact in the topmost layer of the wetland sediment. Oxygen-consuming processes in this zone may outrun diffusion processes of atmospheric oxygen by far. These findings correspond well to results of previous studies, showing the predominance of low oxygen concentrations in horizontal subsurface flow CW (Garcia et al., 2004) and the decrease of oxygen concentrations with increasing depth (Chimney et al., 2006).

The redox potential in the wetlands, denoted as E_h , increased from inflow values between -10 and 0 mV up to mean values of 100 mV in the planted and 50 mV in the unplanted system already within the first 0.5 m of the flow path for all investigated

depths (Fig. 4). Relative redox stability at a higher level ($E_h=65-90$ mV) was observed throughout the planted system compared to the unplanted system ($E_h \leq 70$ mV) even though the same low level of oxygen concentrations prevailed in both systems after 2 m of the flow path (Fig. 3). Thus, the generally higher redox potentials observed in the planted compared to the unplanted wetland support the hypothesis that the planted wetland is indeed the more oxidized system, stressing the importance of the plants for enhancing aerobic microbial degradation processes.

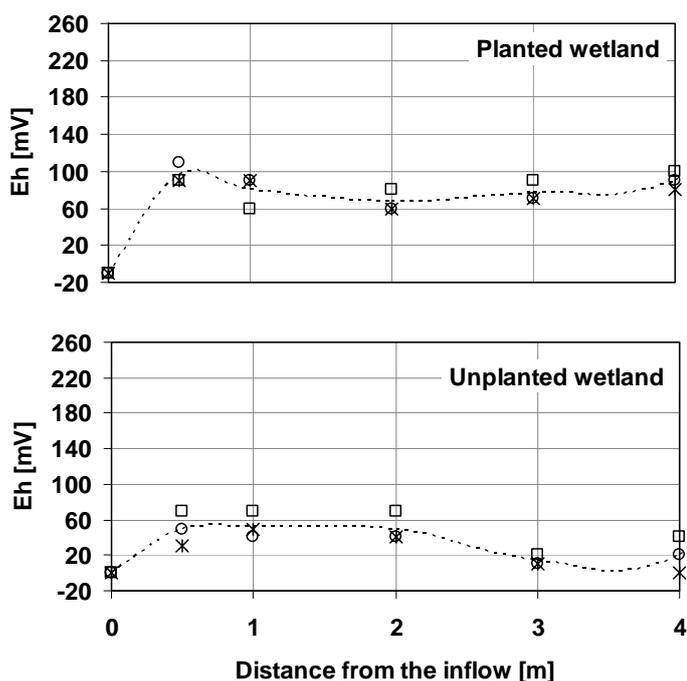


Figure 4: Redox potentials of the pore water depending on the depth of the wetland and the distance from the inflow; (\square) 0.3 m; (\circ) 0.4 m; (\times) 0.5 m; (---) mean.

Ferrous iron concentrations increased from initial concentrations below 1 mg L^{-1} to levels of 35 mg L^{-1} in the planted and to 25 mg L^{-1} in the unplanted wetland (Fig. 5). In the planted wetland, ferrous iron concentrations increased continuously between the inflow and outflow whereas a discontinuous mobilization was observed in the unplanted wetland. The most intensive mobilization occurred here between 0 and 1 m as well as between 2 and 3 m. The increase of concentrations can be attributed to microbial reduction of ferric iron because the filter material used for the wetlands contained up to $13 \text{ g iron kg}^{-1}$ dry weight. Microbial reduction of ferric iron (ferrihydrite) is possible at redox potentials below $+100$ mV (Straub et al., 2001) and can thus occur under the redox conditions observed in our wetland (Fig. 4). The process of ferric iron reduction

is probably coupled to the oxidation of organic compounds. Considering the average final concentrations of ferrous iron and MCB detected in the planted wetland, approximately 29% of the total MCB eliminated may in theory have been oxidized to CO_2 by microbial iron reduction (calculation based on the stoichiometry of the hypothetical redox reaction between ferric iron and MCB). However, the anaerobic oxidation of chlorobenzenes coupled to ferric iron reduction has not yet been described. The more intense formation of ferrous iron in the planted wetland can be explained by enhanced availability of ferric iron for microbial oxidation processes due to Fe-chelation by plant exudates and dead plant matter. Citrate and malate are excreted by plant roots and are potent iron complexers (Jones, 1998).

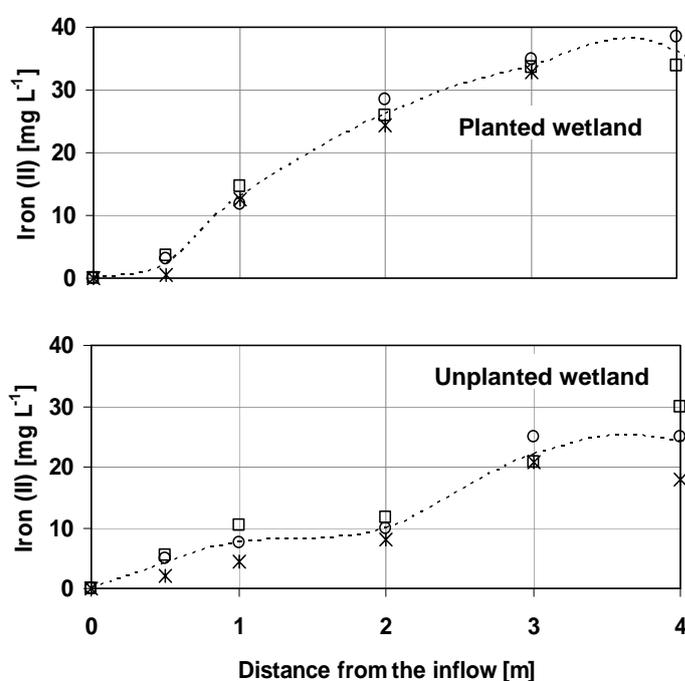


Figure 5: Ferrous iron concentrations of the pore water depending on the depth of the wetland and the distance from the inflow; (□) 0.3 m; (○) 0.4 m; (×) 0.5 m; (---) mean.

Lovely et al., (1996) observed that microbial oxidation of aromatics can be coupled to the reduction of chelated ferric iron species for example in humic acid complexes.

Redox potential, oxygen and ferrous iron concentration dynamics show that a relevant oxygen limitation and microbial ferric iron reduction can be considered in both wetland systems. The oxygen limitation may lead to limited microbial chlorobenzene oxidation and potentially to alternative anaerobic oxidation processes of chlorobenzenes. However, no geochemical indicators of anaerobic electron-accepting processes other

than ferric iron reduction could be detected. As no concentration decrease of sulphate and no sulphide formation were observed along the flow path of both the planted and unplanted wetland (data not shown), relevant microbial sulphate reduction can be excluded. This is in accordance with the occurrence of iron reduction in the pore water, as ferric iron is a more energetically favourable electron acceptor than sulphate.

The physiological condition of the plants and the plant density on the planted wetland were estimated in order to detect correlations between plant activity and contaminant removal. In fact, not the entire plant stock showed optimum development. The effective removal of chlorobenzenes on the first 2 m of the flow path and its near steady state on the second half corresponds to a gradient of plant density in the wetland. Considering all shoots higher than 0.1 m, the plant density decreased with distance from the inflow from 2260 shoots m^{-2} at 0.5 m to 1020 m^{-2} at 2 m down to 900 m^{-2} at 3.5 m. The average growth height decreased from 1.1 m to 0.8 m which can be attributed to nutrient limitation. The concentrations of the single nitrogen source ammonia and of phosphate in the inflow water were rather low (Tab. 1). Ammonia concentrations strongly decreased along the flow path of the planted wetland, whereas in the unplanted wetland no decrease was observed. Phosphate concentrations decreased more rapidly on the first 2 m of the planted wetland compared to the unplanted wetland. Nutrient limitation of plant and microbial biomass growth, presumably leading to reduced oxygen and organic carbon supply by the plants and reduced microbial activity, is likely to impair chlorobenzene degradation in the planted wetland system. High plant density and activity was correlated to a high removal of chlorobenzenes which gives strong evidence for the enhancement of removal efficiency by the plants. Optimised nutrient supply is therefore expected to improve the treatment performance of the wetland system.

Conclusions

The presented data show that the planted CW is effective in removing MCB and 1,4-DCB from contaminated groundwater. However, concentrations of 1,2-DCB in the $\mu\text{g L}^{-1}$ range were not affected. Aerobic microbial degradation of MCB and 1,4-DCB are considered to be dominant removal processes. Strong evidence for anaerobic microbial reduction of ferric iron was observed in the wetlands; this may be linked to

chlorobenzene oxidation or to the decomposition of organic matter. Anaerobic microbial chlorobenzene degradation processes, possibly coupled to ferric iron reduction, have to be investigated in additional experiments.

Microbial investigations conducted with liquid samples from the CW revealed the presence of considerable numbers of aerobic MCB degraders and the high MCB degradation ability of these organisms under aerobic conditions. Thus, high potential for aerobic microbial chlorobenzene removal exists, leading to high removal rates if appropriate conditions prevail in technical-scale CW.

The constitution of the plants and their nutrient supply affect the microbial removal processes. Nutrient limitation in the wetland strongly limits plant development and thus also microbial degradation processes. The observed spatial gradients of efficiency (highest removal on the first 2 m of the flow path at 0.3 m depth) and the nutrient limitation suggest potentials for improved treatment of contaminated groundwater. A more aerobic wetland design (e.g. an intermittently charged vertical flow wetland) can be recommended since the removal efficiency is low in the deeper, more anaerobic zones of the wetland sediment. Further investigations should be focused particularly on anaerobic processes of microbial chlorobenzene degradation, plant uptake, phytovolatilization and soil emission of chlorobenzenes. Since rising groundwater tables may transport pollutants and their metabolites to near-surface layers at contaminated megasites, the construction of near-natural wetlands may provide an effective remediation approach for endangered river flood plains.

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2.2 Chlorobenzenes removal efficiencies and removal processes in a pilot-scale constructed wetland treating contaminated groundwater

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Abstract

Low-chlorinated benzenes (CBs) are widespread groundwater contaminants and often threaten to contaminate surface waters. Constructed wetlands (CW) in river floodplains are a promising technology for protecting sensitive surface water bodies from the impact of CBs. The efficiency and seasonal variability of monochlorobenzene (MCB), 1,4-dichlorobenzene (1,4-DCB) and 1,2-dichlorobenzene (1,2-DCB) removal, the impact of planting and soil-borne gaseous MCB emissions were investigated over the course of one year in a vegetated pilot-scale CW and in an unplanted reference plot (UR). Considerable annual mean concentration decreases of MCB and 1,4-DCB were determined. However, annual mean 1,2-DCB removal was observed only in the upper filter layer. Planting (*Phragmites australis*) had a statistically significant beneficial effect on removal. The CBs removal efficiency in the CW generally decreased with depth, and seasonal variations of removal were evident, with less concentration decrease in the summer season. Load removal efficiencies of 59-65% ($262\text{-}358\text{ mg m}^{-2}\text{ d}^{-1}$) for MCB, 59-69% ($4.0\text{-}5.1\text{ mg m}^{-2}\text{ d}^{-1}$) for 1,4-DCB and 29-42% ($0.6\text{-}2.1\text{ mg m}^{-2}\text{ d}^{-1}$) for 1,2-DCB were observed in June and July. Soil-borne volatilization of MCB accounted for 2-4% of the total amount removed. Simple cover layers of organic materials on the filter surface were suitable for MCB emission reduction.

Model calculations were carried out to estimate the removal of MCB from the CW and UR by various processes: microbial degradation, volatilization, and plant uptake. Microbial degradation was the dominating process, but plants were important for MCB removal due to root oxygen release into the rhizosphere, water table fluctuations and direct plant uptake.

Keywords: Phytoremediation, monochlorobenzene, dichlorobenzene, *VOC* emissions, modeling, oxygen input

1. Introduction

Low-chlorinated benzenes such as MCB and DCBs are important solvents in chemical industries (van Agteren et al., 1998; WHO, 2004). Their production and use led to widespread groundwater contaminations (Field and Sierra-Alvarez, 2008) causing environmental hazards due to their toxicity and persistence. Under anaerobic groundwater conditions, microbial degradation of MCB and DCBs is very slow, leading to accumulation in contaminated aquifers (Heidrich et al., 2004). Furthermore, sites initially contaminated with highly chlorinated benzenes or lindane show accumulation of MCB and DCBs caused by partial reductive dechlorination (Ramanand et al., 1993). In contrast, microbial degradation of low-chlorinated benzenes under aerobic conditions is fast and efficient via dioxygenase-catalysed pathways, as is further degradation of the chlorinated aromatic intermediates via ortho-ring cleavage (Reineke and Knackmuss, 1984; van Agteren et al., 1998; Field and Sierra-Alvarez, 2008).

Chemical industries are often located in the vicinity of rivers due to their dependence on water for production, cooling, and transportation. Contaminated groundwater at these sites can thus interact with surface water bodies or discharge directly into river floodplains (Kalbus et al., 2006), as has been observed e.g. at the Bitterfeld industrial area in Germany (Heidrich et al., 2004).

Active hydraulic groundwater remediation technologies (pump-and-treat) are useful for plume containment (Travis and Doty, 1990; Rivett et al., 2006), but are inefficient and expensive when treating large volumes of groundwater with low contaminant concentrations (Weiß et al., 2001), in particular when contaminated groundwater infiltrates into river floodplains. Constructed wetlands (CW) or engineered natural wetlands for the removal of organic contaminants from groundwater provide a cost-effective remediation approach (Susarla et al., 2002; Williams, 2002; Haberl et al., 2003; Amon et al., 2007; Imfeld et al., 2008). Natural wetlands may already be present in river floodplain areas, and could be modified for groundwater treatment under controlled and optimized conditions.

The removal of volatile organic compounds (VOC) in wetlands is associated with various processes: microbial degradation in the rhizosphere, (phyto)-volatilization, sorption, plant uptake and metabolization (Susarla et al., 2002; Imfeld et al., 2009;

Weishaar et al., 2009). In complex wetland systems, several processes are potentially involved in the removal and create the observed removal pattern, but the removal processes have rarely been balanced or modelled. Oxidative and reductive microbial degradation are considered the most important processes and are desired for destruction of hazardous contaminants. Favourable conditions for microbial growth, activity and diversity prevail in helophyte rhizospheres, leading to the high degradation potential of these natural environments (Walton and Anderson, 1990; Stottmeister et al., 2003).

Wetland treatment of waters contaminated with VOC has been investigated in several studies with chlorinated ethenes (Ma and Burken, 2003; Kassenga et al., 2003; Amon et al., 2007; Tawney et al., 2008; Imfeld et al., 2008) and chlorinated ethanes (Lorah and Voytek, 2004). However, studies on low-chlorinated benzenes are rare. MacLeod et al. (1999) found that volatilization was a major removal process for MCB in laboratory wetland microcosms planted with reed (33% of the ^{14}C MCB added; only 27% was mineralized). Jackson et al. (2000) report complete reductive dechlorination of MCB and DCBs in a bottomland hardwood wetland. Previous studies of the Bitterfeld wetland evidenced aerobic as well as anaerobic microbial degradation of MCB (Braeckevelt et al., 2007; 2008). The operation and efficiency of a larger-scale CW treating a range of VOC including 1,4-DCB have been described by Keefe et al. (2004). However, middle- and long-term operation and efficiency of large-scale CW treating CB-contaminated waters under realistic environmental conditions, including seasonal variability in temperate climates, have not yet been described in the literature. Volatilization is a potential VOC elimination process in CW (Ma and Burken, 2003; Eke and Scholz, 2008; Reiche et al., 2010) which is also relevant for CB (MacLeod et al., 1999; Keefe et al., 2004) but has never been measured in a technical-scale CW. Hence, the lack of reliable risk assessment for air pollution currently discourages regulatory acceptance of VOC phytoremediation (McCutcheon and Rock, 2001; Reiche et al., 2010).

Therefore, the goal of this study was to investigate the annual average efficiency and the seasonal variability of MCB, 1,2-DCB and 1,4-DCB removal in a pilot-scale CW under the conditions of the temperate climate zone. To evaluate the effect of planting on the remediation process, a *Phragmites*-planted CW and an unplanted reference plot were investigated in parallel. Modeling approaches were applied in order to assess various removal processes for the main contaminant MCB. Soil-borne gaseous emissions were

monitored, and options for minimizing VOC emissions from the CW with organic cover layers were tested.

2. Materials and Methods

2.1 Site description and experimental setup

The study was carried out at the SAFIRA research site in Bitterfeld, Germany, where a pilot-scale horizontal subsurface flow (HSSF) CW planted with *Phragmites australis* and an unplanted reference plot (UR) of identical design were set up in 2003 (Braeckevelt et al., 2007; 2008). The local groundwater used for loading the systems contained average concentrations of $17.6 \pm 3.3 \text{ mg L}^{-1}$ MCB and $0.27 \pm 0.04 \text{ mg L}^{-1}$ 1,4-DCB (Nov 04-Oct 05); the 1,2-DCB concentrations were $0.2 \pm 0.01 \text{ mg L}^{-1}$ (Nov 04-Jun 05) and $0.05 \pm 0.005 \text{ mg L}^{-1}$ (after June 05). The systems (with the dimensions 6 m length \times 1 m width \times 0.5 m depth) were each filled with filter material for the first 5 m², leaving 1 m² at the outflow as an open water compartment (OWC). The quaternary aquifer material from the site was used as filter material. It consisted of $\sim 67\%$ gravel and $\sim 25\%$ mica sand (k_f value $2.1 \times 10^{-3} \text{ m s}^{-1}$, porosity 0.35, TOC 1.5 - 2.0% and iron content 0.8–1.3%, w/w). Both systems were continuously supplied with contaminated groundwater at a flow rate of 4.7 L h^{-1} . The maximum water level was adjusted to 0.4 m. The systems were allowed to adjust for 20 months to ensure steady-state conditions before the current investigations were carried out between November 2004 and October 2005.

2.2 Water sampling and analysis

Pore-water samples for the evaluation of CB concentrations were taken from the filters at 0.5, 1, 2, 3 and 4 m distance from the inflow, each at 0.3, 0.4 and 0.5 m depths. The OWCs were sampled from 0.4 m depth only. Water sampling at various depths was carried out using stainless steel lancets (3.5 mm ID) and peristaltic pumps with a flow rate of 0.078 L min^{-1} . Water samples were stored without headspace at 4°C until analysis. 20 sampling events (each taking place over a period of 4-5 hours) were carried out at regular intervals (2-4 weeks). CBs in the liquid phase were analyzed in duplicates by means of headspace GC-FID, as described previously (Braeckevelt et al., 2008).

Chemical oxygen demand (COD) was determined with photometric cuvette tests (LCK 414, Hach Lange, Germany).

To test the significance of differences between the CW and UR in the annual CB concentration data sets, non-parametric Wilcoxon-Mann-Whitney two-sample rank-sum tests were carried out, as normal distribution could not be assumed (one-sample Kolmogorov-Smirnov tests). The PASW Statistics 17.0 program was used.

Precipitation and ambient pressure together with soil and air temperatures were monitored over the entire sampling period. Outflow volumes were determined by flow meters and recorded at regular intervals in June and July 2005.

2.3 Gas sampling and analysis

Gaseous emissions from the sediment surface of the CW and UR were determined at 0.5 m, 2.0 m and 3.5 m distance from the inflow point, as well as from the OWC for MCB as the main contaminant. Four replicate gas samplings and measurements were carried out in June and July 2005. A cylindrical flow-through test chamber (glass, ID=135 mm, V=1.65 L) was placed upon the sediment surface and inserted to a depth of 0.03 m, covering an area of 0.0143 m². To determine exclusively soil-borne emissions, CW plants were shortened only on the sampling area to fit under the test chamber. Ambient air was cleaned with an activated carbon filter and primed through the test chamber with a flow rate of 12 L h⁻¹ using a gas sampling device (DESAGA GS 312, Sarstedt Group, Germany). At the outflow of the test chamber, samples were collected on Tenax TA (150 mg) filled into 110 mm×8 mm glass tubes (SKC, Eighty Four PA, USA). The sample volume was 12 L. Potential substance breakthrough was monitored by a second tube put in series, but was never observed. Samples were analyzed using a thermo-desorption GC/MS system (EM 640/S, Bruker, Germany) equipped with a DB1 column (30 m×0.25 mm ID×1µm film). The desorption step was carried out at 250°C (180 sec) and the following temperature program was applied for GC analysis: 40°C (3 min), 15 K min⁻¹ to 250°C (1 min). Injection was splitless (120 sec) with an inlet temperature of 260°C and Helium as the carrier gas. Methods used in testing of emission reduction layers (bark mulch and activated carbon) can be found in the supplementary material (Appendix A).

2.4 Calculation of oxygen diffusion into the wetland

According to the “two-film” theory, the exchange of volatile and gaseous compounds (such as MCB and oxygen) between water and air is mainly limited by resistance on the water side (Trapp and Matthies, 1998). However, the water in the CW is not well-mixed, and thus oxygen transport into the CW is limited by (slow) diffusion in water. If the initial concentration of oxygen in the CW is zero, and the resistance of the gaseous layer is neglected, the initial and boundary conditions for the diffusion-advection equation are $C = 0$ for $t \leq 0$ and $z \geq 0$, and $C = C_0$ for $t > 0$ and $z = 0$, where C_0 is the oxygen concentration at the surface of the CW (oxygen saturation). A value of 10 mg L^{-1} was chosen (c_{sat} for $T = 14^\circ\text{C}$). The solution of the diffusion-advection equation with these boundary conditions is (Bear, 1972, cited in Trapp and Matthies, 1998) Eq. 1

$$C(z, t) = \frac{C_0}{2} \left\{ e^{-z\sqrt{\frac{k}{D}}} \operatorname{erfc} \frac{z - t\sqrt{4kD}}{\sqrt{4Dt}} + e^{z\sqrt{\frac{k}{D}}} \operatorname{erfc} \frac{z + t\sqrt{4kD}}{\sqrt{4Dt}} \right\} \quad (1)$$

where k is the consumption rate coefficient of oxygen, z is the depth below surface and t is the time. D is the effective diffusion coefficient of oxygen in water and was estimated as $D = D_{O_2} \times \tau$ where D_{O_2} is the diffusion coefficient of molecular oxygen in water ($2.0 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$; Trapp and Matthies, 1998), and τ is the tortuosity coefficient estimated from the porosity of the filter material as $\tau = 0.25$ (according to Jury et al., 1983). The flux across the top water layer (in $\text{g m}^{-2} \text{ s}^{-1}$) is calculated as $F = (D/z_1) \times (C_0 - C_1)$ where z_1 is an arbitrary depth close to the surface and C_1 is the associated concentration. The flux was calculated for $z_1 = 0.005 \text{ m}$ in steps of 0.02 d and summed over time. In the absence of degradation ($k = 0$), an analytical solution for the flux is given by $F = C_0 \times \sqrt{D/\pi t}$ (Carslaw and Jager, 1946).

Another method to determine the flux of oxygen is to derive it from the measured volatilization of MCB. Since diffusion is a non-directed process, MCB and oxygen have to overcome the same resistances for an exchange. Tortuosity and diffusion length are independent of the compound. The compound-specific parameters are the diffusion coefficient D and the concentration gradient. The diffusion coefficient of MCB was

calculated from $D_{MCB} = D_{O_2} \times \sqrt{M_{O_2} / M_{MCB}}$ (Trapp and Matthies, 1998) where M_{O_2} and M_{MCB} are the molar masses of O_2 and MCB.

3. Results and Discussion

3.1 Annual mean concentration profiles

A simple statistical representation of the annual residual CB concentration data sets, similar to a box plot, is shown in Fig. 1 for the CW and UR. It allows an assessment of the annual median and mean removal efficiencies according to the distance from the inflow and depth of the filter, as well as an evaluation of the variability in the annual data sets.

In the CW, annual mean [median] concentration decrease between the inflow and 0.5 m was 43% [30%], 32% [20%] and 25% [15%] for MCB, and 31% [22%], 24% [20%] and 23% [18%] for 1,4-DCB at 0.3, 0.4 and 0.5 m depth, respectively (Fig. 1). No considerable annual mean concentration depletion occurred between 0.5 and 1 m distance from the inflow for all investigated depths, while distinct concentration decreases were evident between 1 and 3 m of the filter passage, where the largest fractions of MCB and 1,4-DCB were removed. Strong correlations between residual concentrations and the filter depth were found mainly from 2 m of the flow path onwards. This was most likely related to more efficient aerobic microbial degradation in the upper filter layer, enabled by oxygen input via helophyte roots and diffusion from the atmosphere. A similar effect was found by Omari et al. (2003) in a CW treating diesel oil, corroborating that removal of aerobically degradable organic contaminants is most efficient in the upper layer of HSSF CW. Volatilization plays a minor role as discussed later. To evaluate the annual mean concentration decrease, the 4 m sampling point was considered as the system outflow, because the OWC exhibited highly different conditions. In 0.3 m depth, high annual mean [median] concentration decreases of 76% [80%] and 68% [69%] were observed for MCB and 1,4-DCB, respectively. Efficiencies decreased with filter depth, reaching only 38% [35%] and 44% [42%] at 0.5 m depth. The impact of filter depth on overall concentration decrease was less pronounced for 1,4-DCB than for MCB; removal was higher for MCB in the upper filter layer but higher for 1,4-DCB in the deepest layer. The result can be

explained by the higher potential of 1,4-DCB for being reductively dechlorinated under anaerobic conditions (Adrian and Görisch, 2002).

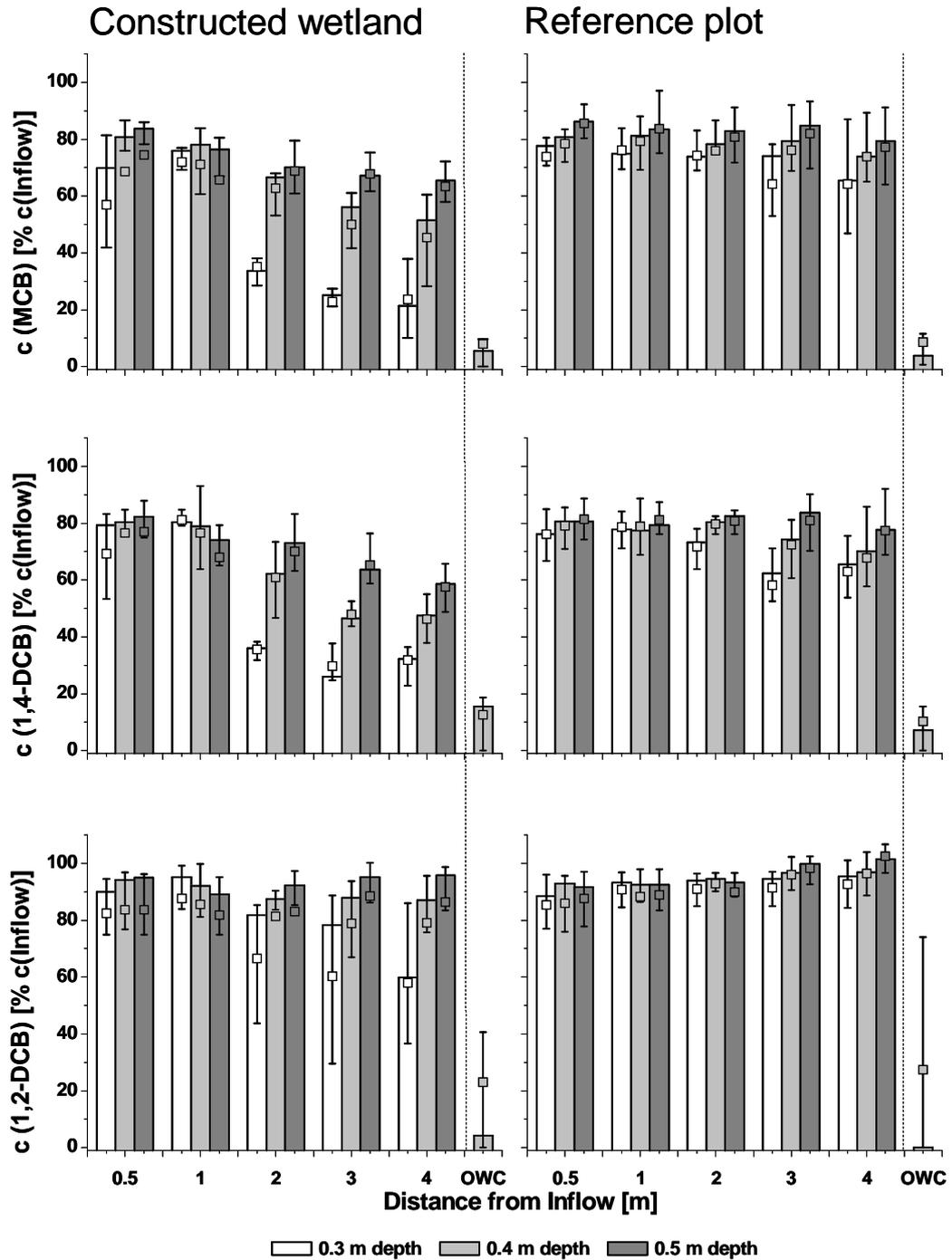


Figure 1: Annual median and mean MCB, 1,2-DCB and 1,4-DCB concentrations (in % of inflow concentrations) in the CW and UR filters in 0.5, 1, 2, 3 and 4 m distance from the inflow at 0.3, 0.4 and 0.5 m depths, and in the OWCs ($n_{CW} = 14$, $n_{UR} = 15$, November 2004 to October 2005). Columns represent the medians, error bars the 25th and 75th percentiles, and squares the means of the annual data sets.

The lower Henry's Law constant of 1,4-DCB, resulting in a lower volatilization (K_{AW} 1,4-DCB = 0.065 L L⁻¹; K_{AW} MCB = 0.15 L L⁻¹, van Agteren et al., 1998), intensifies this effect.

In the UR, annual mean [median] concentration decrease between the inflow and 0.5 m was 27% [23%], 22% [19%] and 15% [14%] for MCB, and 5% [5%], 0% [0%] and 0% [0%] for 1,4-DCB at 0.3 m, 0.4 m and 0.5 m depth, respectively. At 0.3 and 0.4 m depth, only a slight decrease of annual mean residual concentrations was observed along the flow path thereafter, and no depletion was evident at 0.5 m depth. Depth profiles were less pronounced in the UR, indicating the prevalence of more uniform conditions in the absence of *Phragmites*.

In the CW, a decrease of annual mean [median] 1,2-DCB concentrations was detectable between the inflow and 4 m only at 0.3 and 0.4 m depth, accounting for 42% [41%] and 20% [14%], respectively, while no decline was observed at 0.5 m depth (Fig. 1). The high variability of the 0.3 m depth annual data set indicates a strong seasonal impact on 1,2-DCB removal in the upper filter layer. Due to the recalcitrance of 1,2-DCB towards biological degradation, plant uptake and (phyto)volatilization (which are mainly active in summer) are suggested to be major removal processes. In the UR, a slight increase in concentrations (associated with a low variability of the annual data set) over the flow path was detected in the annual mean and median. Since the formation of the compound is unlikely, this effect can only be explained by water loss from the system (see Section 3.4).

The results suggest a strong impact of the plants on residual concentrations and the removal process. Wilcoxon-Mann-Whitney matched-pairs tests, which have been suggested by other authors for this purpose (Dornelas et al., 2009), were used to assess the significance of differences between the CW and UR. Tests of the 4 m residual concentration [%] data sets of one year showed highly significant differences between the CW and UR for all investigated depths for MCB (U = 23 (0.3 m), 20 (0.4 m), 53 (0.5 m)), 1,4-DCB (U = 20 (0.3 m), 28 (0.4 m), 29 (0.5 m)) and 1,2-DCB (U = 28 (0.3 m), 46 (0.4 m), 47 (0.5 m)) ($n_{CW} = 14$, $n_{UR} = 15$; $P < 0.025$ two-tailed). In a study with trichloroethene, removal enhancement by plants was attributed to enhanced microbial degradation in the rhizosphere (Walton and Anderson, 1990). Plants are well

known to increase removal of COD and BOD (Wang et al., 2009; von Sperling et al., 2010) as well as organic chemicals (Omari et al., 2003; Grove and Stein, 2005; Tee et al., 2009) in CW. An enhancing effect of *Phragmites* on MCB mineralization (27 vs. 16% of added MCB) was also shown by MacLeod et al. (1999) in a wetland microcosm. Plant uptake, metabolization and phytovolatilization may also contribute to creating the observed differences in removal, as reported for chlorinated ethenes (Newman et al., 1997; Burken and Schnoor, 1998).

In both the CW and the UR, most of the residual CBs concentrations were removed in the OWC, with the highest remaining concentrations of 1,2-DCB (20-30%). Due to direct contact between water and atmosphere, oxygen diffusion into the OWC was higher than into the filters supporting aerobic microbial degradation, and volatilization was enhanced, too (see Section 3.4).

3.2 Annual patterns of CBs concentration decrease

In particular in the temperate climate zone, the effects of seasonal variations on removal efficiency are crucial for CW performance (Williams, 2002). Seasonal climatic variations influence CB removal directly, i.e. temperature affecting microbial degradation and gaseous emissions, and indirectly by causing fluctuating plant activity levels (Wittgren and Mæhlum, 1997; Kadlec and Reddy, 2001). To assess seasonal variations, overall mean concentration decreases (mean of three monitored depths) of MCB, 1,4-DCB and 1,2-DCB between the inflow and 4 m points of the CW and UR are shown over the period of one year (Fig. 2).

MCB and 1,4-DCB overall mean concentration decrease in the CW was highest in winter, decreasing from 55-80% in December-April to 40-60% in May-September (Fig. 2). In particular for MCB, a distinct downward trend was observed between February and June, and mean concentration decrease stabilized on a low level in the later summer. Concentration decrease increased to 60-65% in October, when soil temperature and plant activity declined. An explanation for this pattern is the higher evapotranspiration from the CW in summer, leading to a concentration of dissolved substances. This effect as has been repeatedly observed in treatment wetlands (Heritage et al., 1995, Lim et al., 2001). In addition, the increase of easily oxidizable organic carbon availability from plant exudates and decomposition of biomass over the course

of the growth period (Briggs et al., 1993; Mann and Wetzel, 1995) may restrain microbial CB oxidation by competition. Oxygen availability in winter is positively affected by higher oxygen solubility at lower water temperatures (e.g. 13.8 mg L⁻¹ at 0.5°C (T_{water} Dec-Feb) and 8.6 mg L⁻¹ at 21°C (T_{water} July)). This may compensate for the lack of active oxygen transport to the rhizosphere during winter (Brix et al., 1996), together with convective flow of air through dead culms driven by Venturi effects (Armstrong et al., 1992). In addition, seasonal dormancy of wetland plants in the winter might reduce the internal plant oxygen demand enough to enhance oxygen availability in the rhizosphere and increase the redox potential and aerobic microbial activity (Stein et al., 2007). In agreement with our results, Kadlec and Reddy (2001) attributed a trend of lower BOD load removal in spring and summer in a number of surface flow treatment wetlands to differences in oxygen transfer.

In the UR system, MCB and 1,4-DCB mean concentration decreases showed a minimum of 0-30% from March to the beginning of June, and reached maxima of 35 to 50% from January to February and of up to 70% from July to August (Fig. 2). Higher concentration decrease in the later summer can be explained by the known positive temperature effect on microbial CB degradation and gaseous emissions. The increase in water loss and in availability of organic carbon during summer was much less pronounced than in the CW, thus playing only a minor role. Low efficiencies in spring and early summer may be due to seasonal changes in the microbial community (Yin et al., 2009), causing the observed lag phase in spring.

The overall 1,2-DCB concentration decrease in the CW showed a maximum of only 15-25% in December-March and very low efficiency in April-June. These findings confirm the lower degradability of 1,2-DCB, compared to 1,4-DCB and MCB, by the indigenous microbial community, as found already by Dermietzel and Vieth (2002) in investigations with Bitterfeld groundwater and aquifer material. It is attributed to sterical and electronical effects associated with vicinal chlorine substituents, being less favourable for enzymatic attack. The sudden increase in concentration depletion between June and July was probably caused in part by an abrupt drop in inflow concentrations due to changes in groundwater flow. In the UR, there was mostly no 1,2-DCB concentration decrease in spring and summer, and only around 10-15% in January-February as well as in October.

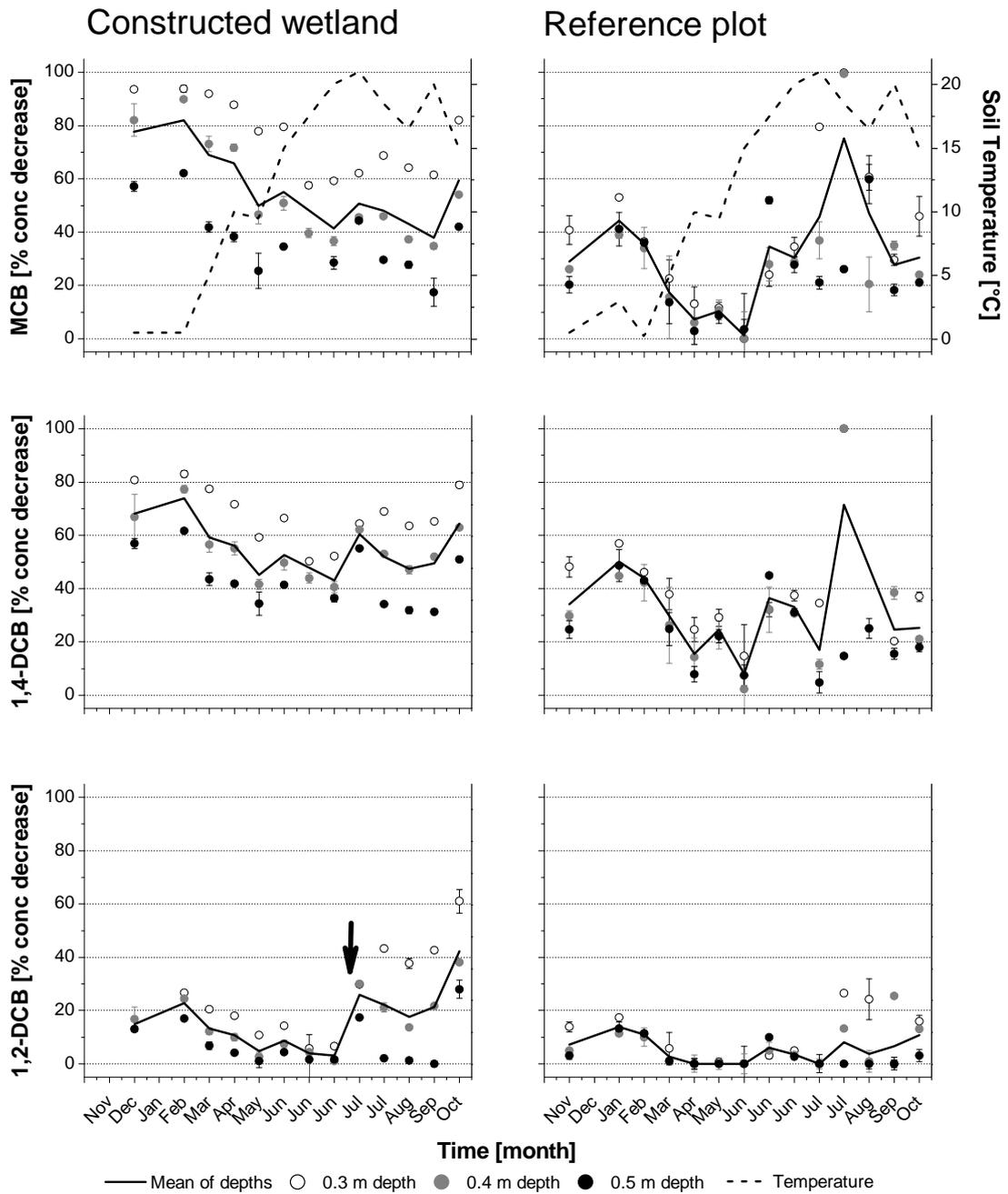


Figure 2: Seasonal variations of MCB, 1,4-DCB and 1,2-DCB concentration decrease between the inflow and 4 m of the flow path at 0.3, 0.4 and 0.5 m depths, and as a mean of concentrations at the three investigated depths, as well as the soil temperature (right Y-axis) in the CW and UR (November 2004 to October 2005). Concentration increase is reported as 0% decrease. Error bars denote standard deviations of replicate determinations. The arrow indicates a sudden decrease in 1,2-DCB inflow concentrations.

3.3 Load removal efficiency

In order to evaluate the CW treatment performance, water losses need to be accounted for when calculating actual load removal efficiencies (Bojcevska and Tonderski, 2007). Thus, water balances were recorded and overall load removals (mean of three investigated depths) were assessed for the CW and UR in June and July 2005, the period when the highest water losses occurred. The CW displayed evapotranspiration rates of up to 59%, while the UR showed a maximum water loss of 22%.

Load removals of MCB and 1,4-DCB in June and July accounted for 59-65% (262-358 mg m⁻² d⁻¹) and 59-69% (4.0-5.1 mg m⁻² d⁻¹) of inflow loads, respectively, and to the best of our knowledge have here been empirically determined for the first time at the pilot-scale. 1,2-DCB removal efficiency was 29-42% (0.6-2.1 mg m⁻² d⁻¹). Thus, the mere consideration of concentration decrease underestimated summertime MCB and 1,4-DCB removal in the CW by 9-23% and 1,2-DCB removal by 17-38% (in reference to the inflow load), underlining the impact of plant transpiration on residual CB concentrations. Hence, load removal was more similar to concentration removal in the UR, with underestimation of removal efficiencies by only 4-11% for all investigated compounds. Load removal was 34-246 mg m⁻² d⁻¹ for MCB, 1.2-3.3 mg m⁻² d⁻¹ for 1,4-DCB and 0-0.8 mg m⁻² d⁻¹ for 1,2-DCB.

3.4 Soil-borne gaseous MCB emissions

Calculation of MCB volatilization fluxes indicated the highest volatilization in the inflow region, where water concentrations were highest, and a decrease of volatilization along the flow path in the CW and UR (Fig. 3). Direct contact between the water phase and atmosphere in the OWC resulted in increased emissions. The high temporal variability of MCB volatilization fluxes is striking and was related to the corresponding weather conditions. Therefore, the data sets should be considered separately. For example, after the heavy rainfall on July 5th 2005, MCB volatilization fluxes were considerably lower than on sunny days (June 27th, July 13th). This was presumably caused by the increase in soil water content, which lowers the effective soil gas diffusion through the vadose zone (Choi and Smith, 2005), and the refreshed retention capacity of the system (adsorption on soil organic matter, solution in uncontaminated rainwater, rising water level). In general, contaminant volatilization from the CW

tended to be more consistent than from the UR, where extreme fluctuations were observed, probably due to buffering of environmental impacts (e.g. surface temperature) by the plant cover (Brix, 1997).

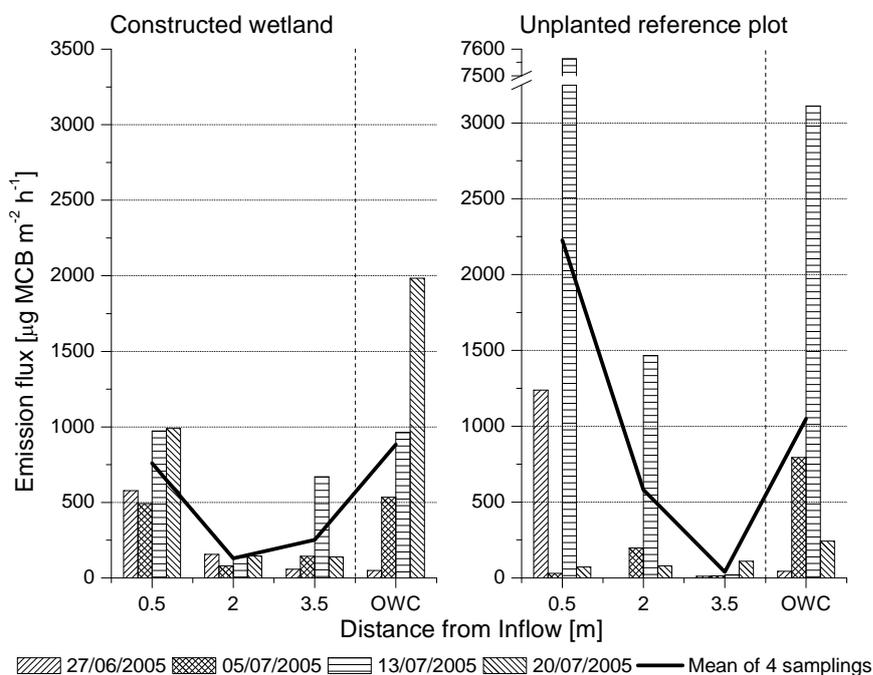


Figure 3: MCB volatilization fluxes from the CW and UR filters, plotted against the distance from the inflow, as well as from the OWCs (4 sampling data sets in June and July 2005). Hypothetical complete emission of the average MCB eliminated daily between the inflow and 4 m in June and July would result in volatilization rates of $13116 \pm 1707 \mu\text{g m}^{-2} \text{h}^{-1}$ and $7098 \pm 3890 \mu\text{g m}^{-2} \text{h}^{-1}$ for the CW and UR, respectively.

Extrapolating the volatilization fluxes over the filter area (4 m^2 ; 1-4 m of the flow path) resulted in MCB emission of 22-54 mg d^{-1} from the CW. This implies a share of emission in overall removal of only 2-4%, showing that emission is a subordinate elimination pathway for MCB in the HSSF CW. These results are contradictory to findings of Keefe et al. (2004) who attributed the predominant part of VOC (1,4-DCB and MCB, among others) removal from a CW to volatilization. However, their CW was partly a surface flow system with enhanced volatilization potential, and the conclusions were drawn exclusively on the basis of model simulations. The presented findings are also in disagreement with the outcome of microcosm experiments where volatilization was the major removal process for MCB (McLeod et al., 1999). On the other hand, Reiche et al. (2010) reported a benzene volatilization of only 2% of the overall removal in summer for a HSSF CW similar to the one used in this study, which is comparable to our data.

The application of additional surface layers was tested successfully in simple field experiments. Bark mulch and granulated activated carbon layers (1-3 cm) were suitable to extensively reduce gaseous emissions of MCB from the wetland surfaces (Appendix A).

3.5 Assessment of removal processes

The results indicate a clear difference between the MCB removal capacities of the CW and the UR. Thus, removal processes were assessed by applying model approaches for MCB as the main contaminant. Due to the changing conditions over space and time, all values are approximate. In order to simplify the approach, the MCB concentration in the systems was generally assumed to be 20 mg L⁻¹ (the max. inflow concentration in June/July). Two hypotheses about the role of plants were tested: I. Plants have only an indirect effect, by changing the conditions in the system and stimulating microbial degradation (by root oxygen release etc.). II. Plants also directly take up and remove MCB (by translocation or degradation).

Reductive dechlorination is a potential anaerobic microbial MCB removal mechanism (Nowak et al., 1996) but is mostly slow and inefficient (Adrian and Görisch, 2002) and was therefore not considered to contribute significantly to the overall removal in the presented model calculations. Thus, oxygen deficiency is the major limitation for microbial degradation. For mineralization of 1 g MCB, about 2 g O₂ are required. Oxygen can enter the system by various pathways (Weishaar et al., 2009): by diffusion from air across the water surface; by transport of air through the aerenchym of *Phragmites* plants; by water level fluctuations; with inflowing water; and with rainwater. The overall oxygen input was evaluated and compared for the CW and UR.

The calculated oxygen concentration profile caused by diffusive oxygen transfer into the filters after 2 days is shown in Fig. 4. Only the top 4-5 cm of the saturated zone are supplied with oxygen by diffusion, and consumption of oxygen does not considerably change this calculated profile. After 5 days, the summed amount of oxygen that entered the wetland by diffusive oxygen flux was 0.14 g m⁻² (28 mg m⁻² d⁻¹). The flux can be compared to the measured surface volatilization of MCB from the system, based on the fact that MCB and oxygen have to overcome the same resistances. The calculated value for D_{MCB} is $1.06 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$. The gradient of oxygen concentration between the water

surface (10 mg L^{-1}) and deeper wetland (0 mg L^{-1}) is half that of MCB (water surface 20 mg L^{-1} and air 0 mg L^{-1}). When entered into the flux equation $F = ((D \times \tau) / z) \times (C_{out} - C_{in})$, it turns out that the resulting fluxes are approximately equal for MCB and O_2 . Measured fluxes of MCB from CW to atmosphere were up to $24 \text{ mg m}^{-2} \text{ d}^{-1}$ (Fig. 3), which is in good agreement with the calculated diffusive oxygen flux ($28 \text{ mg m}^{-2} \text{ d}^{-1}$).

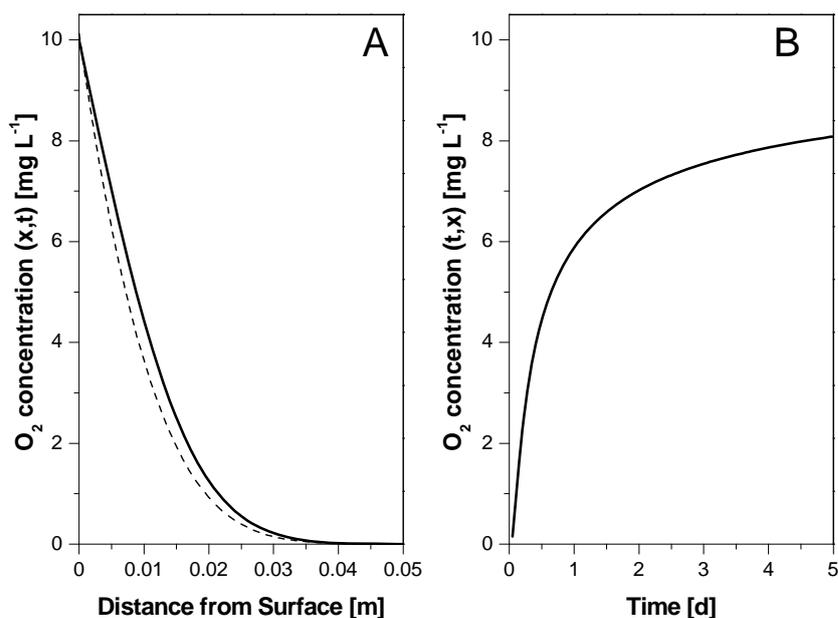


Figure 4: Calculated oxygen concentration profile due to diffusion into the saturated zone of the CW. A) Oxygen concentration versus distance from the CW surface, $t = 2 \text{ d}$. Solid line: no oxygen consumption. Dotted line: with oxygen consumption, $t_{1/2}(\text{O}_2) = 3 \text{ d}$. B) Oxygen concentration versus time, $x = 5 \text{ mm}$.

Root oxygen release of *Phragmites* has been estimated in several studies, with reported oxygen fluxes in the range of 1 to $12 \text{ g m}^{-2} \text{ d}^{-1}$ (Lawson, 1985; Armstrong et al., 1990; Gries et al., 1990; Brix et al., 1996). The flux is advective and driven by pressure difference, thermal expansion of gas, and wind. In addition, air (and thus oxygen) can be transported into the systems by diurnal water table fluctuations (Weishaar et al., 2009) which were measured in July-September. For the CW, fluctuations were $\pm 9.5 \text{ cm}$ per day, for the UR only about $\pm 1.5 \text{ cm}$. With a porosity of the filter material of 0.35, and each m^3 of air containing 300 g oxygen, these fluctuations could result in introduction of maximum 10 g (CW) and 1.6 g (UR) $\text{O}_2 \text{ m}^{-2} \text{ d}^{-1}$ into the systems. Oxygen input with the essentially anaerobic groundwater feed was not observed. The average rainfall in summer (June/July) was $2.1 \text{ L m}^{-2} \text{ d}^{-1}$. Assuming that rainwater is

oxygen-saturated (8 to 10 mg L⁻¹), the input of oxygen with rain was up to 21 mg O₂ m⁻² d⁻¹. The overall oxygen input into the systems is summarized in Tab. 1. The largest oxygen input is caused by water level fluctuations and plant root release in the CW. In the UR, oxygen input is generally much lower than in the CW.

Table 1: O₂ input through the various pathways into the CW and UR (mg m⁻² d⁻¹) in June/July.

	O ₂ input [mg m ⁻² d ⁻¹]	
	Constructed wetland	Reference plot
Diffusion	28	28
Water level fluctuations	10 000	1 600
Rain	21	21
Aerenchym of plants	1000 - 12 000	-
Sum	11 049 - 22 049	1649

Beside oxidation, volatilization and plant uptake were taken into account as MCB removal processes. Volatilization from the soil surface has been measured in this study. *Phragmites* vegetation was considered to contribute to MCB removal via two pathways, namely MCB flux through the aerenchym and MCB translocation into the plants with the transpiration stream.

Simultaneous to the flux of oxygen into the root zone, an escape of CO₂ and methane through the aerenchym takes place (Brix et al., 1996). Thus, an escape of MCB is also likely. Applying the MCB air-water partition coefficient of 0.15 L L⁻¹ (van Agteren et al., 1998), the gas phase MCB concentration in the root zone is 3 mg L⁻¹. This is a factor of 100 lower than the oxygen concentration. The MCB flux through the aerenchym then accounts for 10 to 120 mg m⁻² d⁻¹. In order to estimate the MCB translocation by *Phragmites australis*, a grass species, the transpiration stream concentration factor (TSCF) was calculated considering empirical relationships found by Burken and Schnoor (1998) (for priority pollutants and VOC / *Populus deltoides*) and Briggs et al. (1982) (for herbicides / *Hordeum vulgare*), and a model introduced by Trapp (2007) (*Malus domestica*). For MCB with a log K_{OW} of 2.98 (van Agteren et al., 1998), all three methods give similar results for TSCF, in the range of 0.43-0.69. Plant transpiration was estimated as the difference between water loss from the CW and UR (15-38 L m⁻² d⁻¹ in June/July). Thus, 130-525 mg MCB m⁻² d⁻¹ was extracted from the

CW by plant-driven translocation. For this assessment it is not relevant whether this fraction is phytovolatilized, remains in the leaves, or is metabolized.

Table 2: Potential MCB removal from the CW and UR through the various pathways and comparison to measured MCB load removal in June/July.

	MCB [$\text{mg m}^{-2} \text{d}^{-1}$]	
	Constructed wetland	Reference plot
Oxidation of MCB	5 525 - 11 025	825
Translocation by plants	130 - 525	-
Escape via aerenchym	10 - 120	-
Emission from filter surface	6 - 14	2 - 70
Sum removal	5671 - 11 684	827 - 895
Measured removal	262 - 358	34 - 246

The uptake is generally overestimated, since MCB concentrations in the filter were mostly lower than 20 mg L^{-1} . Furthermore, oxidation of MCB in the rhizosphere will reduce plant uptake and translocation.

All potential MCB removal pathways are summarized in Tab. 2. Oxidation provides the highest potential for MCB removal, followed by plant uptake. As discussed above, volatilization from the filter surface is not significant. However, the observed overall MCB removal is much smaller than the potential removal, which is due to the fact that MCB is not the only oxygen-consuming compound in the CW. In summer, COD was 110-180 and 70-160 mg L^{-1} in the CW and UR, respectively, while inflow COD was only 31 mg L^{-1} . CW not only remove but also produce new carbon load in the form of dissolved organic matter (Wei et al., 2009) from plant exudates and decay of plant material as well as soluble microbial products (Wei et al., 2009). These organic compounds may lead to a considerable additional oxygen demand and are probably more readily degradable than MCB. Soluble microbial products provide the majority of COD in the effluents of biological treatment systems (Barker and Stuckey, 1999), also explaining COD production in the UR.

Finally, both hypotheses on the effect of the plants can explain the different removal capacities of the CW and the UR. There is much higher oxygen input into the CW, both due to stronger water level fluctuations and to plant root release, but also translocation

by plants and escape via the aerenchym could contribute to creating the observed difference.

4. Conclusions

The presented study shows the long-term efficiency of HSSF CW for the removal of MCB and 1,4-DCB from groundwater in the temperate climate zone. Implications for system layout can be derived from the results, suggesting that shallow systems will be more efficient for the removal of CBs. The technology proved to be a promising approach to protecting rivers and surface water bodies against the impact of CB-contaminated groundwater. Considerable annual mean concentration decrease of MCB and 1,4-DCB was observed, depending on filter depth and distance from the inflow. Seasonal variations in concentration decrease occurred for all compounds, underlining the importance of considering annual means for the evaluation of system performance. MCB and 1,4-DCB concentration decrease tended to be more pronounced during winter. The calculation of load removals revealed high removal efficiencies for both compounds also during summer if water balances are taken into account. In contrast, the elimination of 1,2-DCB in the CW was generally less efficient. Soil-borne volatilization was only a subordinate elimination process for MCB and could be abated by simple bark mulch and activated carbon covers. The enhancing effect of plants on CB removal was shown to be highly significant. Model calculations showed the MCB removal potential to be far in excess of the actual removal, particularly in the CW where oxygen input should be sufficient for complete degradation of MCB. Oxygen limitations may be caused by the presence of other easily degradable carbon compounds, competing with the CBs for the electron acceptor. The removal potential was much lower in the UR, which is in accordance with the actual data.

Appendix A. Supplementary material: Testing of simple emission reduction layers

Application of artificial surface layers (bark mulch and activated carbon) was tested in basic field experiments on the reference plot as a potential possibility to further reduce direct volatilization from the soil.

Materials and Methods

The reduction of emissions was tested by covering areas of 0.4 m×0.4 m of the unplanted system with adsorptive layers of bark mulch (0.03 m height) and activated carbon (0.01 m height), using an uncovered area as reference. Starting directly after placement of the layers, soil gas emissions from the covered and reference areas were monitored during periods of 64 (bark mulch) and 140 h (activated carbon). The flow through test chambers described in chapter 2.3 and a vacuum pump (flow rate 10 L h⁻¹) were used. A branch current (3.6 L h⁻¹) downstream the test chamber was used for continuous sampling and analysis with an on site thermo-desorption GC/FID system (Airmo BTX HC 1000, AAA Technologies, Essen, Germany). Gas phase concentrations were monitored three times for periods of 2-2.5 h. The sample volume was 0.36 L. Thermodesorption was carried out at 350°C (360 s). Chromatographic separation was performed on a DB-642 column (J&W Scientific, Folsom, USA) (10.3 m×0.2 mm×1.12 µm) with the following temperature program: 60°C (0.5 min), 25 K min⁻¹ to 160°C (1.5 min). The FID temperature was 150°C.

Results and Discussion

Selected results of the emission reduction tests (Table A.1) underline the ability of bark mulch and activated carbon to extensively reduce gaseous emissions of MCB from wetland surfaces. Emission reduction was greater than 95% after 65 and 135 h for the bark mulch and activated carbon layer, respectively. Taking into account the short period of exposure, the emission reduction effect is probably mostly due to sorption.

Bark mulch and activated carbon cover layers showed promising potential to avoid MCB emissions, if needed. In cases where VOC emission problems are expected (impacts on sensitive recipients, particularly toxic/carcinogenic contaminants), the described simple cover layers could represent a practicable remediation strategy. However, long-term evaluation should be carried out before this kind of layers is implemented in CW applications.

Table A.1: Reduction of MCB emission from the unplanted reference plot surface by artificial layers of bark mulch (b.m.) and activated carbon (a.c.) in comparison to an uncovered reference area (u.r.).

0.03 m bark mulch layer				0.01 m activated carbon layer			
time	Emission	Emission	reduction	time	Emission	Emission	reduction
	b.m.	u.r.			a.c.	u.r.	
[h]	[$\mu\text{g m}^{-2} \text{h}^{-1}$]	[$\mu\text{g m}^{-2} \text{h}^{-1}$]	[%]	[h]	[$\mu\text{g m}^{-2} \text{h}^{-1}$]	[$\mu\text{g m}^{-2} \text{h}^{-1}$]	[%]
40	7.0	460	98.5	90	24.0	1790	98.7
65	6.1	1680	99.7	135	13.3	364	96.4

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2.3 Assessment of *in situ* biodegradation of monochlorobenzene in contaminated groundwater treated in a constructed wetland

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Abstract

The degradation of monochlorobenzene (MCB) was assessed in a constructed wetland (CW) treating MCB contaminated groundwater using a detailed geochemical characterization, stable isotope composition analysis and *in situ* microcosm experiments. A correlation between ferrous iron mobilization, decreasing MCB concentration and a change in carbon isotope composition was visible at increasing distance from the inflow point, indicating biodegradation of MCB in the wetland. Additionally, *in situ* microcosm systems loaded with ¹³C-labelled MCB were deployed for the first time in sediments to investigate the biotransformation of MCB. Incorporation of ¹³C-labelled carbon derived from the MCB into bacterial fatty acids substantiated *in situ* degradation of MCB. The detection of ¹³C-labelled benzene indicated reductive dehalogenation of MCB. This integrated approach indicated the natural attenuation of the MCB in a wetland system. Further investigations are required to document and optimise the *in situ* biodegradation of MCB in constructed and natural wetland systems treating contaminated groundwater.

Keywords: Constructed wetland; Monochlorobenzene; Biodegradation; *In situ* microcosms; Isotope fractionation.

Introduction

Monochlorobenzene (MCB) is encountered worldwide as a groundwater pollutant, and persists in the essentially anaerobic aquifer at the large-scale contaminated site in Bitterfeld, Germany (Heidrich et al., 2004; Wycisk et al., 2004). In recent years, interest has grown in using phytoremediation processes for the elimination of recalcitrant organic substances from waste- and groundwater (Macek et al., 1998; Schnoor et al., 1995; Shimp et al., 1993; Trapp, 2000) including chloroaromatics (Gilbert and Crowley,

1997). Wetland systems represent an effective and inexpensive option to treat groundwater polluted with organic compounds by taking advantage of the geochemical and biological processes (e.g. Baker, 1998; Dunbabin and Bowmer, 1992; Gumbrecht, 1993). Indeed, rapid degradation of chlorinated organics has been observed in the rhizosphere (Anderson and Walton, 1995; Jordahl et al., 1997; Lorah and Olsen, 1999; Pardue et al., 1996).

While aerobic degradation of MCB has been well studied (e.g. Van Agteren et al., 1998), only some evidence for MCB transformation under anoxic conditions has been presented yet and the degradation pathway is unknown (Kaschl et al., 2005; Liang and Gribic-Galic, 1990; Nowak et al., 1996). Moreover, only very few studies focus on the anaerobic microbial transformation of MCB under field conditions. Recently, indications of anaerobic MCB degradation taking place in the Bitterfeld contaminated aquifer were provided on the basis of isotope fractionation patterns (Kaschl et al., 2005). Kinetic isotope fractionation processes have been employed to demonstrate the biological transformation of various contaminants (Richnow et al., 2003a,b; Sherwood Lollar et al., 2001; Song et al., 2002). A substantial enrichment of ^{13}C in the non-degraded fraction in the course of a contaminant plume indicates microbial degradation, as dilution and sorption do not affect the isotope composition of contaminants significantly (Harrington et al., 1996; Schüth et al., 2003; Slater et al., 2000). Combining stable isotope composition analysis with information obtained from simple *in situ* microcosm experiments (BACTRAPs) using isotope labelled substrate may provide a suitable approach to qualitatively support *in situ* biotransformation and to monitor spatial and temporal natural attenuation processes. Previously, BACTRAPs were exclusively installed in groundwater monitoring wells (Geyer et al., 2005; Kästner et al., 2006; Stelzer et al., 2006) and were deployed in sediment for the first time in the framework of this study.

For the assessment of *in situ* biodegradation in constructed wetlands (CW) and wetlands treating contaminated groundwater, it may be necessary to use several methods providing more than one line of evidence. A combined approach may be of additional benefit in particular when systems are complex, possess several compartments and convincing evidence is required. Moreover, a better understanding of the controlling geochemical processes in wetland systems is necessary to reliably predict the retention

and transformation of contaminants. In this study, we evaluated the natural attenuation of MCB in a CW treating MCB contaminated groundwater using a detailed geochemical characterization, stable isotope composition analysis and *in situ* microcosm experiments. The spatial variations of geochemical parameter were studied with the help of multivariate statistics to investigate the main processes controlling the wetland system. The concentration and carbon stable isotope composition of MCB was analysed to monitor the *in situ* contaminant degradation and *in situ* microcosms were used to provide qualitative evidences of *in situ* biotransformation of MCB.

2. Materials and methods

2.1. Design and characteristics of the wetland

The pilot-scale CW at the experimental site in Bitterfeld was set up in December 2002. The horizontal subsurface flow wetland consisted of a stainless steel tank divided into two segments. Each segment was 6 m × 1 m and was filled to an average depth of 0.5 m with autochthonous quaternary aquifer material consisting predominantly of Bitterfeld mica sand (25%) and gravel (67%), which was embedded in lignite (10%) with an effective porosity of 28% (Vogt et al., 2002). The hydrogeochemical characteristics of the study site and the filling material originating from the local aquifer are described in previous studies (e.g. Vogt et al., 2002; Weiss et al., 2001). One of the segments was planted with common reed (*Phragmites australis*, Cav.), whereas the other side was left unplanted. In both segments, a 1 m long open water pond at the outflow side allows direct contact between the atmosphere and the water surface. The water level was maintained at approximately 10 cm below the surface of the wetland. The groundwater was collected from the MCB contaminated aquifer and conveyed from 16 to 22 m depth directly to the wetland. Both segments were operated in a flow-through mode at a flow rate of 4.7 L h⁻¹, corresponding to a retention time of 6 days.

2.2. Sampling

In the period from April to September 2005, pore water samples were collected five times (d0, d53, d66, d143, d172) in order to investigate the geochemical processes and the contaminant behaviour in the wetland system. The pore water was collected in both segments along a transect from the inflow up to the outflow of the wetland, at

respectively 0 (inflow valves), 0.5, 1, 2, 3 and 4 m using a stainless steel lance. At each sampling point, three depths, 30, 40 and 50 cm were systematically investigated. Water samples were also collected at the ponds (6 m).

In addition, to assess the *in situ* biodegradation using isotope composition analysis, pore water samples from both segments were collected at day 0 and day 53, at 0, 1 and 3.5 m along the wetland at 0.5 m depth, as well as in the ponds.

2.3. Physico-chemical and geochemical parameters of the pore water samples

The redox potential was measured on-line in the field using a SenTix ORP electrode (PT 1000, PreSens, Regensburg, Germany). The temperature was determined by a temperature sensor (PT 1000, PreSens, Regensburg, Germany). Samples for the pH and ions analysis were filtered through a syringe filter (5 μm , Ministart NML, Sartorius) for particle removal. The pH value was measured with a SenTix41 electrode with pH 537 Microprocessor (WTW, Weilheim, Germany). Oxygen measurement was carried out using an optical oxygen trace sensor system (oxygen meter Fibox-3-trace and flow-through cell type sensor FTC-TOS7) with automatic temperature compensation (temperature sensor PT 1000) (PreSens, Regensburg, Germany). For the analysis of Mn(II), total iron and Fe(II), hydrochloric acid was added and samples were diluted with deionised water (1:10, v:v). Total iron and Mn(II) concentrations were analysed by atomic emission spectrometry with ICP excitation and CCD detection (Spectro Ciros Vision CCD, Spectro Analytical Instruments, Kleve, Germany). Photometric analysis of ferrous iron was carried out at 562 nm after derivatisation with ferrocine using a Cadas 100 photometer (Hach Lange, Düsseldorf, Germany). Chloride and sulphate concentrations were determined by ion chromatography (DX 500) with conductivity detection (CD 20) and an IonPacAG11 (4 \times 250 mm) column (Dionex Corporation, Sunnyvale, USA). For the analysis of sulphide concentrations samples were spiked with sulphide anti-oxidant buffer (200 ml L⁻¹ 10 M NaOH, 35 g L⁻¹ ascorbic acid, 67 g L⁻¹ EDTA) (1:1, v:v) and measured with an ion selective Ag/S 500 electrode and reference electrode R 503 (WTW, Weilheim, Germany).

2.4. Analysis of benzene, MCB and metabolites

Pore water samples for the analysis of benzene and MCB concentrations were collected in 20 mL glass flasks (Supelco, Bellefonte, USA), and sealed with PTFE-lined septa. Sodium azide solution was added to the samples to inhibit microbial activity. Benzene and MCB concentrations were quantified by automatic headspace gas chromatography using an HP 6890 gas chromatograph with flame ionisation detector (Agilent Technologies, Palo Alto, USA). For headspace analysis a volume of 1000 μL was injected at an injection temperature of 250°C with split 1:5 (measurements in duplicates). The chromatographic separation was achieved on an HP-1 capillary column (Agilent Technologies, Palo Alto, USA) (30 m \times 0.32 mm \times 5 μm) with the following oven temperature program: 45°C (1 min), 20 K min^{-1} to 200°C (2.5 min), 65 K min^{-1} to 250°C (1 min) and a detector temperature of 280°C.

For the determination of the carbon isotope composition of MCB, 1 L glass bottles (Schott, Mainz, Germany) containing NaOH pellets to prevent microbial growth were filled completely with groundwater, stored at 4°C and extracted within 24 h using 2 mL n-pentane as described previously (Richnow et al., 2003b). The analysis of volatile metabolites obtained in the *in situ* microcosm experiments was carried out using an HP 6890 gas chromatograph with HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, USA). Aliquots of 1 mL liquid samples were injected at a temperature of 280°C with split 1:40 and separated on a Zebron BPX-5 column (30 m \times 0.32 mm \times 0.25 μm) (Phenomenex, Torrance, USA). The following oven temperature program was applied: 40°C (2.5 min), 10 K min^{-1} to 70°C (0 min), 60 K min^{-1} to 280°C (4 min).

2.5. *In situ* microcosms (BACTRAPs)

2.5.1. Preparation of microcosms and derivatisation of fatty acids

The *in situ* microcosms were prepared as described previously (Stelzer et al., 2006). Different sets of *in situ* microcosm experiments were prepared. One set was loaded with [$^{13}\text{C}_6$]-labelled MCB (Cambridge Isotope Laboratories, Andover, USA) and another one with natural abundance MCB. A third set was kept unloaded to observe the background effects. The loading was done by vapor exposure under reduced pressure. The microcosms were put in small glass containers together with approximately 40 mg of

either labelled or non-labelled MCB per g Bio-Sep®. The gas phase was removed from the containers by a vacuum pump until the organics started boiling, and the outlet valve was then closed. Microcosms were held under these conditions for 48 h. The microcosms were deployed at 1.5, 2.5 and 4.5 m from the inflow in both planted and unplanted segments at 50 cm depth. The microcosms were collected after 6 weeks and fatty acid extraction was carried out according to Bligh and Dyer (1959). The derivatisation to obtain fatty acid methyl esters (FAME) was done according to Thiel et al. (2001). After evaporation to complete dryness and addition of heneicosanoic acid methyl ester (C21:0) as an internal standard the FAME fraction was dissolved in n-hexane for further identification, structural characterization and carbon isotope composition analysis.

2.5.2. GC-MS analysis

For identification and structural characterization of FAME, an HP 6890 gas chromatograph coupled with an HP 5973 quadrupole mass spectrometer (Agilent Technologies, Palo Alto, USA) was used. The FAME were separated on a Zebron BPX-5 column (30 m × 0.32 mm × 0.25 μm) (Phenomenex, Torrance, USA) with the following temperature program: 70°C (1 min), 20 K min⁻¹ to 130°C, 2 K min⁻¹ to 150°C (5 min), 2 K min⁻¹ to 165°C (5 min), 2 K min⁻¹ to 230°C, 20 K min⁻¹ to 300°C (5 min). FAME were identified by comparison with the retention time and mass spectra of an authentic standard mix (bacterial acid methyl esters mix, Sigma Aldrich, Germany) and quantified relatively to the internal standard.

2.6. *Isotopic composition analysis*

The carbon isotope composition of MCB and the FAME was measured with a gas chromatography-combustion-isotope ratio mass spectrometry system (GC-C-IRMS) consisting of a GC unit (HP 6890, Agilent Technologies, Palo Alto, USA), a combustion device (Finnigan MAT GC III, Thermo Finnigan, Bremen, Germany) with water-removal assembly (Nafion® membrane, 50 cm long, T = 0°C) and a mass spectrometer (Finnigan MAT 252; Thermo Finnigan, Bremen, Germany), as previously described (Richnow et al., 2003a). Helium was used as carrier gas at a flow rate of 1.5 mL min⁻¹.

Stable isotope samples were measured in triplicates and the analyses were carried out immediately after each sampling. Aliquots of 1 mL were injected at 250°C with split 1:10 to the GC-C-IRMS and separated on a capillary column (Zebron ZB-1, 60 m × 0.32 mm × 1 µm; Phenomenex, Torrance, USA). The following chromatographic conditions were applied: injector temperature 250°C, oven temperature program: 40°C (1 min), 4 K min⁻¹ to 150°C, 20 K min⁻¹ to 250°C (2 min). The carbon isotope composition is reported in the delta notation as δ¹³C values [‰] relative to Vienna Pee Dee Belemnite Standard (V-PDB, IAEA-Vienna) (Eq. 1) (Hoefs, 1997).

$$\delta^{13}\text{C}[\text{‰}] = \left(\frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{Sample}}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{Standard}}} - 1 \right) \times 1000 = \left(\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right) \times 1000 \quad (1)$$

Eq. (2) is applied to calculate the remaining substrate fraction (f_t) using the isotope fractionation factor (αC).

$$f_t = \frac{R_t}{R_0} \exp\left(\frac{1}{1/\alpha C - 1}\right) \quad (2)$$

The R_t and R_0 give the isotope composition of MCB at time t and zero. Eq. (3) is applied to calculate the percentage of biodegradation of the residual substrate fraction (B_t).

$$B[\text{‰}] = (1 - f_t) \times 100 \quad (3)$$

For the separation of the FAME fractions, a Restek RTX 5 column (60 m × 0.32 mm × 0.1 µm; Restek, Bellefonte, USA) was used with the same temperature program applied for the GC-MS-analysis of FAME. Aliquots of 1 mL were injected with split 1:5. The methylation of fatty acids for gas chromatographic analysis introduces an additional carbon atom into the structure of the fatty acid molecules which affects its isotopic composition. Therefore, the isotope signature of fatty acids (δ¹³C_{FA}) was corrected for the isotope effect upon derivatisation to FAME with methanol as described previously (Abraham et al., 1998; Abrajano et al., 1994; Goodmann and Brenna, 1992). The methanol used for the derivatisation had an isotope signature of -38.2‰.

2.7. Statistical analysis

Statistical analyses were carried out using the R Software (R, Version 2.1.1, 2005). Statistical significance of the difference in geochemical parameters as well as concentrations and isotopic compositions of MCB between the planted and unplanted segment was determined with the unpaired Wilcoxon or the Kruskal-Wallis rank sum tests. Correlation analyses were carried out using the Spearman rank sum coefficient. Principal component analysis (PCA) was used to analyse the relationship between the different samples with reference to their respective pore water parameters. The sampling location corresponds to the object and the chemical parameters to the descriptors (represented by the vectors) of the multivariate analysis. The PCA were scaled as correlation biplots.

3. Results

3.1. Characterization of pore water chemistry

3.1.1. Distribution of MCB and benzene

The MCB concentration was measured as a function of the distance from the inflow point in both the planted and unplanted segment (Table 1). The average concentration of MCB ranged from 14.4-17.7 mg L⁻¹ at the inflow down to 2.0-2.2 mg L⁻¹ in the ponds for the planted and the unplanted segment, respectively. No significant difference in MCB concentration among the three depths over the study period was generally observed ($p < 0.05$). Benzene was found in low concentration in both segments ($< 26 \mu\text{g L}^{-1}$), with generally higher concentration values in the unplanted segment (Table 1).

3.1.2. Pore water geochemistry

The evolution of oxygen, redox potential, manganese, sulphide, sulphate, ferrous iron and total iron were monitored along the flow path at three depths in order to characterise the geochemical conditions prevailing in the wetland system. The average values of the three depths investigated at each sampling point (0, 0.5, 1, 2, 3, 4 m from the inflow) were computed (Table 1). To investigate the differences between the sampling locations and to explore existing gradients, the data sets were analysed by principle component analysis, separately for the unplanted and the planted segments (Fig. 1).

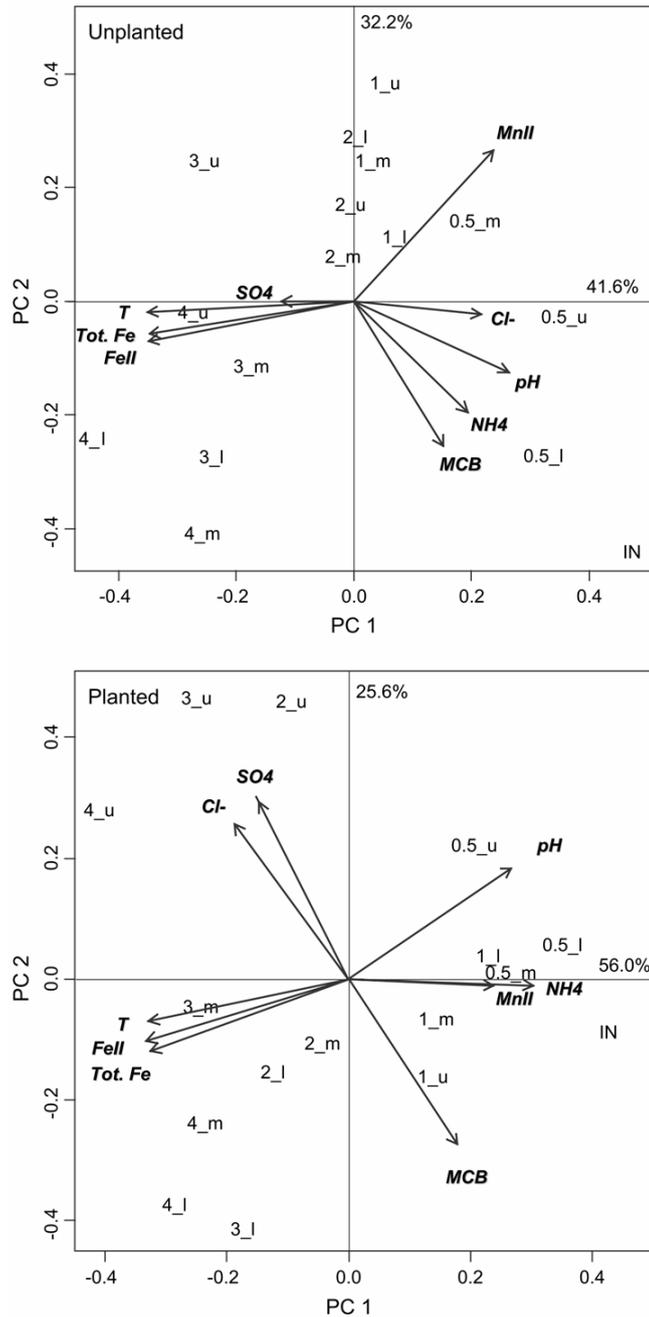


Figure 1: Ordination plot generated by principal component analysis representing the relationship between the sampling locations and the average geochemical parameters measured in both the planted (a) and the unplanted (b) experimental wetlands over the study period. Description vectors correspond to: $FeII$, ferrous iron; $Tot.Fe$, total iron ($Fe(II) + Fe(III)$); MCB , monochlorobenzene; $MnII$, manganese(II); NH_4 , ammonium; T , temperature. Objects correspond to: 0.5-4, distance [m] from the inflow point; u, upper depth (30 cm); m, medium depth (40 cm); l, lower depth (50 cm). Values on the axes indicate % of total variation explained by the axes (PC 1, principal component axis 1; PC 2, principal component axis 2).

Table 1: Geochemical characterization, MCB and benzene concentrations of samples collected from (a) unplanted segment and (b) planted segment. Values represent the depth profiles average in the soil compartments (0.5-4 m) and average (0 m and pond) over the study period (13/04/05 to 29/09/05). Standard deviation is indicated in parentheses.

Sampling point [m from inflow]	pH	Temp. °C	O ₂ [mg L ⁻¹]	Eh [mV]	Fe(II) [mg L ⁻¹]	Sulphate [mg L ⁻¹]	Cl ⁻ [mg L ⁻¹]	Benzene [µg L ⁻¹]	MCB [mg L ⁻¹]
(a)									
0	7.1 (3.6) ^d	14.8 (8.5) ^d	0.04 (0.02) ^c	38 (37) ^d	0.18 (0.23) ^c	968.6 (401.8) ^c	280.4 (116.3) ^c	25.6 (3.6) ^c	17.73 (7.98) ^c
0.5	7.0 (2.2) ^b	16.3 (5.4) ^a	0.05 (0.04) ^b	61 (26) ^b	2.43 (1.83) ^a	969.5 (253.4) ^a	272.8 (68.9) ^a	21.2 (6.1) ^a	14.58 (5.04) ^a
1	6.7 (2.1) ^a	17.1 (5.4) ^a	0.04 (0.02) ^a	84 (38) ^a	9.58 (5.80) ^a	966.6 (252.7) ^a	269.3 (67.6) ^a	23.3 (3.9) ^a	14.50 (5.11) ^a
2	6.9 (2.0) ^a	17.8 (5.5) ^a	0.04 (0.04) ^a	70 (41) ^a	12.40 (6.74) ^a	973.2 (253.8) ^a	267.2 (67.6) ^a	22.2 (5.4) ^a	14.47 (5.23) ^a
3	6.8 (1.9) ^a	18.4 (5.5) ^a	0.04 (0.03) ^a	57 (34) ^a	21.12 (7.45) ^a	974.4 (254.1) ^a	261.2 (71.1) ^a	22.5 (5.5) ^a	14.95 (5.56) ^a
4	6.8 (1.9) ^a	18.9 (5.6) ^a	0.04 (0.02) ^a	57 (37) ^a	30.23 (7.08) ^a	973.2 (256.1) ^a	267.3 (72.0) ^a	21.1 (6.3) ^a	14.43 (5.53) ^a
Pond	6.8 (3.0) ^d	20.9 (8.4) ^d	n.a.	250 (44) ^d	3.06 (8.81) ^c	985.7 (403.0) ^c	287.3 (117.6) ^c	1.8 (3.0) ^c	2.01 (7.90) ^c

n.a., Not assessed; ^aProfile average of five sampling dates (n = 15); ^bProfile average of four sampling dates (n = 12); ^cAverage of five sampling dates (n = 5); ^dAverage of four sampling dates (n = 4); ^eAverage of three sampling dates (n = 3).

Table 1 (continued)

Sampling point [m from inflow]	pH	Temp. °C	O ₂ [mg L ⁻¹]	Eh [mV]	Fe(II) [mg L ⁻¹]	Sulphate [mg L ⁻¹]	Cl ⁻ [mg L ⁻¹]	Benzene [µg L ⁻¹]	MCB [mg L ⁻¹]
(b)									
0	6.8 (3.1) ^d	14.8 (8.5) ^d	0.04 (0.02) ^c	35 (40) ^d	0.20 (0.21) ^c	977.0 (399.8) ^c	261.8 (109.2) ^c	24.0 (6.9) ^c	14.44 (6.42) ^c
0.5	6.8 (2.2) ^b	15.6 (5.5) ^a	0.13 (0.05) ^b	47 (42) ^b	5.73 (3.62) ^a	994.2 (250.7) ^a	257.3 (68.5) ^a	11.8 (5.5) ^a	8.71 (7.67) ^a
1	6.8 (2.1) ^a	16.6 (5.4) ^a	0.12 (0.09) ^a	54 (40) ^a	13.34 (7.48) ^a	979.7 (247.8) ^a	256.4 (67.8) ^a	15.7 (6.6) ^a	9.78 (7.25) ^a
2	6.7 (2.0) ^a	17.6 (5.4) ^a	0.05 (0.02) ^a	46 (36) ^a	25.48 (7.60) ^a	1006.2 (248.0) ^a	266.3 (68.2) ^a	11.9 (8.8) ^a	8.20 (6.57) ^a
3	6.7 (1.9) ^a	18.0 (5.4) ^a	0.05 (0.03) ^a	51 (34) ^a	36.57 (7.33) ^a	999.7 (248.0) ^a	271.1 (69.0) ^a	11.3 (8.9) ^a	7.94 (6.07) ^a
4	6.5 (1.9) ^a	18.8 (5.6) ^a	0.03 (0.02) ^a	57 (34) ^a	37.40 (8.94) ^a	998.4 (259.2) ^a	265.8 (73.7) ^a	10.2 (8.0) ^a	7.73 (5.86) ^a
Pond	6.8 (3.1) ^d	20.5 (8.1) ^d	4.85 (1.89) ^c	204 (43) ^d	2.42 (9.28) ^c	1068.2 (397.4) ^c	274.8 (110.7) ^c	0.2 (0.4) ^c	2.20 (7.77) ^c

n.a., Not assessed; ^aProfile average of five sampling dates (n = 15); ^bProfile average of four sampling dates (n = 12); ^cAverage of five sampling dates (n = 5); ^dAverage of four sampling dates (n = 4); ^eAverage of three sampling dates (n = 3).

In both cases, the vectors representing the temperature, Fe(II), and total Fe parameters were orientated in the same direction (positive correlation), in the opposite direction with regards to vectors representing manganese and ammonium (negative correlation), and are perpendicular to the MCB vector (absence of correlation). The orthogonal projection of an object on a descriptor allows approximating the correlation between that object and the descriptor. For both segments, samples from the inflow part of the wetland (0.5-1 m) were associated with MCB, manganese and ammonium, whereas samples from the outflow (3 and 4 m) were generally associated with total Fe and Fe(II). Indeed, the Fe(II) concentration at the inflow was below 0.5 mg L^{-1} and systematically increased along the flow path in both planted and unplanted segment, indicating ferrous iron mobilization. A clear shift in the parameters characterising predominantly the trends of variation of the samples along the flow path is operating on the first principal component. Indeed, the axis I corresponds to a spatial gradient from the inflow to the outflow of the system, and separates sampling sites accordingly (from the right to the left). The variations of geochemistry along the flow path contributed more in characterising the samples than the variations occurring along the vertical profile.

The maximum concentration of Fe(II) reached an average value of 37.4 mg L^{-1} at 4 m from the inflow of the planted segment. The levels of total dissolved iron detected throughout the experimental period ranged from 0.8 to 50.6 mg L^{-1} . Interestingly, Fe(II) mobilization was more important in the planted than in the unplanted segment. Correlation analysis revealed, however, very similar Fe(II) mobilization patterns in both segments over the study period (Spearman's rank correlation coefficient, $\rho > 0.9$; $p < 0.01$). Iron reduction was apparently a relevant process at the three depths. Due to the high background concentration of sulphate in the supplied groundwater ($700\text{-}1100 \text{ mg L}^{-1}$), a sensitive analysis of the microbial sulphate reduction on the basis of sulphate concentration was not possible. However, trace concentrations of sulphide ($<3 \text{ } \mu\text{g L}^{-1}$) were detected along the horizontal transect, indicating sulphate reduction. Mn(II) concentrations systematically ranged below 0.2 mg L^{-1} , suggesting a low relevance of Mn as electron acceptor in the wetland (data not shown). The redox potential displayed averages of values ranging from 35 mV at the inflow to 250 mV in the ponds, and maximal concentration of oxygen in the soil compartments was 0.12 mg L^{-1} , indicating

the prevalence of anoxic conditions in the soil compartments and oxic conditions in the ponds, respectively. The dissolved organic and total carbon concentrations ranged from 0.24 to 0.45 mM and from 2.27 to 2.41 mM in the unplanted and planted segments, respectively (data not shown). Nitrate concentrations were assessed in previous studies and ranged systematically under the detection threshold in the groundwater supplied to the wetland (Vogt et al., 2002), and nitrate may therefore not represent a relevant electron acceptor. The chloride concentrations did not show any significant variation along the transect.

3.2. Carbon isotopic composition of MCB

The concentration and isotopic composition ($\delta^{13}\text{C}$) of MCB were plotted as a function of the distance from the inflow (Fig. 2). A decrease in MCB concentrations over the flow path was systematically associated in both segments with a significant enrichment in ^{13}C . In the planted segment, MCB showed a maximal isotope shift of 0.6‰, whereas it reached 0.9‰ in the unplanted segment. This indicates that MCB is subjected to *in situ* biodegradation in both segments. Assuming an isotopically homogenous source of MCB, these values are slightly higher than the typically defined analytical error of 0.5‰ associated with compound specific isotope analysis (Dempster et al., 1997; Mancini et al., 2002).

A quantitative assessment of *in situ* MCB biodegradation requires a fractionation factor representing the *in situ* conditions. Fractionation factors for aerobic MCB degradation are available (Kaschl et al., 2005), whereas factors retrieved under laboratory conditions for anaerobic MCB degradation are missing. Although MCB degradation under anaerobic conditions is expected in the wetland, Eq. (3) allows estimating the significance of degradation along the flow path throughout the wetland applying the highest isotope fractionation factors (αC) retrieved for aerobic MCB degradation by a dioxygenase reaction pathway ($\alpha\text{C} = 1.0004$) (Kaschl et al., 2005). The quantification was carried out based on the isotope signature of MCB measured at the planted side at day 0. The lowest $\delta^{13}\text{C}$ value measured (inflow point, 0 m: MCB $\delta^{13}\text{C} = -27.0\text{‰}$) was used as the initial isotope composition of the source (R_0). The estimated percentage of biodegradation was 60% of the inflowing MCB mass at 3.5 m, whereas the observed contaminant mass decrease reached only 38% at 4 m from the inflow. Processing of

isotope and concentration data of the second sampling campaign gave almost identical results.

The use of too low fractionation factors for the quantification of MCB degradation would lead to an overestimation of the effective MCB mass depletion at the planted segment. The observed absence of significant MCB mass depletions along with a higher isotopic shift at the unplanted segment obviously direct to the same conclusion.

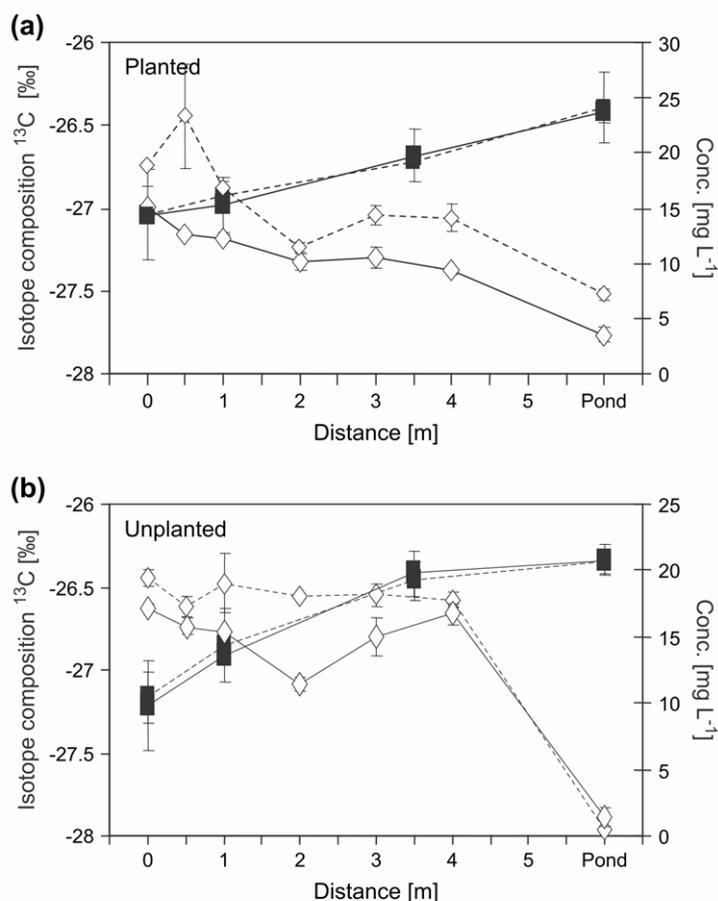


Figure 2: Concentration and isotopic composition of MCB (diamond, concentration; squares, isotopic composition) in both planted (a) and unplanted (b) segments and for both sampling dates (line, 14/04/2005; dashed, 09/05/2005). Error bars show the standard deviation.

Therefore, higher fractionation factors are expected, which would be more in concordance with the observed MCB concentration values. The use of higher isotope fractionation factors would point to a dominating anaerobic fractionation process, which is compatible with the observation of anoxic conditions in the wetland. The fractionation factors would then be comparable to the ones retrieved by Kaschl et al. (2005) ($\alpha_C = 1.0005$) in the local anaerobic aquifer with MCB contamination or by

Griebler et al. (2004) and Mancini et al. (2003), obtaining significantly higher fractionation factors for the anaerobic degradation of benzene or trichlorobenzenes.

3.3. *In situ* microcosm experiment

[¹³C₆]-labelled MCB was used as stable isotope tracer in the *in situ* microcosm experiment in the wetland. The BACTRAPs were incubated directly in both, soil compartments, at several distances from the inflow, as well as in the ponds. The incorporation of ¹³C derived from the labelled substrate into bacterial fatty acids provided evidence for MCB degradation in the wetland system. In addition, [¹³C₆]-benzene, a possible intermediate, found on the BACTRAPs from both segments demonstrated the occurrence of reductive dehalogenation of MCB (Fig. 3). Even though the employed [¹³C₆]-MCB contained 0.02% of [¹³C₆]-benzene as impurity, significantly higher amounts of [¹³C₆]-benzene were detected by GC-MS.

3.3.1. Fatty acid composition

The composition of total fatty acid fractions extracted from *in situ* microcosms was compared to investigate variations in the microbial community. No systematic differences were observed between the oxic ponds and the anoxic segments of the wetland. However, the lowest quantity of fatty acids was retrieved from the microcosms exposed at 4.5 m in the soil compartments, and the highest accumulated biomass was retrieved from the microcosms deployed in the pond of the unplanted side of the wetland (Fig. 4).

The fatty acid patterns were dominated by high amounts of the saturated hexa- (C16:0) and octadecanoic (18:0) and the monounsaturated hexa- (C16:1) and octadecenoic (C18:1) acids which are common fatty acids in bacteria. The tetra-(C14:0), pentadecanoic (C15:0) and eicosanoic (C20:0) acids, the iso and anteiso isomers of C15:0 as well as the unsaturated C18:2 were present in lower abundance and could not be detected in all samples (Fig. 4).

The variation of the geochemical parameters down gradient of the inflow was not related to a distinct change in fatty acid composition in both segments. No systematic differences in the fatty acid patterns were found between the planted and unplanted segments. Globally, no clear indications of microbial community changes were obtained

on the basis of the fatty acid composition. In this experiment, fatty acid patterns were probably not sensitive enough to reflect changes in the microbial communities as a function of variation in geochemical conditions within the CW.

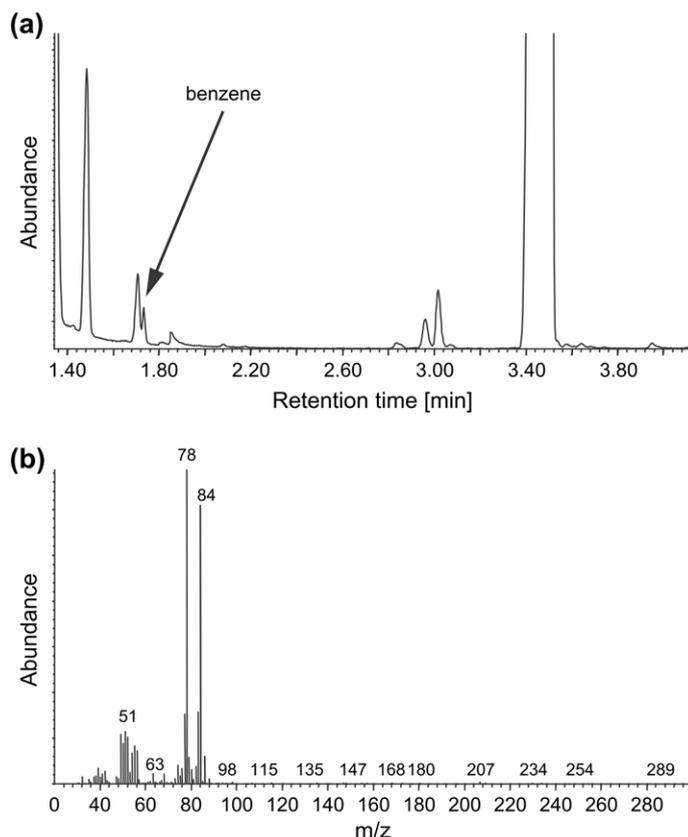


Figure 3: GC-MS chromatogram (total ion current) (a) of the *in situ* microcosm extract showing the ^{13}C -labelled benzene metabolite at retention time of 1.73 min and corresponding mass spectrum (b) with $m/z = 84$ for $[^{13}\text{C}_6]$ benzene and $m/z = 78$ for $[^{12}\text{C}]$ benzene.

3.3.2. Isotope signatures of fatty acids

The total lipid fatty acids extracted from the *in situ* microcosms displayed some differences in the incorporation of ^{13}C into fatty acids. This was particularly obvious when comparing the samples from the soil compartments and the ponds. The isotope composition of fatty acids ($\delta^{13}\text{C}_{\text{FA}}$) extracted from the BACTRAPs amended with $[^{13}\text{C}_6]$ -MCB ranged between -39‰ and 7244‰ (Table 2). An enriched ^{13}C signature of fatty acids ($>0\%$) can only stem from the microbial utilisation of $[^{13}\text{C}_6]$ -MCB as a carbon source. In contrast, fatty acids with an isotope signature lower than -20‰ showed the typical natural abundance of $\delta^{13}\text{C}_{\text{FA}}$ found in soil and aquifer material (Pelz et al., 2001; Pombo et al., 2002).

Fatty acids derived from parallel microcosms with non-labelled MCB or from non-amended *in situ* microcosms displayed an isotope signature ranging from -24‰ to -54‰ (data not shown). This represents a typical isotope composition of lipids derived from microorganisms feeding on organic substrates with natural isotope composition.

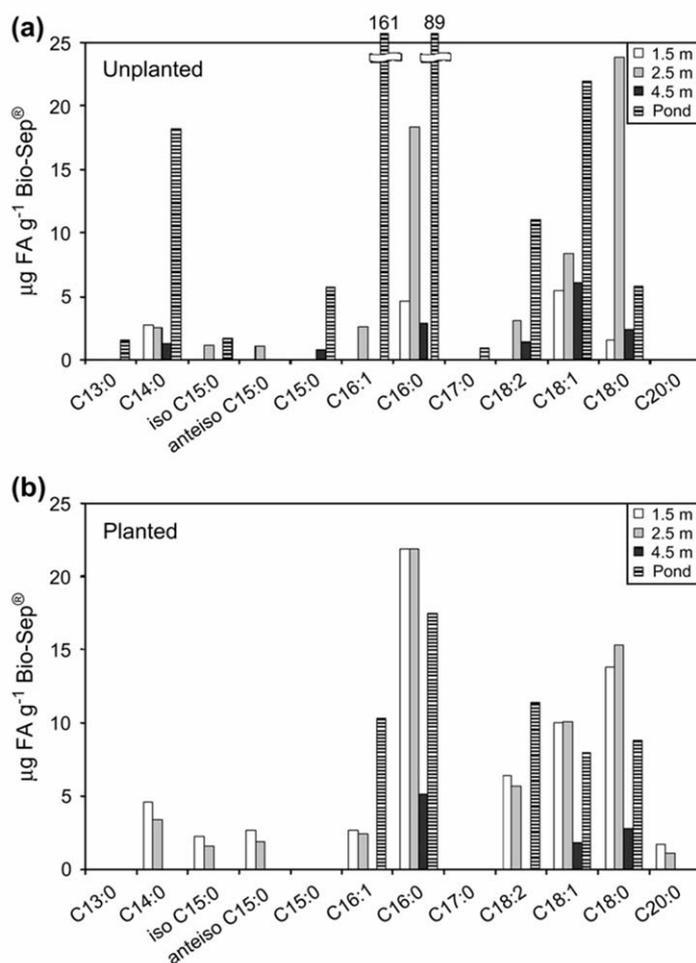


Figure 4: Absolute abundance of extracted fatty acids [$\mu\text{g FA per gram Bio-Sep}^{\text{®}}$ beads] from *in situ* microcosms exposed in the soil compartment at different distance from the inflow and ponds at the unplanted (a) and planted (b) segments of the CW.

Generally, all fatty acids extracted from the *in situ* microcosms exposed in the ponds showed enrichment in ^{13}C (65‰ to 7244‰), which was generally of higher intensity than in the soil samples (-39‰ to 1832‰). In contrast, only fatty acids with up to 16 carbon atoms showed ^{13}C incorporation in the soil samples with highest enrichment in C16 species. Fatty acids with longer carbon chains such as C18:0 or C18:1 displayed no enrichment in ^{13}C (-22‰ to -39‰), indicating that the microbial community was not exclusively growing on the [$^{13}\text{C}_6$]-MCB. Comparing the samples from the planted and

unplanted segments no significant differences in $\delta^{13}\text{C}_{\text{FA}}$ were observed. Therefore, the putative impact of plants on the microbial community involved in MCB degradation could not be assessed using our test system.

Table 2: Carbon isotope composition of fatty acids extracted from in situ microcosms incubated for 6 weeks with $^{13}\text{C}_6$ labelled monochlorobenzene at 1.5, 2.5 and 4.5 m from the inflow point as well as at the pond.

[m] from inflow	Planted				Unplanted			
	$\delta^{13}\text{C}_{\text{FA}}$ [‰]				$\delta^{13}\text{C}_{\text{FA}}$ [‰]			
	1.5	2.5	4.5	Pond	1.5	2.5	4.5	Pond
Fatty Acid								
C13:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	118
C14:0	-6	40	n.d.	n.d.	138	204	116	238
iso C15:0	121	89	n.d.	n.d.	n.d.	742	n.d.	1597
anteiso C15:0	23	68	n.d.	n.d.	n.d.	3	n.d.	n.d.
C15:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-37	252
C16:1	411	453	n.d.	7244	n.d.	1832	n.d.	806
C16:0	2	9	93	1711	260	95	192	639
C17:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	242
C18:2	-31	-30	n.d.	65	n.d.	-27	-30	216
C18:1	-30	-29	-28	276	-23	-23	-24	175
C18:0	-27	-28	-24	449	-22	-27	-26	266
C20:0	-37	-39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected

Some fatty acids can be used as biomarker to identify specific groups of microorganisms (Kaur et al., 2005; Zelles, 1999). In some of the samples labelled iso and anteiso branched fatty acids with 15 carbon atoms could be identified, indicating that Gram-positive bacteria were involved in the biodegradation of MCB. Labelled C18:2 was only found in samples of the ponds. Linoleic acids (C18:2) can serve as a biomarker for fungi or other eukaryotic organisms (Lösel, 1988) and their presence may lead to the hypothesis that grazing organisms, not involved in the biodegradation of MCB, may feed on the microbial biofilm. However, the applied GC-MS procedure did not allow conclusive identification of the position of the double bonds.

4. Discussion

The geochemical parameters indicated the overall prevalence of anoxic conditions associated with iron mobilization in the soil parts of the wetland, whereas an aerobic milieu characterised the ponds. In the *in situ* microcosm experiments, the level of incorporation of labelled carbon into bacterial biomass was used as direct indicator of *in situ* MCB degradation. Interestingly, the analysis of the BACTRAPs incubated in the ponds revealed fatty acids patterns and ^{13}C incorporation levels differing from the ones retrieved from the soil compartments. The higher ^{13}C incorporation level observed in the ponds of both segments is indicative of a more effective microbial transformation of the [$^{13}\text{C}_6$]-MCB under the prevailing aerobic conditions. Along with a significant accumulation of biomass on the microcosms retrieved from the ponds, these results suggest that some change in the microbial community dynamics may operate between the anoxic soil compartments and the more aerobic ponds. Moreover, the fact that all the extracted fatty acids showed incorporation of ^{13}C suggests that the microbial community established on the microcosms was mostly involved in contaminant degradation. However, it should be considered that cross-feeding by metabolites or recycling of dead biomass within the biofilm may also channel labelled carbon into individual members of the microbial community, explaining the variation in the labelling of specific fatty acids. In general, fatty acids displaying a higher incorporation of ^{13}C were very likely synthesised by organisms feeding on [$^{13}\text{C}_6$]-MCB, whereas organisms synthesising non-labelled fatty acids were likely not involved in the degradation of the labelled MCB and used different carbon sources. The analysis of the composition of total fatty acid fractions showed that this method might not be sensitive enough for investigating detail changes in microbial communities between soil and water compartments as well as down gradient the flow path.

Additionally, the MCB degradation processes in the wetland were investigated by carbon stable isotope composition analysis. A correlation between decreasing MCB concentration and a shift in the carbon isotope signature towards the heavier isotope was visible along the flow path, suggesting the degradation of MCB. Toxic effects of MCB on the MCB-degrading population can be reasonably excluded at the observed range of concentration values (Fritz et al., 1992; Vogt et al., 2002, 2004).

In the transition from the soil compartments to the ponds, a substantial contaminant mass depletion without concomitant isotope enrichment was observed. In this open system, it is likely that part of the contaminant may partition into the atmosphere, affecting the MCB concentration values without generating a significant isotope shift. Moreover, under oxic-prevailing conditions, several bacteria have the ability to use MCB as sole carbon and energy source, and may putatively adopt the well-known and described aerobic degradation pathways (Van Agteren et al., 1998). These bacteria may degrade MCB at faster rate than the degrading bacteria associated with anoxic conditions, contributing to the observed contaminant mass decrease. These oxygen-driven degradation reactions would lead to MCB mass decrease without a significant associated isotope effect (Kaschl et al., 2005). In parallel, biogeochemical processes such as oxidation of ferrous iron or mineral surfaces may compete for oxygen (Ehrlich, 1998; Søgaard et al., 2001; Warren and Haack, 2001), leading to transient conditions and oxygen gradients, which may affect the composition of the existing microbial community and rate of degradation reactions. However, the anaerobic degradation pathway of MCB is not elucidated yet.

In the anoxic soil compartments, two major hypothetical degradation pathways would come into consideration: (1) reductive dechlorination of MCB; and (2) degradation of MCB as an electron donor molecule. First, an initial dechlorination of MCB, followed by the degradation of benzene, is likely to occur in the soil compartments. Indeed, the presence of ^{13}C -labelled benzene detected in all the microcosms along with the detection of low benzene concentrations suggested that MCB is degraded reductively to benzene under anoxic conditions prior mineralization. This pathway would suggest a similar pattern as observed previously by Nowak et al. (1996). The pH values were, however, constant, and the relatively high background level of chloride in the groundwater hindered the direct verification of MCB degradation by an increasing chloride concentration along the flow path. Bacterial breakdown of benzene could lead to the formation of benzoate or phenol as intermediates (Chakraborty and Coates, 2005; Edwards and Grbić-Galić, 1992; Lovley, 2000; Phelps et al., 2001; Ulrich et al., 2005), which were not detected in this study. Soluble organic carbon species, which could serve as electron donors for dechlorination processes, were found in low concentration in the unplanted segment, which might effectively limit the extent of degradation.

Conversely, dechlorination activity in the planted segment may be partly related to the abundance of hydrogen and reduced organic acids such as acetate and propionate (Holliger et al., 1992; Middeldorp et al., 1997).

Reductive dechlorination reaction of MCB to benzene is expected to be associated with a pronounced primary isotope effect (Griebler et al., 2004). In contrast, the isotope composition presented in this study displayed a slight but significant enrichment ranging from 0.4 to 0.7 δ units, in the soil compartments of the planted and the unplanted segments, respectively. However, the isotope effect at zones of preferential *in situ* degradation reaction can be substantially higher. If other non-fractionating processes such as sorption and volatilization also contribute to a decrease in MCB concentrations, the isotope effect upon *in situ* degradation is expected to be relatively high. The observed isotope effect points to dominating anaerobic processes, as inferred by the estimation of biodegradation levels over the flow path. In the planted segment, the oxygen supplied by the plant at the rizosphere level may favour the establishment of aerobic zones. Although this process is not relevant for the electron budget, oxygen may contribute to the MCB degradation reactions, leading to MCB decrease without concomitant isotope effect. A fractionating anaerobic process and a less fractionating aerobic process may both contribute to *in situ* degradation, resulting in a mixed overall fractionation at the planted segment. Conversely, the isotopic composition shift observed at the unplanted segment suggests the occurrence of a more fractionating process.

Alternatively, MCB may be degraded as an electron donor molecule under ferric iron- or sulphate-reducing conditions. Anaerobic oxidation of benzene under these conditions has been previously observed (Anderson et al., 1998; Anderson and Lovley, 2000). For instance, geochemical footprints of iron reduction processes were found, and the ferrous iron mobilization was increasing as a function of the flow path. Dissolved Fe in pore waters can be a result of different processes such as Fe(III) reduction (Lovley, 1991, 1997), pyrite oxidation (Lord and Church, 1983), or Fe complexation (Luther et al., 1996). Fe(II) may be precipitated with sulphide originating from sulphate reduction activity or form complexes. Therefore the concentrations may not reflect the true extent of iron reduction in the presence of sulphate reduction. A low extent of sulphate reduction processes and the availability of reactive iron may prevent accumulation of

H₂S in the near-neutral conditions of the wetland. A MCB mineralization by sulphate reduction contributing to the contaminant mass decrease is feasible and may be possible. However, due to the high concentrations of sulphate in the supplied water, a reliable estimation of the extent of sulphate reduction could not be carried out. However, a reduction of ferric iron directly linked stoichiometrically to MCB oxidation in the wetland may theoretically account for a MCB mass decrease of about 18% between the inflow and the outflow of the system at both the planted and unplanted segment.

Additionally, other unknown degradation pathways cannot be excluded. These hypotheses will require further detailed investigations, along with further isolation and identification of microorganisms involved in the anaerobic MCB degradation in wetlands treating MCB contaminated water.

5. Conclusion

The integrated approach provided evidence for *in situ* MCB biodegradation in both, soil compartments and ponds of the planted and unplanted segments of a horizontal subsurface flow CW. This was supported by isotopic fractionation analysis, combined with *in situ* microcosm experiments, which can be utilized to document further the *in situ* degradation of MCB and other contaminants in wetland systems. Further investigations to elucidate the microbial degradation of MCB, facilitated by an integrated approach and combined with a high resolution sampling, are required to evaluate zones of enhanced *in situ* biodegradation of MCB and to optimise wetland systems treating contaminated groundwater.

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3 Simultaneous treatment of monochlorobenzene and perchloroethene

3.1 Adaptation of a constructed wetland to simultaneous treatment of monochlorobenzene and perchloroethene

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Abstract

Mixed groundwater contaminations by chlorinated volatile organic compounds (VOC) cause environmental hazards if contaminated groundwater discharges into surface waters and river floodplains. Constructed wetlands (CW) or engineered natural wetlands provide a promising technology for the protection of sensitive water bodies. We adapted a CW able to treat monochlorobenzene (MCB) contaminated groundwater to a mixture of MCB and tetrachloroethene (PCE), representing lowly and highly chlorinated model VOC. Simultaneous treatment of both compounds was efficient after an adaptation time of 2.5 years. Removal of MCB was temporarily impaired by PCE addition, but after adaptation a MCB concentration decrease of up to 64% (55.3 μM) was observed. Oxygen availability in the rhizosphere was relatively low, leading to sub-optimal MCB elimination but providing also appropriate conditions for PCE dechlorination. PCE and metabolites concentration patterns indicated a very slow system adaptation. However, under steady state conditions complete removal of PCE inflow concentrations of 10-15 μM was achieved with negligible concentrations of chlorinated metabolites in the outflow. Recovery of total dechlorination metabolite loads corresponding to 100%, and ethene loads corresponding to 30% of the PCE inflow load provided evidence for complete reductive dechlorination, corroborated by the detection of *Dehalococcoides sp.*.

Keywords: Phytoremediation, chlorinated solvents, groundwater-surface water interface, reductive dechlorination, microbial degradation

Introduction

Widespread groundwater contaminations with chlorinated ethenes and chlorinated benzenes are encountered in the industrialized countries of Europe and Northern America, due to their extensive production and use (McCarty, 1993; Field and Sierra-Alvarez, 2008; Kaschl *et al.*, 2005). At these sites, mixed contaminations with different chlorinated organic compounds resulted from various industrial activities for decades, and also from partial degradation in the aquifer (Heidrich, Weiss, and Kaschl, 2004; Moran, Zogorski, and Squillace, 2007; Persson *et al.*, 2008). These multi-component contaminations impede remediation approaches in the industrial area of Bitterfeld in Eastern Germany (Weiß *et al.*, 2001) and at several other sites (Kao and Prosser, 1999). Industrial sites are often located in the vicinity of rivers, due to the dependence of chemical industries on water availability for production and transportation. Surface-near groundwater at these sites may be in contact with surface waters, or may discharge directly into river floodplains and natural wetlands (Lorah and Olsen, 1999; Bankston *et al.*, 2002; Pardue, 2002; Kalbus, Reinstorf, and Schirmer, 2006; Tawney, Becker, and Baldwin, 2008). The contamination of sensitive ecosystems is the resulting problem, which is also encountered at the Bitterfeld site with the nearby river Mulde (Heidrich *et al.*, 2004).

Monochlorobenzene (MCB) and perchloroethene (PCE) are widespread representatives of these classes of groundwater contaminants, and thus are appropriate model compounds for highly and lowly chlorinated contaminants in experimental approaches. MCB bears only one chlorine substituent, is readily biodegradable via oxidation under aerobic conditions (Field and Sierra-Alvarez, 2008), and tends to accumulate in anaerobic aquifers (Heidrich *et al.*, 2004; Kaschl *et al.*, 2005). The fully chlorinated solvent PCE is only degradable via initial reductive dechlorination under anaerobic conditions (McCarty, 1993; Maymó-Gatell, Anguish, and Zinder, 1999). The only known group of microorganisms able to reductively dechlorinate PCE completely to ethene, using exclusively hydrogen as an electron donor, is *Dehalococcoides sp.* (Maymó-Gatell *et al.*, 1999). Several other organisms, such as *Dehalobacter*, *Desulfuromonas* and *Desulfitobacterium sp.*, dechlorinate PCE to trichloroethene (TCE) and dichloroethenes (DCEs), with *cis*-DCE being the dominant degradation product (Sung *et al.*, 2003; Yoshida *et al.*, 2007; Huang and Becker, 2009). The dechlorination

products TCE, DCEs and vinyl chloride (VC) are increasingly (in the given order) susceptible to microbial transformation under aerobic conditions, which is largely cometabolic for TCE and cis-DCE (Broholm *et al.*, 2005; Fathepure *et al.*, 2005; Olaniran, Pillay, and Pillay, 2008). A key mechanism viable at aerobic/anaerobic interfaces and in wetland rhizospheres is the aerobic cometabolism of TCE, cis-DCE and VC by methanotrophs (Lorah and Olsen, 1999; Chu and Alvarez-Cohen, 2000; Amon *et al.*, 2007). Cis-DCE, trans-DCE and VC are only slowly reductively dechlorinated (McCarty, 1993), often leading to undesirable accumulation in contaminated anaerobic aquifers (Broholm *et al.*, 2005).

Constructed wetlands (CW) and engineered plots of natural wetlands are a promising simple and near-natural technology to treat groundwater contaminated by chlorinated organics and to protect the adjacent river floodplains from contamination. The approach has recently attracted considerable interest: CW treatment of waters contaminated with chlorinated benzenes (Jackson, 1999; Leppich, 1999; MacLeod, 1999; Keefe *et al.*, 2004; Braeckevelt *et al.*, 2007; 2008) and chlorinated ethenes (Bankston *et al.*, 2002; Pardue, 2002; Kassenga *et al.*, 2003; Ma and Burken, 2003; Amon *et al.*, 2007; James *et al.*, 2009) has been in the focus of several studies. The characteristics of the wetland rhizosphere as a reactive zone make CW particularly suited for treatment of multi-component contaminated groundwaters where reductively and oxidatively degradable contaminants are present as a mixed contamination (Faulwetter *et al.*, 2009). The root-zone environment is in general beneficial for microbial growth and activity (Walton and Anderson, 1990; Anderson, Guthrie, and Walton, 1993; Stottmeister *et al.*, 2003). In particular, the mosaic structure of aerobic zones near helophyte root surfaces and at interfaces with the unsaturated zone, and anaerobic zones in the bulk soil, constitutes the potential of this technology for the simultaneous treatment of contaminants requiring both oxidative and reductive degradation processes.

Therefore, the aim of this study was to investigate the treatment of groundwater with a mixed contamination of MCB and PCE in a pilot-scale CW. The CW had already been adapted to MCB contamination when PCE was added as a second contaminant. The objectives were to test the hypothesis that CW have the potential for simultaneous efficient reductive dechlorination and oxidation, and to evaluate the adaptation of the system to a mixture of the contaminants. Particular focus was laid on the development

of PCE degradation and metabolite patterns, the potential to achieve complete dechlorination to the non-toxic end product ethene, and the time period necessary in order to obtain steady-state system performance. In addition, the effect of sorption on contaminant removal and the influence of the main electron acceptors oxygen, iron(III) and sulphate were investigated.

Materials and Methods

Site description and experimental setup

The SAFIRA site for groundwater remediation research in regionally contaminated aquifers was established in 2003 (Weiß *et al.*, 2001). It is located in the south-east of the town of Bitterfeld in Saxony-Anhalt, Germany: an important industrial site for more than one hundred years. The groundwater in the region is heavily impacted by chlorinated aliphatic and aromatic as well as BTEX contaminants, originating from extensive activities in multiple areas of the chemical industry (Heidrich *et al.*, 2004). At the site a pilot-scale CW was built in March 2003 to investigate the remediation of chlorinated-benzene-contaminated groundwater in wetland systems (for details see Braeckevelt *et al.*, 2007; 2008). The CW was designed to be operated in horizontal subsurface flow (HSSF) mode and consisted of a 6 m×1 m×0.7 m stainless-steel container filled with the local aquifer material (36% gravel, 58% sand, 0.8–1.3% iron, 1.5-2.0% TOC containing residues of local lignite) to a height of 0.5 m and a length of 5 m. The last meter was constructed as an open water compartment (OWC) to simulate the littoral zone of a river. Anaerobic, contaminated groundwater from a well installed in 22 m depth in the local quarternary aquifer was supplied to the wetland continuously at a flow rate of 4.7 L h⁻¹. During the study period the concentration of the main contaminant MCB in the local groundwater was 70-140 µM, with lower concentrations of 1,4-DCB (1.3-2.7 µM) and 1,2-DCB (0.3-0.5 µM). The groundwater was rich in sulphate (800-900 mg L⁻¹), calcium (350-380 mg L⁻¹) and chloride (220-360 mg L⁻¹). Further information on water quality parameters and inorganic constituents of the groundwater are given in the supporting information (Table S1). PCE was added to the wetland inflow as an additional contaminant at a concentration of 6-15 µM. A metering pump was used to continuously add the PCE stock solution to the inflow tube. The

stock solution was a saturated solution of PCE in water, which was kept in a continuously stirred stainless steel tank overlaying a neat PCE phase. The wetland system was planted with common reed (*Phragmites australis*). The water level was adjusted to 0.4 m by float valves in the OWC, and volumes pumped off were measured by a flow meter in order to enable the determination of water and degradation mass balances. The theoretical hydraulic retention time in the filter was 119 h. The system had already been operated with the local groundwater for 3 years before PCE was added and the current investigations were carried out between May 2006 and December 2008.

Water sampling and analysis

Pore water for the determination of contaminant concentrations and redox parameters was obtained from the middle of the wetland at 0.5, 1, 2, 3 and 4 m distance from the inflow in 0.3, 0.4 and 0.5 m depth of the filter bed. The OWC was only sampled in 0.4 m depth. Water sampling at different depths was carried out using stainless steel tubes (3.5 mm inner diameter) connected to peristaltic pumps with a flow rate of 78 ml min⁻¹. All water samples for chemical analysis were stored without headspace at 4°C until analysis. In total, 32 samplings were carried out over the study period.

Organic contaminants in the liquid phase were analyzed in duplicates by means of headspace GC-FID (7890 A with G1888 headspace autosampler, Agilent Technologies, USA) using a HP-1 column (30 m×0.32 mm×5 µm). The following temperature program was applied: equilibration at 60°C (60 min), GC oven program 45°C (1 min), 20 K min⁻¹ to 200°C (2.5 min), 65 K min⁻¹ to 250°C (1 min). The detector temperature was 280°C and helium carrier gas flow was 1.7 mL min⁻¹. Aliquots of 1 mL were injected with a split of 1:5 at an injector temperature of 250°C.

The photometric detection of ferrous iron was carried out as described previously (Lovley and Phillips, 1986). For the analysis of sulphide concentrations, samples were stabilized according to manufacturer's instruction and measured with an ion-selective Ag/S 500 electrode and R 503 reference electrode (WTW, Germany).

Oxygen measurement

Oxygen measurement was carried out using optical oxygen sensor systems (Fibox-3, sensor spots type PSt3) with automatic temperature compensation (PT 1000 temperature

sensor) (PreSens, Germany). The oxygen sensor spots and temperature sensors were mounted on stainless steel drilling rods equipped with a lost cone and retrievable protective casing. They were connected to the measuring device with polymer optical fibres and were permanently installed in the filter at 0.5, 1, 2 and 4 m distance from the inflow. At each distance, two sensor rods were installed in parallel, one rod covering nearly the complete filter depth (0, 0.059, 0.119, 0.178, 0.237, 0.296, 0.355 and 0.415 m depth) and the other only the upper filter layer (0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, 0.18 and 0.2 m depth). At the 0.5 m sampling point only the former layout was used. The data presented here were obtained between May and July 2008.

Soil sampling and analysis

Soil profile samples were taken at 0.5, 2 and 4 m distance from the inflow in November 2006 (6 months after PCE addition) in order to determine MCB and PCE adsorbed to the soil, the oxalate-extractable ferric iron content and the amount of root biomass. Plastic liner probes (ID 33 mm) were pushed into the soil in order to obtain filter profiles with a sample fresh weight of 0.45-0.53 kg. The samples were stored at -20°C prior to sample processing in the anaerobic glove box. Profiles were divided into three sections of equal length, representing the upper, middle and lower section (approximately 0.17 m) of the filter profile.

For determination of MCB and PCE adsorption, duplicate samples of 1 g fresh soil each were extracted in 4 ml dichloromethane (containing 0.2 g L⁻¹ n-octane as an internal standard) by ultrasonication for 15 min prior to shaking for 24 h at 20°C. Concentrations were determined using a GC-MS system (HP 6890 Series, MSD HP 5973, Agilent technologies, Palo Alto, USA) with a BPX-5 column (30 m×0.32 mm×0.25 µm, SGE Analytical Science, Victoria, Australia) and the following temperature program: 40°C (4 min), 15 K min⁻¹ to 150°C, 40 K min⁻¹ to 300°C (2 min). Oxalate extractable ferric iron is a good measure for microbially reducible ferric iron forms (van Bodegom, van Reeve, and Denier van der Gon, 2003). Ferric iron contents were determined photometrically in duplicates after anaerobic oxalate extraction using the method proposed by Phillips and Lovley (1987). Roots were separated from the soil manually and dried at 105°C to constant weight. Dry

weight was determined by drying soil samples to constant weight at 105°C, obtaining a ratio of fresh weight:dry weight = 1:0.73.

Molecular biology

Taxon-specific PCRs were carried out on groundwater inflow and CW water samples to determine the presence of *Dehalococcoides sp.*, using the primers and method suggested by Löffler *et al.* (2000), after extraction of genomic DNA with the FastPrep bead-beater technique (FastDNA Kit, QBiogene, USA).

Data analysis

All organic analyses were performed in duplicates; data presented are sample means. In order to test differences in MCB concentration data sets before and after PCE addition, hierarchical cluster analyses (between-groups linkage, euclidian distance) were carried out using the PASW Statistics 17.0 program (SPSS Inc./IBM, USA). Contour plots of oxygen concentrations in the wetland filter were realized using the OriginPro 8G software (OriginLab, USA), applying kriging correlation as a gridding method.

Results and Discussion

The presented study provides results on the adaptation of a pilot-scale CW system to the simultaneous treatment of MCB and PCE, with particular focus on the temporal development of PCE degradation and metabolite patterns. At the beginning of the experimental period, MCB removal was already established under steady-state conditions with summertime elimination rates of up to 3196 $\mu\text{mol m}^{-2} \text{d}^{-1}$ as published elsewhere (Braeckevelt *et al.*, 2007; 2008; 2011).

MCB removal and impact of PCE addition on MCB elimination

The concentration profile of MCB in the CW is shown in Table 1 for the year 2008. Concentration removal was most efficient in 0.3 m depth, with an annual mean concentration reduction of 64% (55.3 μM) between the inflow and 4 m. Annual mean concentration decrease was less pronounced in 0.5 m depth, accounting for only 29% (24.9 μM).

Table 1: Annual mean MCB concentrations and standard deviations in the CW profile (2008).

Distance [m]	c(MCB) [μ M]		
0 (Inflow)		86.9 \pm 7.9	
	0.3 m depth	0.4 m depth	0.5 m depth
0.5	78.8 \pm 12.5	84.2 \pm 11.3	81.5 \pm 15.8
1	68.5 \pm 18.0	76.5 \pm 10.8	72.0 \pm 11.8
2	28.0 \pm 19.5	52.6 \pm 18.9	67.6 \pm 9.1
3	26.1 \pm 16.6	54.5 \pm 18.8	63.6 \pm 14.3
4	31.6 \pm 12.6	54.2 \pm 14.5	62.0 \pm 12.8
6 (OWC ^a)		18.2 \pm 15.5	

^a Open Water Compartment

The decrease of MCB concentrations was slightly lower than in 2005 (before PCE addition) when annual mean concentration decrease was found to be 76% in 0.3 m and 36% in 0.5 m depth (Braeckevelt *et al.*, 2010). Presumably, this was due to the presence of PCE and its metabolites resulting in competition for electron acceptors and nutrients, and in changes in the microbial community being potentially disadvantageous for MCB oxidizers.

In order to evaluate how the PCE addition influenced MCB removal, a hierarchical cluster analysis was performed using summer concentration data sets of the years 2005-2008 (Fig. 1). The data sets from later summer 2006 (07-08/2006), after PCE addition in May, form a cluster clearly segregated from the other data sets. During this period, MCB removal was in fact strongly impaired by the presence of the additional contaminant PCE. However, in a lag phase directly after PCE addition (05-06/2006), no changes became evident yet (Fig. 1). The observed effect probably resulted from several mechanisms, e.g. toxic impacts on the previously unadapted microbial communities as well as physico-chemical processes. Sorption equilibriums existing under stable conditions were disturbed by PCE addition, since competitive sorption can differ substantially from single-compound sorption (Stuart, Bowlen, and Kosson, 1991). This may have resulted in displacement of MCB sorbed to the soil in the process of PCE sorption (Table 2), leading to transiently higher residual MCB concentrations, as has been shown for other contaminants (White *et al.*, 1999). Sorption of approximately 15 kg PCE could have led to desorption of a considerable amount of MCB (see PCE concentrations and PCE sorption).

However, no substantial differences between data sets of the summer 2005, before PCE addition, and those of the summers 2007 and 2008 were found using the applied cluster method. This indicates that PCE addition only temporarily resulted in much higher residual MCB concentrations and caused – if at all - only minor changes in overall MCB removal in the long run. However, it is likely that PCE addition also caused changes in the microbial degradation pathways that can not be observed with the applied methods.

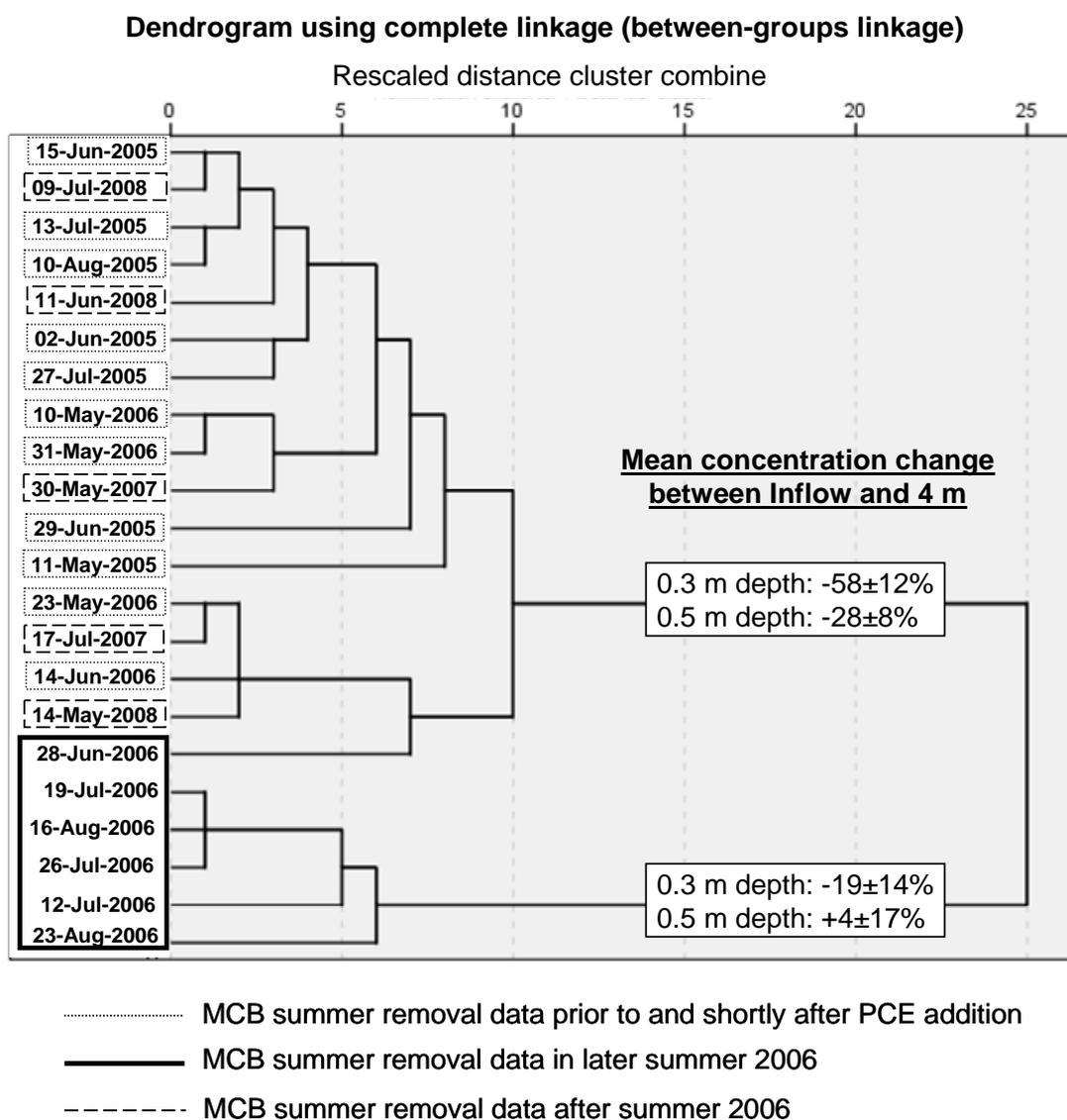


Figure 1: Hierarchical cluster analysis of summertime MCB concentration data sets for the years 2005-2008 (concentrations in 0.3, 0.4 and 0.5 m depth of the CW filter and at 0.5, 1, 2, 3 and 4 m distance from the inflow, in % of inflow concentrations).

For example, previous evidence has shown that MCB can serve as an electron donor in TCE biodegradation, both in aerobic cometabolism and reductive dechlorination (Kao and Prosser, 1999).

PCE concentrations and PCE sorption

The evolution of PCE and metabolite concentration patterns was monitored in the CW for a period of 2 ½ years after PCE addition. For a better overview, the development of the system was divided into two phases, phase 1 covering the first 11 months after addition of PCE (Fig. 2) and phase 2 the following 20 months (Fig. 3). PCE was rapidly eliminated in the filter during the weeks following the onset of PCE addition, mainly in 06-07/2006 (Fig. 2), but no significant concentrations of PCE degradation products were detected. From 08/2006 onwards, the PCE concentration decline became less steep and the PCE plume moved further into the filter. This effect was presumably caused by initial sorption of PCE to the previously unexposed soil. Sorption equilibrium was apparently reached quickly, given the observed filter breakthrough between 10/2006 and 04/2007 with considerable PCE concentrations at the 4 m point. Soil profiles were extracted for sorbed organic compounds 6 months after PCE addition (Table 2).

Table 2: MCB and PCE adsorbed to the soil, root biomass, and oxalate-extractable ferric iron in the filter profile at 0.5, 2 and 4 m distance from the inflow, 6 months after PCE addition.

Distance [m]	Depth [m]	c(MCB) [mg kg ⁻¹ _{dw}]	c(PCE) [mg kg ⁻¹ _{dw}]	Roots [mg kg ⁻¹ _{dw}]	Iron(III) [g kg ⁻¹ _{dw}]
0.5	0.3	79.4±3.6	9.9±0.7	50.6	1.10±0.32
	0.4	33.4±7.1	5.3±0.8	3.6	0.84±0.04
	0.5	39.3±9.4	6.5±2.0	0.4	0.77±0.16
2	0.3	11.9±2.4	2.1±0.4	18.6	2.25±0.93
	0.4	70.3±38.4	6.5±3.6	4.8	1.08±0.08
	0.5	61.1±19.0	4.5±1.6	2.1	1.27±0.58
4	0.3	9.8±7.0	0	8.9	3.26±0.47
	0.4	11.3±3.4	0.1±0.1	6.3	2.13±0.11
	0.5	22.5±7.1	1.0±0.3	6.8	1.68±0.43

PCE concentrations were highest near the inflow and declined strongly towards the outflow, with very low concentrations at 4 m. Reflecting the much longer exposure time, PCE soil concentrations were approximately one order of magnitude lower than MCB concentrations. In addition, the lower log K_{OC} of PCE (log apparent K_{OC} =1.9-2.56, European Chemicals Bureau, 2000) compared to MCB may affect the observed concentration distribution. For Bitterfeld lignite, which constitutes an important source of organic carbon in the CW filter, a log apparent K_{OC} of at most 2.6 was determined for MCB (Dermietzel and Christoph, 1997).

An estimation of PCE adsorption on the total filter material of the CW resulted in approximately 15 kg PCE after 6 months of PCE feeding, representing 30-33% of the total PCE load added during this period. Taking into account the observed breakthrough and the low concentrations of degradation products, it can be assumed that in this adaptation stage of the system, the microbial community was not yet adjusted to PCE dechlorination. In summer 2007, the PCE concentration patterns in the CW stabilized, resulting in complete removal within the first 1 m (0.3 m depth) or 2 m (0.5 m depth) of the flow path (Figure 3).

Temporal development of PCE degradation patterns

Products of reductive PCE dechlorination were already observed a few weeks after PCE addition. In the first phase (~ 6 months), TCE occurred in ever higher concentrations from one sampling to the next, with concentration increase up to 2 or 3 m along the flow path and subsequent decrease towards the outflow (Fig. 2). TCE concentrations were higher in the deeper filter zone (0.5 m depth), resulting from the more reduced conditions in the anaerobic deeper filter layers (see Fig. 4). Only trace concentrations of cis-DCE were detected in 2006. After 10 months of operation, the cis-DCE production showed a pronounced increase in spring 2007 with a clear advance in the deeper filter layers (Fig. 2 and 3). In addition, first traces of ethene were detected, providing evidence for complete reductive dechlorination of PCE. At the same time, TCE concentration levels building up during the filter passage diminished. From summer 2007 onwards, TCE concentrations already dropped down to very low levels after 0.5-1 m, particularly in 0.3 m depth.

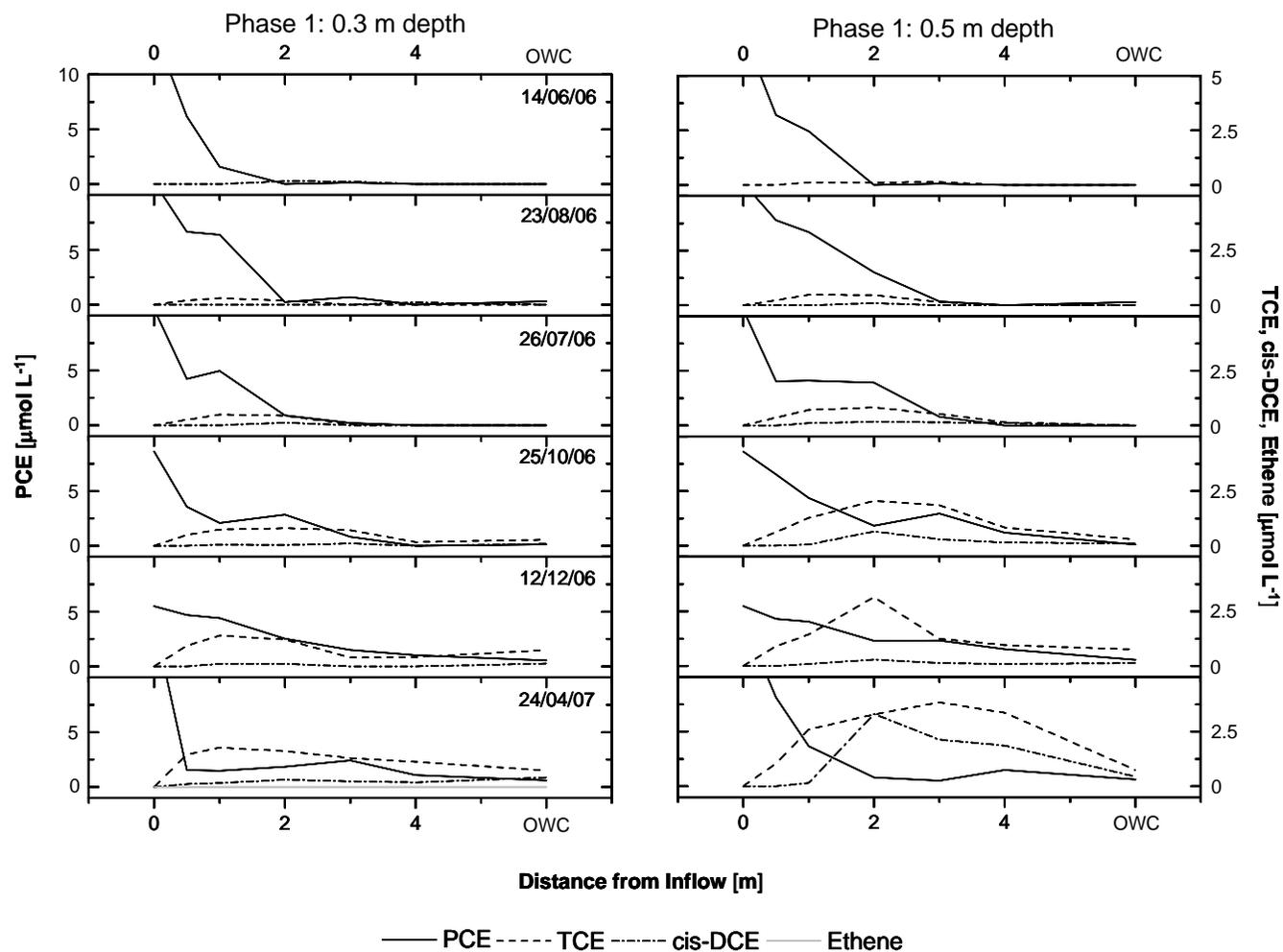


Figure 2: PCE removal and formation of metabolites over the course of the CW flow path in 0.3 and 0.5 m depth: Phase 1 (06/2006-04/2007), prior to start of VC production (PCE inflow concentrations were 10-15 μM).

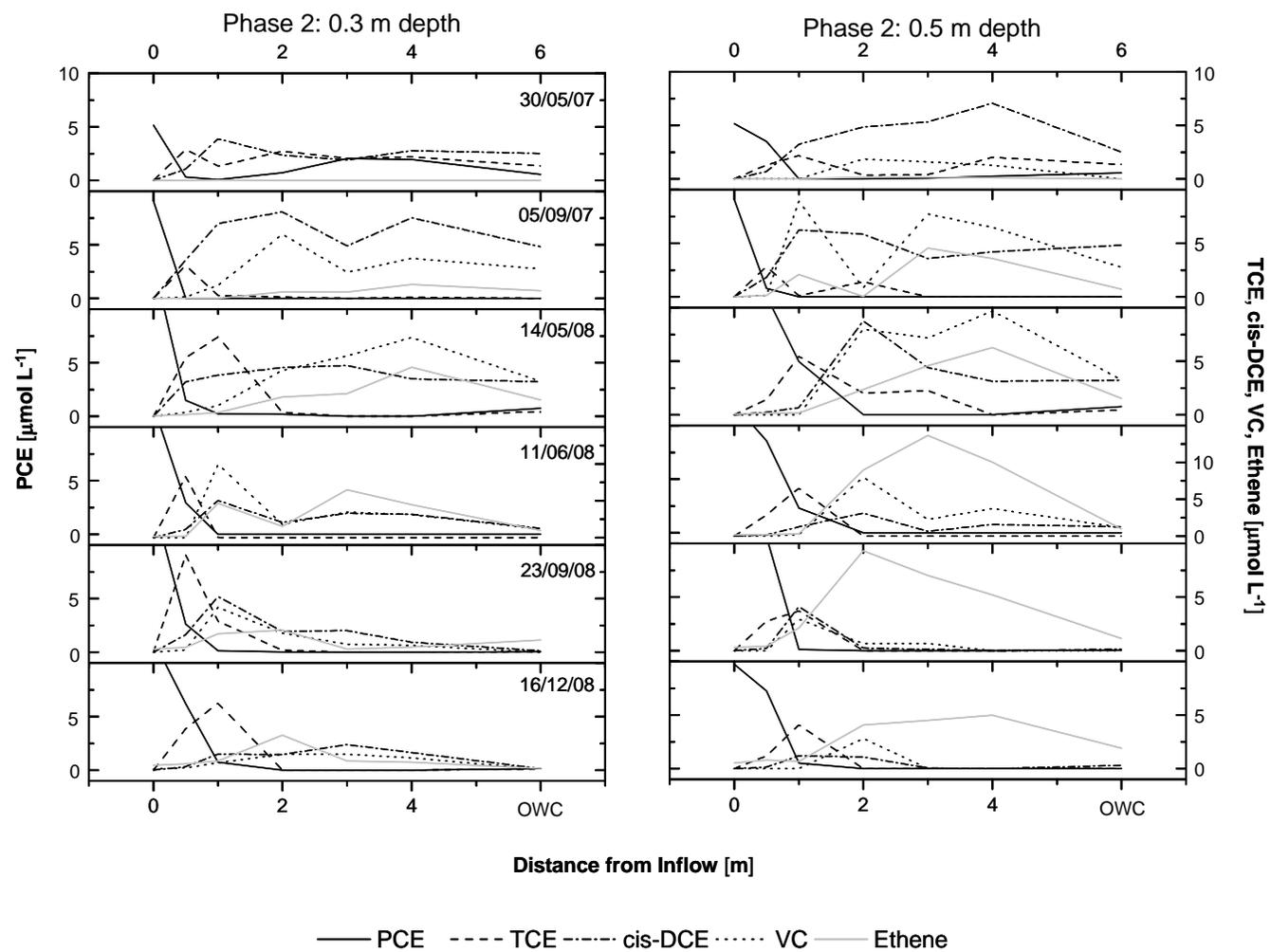


Figure 3: PCE removal and formation of metabolites over the course of the CW flow path in 0.3 and 0.5 m depth: Phase 2 (05/2007-12/2008), after start of VC production (PCE inflow concentrations were 10-15 μM).

In 0.5 m depth, considerable amounts of TCE were still detected between 2-4 m. Production of VC started during 05/2007 and 09/2007 in 0.3 and 0.5 m depth, respectively, and became very pronounced in summer 2007. Between autumn 2007 and spring 2008, conditions well-known in chlorinated ethene-contaminated aquifers occurred (Broholm *et al.*, 2005): high residual concentrations of cis-DCE (up to 5 μM) and VC (up to 3 μM) were still present in the system outflow. This was caused by the slower reductive dechlorination of the DCEs and VC compared to PCE and TCE (Broholm *et al.*, 2005), and also reflected the fact that the microbial community was not yet capable of complete PCE dechlorination. Presumably the lack of oxygen was responsible for the low activity of the potentially efficient aerobic microbial degradation of cis-DCE and VC (Field and Sierra-Alvarez, 2004) (Fig. 4). The described conditions are rather undesirable because both cis-DCE and VC are harmful to human health and the environment: VC is even a known human carcinogenic (Lee *et al.*, 2002).

Ethene concentrations became more pronounced after 09/2007 with production beginning at 0.5-1 m of the filter passage. Ethene concentrations were considerably higher in the deeper filter zone, and did not level off towards the end of phase 2 as observed in the upper filter zone. Ethene concentrations of up to 15 μM were observed in the deeper filter zone due to elevated dechlorination activity together with the absence of significant gaseous emissions and aerobic ethene degradation. From 06/2008 onwards, cis-DCE and VC concentrations decreased within the second half of the filter passage and concentration maxima moved towards the inflow of the system. In 12/2008, very low cis-DCE and VC concentrations were detected throughout the filter. PCE and all dechlorination products were eliminated practically to zero in the system outflow. At the 4 m sampling point, trace residual concentrations of cis-DCE and VC remained only in the upper filter layer, whereas no chlorinated PCE metabolites could be detected after 3 m in the deeper filter zone.

A degradation mass balance was calculated using data from the period 07-12/2008 for various points along the filter passage. After 1 m of the CW flow path, up to 100% of PCE inflow load ($1250 \pm 260 \mu\text{mol day}^{-1}$) was found as TCE, DCEs, VC and ethene, indicating complete dechlorination. A maximum of 30% was recovered as ethene. After 4 m of the flow path, only 29% of PCE inflow load was found as degradation products,

with ethene as the major metabolite (26%). The incomplete mass balance in this CW system can be explained by the fast conversion of ethene to CO₂ or other organic metabolites, accompanied by an expected fast loss to the atmosphere. Nevertheless, the observed metabolite patterns, and the very low amounts of chlorinated metabolites detectable after 4 m, demonstrate that a predominant part of the PCE load was actually dechlorinated to ethene.

Degradation and efficient removal of cis-DCE (Kassenga *et al.*, 2003; 2004; Tawney *et al.*, 2008) and TCE (Lorah and Olsen, 1999; Bankston *et al.*, 2002) in CW has been shown repeatedly. These studies mostly worked with upflow vertical systems (Lorah and Olsen, 1999; Kassenga *et al.*, 2003) or microcosm experiments (Bankston *et al.*, 2002; Kassenga *et al.*, 2004; Tawney *et al.*, 2008) with limited comparability to the study presented here. The distribution of TCE and metabolites along the flow path reported by Lorah and Olsen (1999) displays strong similarities to the patterns observed in this study, in particular at the end of phase 1 in the deeper filter zone. However, very few studies actually deal with the treatment of PCE in CW. Mastin *et al.* (2001) reported similar removal efficiency of 99% for PCE in a vertical upflow anaerobic wetland, but without any information on metabolite formation. In a study by Amon *et al.* (2007), an upflow vertical wetland (670 m², 1.14 m depth) was fed with PCE (0.205 µM) contaminated groundwater for 21 months of operation. Similar to our study, a rather slow development of sequential PCE dechlorination was observed and the treatment capacity was probably still developing at the end of the study period, underlining the very slow adaptation to PCE dechlorination. VC concentrations detected were low in comparison to PCE inflow concentrations. It was assumed that either further degradation or undesirable venting to the atmosphere (facilitated by surface discharge) was responsible for complete removal of VC. However, no ethene production was reported by the authors (Amon *et al.*, 2007). Strong ethene production clearly indicated complete detoxification of PCE and probably *Dehalococcoides* activity in the present study. By means of taxon-specific PCR, *Dehalococcoides sp.* were shown to be present in the groundwater inflow and in the OWC of the CW. However, PCR was not successful with soil samples from the CW filter, probably due to high amounts of humic substances disturbing the process.

Redox and electron acceptor conditions

The oxygen contour map (Fig. 4) shows that the CW filter was effectively anoxic below 0.1-0.15 m depth. Only the uppermost layer of the filter, including an unsaturated zone of approximately 0.1 m thickness, showed detectable oxygen concentrations. This was considered to be an effect of diffusion from the atmosphere and higher root density in the upper filter zone (Table 2). Close to the inflow, oxygen concentrations were slightly higher due to high plant density. This is clearly reflected by the root density in 0.3 m depth, being considerably higher at the 1 m sampling point than at 2 and 4 m (Table 2). As expected, oxygen penetration depth into the filter increased between 1 and 4 m, as a result of sustained oxygen diffusion.

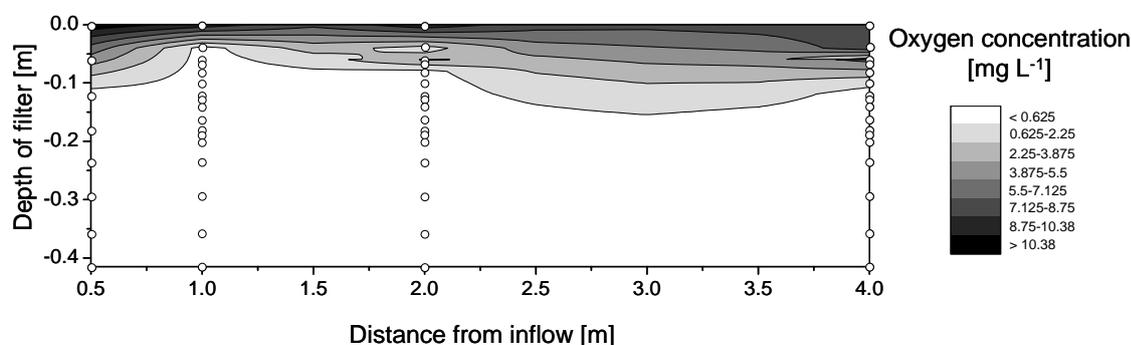


Figure 4: Contour plot of oxygen concentrations in the CW filter, applying kriging correlation as a gridding method ($n=9$, May-July 2008). White dots represent oxygen measurement locations.

Sulphate availability in the CW was high, with groundwater inflow concentrations of 800-900 mg L⁻¹. Sulphide concentrations in the inflow were ≤ 0.5 mg L⁻¹ in spring-autumn 2006 and up to 9 mg L⁻¹ in spring-autumn 2008. In 0.3 m depth, a rise in sulphide concentrations during the filter passage was observed only in summer 2006, accounting for a concentration increase of below 1 mg L⁻¹. In the deepest, most anaerobic filter zone (0.5 m depth), a sulphide concentration increase over the filter passage of up to 5 mg L⁻¹ was observed both in summer 2006 and 2008. These results show the presence of sufficiently reducing zones for dissimilatory sulphate reduction (redox potentials in the CW filter: -110 – 150 mV, data not shown). Stronger sulphide production in summer can be explained by the increasing availability of organic carbon from root exudation, lower redox potentials in the root zone, and the higher temperature (Mann and Wetzel, 1995; Stein *et al.*, 2007).

Ferric iron is also an important electron acceptor in the CW system, as known from other wetland soils (Kadlec and Wallace, 2008), and Fe(II) mobilization was observed in the CW ever since operation started (Braeckevelt *et al.*, 2007; 2008). Oxalate-extractable ferric iron, as a proxy for microbially reducible iron(III), amounted to 0.8-1.1 g kg_{dw}⁻¹ at 0.5 m and 1.7-3.3 g kg_{dw}⁻¹ at 4 m distance from the inflow (Table 2). It can be concluded that considerably more ferric iron originating from the filter material was reduced at the beginning of the filter passage, where contaminant concentrations were highest, creating the observed spatial inhomogeneity. There has been evidence for ferric iron reduction coupled to MCB oxidation (Dermietzel and Vieth, 1999; Vogt *et al.*, 2002), but the final proof is still lacking. After showing stable patterns from 2005 until July 2006 with ferrous iron concentrations of 35-40 mg L⁻¹ at the 4 m sampling point, ferrous iron formation began to decline constantly in late summer 2006. Mobilization only resulted in ferrous iron concentrations below 10 mg L⁻¹ at 4 m in winter 2008. The observed concomitance with the start of PCE addition suggests that the presence of chlorinated ethenes affected ferric iron reduction, presumably due to a competition for electron donors. This hypothesis is supported by the fact that some organisms can use ferric iron as well as PCE or TCE as electron acceptors (Krumholz, Sharp, and Fishbain, 1996). In addition, persisting ferrous iron mobilization may have generally depleted microbially reducible ferric iron in the system over time.

Conclusions

The presented study evaluated for the first time the potential of simultaneous treatment of MCB and PCE, representing highly and lowly chlorinated compounds, in a pilot-scale horizontal subsurface flow CW. The system served as a simulation of a surface-near groundwater aquifer discharging into a natural wetland, and as a technical CW system for the investigation of chlorinated VOC removal.

The results showed that CW are suitable for efficient simultaneous treatment of MCB- and PCE-contaminated waters, thus being a promising approach for the remediation of groundwater with mixed chlorinated VOC contaminations. Simultaneous treatment of MCB and PCE resulted in slightly lower removal efficiencies for MCB in comparison to the treatment of MCB alone, but MCB removal could still be efficiently maintained. Sub-optimal MCB removal prior to as well as after PCE addition generally resulted

from the lack of oxygen in the deeper anaerobic filter layers. Additionally, evidence has been found that changes in electron acceptor conditions (ferric iron and sulphate) may have resulted from PCE addition.

We were able to elucidate the development of PCE elimination potential and the evolution of dechlorination metabolites formation. The degradation potential for PCE developed very slowly and continuous changes in metabolite patterns occurred over the course of 2 ½ years. After the adaptation time, steady-state elimination of PCE and the dechlorination products was finally achieved. Ethene production and the presence of *Dehalococcoides sp.* clearly showed that VC was predominantly dechlorinated, and not lost by emission to the atmosphere.

In principle, the results obtained in the pilot-scale CW can be transferred to other conditions and mixtures of highly and lowly chlorinated contaminants, since redox gradients in CW rhizospheres provide suitable niches for a multitude of microbial processes. However, the mutual interference of different contaminants might differ in other cases. CW are suggested to be a potential treatment technology for chlorinated-VOC-contaminated groundwater wherever the necessary space is available. They are of particular interest for the treatment of surface-near groundwater discharging into natural river floodplains or wetlands.

Supporting information

Further information on water quality parameters and inorganic constituents of the groundwater feed supplied to the CW are given in Table S1.

Table S1: Water quality parameters and inorganic constituents of the groundwater feed supplied to the CW; means and standard deviations (for E_h : ranges) of representative control measurements carried out in spring ($n=2$) and autumn ($n=2$) of each year.

	Year		
	2006	2007	2008
Ca^{2+} [mg L ⁻¹]	379±9	NV ^a	357±7
K^+ [mg L ⁻¹]	147±10	160±1	169±7
Na^+ [mg L ⁻¹]	134±2	NV ^a	144±12
Mg^{2+} [mg L ⁻¹]	47±0.5	NV ^a	47±0.8
NH_4^+ [mg L ⁻¹]	1.9±1	2.7±0	28±1.8 ^b
SO_4^{2-} [mg L ⁻¹]	886±76	813±9	790±29
Cl^- [mg L ⁻¹]	222±9	305±15	359±21
PO_4^{3-} [mg L ⁻¹]	5.0±0.1	6.4±0.5	5.8±0.3
S^{2-} [mg L ⁻¹]	0.8±0.6	NV ^a	7.9±2
Fe_{total} [mg L ⁻¹]	1.1±0.8	0.4±0.1	0.3±0.1
O_2 [mg L ⁻¹]	0.15±0.19	0.15±0.05	NV ^a
σ [mS cm ⁻¹]	2.8±0.1	3.1±0	3.2±0.3
pH	7.1±0.8	6.7±0.2	6.8±0.4
E_h [mV]	-36 – 84	-95	-113 – -66

^a no value; ^b fertilizer addition

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4 Engineering approaches to optimization of monochlorobenzene and perchloroethene removal

4.1 Removal of monochlorobenzene and perchloroethene in wetland rhizosphere model systems

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Abstract

Constructed wetlands (CW) are a promising technology to protect river floodplains against the impact of contaminated groundwater. They are suitable for the treatment of waters contaminated by monochlorobenzene and perchloroethene. However, the removal performance differs with operation conditions and general, transferable performance data are not yet available. In this study, removal efficiencies were determined and dominant removal processes for monochlorobenzene and perchloroethene were evaluated under various operation conditions in helophyte rhizosphere reactors.

Monochlorobenzene removal was very efficient (>99%) under low carbon load (overall oxic) and moderate carbon load (overall reduced) conditions. Higher loads of easily degradable carbon (300 mg L⁻¹ acetate) impaired monochlorobenzene elimination (removal of 72-96%). Microbial reductive dechlorination of perchloroethene was not detected in the rhizosphere under low carbon load, sulphate reduction, and high carbon load conditions. Nonetheless, considerable amounts of perchloroethene were eliminated (79-87%), presumably by plant uptake and phytovolatilization. Under fluctuating moderate carbon load conditions, perchloroethene dechlorination was initiated, and trichloroethene and cis-dichloroethene production showed that minimum 10% of the perchloroethene inflow load was dechlorinated. Sulphate reduction and associated sulphide toxicity showed to constitute a hazard for CW treatment of sulphate containing groundwater contaminated by chlorinated volatile organic compounds, causing a

decrease in removal efficiencies by 50% and 20% for monochlorobenzene and perchloroethene, respectively.

Keywords: Phytoremediation; removal efficiency; volatile organic compounds (VOC)

1. Introduction

In recent years, natural and constructed wetlands (CW) have attracted considerable interest as cheap and simple treatment systems for waters contaminated by xenobiotic organic chemicals [1-3]. Several studies have dealt with treatment of petroleum hydrocarbons [4, 5], BTEX [6-8] and chlorinated volatile organic compounds (VOC) [9-12]. CW are an alternative to conventional pump-and-treat technologies for remediation of large scale groundwater contaminations, in particular for the lower concentration range where pump-and-treat applications become increasingly unsustainable [13, 14]. Even though complete remediation of large scale contaminated aquifers is mostly not feasible [13], measures for risk mitigation and safeguarding of adjacent river floodplains and surface water bodies are often indispensable [15]. Due to the natural occurrence of wetlands in groundwater discharge areas and the ability of being integrated into natural landscapes, CW are a promising technology for protection of sensitive aquatic ecosystems against the impact of contaminated groundwater.

Monochlorobenzene (MCB) and perchloroethene (PCE) are among the environmentally most relevant chlorinated VOC, and are priority groundwater pollutants worldwide as a result of their substantial production and use [16-18]. They represent two important contaminant classes with discriminative properties: MCB is a monosubstituted aromatic compound readily biodegradable under aerobic conditions [18], but mostly persistent in anaerobic aquifers [17, 19]. In contrast, PCE is a fully chlorinated alkene and requires initial reductive dechlorination for its successful degradation [16, 20]. For reductive dechlorination of chlorinated ethenes, microorganisms need electron donors like molecular H₂ or organic acids (e.g. acetate, lactate) [20, 21]. Organic acids are provided in wetland rhizospheres by rhizodeposition, and the fermentation of organic root exudates may lead to H₂ production [22, 23]. Main removal processes for chlorinated VOC in CW are reported to be microbial degradation and (phyto)volatilization, and for chlorinated ethenes also plant uptake and metabolization [3, 24]. Vertical upflow CW have been shown to be effective for the treatment of waters polluted by PCE: Mastin *et*

al. [25] reported a removal efficiency of 99% in such a system but without any information on metabolite formation, and therefore giving no indication of dominant removal processes. Amon *et al.* [26] observed efficient removal of PCE and slow development of sequential PCE dechlorination, and reported some vinyl chloride, but no ethene production. MCB removal has been investigated in a number of different wetland systems. Using laboratory wetland microcosms planted with reed, MacLeod *et al.* [27] found that 33% of added MCB were volatilized and 27% were mineralized. A large-scale CW consisting of alternating deep and shallow vegetated zones treating a range of VOC also removed low concentrations of MCB, and volatilization modelling was successfully applied to explain observed removal [28]. In a pilot-scale horizontal subsurface flow CW, evidence for aerobic as well as anaerobic microbial degradation of MCB was found, and removal efficiencies of up to 71% in the upper filter layer were reported [29, 30], while emission measurements showed volatilization to be only a minor removal process [31].

Even though several studies have dealt with the removal of chlorinated VOC in CW, general, transferable concepts to control the removal performance are very rare. Reported removal efficiencies and the contributing processes differ strongly with the type of CW and various operation parameters. Therefore, the aim of this study was to determine removal efficiencies for MCB and PCE treatment in CW under defined, reproducible rhizosphere conditions, and to assess dominant removal processes. Helophyte rhizosphere reactors (Planted Fixed-Bed Reactors [PFR]) were used for this study, as a lab-scale testing unit representing the wetland root zone [32]. The macro-gradient free operation mode allowed precise determination and regulation of overall rhizosphere conditions (redox potential, oxygen concentration, pH, organic carbon content etc.). However, micro-gradients near root surfaces and associated with biofilms were still viable, enabling different microbial and redox processes simultaneously in the rhizosphere. The PFR systems are valuable tools for basic investigations on redox and oxygen dynamics, sulphur, nitrogen and carbon cycles in wetland rhizospheres [32-34].

Two rhizosphere reactors were run in parallel, of which reactor A was fed with MCB and reactor B with PCE. Several basic operation conditions were investigated that are supposed to be relevant in CW treatment of chlorinated VOC. Experiment 1: low organic carbon load of the feed. Groundwater often does not contain any additional

carbon source except the chlorinated contaminants. Therefore, the impact of root exudates as the only relevant carbon source on rhizosphere redox conditions and removal success was investigated. Experiment 2: sulphate reducing conditions. Chlorinated VOC contaminated groundwater may contain considerable amounts of sulphate [30, 35, 36], suggesting sulphate reducing conditions to be potentially relevant in CW treatment. Experiment 3: high load of easily degradable organic carbon. Experiment 4: fluctuating moderate load of easily degradable organic carbon. Typical reduced wetland rhizosphere conditions due to high organic carbon availability are expected to support reductive dechlorination, and auxiliary carbon addition may even be necessary for treating waters containing only reducible contaminants such as PCE. However, high organic carbon loads can in turn impair the removal of oxidizable compounds such as MCB due to competition for electron acceptors. With regard to CW treatment of mixed groundwater contaminations, it is important to assess the impact of various organic carbon concentrations on removal of highly and lowly chlorinated VOC.

2. Material and Methods

2.1 Experimental setup and operation parameters

The design and operation principles of the PFR have been published in detail elsewhere [32-34]. Briefly, the reactors were operated under macro-gradient free conditions, achieved by an internal circulation flow of the water in the gravel bed. The reactors consisted of cylindric glass tanks (diameter 0.28 m, height 0.3 m, volume 18.5 L) filled with gravel (size 2-4 mm, total pore volume 10-11 L). The reactors were closed by a Teflon lid with 5 plant openings and were planted with *Juncus effusus*. The hydraulic retention time was adjusted to 6-7 days (inflow rate 1.7 L d⁻¹) and the recirculation flow rate to 69.1 L d⁻¹. The feed was pumped from a 13 L glass storage container kept in the dark. Nutrients were added directly to the inflow tank in Exp. 1 and 2. In order to avoid microbial colonisation of the feed system, nutrients and acetate were directly added to the inflow via syringe pumps (Serie 200, KD Scientific Inc., USA) in Exp. 3 and 4. Chlorinated organics were added to the inflow tank directly in Exp. 1-3, and via syringe pumps and gas tight glass syringes (100 mL, SGE Analytical Science, Australia) in Exp.

4. Contaminant loss from the inflow tank and syringes between weekly refills has been determined and accounted for in all calculations of inflow loads.

Four different operation conditions (see Section 1) were investigated in reactor A receiving MCB and reactor B receiving PCE as a contaminant. Target operation conditions were adjusted by the following parameter settings: low carbon and high sulphate load, low buffer capacity (Exp. 1), low carbon and high sulphate load, high buffer capacity (Exp. 2, sulphate reduction), high carbon and contaminant load, high buffer capacity (Exp. 3), fluctuating moderate carbon load, moderate contaminant load, high buffer capacity (Exp. 4). The composition of model groundwater in the experiments is summarized in Table 1. For long-term support of plant and microbial growth, 1 mL L⁻¹ of a trace metal solution was added to the feed, containing (in g L⁻¹): Na-EDTA 1; FeSO₄*7 H₂O 1; MnCl₂*2 H₂O 0.8; CoCl₂*6 H₂O 1.7; CaCl₂*2 H₂O 0.7; ZnCl₂ 1; CuCl₂*2 H₂O 1.5; NiCl₂*6 H₂O 0.3; H₃BO₄ 0.1; Na₂MoO₄*2 H₂O 0.1; Na₂SeO₃*5 H₂O 0.02. Prior to the start of each experiment, the systems were inoculated with chlorinated hydrocarbon contaminated groundwater (25-50 L), and extracts of sediment from a CW treating the same contaminated groundwater, from a site in Bitterfeld/Germany [29]. Extracts of 1 kg sediment per reactor were obtained according to a method adapted from Bakken [37] and Kindler *et al.* [38]. After setup and planting, the reactors were operated for several weeks prior to the start of data acquisition. Experiments were carried out under defined conditions of an average Middle European summer day [32]. Transpiration was assessed weekly by determining inflow and outflow water volumes.

2.2 Water sampling and analysis

Redox potential (electrode 2ME-2G-PtK-1, Jumo, Germany), dissolved oxygen (electrode Sipan 34, 7MA3100-8EF, Siemens, Germany) and pH (electrode 2GE-2-G-U-1, Jumo, Germany) were continuously determined in the recirculation flow. Inflow and outflow water samples of approx. 0.2 L were obtained by weekly sampling in order to determine the concentration of chlorinated organics, acetate, COD and sulphide. Chlorinated organics were quantified by GC-FID (HP 7694 Autosampler, HP 5890 GC-FID Series II, Agilent Technologies, USA) after addition of NaN₃ (65 mg L⁻¹) to prevent further microbial activity. The analytes were separated on a CP-Sil 5CB column

(25 m×0.32 mm×5 μm) (Chrompack/Varian Inc., USA) with the following temperature program: 35°C (3 min), 8 K min⁻¹ to 90°C (4 min), 6 K min⁻¹ to 120°C, 15 K min⁻¹ to 250°C. The data presented are means of duplicate samples. Acetate concentrations were determined using ion chromatography with conductivity detection (DX 100, Ion Pac AS4A-SC column and Ion Pac AG4A-SC guard column, anion self-regenerating suppressor ASRS-Ultra4-mm, Dionex, Germany).

Table 1: Model groundwater composition in the different experimental phases (Experiment 1-4) for reactor A fed with MCB and reactor B fed with PCE.

Experiment	1	2	3	4
Time period	05/01-24/03/07	24/03-30/05/07	09/10/07- 05/02/08	05/05-28/07/09
Days of operation	78	67	119	91
Reactor A				
MCB [mg L ⁻¹]	16.6	16.6	16.6	8
Acetate [mg L ⁻¹]	0	0	300	100 to 0
Reactor B				
PCE [mg L ⁻¹]	4.8	4.8	8.5	3
Acetate [mg L ⁻¹]	0	0	300	100 (transient 150)
Both reactors				
NH ₄ ⁺ [mg L ⁻¹]	5	5	5	5
PO ₄ ³⁻ [mg L ⁻¹]	5	5	6.6	6.6
SO ₄ ²⁻ [mg L ⁻¹]	250	250	0.23 ^a	0.23 ^a
Cl ⁻ [mg L ⁻¹]	206.9	206.9	6.9 ^a	6.9 ^a
Br ⁻ [mg L ⁻¹]	0	0	43.3	43.3
Na ⁺ [mg L ⁻¹]	150	177.4	144	171.7
Mg ²⁺ [mg L ⁻¹]	45	45	2	2
Ca ²⁺ [mg L ⁻¹]	30	30	2	2
K ⁺ [mg L ⁻¹]	4	4	5.4	5.4
HCO ₃ ⁻ [mg L ⁻¹]	0	72.6	72.6	145.2

^afrom trace metal solution

COD and sulphide concentrations were measured using photometric test kits and a Cadas 100 photometer (COD: LCK 314, 414 and 514; Sulphide: LCW053; Hach Lange, Germany).

3. Results and Discussion

3.1 Experiment 1: Low organic carbon load

Redox potentials in the reactors stabilized on a high positive level, ranging between 600-700 mV in reactors A and B fed with MCB and PCE, respectively (Fig. 1). Oxygen concentrations were between 0.5 and 2 mg L⁻¹. Considerable diurnal oxygen fluctuations but only weak redox fluctuations were observed (Fig. 1).

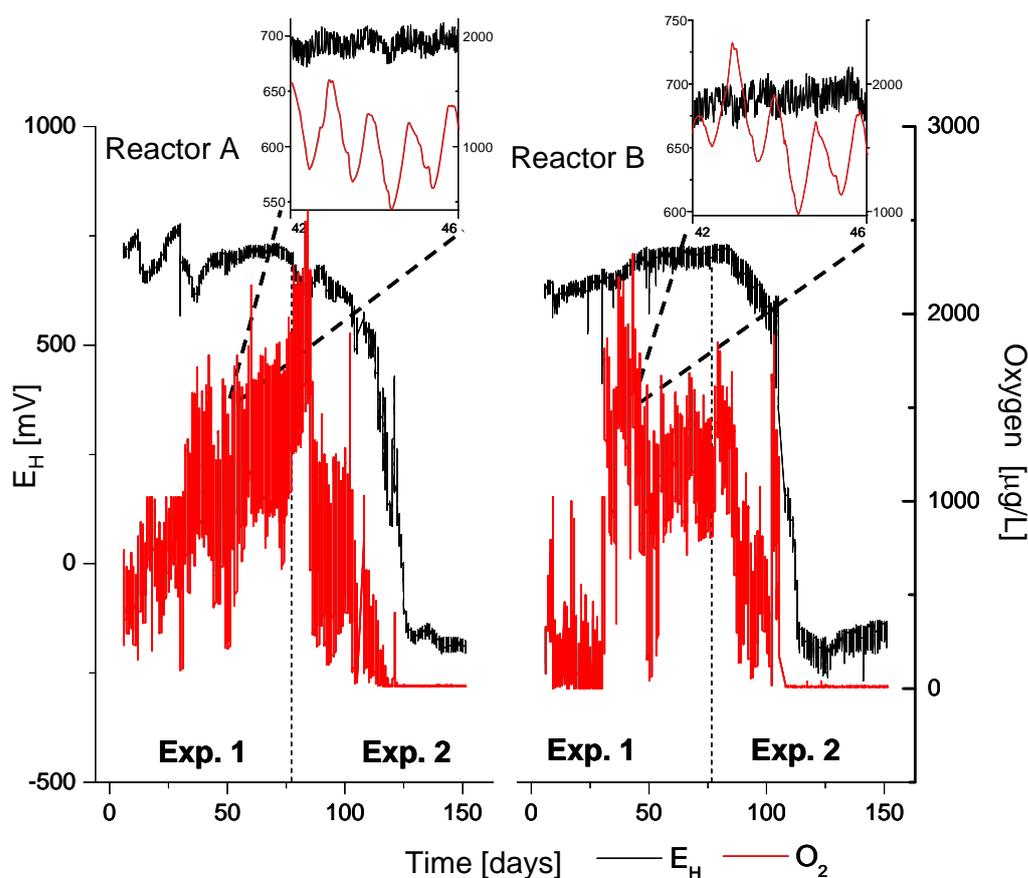


Figure 1: Redox potential and oxygen concentrations in the Exp. 1 and 2 in reactor A fed with MCB and reactor B fed with PCE.

Diurnal oxygen concentration and redox potential fluctuations have been observed in the rhizosphere of helophytes, and it was demonstrated in PFR that root oxygen release is influenced by light intensity [33, 39, 40]. Due to the low carbon load, oxygen concentrations did not decrease to zero during the night, suggesting that excess oxygen was present in the root zone. Sulphate concentrations were high (Table 1), but microbial sulphate reduction was suppressed by the low carbon availability and the low pH establishing in both reactors. pH decreased from neutral at the beginning of operation to

3.9 in reactor B and 3.7 in reactor A. Many common sulphate reducing bacteria have a circumneutral or slightly alkaline pH optimum [41], and inhibition by H_2S and organic acids has been observed at low pH [42]. Rhizosphere acidification might have been caused by a combination of several processes, including root release of protons and organic acids [43-45], microbial oxidation of organic matter [46, 47], a poor buffer capacity and the use of ammonium as a nitrogen source.

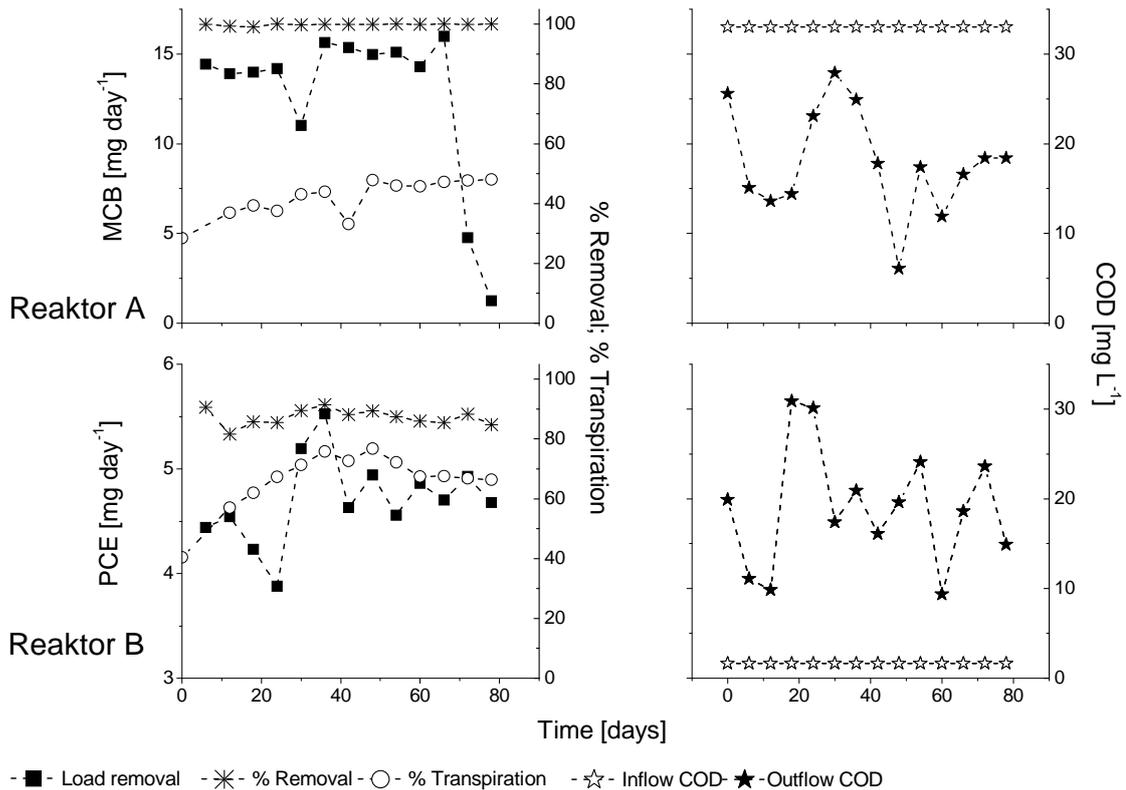


Figure 2: Removal of MCB and PCE in Exp. 1: Low organic carbon load.

The prevailing oxygen excess conditions showed to be ideal for removal of MCB, which is preferably degraded oxidatively. Load removal was virtually 100% (mostly around 15 mg d⁻¹) throughout the experiment (Fig. 2). Transpiration rate increased over time, confirming that plant biomass was in good health. COD removal was not complete, in spite of the low inflow COD and total removal of MCB. Formation of soluble microbial products and products of plant biomass decay probably lead to the observed outflow COD, as has been shown for CW and other biological water treatment systems [48, 49]. MCB removal was due to microbial degradation and phytovolatilization [3], as soil bound emissions were largely minimized by the teflon

cover plate. As a precondition for phytovolatilization, plant uptake was estimated according to Eq. 1 [50],

$$Uptake = TSCF \times Trans \times c_{solution} \quad (1)$$

where $TSCF$ is the transpiration stream concentration factor, $Trans$ is the transpiration rate and $c_{solution}$ is the effective contaminant concentration of the solution. $TSCF$ was calculated from the octanol water partition coefficient K_{OW} using an empirical relationship [50]. Application of a $\log K_{OW}$ for MCB of 2.98 [51], an inflow concentration of 16.6 mg L^{-1} and a transpiration rate of $0.8\text{-}0.9 \text{ L d}^{-1}$ resulted in the estimation that maximum $9\text{-}10 \text{ mg MCB d}^{-1}$ could have been taken up by the vegetation. The comparison to the overall removal rate of 15 mg d^{-1} shows that uptake by plants may constitute an important process for the removal of MCB; but there must also be others. In addition, plant uptake is likely to be heavily overestimated by this assessment, as pore water concentrations in the reactor are much lower than inflow concentrations. Hence, the highly efficient MCB removal was probably largely due to microbial oxidation under the prevailing oxic conditions.

Elimination of PCE was mostly between $80\text{-}90\%$ ($4\text{-}6 \text{ mg d}^{-1}$), with a slight increase after 30 days (Fig. 2). An increase in transpiration rate over time was also observed ($> 60\%$ after 30 days). In spite of a negligible inflow COD, the outflow COD was similar to that determined in reactor A. No PCE dechlorination products were found as expected for the overall oxic redox conditions. Obviously, root exudates did not create sufficient oxygen demand to balance root oxygen input, and were not suitable to serve as electron donors in the reductive dechlorination of PCE. The considerable removal of PCE observed under the present conditions is striking, considering that reductive dechlorination to trichloroethene (TCE) is the only known microbial PCE degradation pathway [16]. Dechlorination to TCE in anaerobic micro-zones and subsequent fast cometabolic oxidative degradation, being an important TCE elimination process in the environment [52], may represent a potential elimination pathway even under overall oxic rhizosphere conditions. However, plant related removal processes like uptake and subsequent metabolization or phytovolatilization probably play a major role in PCE removal in the rhizosphere model system. This has been observed repeatedly for TCE in phytoremediation applications [53-55]. Emission measurements provided first

indications that gas phase PCE concentrations in the vicinity of the reactors were considerable (data not shown). Theoretical plant uptake of PCE was calculated as described above, with a log K_{OW} of 2.53 [56], a PCE inflow concentration of 4.8 mg L^{-1} , and a transpiration rate of 1 L d^{-1} . A potential maximum plant uptake of $\sim 4 \text{ mg d}^{-1}$ was found, showing that a major fraction of the PCE removed could have been eliminated via plant uptake.

3.2 Experiment 2: Low organic carbon load and sulphate reduction

To prevent acidification of the pore water and enable sulphate reduction, buffering with NaHCO_3 was applied from day 78 of Exp. 1, which was considered day 0 of Exp. 2 (Table 1), causing an immediate response of redox potential in both reactors (Fig. 1). After initial moderate decrease until day 25-30, the redox potential dropped suddenly and stabilized fluctuating between -125 and -250 mV . Thus, redox conditions were appropriate for dissimilatory microbial sulphate reduction ($< -150 \text{ mV}$) [57, 58]. After a short lag phase, no oxygen was detectable in the pore water of both reactors.

Load removal of MCB decreased from nearly 100% (16 mg d^{-1}) at the start of the experiment to 50% (8 mg d^{-1}) after 60 days (Fig. 3). Toxic impacts of dissolved sulphide on plant health were evident since the transpiration rate dropped to below 10%. Considerable sulphide concentrations were observed in reactor A beyond day 40 reaching $\sim 25 \text{ mg L}^{-1}$ at the end of the experiment. This was accompanied by an increase in outflow COD to over 400 mg L^{-1} which was most likely constituted by reduced sulphur species formed by sulphate reduction, and the leaching of carbon compounds from dead biomass due to sulphide induced necrosis [59, 60]. Sulphide concentrations of 45 mg L^{-1} have been shown to be highly toxic to *Phragmites* [60], and $20\text{-}25 \text{ mg L}^{-1}$ had adverse effects on the transpiration rate of *Juncus effusus* [61]. The observed decrease in MCB removal efficiency was presumably caused by a lower plant activity, which in turn negatively affected microbial activity. In addition, direct inhibition of microbial degradation processes [42, 58, 62] and the establishment of reducing conditions may have caused less efficient microbial MCB elimination. MCB degradation by microorganisms adapted to sulphate reducing conditions could still have been viable under the present conditions. However, dissimilatory sulphate reduction coupled to MCB oxidation has not been conclusively shown yet [63, 64]. In spite of the

overall reduced conditions, it is assumed that oxic micro-zones around the roots still existed and thus enabled MCB oxidation to a certain extend.

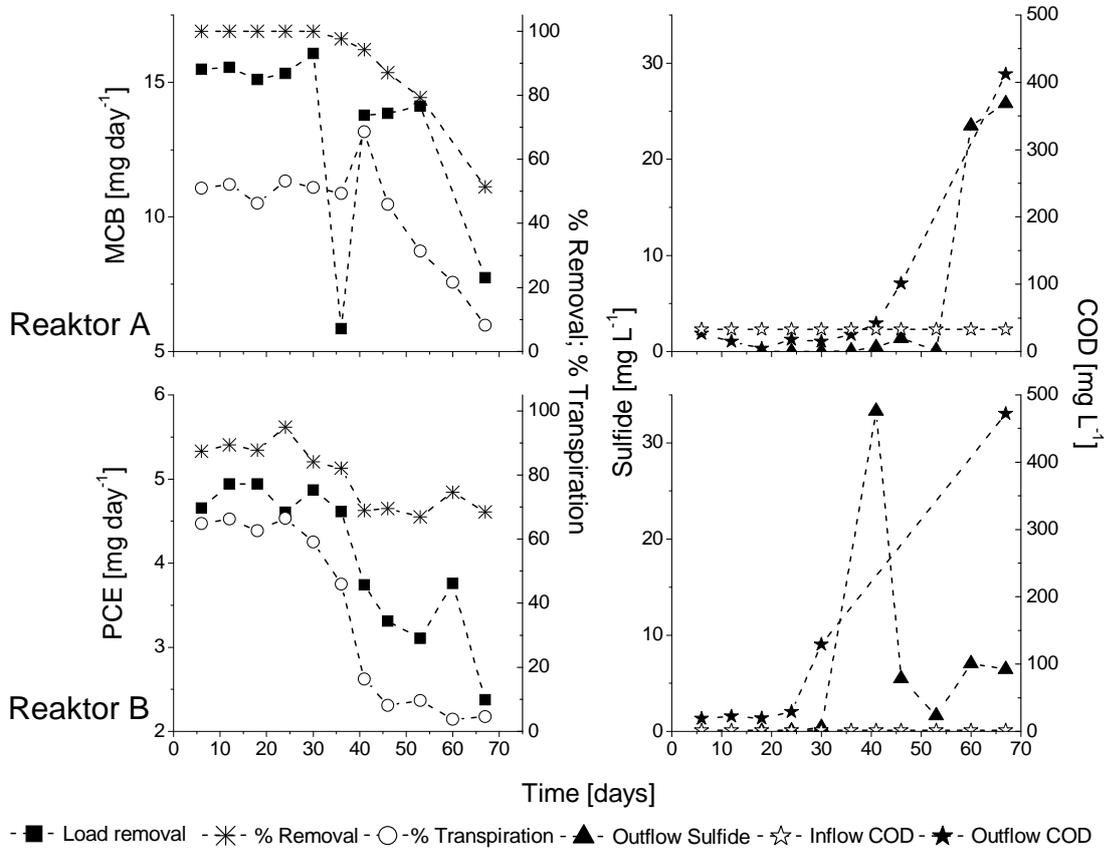


Figure 3: Removal of MCB and PCE in Exp. 2: Low organic carbon load and sulphate reduction.

PCE removal efficiency was also negatively affected under sulphate reducing conditions. However, the load removal decrease was less pronounced than for MCB dropping from 90% at the beginning to ~70% at the end of the experiment (Fig. 3). Transpiration was lowered to <10% after day 47 in concomitance with sulphide concentrations of up to 32 mg L⁻¹ and COD levels of ~470 mg L⁻¹. Similar to Exp. 1, no dechlorination products were detected, even though reduced conditions prevailed. Sulphate reduction has been found to compete with and to inhibit reductive dechlorination [36, 65,]. However, both processes may also proceed simultaneously, and some sulphate reducers have been shown to be capable of dehalorespiration [66].

The apparent response of MCB removal to high sulphide concentrations provides additional evidence for MCB removal processes being mostly biological and oxygen-dependant (50% reduction of removal efficiency in comparison to Exp. 1). In contrast,

biotic removal processes susceptible to inhibition by sulphate reduction were not dominant in PCE removal, with only 20% reduction of removal efficiency.

3.3 Experiment 3: High organic carbon load

Both reactors were operated with high additional carbon load (300 mg acetate L⁻¹). Redox potential decreased from -20 mV at the beginning to -175 – -230 mV and from +100 mV to -155 – -210 mV in reactors A and B, respectively. In both reactors, oxygen concentrations were <0.02 mg L⁻¹ without distinct fluctuations and the pH was 6.5-7 throughout the experiment. Day-night redox fluctuations accounted for 20-25 mV in reactor A and 30-40 mV in reactor B.

Two distinct phases of MCB removal were observed (Fig. 4). Removal was instable up to day 50 with load removals between 2 and 7 mg d⁻¹ (25-61%). Apparently, a lag phase occurred after start of operation. Acetate was presumably consumed more readily by the initial microbial population, so that MCB removal was inefficient at first. Subsequently, the high load of acetate supported microbial biomass growth leading to an increase in acetate removal from 330 to 510 mg d⁻¹ after day 56. MCB load removals also increased to 9-10 mg d⁻¹ (72-96%). Thus, microbial MCB degradation processes established in the reduced rhizosphere after the observed lag phase and were efficient even with high additional loads of easily degradable carbon. However, removal efficiency never reached the capacity observed under oxygen excess conditions (see 3.1).

PCE elimination was more uniform over the study period with load removals of 5-7 mg d⁻¹ (69-96%). No clear trend could be observed and no dechlorination products were determined. Thus, even under the reduced conditions prevailing in the reactor and in the absence of sulphate reduction, probably no dechlorination occurred. In spite of the inoculation of the reactor, the absence of reductive dechlorination may have been connected to the absence of suitable dechlorinators or the necessity for a longer adaptation time due to their low growth rates. However, a considerable amount of PCE was still eliminated. Theoretical maximum plant uptake of PCE was calculated as 2.4 mg d⁻¹, approximately 30-50% of the PCE removed. Non-plant-mediated PCE volatilization may have been more relevant than expected and could have been involved in removal of the remaining PCE fraction. An increase in removal was observed for

both COD and acetate showing that microbial biomass had increased and adapted to acetate utilization.

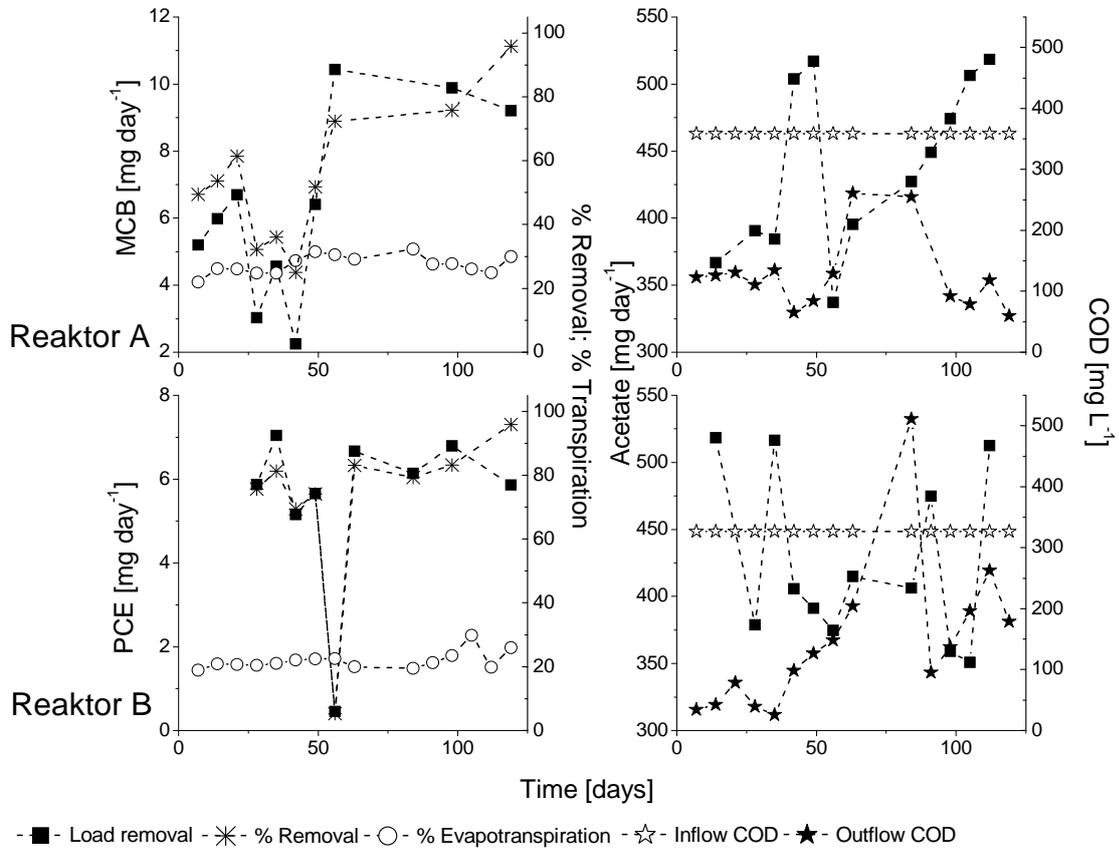


Figure 4: Removal of MCB and PCE in Exp. 3: High organic carbon load.

3.4 Experiment 4: Fluctuating moderate organic carbon load

In both reactors, operation started with a moderate carbon load of 100 mg acetate L⁻¹. The general goal was to create conditions supporting dechlorination of PCE as well as oxidation processes of MCB and partly dechlorinated chloroethenes. Besides, initial addition of easily degradable carbon should support biomass growth, resulting in higher removal potential compared to operation without initial carbon addition (Exp. 1). Fluctuations of carbon load were applied to determine the redox state most conducive for elimination of the two contaminants, and to investigate system response to changes in the carbon regime.

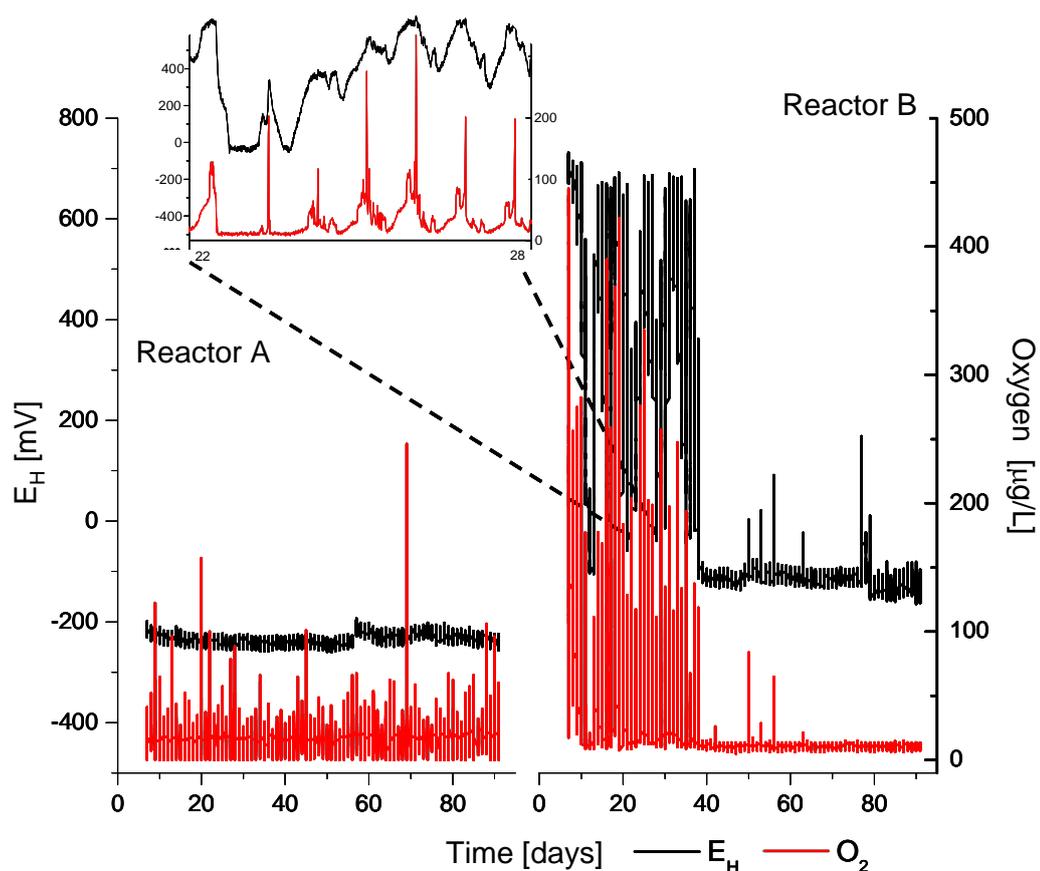


Figure 5: Redox potential and oxygen concentrations in reactor A fed with MCB and reactor B fed with PCE in Exp. 4.

In reactor A, acetate loads were decreased from 100 to 0 mg L⁻¹ in the course of the experiment. No strong redox and oxygen concentration fluctuations were observed. Redox potential was between -200 and -260 mV, and oxygen concentrations were up to 0.1 mg L⁻¹ throughout the experiment (Fig. 5). No changes were observed with the gradual decrease of acetate concentration to 0 (day 37 - 63), indicating a high stability of the rhizosphere redox state.

While decreasing the acetate inflow concentration had no detectable effect on redox conditions a beneficial effect on MCB removal was evident (Fig. 6). After a decline in removal at the beginning of the experiment an immediate response was observed when acetate concentration was lowered in a first step on day 37. Load removal converged to 9-10 mg d⁻¹ (99.5%) after day 70 and percentage load removal was comparable to that determined in Exp. 1, even under the prevailing overall reduced conditions (Fig. 5). Comparing the early phases of Exp. 3 and 4, it is obvious that the higher acetate load

(300 mg L⁻¹ instead of 100 mg L⁻¹) initially impaired MCB removal more profoundly in Exp. 3.

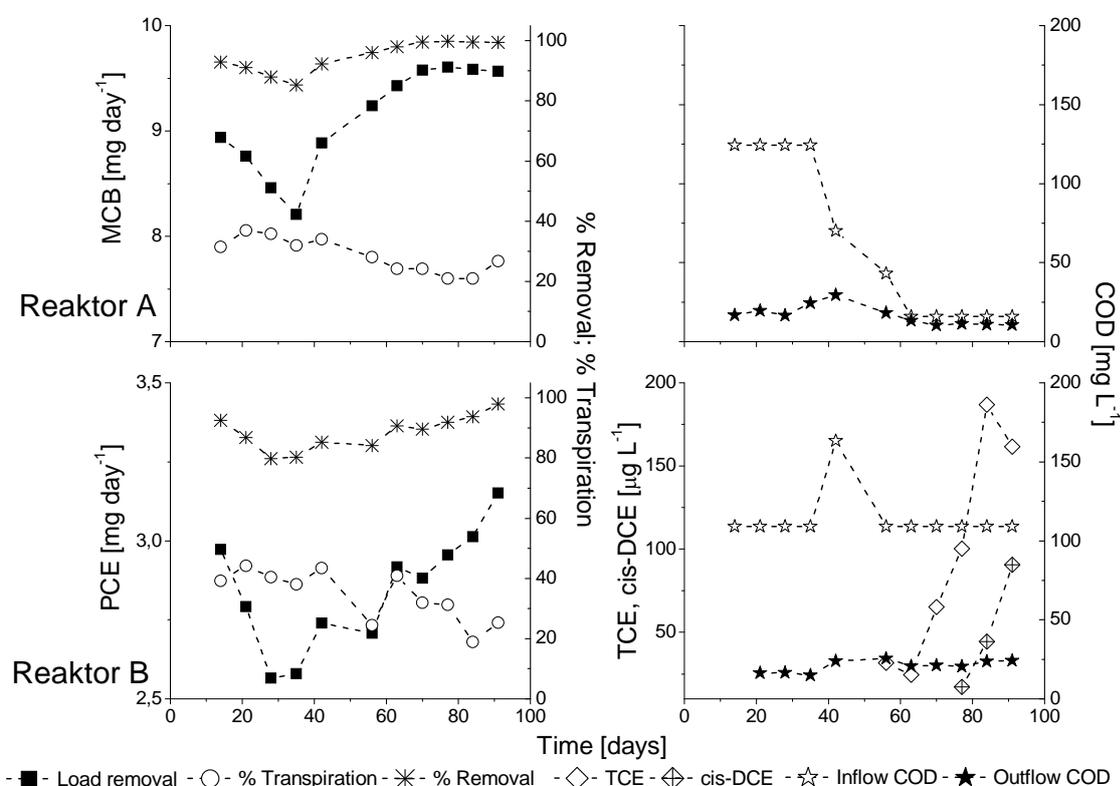


Figure 6: Removal of MCB and PCE in Exp. 4: Fluctuating moderate organic carbon loads. Reaktor A: Acetate concentration decreased from 100 to 0 mg L⁻¹ (day 37-63); Reaktor B: Acetate concentration 100 mg L⁻¹, transiently elevated to 150 mg L⁻¹ (day 37-48).

In reactor B, redox conditions stabilized on a high level during the first 35 days of operation (Fig. 5). Pronounced diurnal redox (0 - 600 mV) and oxygen concentration fluctuations (0 - 0.3 mg L⁻¹) were observed. When carbon load was transiently elevated to 150 mg L⁻¹ for 11 days (day 37-48) to bring down the redox potential to a level suitable for reductive dechlorination, it dropped to ~ -100 mV (Fig. 5). However, only weak diurnal fluctuations were observed and redox potential did not increase again after acetate loading was set back to 100 mg L⁻¹. Also distinct oxygen concentration fluctuations terminated as a result of the transient carbon load elevation (Fig. 5). Evidently, the rhizosphere redox state switched over to a new equilibrium and the changeover was not reversible by returning to the original carbon load.

After an initial lag phase, an increase in PCE load removals was observed, in contrast to Exp. 3, where both higher acetate and PCE loads were applied. Between day 30 and 91, load removal steadily rose from 2.6 to 3.1 mg d⁻¹ (80 to 98%) and COD removal was

efficient throughout the experiment. Apparent adaptation to PCE removal may have been facilitated by the lower inflow concentrations considering that PCE is a potential toxin for microbes [67]. PCE dechlorination was evidently triggered by the period of elevated acetate concentration and the drop in redox potential, and TCE was determined in the pore water from day 50 onwards. TCE concentration reached nearly 0.2 mg L^{-1} at the end of the experiment. After day 77, also cis-dichloroethene (cis-DCE) was detected with concentrations of $\sim 0.1 \text{ mg L}^{-1}$. With a PCE inflow load of 5.2 mg d^{-1} ($0.031 \text{ mmol d}^{-1}$) and assuming simultaneous outflow loads of $0.24 \text{ mg TCE d}^{-1}$ ($0.0018 \text{ mmol d}^{-1}$) and $0.12 \text{ mg cis-DCE d}^{-1}$ ($0.0012 \text{ mmol d}^{-1}$), about 10% of the added PCE were proven to be dechlorinated. PCE dechlorination was not yet complete and dechlorination efficiency may increase after further system adaptation. Long adaptation periods have already been observed in pilot-scale CW systems treating PCE [26, 68].

4. Conclusions

It has been shown that MCB removal can be very efficient in rhizosphere systems, both under overall oxic and overall reduced conditions reaching over 99% removal. Higher loads of easily degradable carbon evidently impaired MCB elimination. Thus, negative impacts of easily degradable organic carbon addition on the removal of compounds primarily degraded via oxidation, such as MCB, should be considered when the supply of electron donors for the treatment of mixed contaminations is intended. However, moderate loads were shown to enable both processes, and also overall reduced redox potential did not generally corrupt microbial MCB removal.

PCE dechlorination was highly difficult to establish in the rhizosphere systems, and was not conclusively detected under oxic conditions, reduced conditions with sulphate reduction, and under reduced conditions with high carbon loads. Nonetheless, relatively high amounts of PCE were eliminated from the system accounting for 79-87% of the inflow loads. Plant uptake and subsequent phytovolatilization, as well as surface volatilization, were considered to be important removal processes for PCE. Under the condition of fluctuating moderate acetate and low PCE concentrations, PCE dechlorination was initiated and TCE as well as cis-DCE were detected as degradation products. By assessment of degradation product outflow loads, 10% of PCE inflow loads were proven to be dechlorinated. Efficiency of dechlorination is expected to

increase in a long-term experiment. Additional investigations should be carried out to define conditions more conducive for complete PCE dechlorination and to assess plant uptake with subsequent phytovolatilization of PCE in rhizosphere systems.

Sulphate reduction was shown to be a hazard for CW treatment of sulphate containing groundwater contaminated by chlorinated VOC resulting in a considerable decrease of removal efficiencies. This potential drawback should be taken into account before setup of CW treatment systems.

An additional insight gained from the experiments with fluctuating carbon loads is that the behaviour of redox potential in the rhizosphere model system – and presumably also in the natural wetland rhizosphere - is highly difficult to predict on the basis of the knowledge available to date. The system may remain in a steady redox state even if the regime is crucially changed, but in other cases it may strongly react towards small and transient changes. Thus, additional investigations should be carried out for improving the knowledge on the factors governing stability of rhizosphere systems.

Symbols and abbreviations used

BTEX, benzene, toluene, ethylbenzene and xylenes; **cis-DCE**, cis-dichloroethene; **COD**, chemical oxygen demand; **CW**, constructed wetland; **GC-FID**, gas chromatography with flame ionisation detector; **K_{ow}**, octanol water partition coefficient; **MCB**, monochlorobenzene; **PCE**, perchloroethene; **PFR**, planted fixed-bed reactor; **TCE**, trichloroethene; **TSCF**, transpiration stream concentration factor; **VOC**, volatile organic compounds.

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Conflict of interest

The authors have declared no conflict of interest.

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5 Summary and concluding remarks

The use of CW for treatment of chlorinated VOC contaminated groundwater is a promising cost-efficient alternative to conventional pump-and-treat technologies, particularly for surface-near groundwater discharging into natural river floodplains or wetlands. CW can be integrated into the natural landscape in floodplain areas, or parts of existing wetlands can be employed in a controlled process. The technology is expected to be particularly suitable for the treatment of groundwater containing both highly and lowly chlorinated (microbially oxidizable and reducible) VOC, due to the inherent gradient characteristics of the wetland rhizosphere. Even though some experience has been gathered concerning CW treatment of chlorinated VOC contaminated waters, established knowledge on treatment performance, the factors governing removal efficiency, and dominant removal processes is scarce. Large scale applications are particularly rare, and performance data are often not transferable due to application of various system types and operation conditions. Treatment of mixed highly and lowly chlorinated VOC contaminations, often requiring simultaneous activity of oxidative and reductive microbial processes, has not been systematically studied.

Therefore, the main aim of this study was to provide general knowledge on removal performance, factors governing efficiency and the role of various removal processes in CW treatment of highly and lowly chlorinated VOC. These were represented by the model compounds MCB and PCE, and treatment performance was studied under the conditions of the temperate climate zone, using HSSF systems and rhizosphere model reactors. Microbial degradation, volatilization and plant uptake were investigated as the main relevant elimination processes, depending on the contaminant characteristics and operation conditions. In addition, the potential of technical and engineering optimizations, namely biostimulation options, was explored. In this final chapter, the results are summarized, recommendations for future research are given, and limitations and practical implications for the implementation of the technology are discussed.

5.1 Pilot-scale study on the treatment of chlorobenzenes in CW

In Chapter 2.1, summer time concentration decrease of MCB, 1,4-DCB and 1,2-DCB from contaminated groundwater in the HSSF CW in Bitterfeld was presented. At inflow concentrations of 20 mg L^{-1} MCB, 0.19 mg L^{-1} 1,2-DCB and 0.25 mg L^{-1} 1,4-DCB, the planted system showed high concentration decrease of MCB and 1,4-DCB already after 2 m of the flow path with averages of 48% MCB and 38% 1,4-DCB over all depths. A depth zonation of efficiencies, with higher concentration decrease in the upper filter layer, was generally observed (concentration decrease of 71% MCB and 62% 1,4-DCB in the upper filter layer). Thus, shallow wetlands may be more suitable and more efficient for removal of chlorobenzenes. The unplanted wetland showed its highest mean concentration decrease only after 4 m of the flow path with 35% for MCB and 32% for 1,4-DCB. However, 1,2-DCB was not considerably removed in either system. The beneficial influence of plants on removal of chlorobenzenes in summer was shown to be significant. The nutrient deficiency in the CW may have affected also microbial degradation processes by limiting plant development. Oxygen limitation prevailed in both systems, but concentrations were higher in the planted CW with redox potentials of 65–90 mV (0–65 mV in the unplanted reference system). Microbial investigations (MPN and aerobic microcosm degradation experiments) conducted with liquid samples from the CW revealed the presence of considerable numbers of aerobic MCB degraders and the high MCB degradation ability of these organisms under aerobic conditions. Thus, high potential for aerobic microbial MCB degradation exists, and high removal rates can be expected if appropriate conditions prevail.

Chapter 2.2 focused on the annual efficiency, seasonal variability and load removal assessment of MCB, 1,4-DCB and 1,2-DCB treatment, the long-term impact of planting, and the soil borne MCB emissions in the HSSF CW in Bitterfeld. Furthermore, model calculations were carried out in cooperation with S. Trapp (Technical University of Denmark, DTU) to estimate the removal of the main contaminant MCB via various processes: microbial degradation, volatilization and plant uptake, in comparison to the elimination in the unplanted reference system. Load removal efficiencies were assessed in summer, and amounted to 59-65% ($262\text{--}358 \text{ mg m}^{-2} \text{ d}^{-1}$) for MCB, 59-69% ($4.0\text{--}5.1 \text{ mg m}^{-2} \text{ d}^{-1}$) for 1,4-DCB and 29-42% ($0.6\text{--}2.1 \text{ mg m}^{-2} \text{ d}^{-1}$) for 1,2-DCB. Seasonal variations of CB removal in the CW were

evident, surprisingly showing more pronounced concentration decrease in winter, which was most likely attributable to seasonal fluctuations in oxygen demand and oxygen availability. These findings underlined the importance of considering annual means for the evaluation of system performance in temperate climates. Considerable annual mean concentration decrease of MCB and 1,4-DCB was determined, while annual mean 1,2-DCB removal was observed only in the upper filter layer, corroborating its recalcitrance in the investigated system. Planting with *Phragmites australis* exerted a statistically significant beneficial influence on removal success in comparison to the unplanted reference system not only in the summer, as already addressed in Chapter 2.1, but also over the course of the year. Emission measurements showed that soil-borne volatilization of MCB accounted for 2-4% of the total amount removed, thus being a subordinate removal process. Simple emission reduction cover layers (bark mulch and activated carbon) on the filter surface were suitable to further reduce MCB emission. These results provide indications that emissions emanating from HSSF CW treating chlorobenzenes are probably of minor relevance, and difficulties for regulatory acceptance will be conquerable. However, further investigations have to be carried out regarding phytovolatilization.

The results of model calculations demonstrated that oxidative microbial degradation had the highest potential for removal of MCB in the CW, followed by plant uptake. The largest oxygen input was caused by water level fluctuations (up to 10,000 mg m⁻² d⁻¹) and plant root release (up to 12,000 mg m⁻² d⁻¹) in the CW. In the unplanted system, calculated oxygen input and overall removal potential were much lower, being in good accordance with the actual data. In general, the calculated MCB removal potential was far in excess of the actually observed removal, probably due to oxygen limitation caused by presence of additional easily degradable carbon compounds. The presented model calculations effectively showed that plants play an important role in MCB removal due to root oxygen release, intensification of water table fluctuations, and direct plant uptake.

Chapter 2.3 focused on the detection and evaluation of *in situ* microbial degradation of MCB in CW, applying an integrated approach that relied on a detailed geochemical characterization with multivariate statistics, stable carbon isotope composition analysis and *in situ* microcosm experiments with ¹³C labelled tracers. Using several lines of

evidence, it could be shown that *in situ* biodegradation of MCB occurred both in the filter and open water compartment of the pilot-scale HSSF CW in Bitterfeld. Stable isotope fractionation analysis revealed that a decrease in MCB concentrations over the flow path was systematically associated with a significant enrichment in ^{13}C , with a maximum isotope shift of 0.6‰ in the planted system and 0.9‰ in the unplanted system. Incorporation of ^{13}C derived from labelled MCB into bacterial fatty acids substantiated *in situ* degradation, and the detection of ^{13}C -labelled benzene indicated reductive dehalogenation of MCB. Ferrous iron mobilization provided evidence for potential anaerobic MCB oxidation coupled to ferric iron reduction. Efficient anaerobic MCB degradation pathways have rarely been described in the literature. However, the gradient environment of a HSSF CW filter showed to enable these processes. Conclusive quantification of microbial degradation could not be achieved, due to the inhomogeneity of the CW system and the lack of an applicable laboratory-derived fractionation factor α for anaerobic MCB degradation. Nevertheless, the application of isotopic fractionation analysis combined with ^{13}C labelled tracers exposed in *in situ* microcosms showed to be a valuable integrated tool to document the *in situ* degradation of organic contaminants in wetland systems.

5.2 Simultaneous treatment of MCB and PCE

The gradient characteristics of the CW rhizosphere make CW particularly suited for treatment of multi-component contaminated groundwaters containing both reductively and oxidatively degradable contaminants. Therefore, Chapter 3 is dedicated to investigating the adaptation of a HSSF CW treating MCB contaminated groundwater to a mixed contamination of MCB and PCE, representing lowly and highly chlorinated VOC. The hypothesis that CW have the potential for simultaneous efficient microbial reductive dechlorination and oxidation was tested, and the adaptation of the system to a mixture of the contaminants was evaluated. Particular focus was laid on the development of PCE degradation and metabolite patterns, the potential to achieve complete dechlorination to ethene, and the time period necessary to obtain steady-state system performance.

Simultaneous treatment of MCB and PCE was efficient after an adaptation time of 2.5 years. Removal of MCB was temporarily strongly impaired by PCE addition. After

adaptation, simultaneous treatment of MCB and PCE resulted in slightly lower removal efficiencies for MCB in comparison to the treatment of MCB alone, but MCB removal could still be efficiently maintained. A MCB concentration decrease of 64% (55.3 μM) in 0.3 m depth and 29% (24.9 μM) in 0.5 m depth was observed. Sub-optimal MCB removal prior to as well as after PCE addition generally resulted from the lack of oxygen in the deeper anaerobic filter layers. The development of PCE and metabolite concentration patterns over the course of 2.5 years indicated slow system adaptation to PCE dechlorination. Under steady state conditions, complete removal of PCE inflow concentrations of 10-15 μM was finally achieved, and outflow concentrations of chlorinated metabolites were negligible. Ethene production and detection of *Dehalococcoides sp.* provided evidence for complete PCE dechlorination, and indicated that vinyl chloride was eliminated from the system via microbial degradation and not via volatilization. Mass balance calculations showed that after a CW filter passage of just 1 m, up to 100% of PCE inflow load (1250 \pm 260 $\mu\text{mol day}^{-1}$) had been dechlorinated and a maximum of 30% was recovered as ethene.

These investigations most importantly showed that HSSF CW are suitable for simultaneous removal of highly and lowly chlorinated VOC, thus being a promising approach for the remediation of groundwater with mixed chlorinated VOC contaminations. The long adaptation time necessary to achieve PCE dechlorination, and recovery of MCB elimination performance, should be considered in planning and implementation of large scale CW for groundwater treatment. In principle, the results obtained can be transferred to other conditions and contaminant mixtures, since redox gradients in CW rhizospheres provide suitable niches for a multitude of microbial processes.

5.3 Engineering approaches to optimization of MCB and PCE removal

CW have been shown to be suitable for the treatment of waters contaminated by highly and lowly chlorinated VOC. However, general, transferable evaluations and concepts to control the removal performance are not yet available. Reported removal efficiencies differ strongly with the type and operation mode of the CW. Therefore, in Chapter 4 removal efficiencies for MCB and PCE treatment in the CW rhizosphere were

determined under defined, reproducible operation conditions. Macro-gradient free helophyte rhizosphere reactors were used as lab-scale testing units representing the wetland root zone, allowing precise determination and regulation of overall rhizosphere conditions. Operation under low carbon load, high carbon load, fluctuating medium carbon load, and sulphate reducing conditions was investigated. MCB removal was very efficient in the rhizosphere, both under low carbon load (overall oxic) conditions and fluctuating moderate carbon load (overall reduced) conditions, reaching over 99% removal. Overall reducing redox potentials did not generally inhibit microbial MCB removal. Higher loads of easily degradable carbon (300 mg L^{-1} acetate) impaired MCB elimination, resulting in lower removal efficiencies of 72-96%. Microbial reductive dechlorination of PCE was very difficult to establish, and was not conclusively detected in the rhizosphere model system under low carbon load, sulphate reduction, and high carbon load conditions. Nonetheless, considerable amounts of PCE were eliminated accounting for mean load removals of 79-87%. Plant uptake and volatilization were considered to be important removal processes for PCE. Under fluctuating moderate carbon load conditions, PCE dechlorination was initiated, and TCE and cis-DCE production provided evidence that minimum 10% of PCE inflow loads was dechlorinated, with efficiencies still increasing at the end of the experiment. Sulphate reduction and associated sulphide toxicity showed to constitute a hazard for CW treatment of sulphate containing groundwater contaminated by chlorinated VOC, causing a decrease in removal efficiencies by 50% and 20% for MCB and PCE, respectively.

To conclude, negative impacts of easily degradable organic carbon addition on the removal of oxidizable contaminants should be considered when the supply of electron donors for the treatment of mixed contaminations is intended. Moderate additional organic carbon loads were shown to enable both MCB and PCE removal processes, indicating a promising optimization potential for CW treatment of groundwater containing mixed contaminations.

5.4 Outlook: Further research needs

In the presented study, CW treatment of chlorinated VOC has shown to be potentially efficient, and optimization potentials are promising. The subject was explored

comprehensively in the lab and pilot-scale, leading also to the identification of important remaining gaps of knowledge.

Microbial biodegradation is generally the main elimination process for organic contaminants in CW, and its potential and versatility in CW treatment of chlorinated VOC could be confirmed in this study. To enhance *in situ* biodegradation, knowledge on degradation pathways is crucial, and may be available from the literature for many contaminants. However, microbial degradation of MCB in CW systems was found to be complex, and did not solely proceed via the well known aerobic degradation pathway. Anaerobic microbial degradation of MCB via reductive dechlorination as well as anaerobic oxidation are not well studied and need to be elucidated in lab-scale experiments, as they play a vital role in HSSF CW. Uptake of chlorinated VOC into wetland plants, and subsequent metabolization and phytovolatilization, have so far been studied mainly in trees (poplar, willow), but only rarely in graminoid helophytes such as *Phragmites* which are often employed in CW. These processes can significantly contribute to removal performance depending on the plant species, compounds, system design and operation mode. This was also indicated by general modelling approaches employed in the presented study, based on log K_{OW} and TSCF relationships. Plant related processes should be thoroughly investigated in the future so that models can be improved and contaminant fractions removed by these processes can be predicted more conclusively.

Biostimulation options were found to hold great potential for performance optimization, and should be investigated in more detail. Other optimization methods, mainly technological and operational adaptations (fill-and-drain modes, intermittently charged VF CW etc.), should be considered and studied in the pilot-scale. Bioaugmentation has been suggested for enhanced chlorinated VOC removal in wetland environments with contaminated groundwater seepage (Majcher et al., 2009), and should be another focus of future investigations.

A further aspect which could not be addressed in the presented work is the sorption capacity of the CW filter material containing lignite (pilot-scale CW Bitterfeld), and the sorption-desorption-kinetics of the organic contaminants. These phenomena are important for VOC treatment in CW equipped with filter materials with a sufficient

sorption capacity. As sorption is temperature dependent, an increase in temperature leads to enhanced desorption, and a decrease in temperature to enhanced sorption of contaminants. However, especially desorption of organic contaminants from soils and sediments can be strongly rate limited (Lee et al., 2002), introducing a delay of system response to changes in temperature. Sorption and desorption phenomena will overlap with other temperature influenced processes altering the contaminant concentration, such as microbial degradation. This complex concomitance of processes may hinder interpretation of the actual observations of concentration dynamics. Therefore, an investigation of sorption capacity and sorption-desorption kinetics is strongly recommended when investigating organic contaminants behaviour in CW.

Further target-oriented research will help to develop the full potential of CW applications for groundwater remediation. In general, it is crucial to retain as much simplicity as possible to implement the idea of a low-tech, cost-efficient treatment method for chlorinated VOC contaminated groundwater.

5.5 Limitations and practical implications for implementation

The results obtained in the presented study demonstrate the potential of the CW technology for treatment of chlorinated VOC, as well as important limitations which have to be taken into consideration. These limitations are mainly connected to spatial inhomogeneities in removal efficiency for different compounds, and generally insufficient oxygen availability. This leads to several implications concerning system design and operation of CW treating (mixed) chlorinated VOC contaminated groundwater.

HSSF CW have been chosen for this application due to their gradient characteristics, enabling different microbial processes to take place simultaneously, their technical simplicity, and their expected low VOC emission potential. However, HSSF CW are oxygen limited, a problem that has to be resolved when treating oxidizable organic pollutants. Low oxygen availability showed to impair chlorobenzenes removal in the Bitterfeld pilot-scale HSSF CW, and also in rhizosphere model systems loaded with high additional carbon loads. Limited aerobic microbial MCB degradation was found to be most likely a result of additional oxygen demand in the system, as a result of carbon

compounds produced by plants and microorganisms. In the pilot-scale CW, also TOC present in the filter material may add to this oxygen demand. A depth zonation of removal efficiency was generally observed, with higher efficiencies in the upper filter layers which are more oxic due to higher root density and diffusion from the atmosphere. Thus, to achieve complete removal of oxidizable VOC, I can recommend more oxic systems on the basis of the presented findings. Shallower systems would improve reactor volume utilization, as deeper zones do not contribute as much to overall removal. An inert, permeable filter material containing no organic carbon compounds, such as gravel, is advantageous. Furthermore, technical optimizations could lead to a better oxygen availability, e.g. operation with enhanced water table fluctuations (fill-drain mode), or vertical down-flow systems with a subsurface loading solution to minimize emissions. In cases where there is no concern regarding VOC emissions (due to low toxicity and/or fast degradation of the compound in the atmosphere), a CW system combined with a long, shallow pond, enhancing aerobic microbial degradation as well as emissions, might be an interesting option.

The observed spatial gradients of efficiency along the flow path in the pilot-scale CW (highest removal on the first 1-2 m) suggest further potentials for treatment improvement. The high removal efficiency close to the inflow may be connected to a higher amount of microbial biomass, due to the higher concentration of contaminants. Additionally, plant growth is stronger near the inflow if the nutrient concentration of the groundwater is low, like it was observed in the pilot-scale CW. This can lead to a higher oxygen and organic carbon input and thus to higher microbial activity in the inflow region. In conclusion, it may be advantageous to use wetlands with a length-to-width ratio smaller than 1 to achieve a higher efficiency, as removal efficiency per m² is considerably greater close to the inflow. This is actually common practice in Germany for HSSF CW (Geller et al., 1990; Geller, 1997).

In the pilot-scale study in Bitterfeld, nutrient limitation has been observed to impair plant development, and therefore also treatment performance. This may be a problem in many cases of CW groundwater treatment, as groundwater is usually not rich in nutrients, if it is not impacted by agricultural runoff or industrial pollution (e.g. ammonia at the Leuna/Germany site (Jechalke et al., 2010)). In such cases of nutrient

deficiency, the use of nutrient supplements is recommended, keeping plant growth and activity at a high level and thus achieving maximum performance of the system.

In treatment of mixed contaminations containing oxidizable and reducible chlorinated VOC, such as MCB and PCE, the simultaneous occurrence of reduced and oxidized zones in the rhizosphere is crucial. Simultaneous removal of MCB and PCE was found to have a good potential in HSSF CW. PCE removal was in fact similarly efficient in the upper and in the deeper filter layers, but differences occurred concerning cis-DCE and VC elimination, and ethene production, which were stronger in the deeper, more anaerobic filter layers. MCB elimination was generally stronger in the upper filter layers than in the deeper filter layers. Thus, simultaneous treatment is potentially efficient, but due to various limitations it may be necessary to find a suitable compromise in treatment of mixed VOC contaminations, depending on the specific treatment goals. It has to be confirmed yet whether simultaneous treatment would be equally successful in a CW operated in vertical flow or fill-and-drain mode.

For real-scale application of the CW technology, estimations of area demand and loading capacity (hydraulic loading rate and inflow contaminant concentrations) are of great importance. CW design is most often based on a) loading specifications, i.e. area-based scaling procedures in which either the hydraulic load or contaminant load is set to values believed to meet performance criteria, or b) exponential decline modelling, mainly volumetric or area-based first-order removal rules such as P-k-C* models (Kadlec and Wallace, 2008). However, a large number of systems should be considered to provide a sound basis for loading criteria and removal rate constants, and allow determining the central tendency of performance for a certain application. In CW treatment of chlorinated VOC, knowledge is to date based on a handful of lab-scale and pilot-scale studies, dealing with different kinds of systems and contaminants. Calculation of rate constants, e.g. using a P-k-C* model, requires additional information on hydraulics in the system in question. In general, wetland systems display large inter- and intra-system variability due to their inherent complexity.

Results of the presented study provide an important general assessment of the potential and efficiency of HSSF CW treatment of MCB and PCE contaminated groundwater, and can serve as a vantage point for implementation considerations. In the Bitterfeld

pilot experiment on treatment of chlorobenzene contaminated groundwater, a hydraulic loading rate of $\sim 30 \text{ L m}^{-2} \text{ d}^{-1}$ and an inlet MCB concentration of $\sim 18 \text{ mg L}^{-1}$ were applied, giving an inlet loading rate of $540 \text{ mg MCB m}^{-2} \text{ d}^{-1}$. Load removals were up to $360 \text{ mg MCB m}^{-2} \text{ d}^{-1}$. In the experiment on simultaneous treatment of PCE and MCB, PCE inlet concentrations were up to 2.5 mg L^{-1} , resulting in an inlet loading rate and load removal rate of $75 \text{ mg m}^{-2} \text{ d}^{-1}$, as PCE was completely removed in the pilot-scale HSSF CW.

CW are an advantageous technology for treatment of contaminated groundwater particularly where the required area is available and accessible, and where extensive groundwater pumping is not necessary to supply the treatment systems, e.g. for the treatment of surface-near groundwater discharging into natural river floodplains or wetlands. If strict discharge criteria have to be met to avert environmental hazards, it is an interesting option to install small scale chemical or physical treatment reactors (e.g. activated carbon adsorption) at the wetland outlet in case performance expectations are not always fulfilled. CW systems, or combined systems including a CW treatment step, may be advantageous in many situations, providing a cheap and simple method of improving water quality before groundwater discharges into receiving water bodies.

6 References

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Publications

Journal publications

Braeckevelt, M., Rokadia, H., Imfeld, G., Stelzer, N., Paschke, H., Kuschk, P., Kästner, M., Richnow, H.-H., Weber, S. (2007). Assessment of *in situ* biodegradation of monochlorobenzene in contaminated groundwater treated in a constructed wetland. *Environmental Pollution* 148: 428-437.

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Appendices

A.1 Gwenaël Imfeld, Mareike Braeckevelt, Peter Kusch, Hans H. Richnow. *Review: Monitoring and assessing processes of organic chemicals removal in constructed wetlands. **Chemosphere 74 (2009) 349–362.***

A.2 Pilot-scale constructed wetland systems

A.3 Model rhizosphere reactors

A.1 Review: Monitoring and assessing processes of organic chemicals removal in constructed wetlands

G. Imfeld, M. Braeckevelt, P. Kusch, H.H. Richnow (2009), Chemosphere 74:349-362.



Review

Monitoring and assessing processes of organic chemicals removal in constructed wetlands

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ABSTRACT

Physical, chemical and biological processes interact and work in concert during attenuation of organic chemicals in wetland systems. This review summarizes the recent progress made towards understanding how the various mechanisms attributed to organic chemicals removal interact to form a functioning wetland. We also discuss the main degradation pathways for different groups of contaminants and examine some of the key characteristics of constructed wetlands that control the removal of organic chemicals. Furthermore, we address possible comprehensive approaches and recent techniques to follow up *in situ* processes within the system, especially those involved in the biodegradation processes.

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1. Introduction

Constructed wetland systems may be converted natural or constructed shallow ecosystems designed to capitalize on intrinsic physical, chemical, and biological processes for the primary purpose of water quality improvement (Hammer et al., 1989). Constructed wetlands consist of four main compartments: plants, sediment and soil, microbial biomass and an aqueous phase loaded with the chemicals, and typically include beds filled with poorly drained graded medium and aquatic plants. These systems are generally coupled to a drainfield or polishing pond, engineered to return the filtered water back to the environment. There are two basic designs for constructed wetlands whose primary purpose is wastewater treatment: subsurface-flow and surface-flow. In the subsurface-flow wetlands (SSF), the water may flow horizontally (parallel to the surface) or vertically (in the majority of cases from the planted layer down) through the matrix and out of the system, whereas the water moves above the substrate surface in surface-flow wetlands (SF). The application and reliability of these systems during domestic sewage treatment has previously been reviewed (Cooper et al., 1996; Sundaravadivel and Vigneswaran, 2001; Griffin, 2003). In recent years however, the applicability of constructed wetland technology (CWT) as a cost-effective and operational alternative to conventional technologies for the elimination of various contaminants of industrial relevance has been explored (Kadlec et al., 2000; Pardue, 2002; WetPol, 2007). In particular, developments have focused on organic chemicals, defined as undesirable substances not normally present in surface or groundwater, or naturally occurring substances at an unusually high concentration and displaying harmful environmental effects. Though this technology has the potential to become an important remediation strategy, its successful application remains challenging. Indeed, numerous environmental factors and system-inherent processes

influence organic contaminant removal efficiency and may complicate the maintenance of acceptable levels of system control. Although several approaches and methods have been described in the literature, the physicochemical and biogeochemical processes associated with the transformation of organic chemicals in constructed wetlands are rarely evaluated. The results of a short literature survey are provided in Table 1. The majority of studies in constructed wetlands are orientated towards efficiency or performance (>20%), whereas studies integrating microbial, molecular or microcosm investigations account for about 11% of the total. The investigation of processes, such as degradation, sorption, and volatilization accounts for another 18%, whereas studies focusing on specific compartments (biofilms, sediment, rhizosphere) amount to less than 25% of the contributions. This brief survey permits depicting overall trends (Table 1), however, it is likely that many studies on related topics do not contain the researched keyword in their title or abstract and have therefore not been included.

Future challenges will surely consist of optimizing CWT for more sustainable and reliable treatment of both industrial and agricultural organic contaminants. This would necessarily imply that the ability to assess design characteristics has reached an acceptable level of understanding, and reliable predictions about the mechanisms associated with organic contaminants removal could be performed. This review focuses on key processes determining the fate of organic chemicals in constructed wetlands and aims to improve their assessment in pilot studies and active treatment plants. It mainly focuses on selected categories of contaminants of worldwide relevance, namely the volatile organic compounds (VOCs), the organochlorines, the PAHs, as well as some pharmaceuticals. In the first part, main physicochemical and biological mechanisms contributing to organic chemical removal in constructed wetland are successively reviewed. Second, relevant characteristics of wetland systems that determine the feasibility

Table 1
Output from a literature search performed using Thomson ISI research tool, with the following variables (Doc type: all document type; language: all languages; databases: SCIEXPANDED, SSCI, A&HCI; Timespan: 1957–2007) on November 13, 2007

	Keywords	Natural wetland		Constructed wetland	
		Relative proportion (%)	Number	Relative proportion (%)	Number
Wetland type	Free-surface	1.2	191	5	102
	Sub-surface	0.8	124	2.3	47
Type of investigation	Performance	5.2	835	22.9	469
	Efficiency	4.9	786	19.1	391
	Microcosm	0.5	81	1.5	30
	Molecular	1.1	176	0.9	19
	Process	5.4	872	8.2	168
Processes	Degradation	3.8	613	5.3	109
	Sorption	1.7	273	5.4	110
	Volatilization	0.7	119	2	40
	Bacterial	2.3	364	5.1	105
Compartment	Microbial	4.4	714	8.1	166
	Plant	21	3392	26	534
	Sediment	11.4	1846	12.9	265
	Rhizosphere	1.5	249	3.2	65
	Total number		16164		2050

Only the titles and abstracts of the articles were searched. Each keyword (wetland, constructed wetland) was additionally combined with a designation embedded in the following categories: wetland type, type of investigation, processes and compartment. The values are provided in percent of the corresponding total number of publications enumerated for each keyword.

and efficiency of organic chemical removal are briefly discussed. The final part addresses approaches to assess processes leading to contaminant depletion in constructed wetlands. It provides some insights into experimental designs necessary for process investigations and succinctly presents traditional and emerging methods that have been proven relevant. Overall, special emphasis is placed on degradation, as it generally represents an expected sink of organic chemicals in constructed wetlands.

2. Removal processes in constructed wetland

Several elimination pathways may occur in a complex constructed wetland system. Kadlec (1992) listed volatilization, photochemical oxidation, sedimentation, sorption and biological degradation as the major processes affecting the organic compound loads in wetlands. Additionally, processes such as plant uptake and phytovolatilization, contaminant accumulation and metabolic transformation may be relevant for some plants and organic chemicals (Susarla et al., 2002). The relative importance of a particular process can vary significantly, depending on the organic contaminant being treated, the wetland type (e.g. SSF or SF, horizontal flow (HF) or vertical flow (VF)) and operational design (e.g. retention time), the environmental conditions, the type of vegetation within the system, as well as the soil matrix. Clear treatment goals and an evaluation of the occurrence and extent of putative removal processes are preliminary requirements for defining appropriate design and operation parameters. This evaluation is particularly critical when targeting organic chemical treatments. The assessment of organic carbon removal in conventional wastewater treatment, mainly based on COD and BOD values, has been well documented since the early 1950s (Vymazal, 2005), but the treatment of organic chemicals in constructed wetlands is still at its infancy. Organic chemicals exhibit a wide range of physico-chemical properties, numerous specific toxicity effects and often a degree of recalcitrance rarely encountered in common contaminants of domestic and agricultural sewage. Therefore, evaluating the physicochemical properties and biological effects of specific groups of organic chemicals with respect to their potential and observed fate in constructed wetlands may help refining artificial wetland design and operation modes. Physico-chemical properties for various organic contaminant groups of interest regarding constructed wetland treatment are listed in Table 2. The relationship between these physico-chemical characteristics and the fate of contaminants in constructed wetland systems is highlighted in the following sections.

2.1. Non-destructive processes

The mere reduction of contaminant concentration within the aqueous phase via non-destructive partitioning processes, such as sorption and volatilization, may only relocate the contamination. Therefore, the mass transfer of contaminants from the aqueous phase to other compartments (soil and atmosphere) has to be considered carefully when evaluating potential environmental hazards.

2.1.1. Volatilization and phytovolatilization

In addition to direct contaminant emission from the water phase to the atmosphere (volatilization), some wetland plants take up contaminants through the root system and transfer them to the atmosphere via their transpiration stream, in a process referred to as phytovolatilization (Hong et al., 2001; Ma and Burken, 2003). In the case of helophytes, this transfer may also occur via the aerenchymatous tissues (Pardue, 2002).

VOCs are defined as substances with a vapor pressure greater than 2.7 hPa at 25 °C (NPI, 2007). The Henry coefficient (H) is ex-

pected to be a valuable indicator for predicting volatilization behavior of organic contaminants. It comprehensively describes the transfer of volatile contaminants from the water phase to the atmosphere. In unsaturated soil zones, additionally the diffusion transport determines effective VOCs emission. A high Henry coefficient is a characteristic of a number of organic contaminant groups frequently treated in constructed wetlands such as chlorinated solvents, BTEX (benzene, toluene, ethylbenzene, xylene) compounds and MTBE (methyl *tert*-butyl ether) (Table 2).

Direct volatilization and phytovolatilization are expected to be moderate for hydrophilic compounds such as acetone (Grove and Stein, 2005) and phenol (Polprasert et al., 1996). In contrast, volatilization may be an important removal process for volatile hydrophobic compounds such as lower chlorinated benzenes (MacLeod, 1999; Keefe et al., 2004), chlorinated ethenes (Bankston et al., 2002; Ma and Burken, 2003) and BTEX compounds (Wallace, 2002). In constructed wetland treatment of MTBE, which is characterized by a moderate Henry coefficient, high water solubility and additionally by strong recalcitrance under anaerobic conditions (Deeb et al., 2000), various processes may result in the release of the compound to the atmosphere. Uptake by the transpiration stream and subsequent phytovolatilization through the stems and leaves may be a major removal process and significantly contribute to contaminant mass loss; additionally, the vegetation increases the upward movement of water into the unsaturated zone, where enhanced volatilization occurs (Hong et al., 2001; Winnike-McMillan et al., 2003). If the atmospheric half-lives of VOCs are reasonably short like the one for MTBE (three days at 25 °C (Winnike-McMillan et al., 2003)), and the toxicological risk is assumed to be low, the water-to-atmosphere contaminant transfer occurring in wetlands may constitute a possible remediation option. However, volatilization of VOCs may also lead to air pollution and to a dispersal of the contaminant in the environment. This fact and the lack of reliable risk assessment currently discourages regulatory acceptance of phytoremediation as a strategy for VOCs removal (McCutcheon and Rock, 2001).

Phytovolatilization may be of particular relevance in SSF systems, where direct volatilization is restrained due to slow diffusion rates of contaminants through the unsaturated zone as well as laminar flow in water saturated soil zones that may result in relatively low mass transfers. Direct contaminant volatilization is expected to be more pronounced in SF wetlands, as water remains in direct contact with the atmosphere (Kadlec and Wallace, 2008).

2.1.2. Plant uptake and phytoaccumulation

Uptake of organic chemicals into plant tissue is predominantly affected by the lipophilic nature of organic pollutants, which can be characterized by the octanol water partition coefficient (K_{ow}) (Ryan et al., 1988). Hydrophobic organics with a $\log K_{ow} > 4$ are believed not to be significantly taken up through the plant cell membrane because of significant retention within the root epidermis (Trapp, 1995), but exceptions may occur. Reed and rice plants have been shown to take up highly lipophilic PCBs (Chu et al., 2006a). Only under the condition of significant contaminant uptake by the vegetation, processes like phytovolatilization, phytoaccumulation and plant metabolic transformation have to be considered as potentially significant removal processes for organic contaminants.

Phytoaccumulation occurs when the sequestered contaminants are not degraded in or emitted from the plant rapidly and completely, resulting in an accumulation within the plant tissue (Susarla et al., 2002). The accumulation of chlorinated contaminants, e.g. PCDD/Fs and chlordanes, has been studied in *Cucurbita pepo* species that seem to have a special uptake mechanism for these contaminants (Campanella et al., 2002; Mattina et al., 2007). Long-term storage of organic chemicals in plant biomass has only been observed for particularly persistent organic

Table 2
Range of values for selected physicochemical properties of various organic contaminant groups of interest regarding treatment in constructed wetland

Organic compound	Physico-chemical properties				Expected processes in constructed wetlands
	S_w^a 25 °C [mg L ⁻¹]	P_v^b 25 °C [hPa]	$\log K_{ow}^c$ (25 °C)	H^d 25 °C [Pa m ³ mol ⁻¹]	
PCBs ¹	5 × 10 ⁻⁸ – 5.9	7 × 10 ⁻⁹ – 5 × 10 ⁻²	3.9–9.6	0.9–97	Sorption(+), microbial degradation, plant uptake, accumulation and metabolism(–) ^{8–13}
PCDD, PCDF ¹	4 × 10 ⁻⁷ – 4.2	1 × 10 ⁻¹² – 3 × 10 ⁻⁴	4.8–11.3	0.3–12.6	Sorption (+), microbial degradation (hypothesised) ⁸
PAH (3–6 rings) ¹	1 × 10 ⁻⁴ – 16.1	1 × 10 ⁻¹⁵ – 1 × 10 ⁻²	3.6–7.6	0.1–24.4	Sorption(+), microbial degradation(+), plant uptake and metabolism(–) ^{10,14–16}
Chlorobenzenes (3–6 Cl substituents) ¹	4 × 10 ⁻³ –52	2 × 10 ⁻¹⁵ – 0.3	4–5.7	41–375	Sorption, sedimentation, microbial reductive dechlorination, volatilisation ^{17–19}
Fuels: kerosene C9–16, diesel C10–19, heavy fuel oil C20–70 ²	≤5 (20 °C)	<1 × 10 ⁻⁴ – 35 (21 °C)	3.3–7.1	6–749805 (20)	Microbial degradation, sorption and sedimentation, volatilisation ^{20–26}
Ibuprofen ^{3–5}	21–49	6 × 10 ⁻⁵ – 3 × 10 ⁻⁴	3.5 (pH 8: 0.5)	2 × 10 ⁻² (cal.) ^e	Microbial degradation, sorption ^{27–29}
Carbamazepin ^{3–6}	17.7	3 × 10 ⁻⁷	2.7	2 × 10 ⁻⁶	Sorption ²⁸
Naproxen ⁴	15.9	n.a.	3.3 (pH 8: –0.3)	n.a.	Microbial degradation, sorption (protonated form) ²⁹
Ketoprofen ⁴	51	n.a.	3.6 (pH 8: –0.4)	n.a.	Sorption (protonated form) ²⁹
Diclofenac ³	2.4	8 × 10 ⁻⁸ (cal.) ^e	4.5 (pH 8: 0.7)	5 × 10 ⁻⁷ (cal.) ^e	Sorption (protonated form) ²⁹
Gasoline C4–12 ^{2,7}	120	35–90 (20 °C)	2.1–4.9	5–334373	Microbial degradation, volatilisation
Chlorobenzenes (1–2 Cl substituents) ¹	31–503	1.3–15.9	2.8–3.5	159–398	Microbial degradation, volatilisation(+), sorption ^{18,19,30–32}
BTEX ¹	131–2167	8.8–131	1.6–3.4	272–959	Microbial degradation, volatilisation(+), sorption ^{30,33–36}
Chlorinated solvents (1–2 C-atoms) ¹	150–20000	4.7–5746	0.6–3.4	25–3080	Plant uptake and metabolism(+), volatilisation(+), phytovolatilisation, microbial degradation(–), sorption ^{36–43}
MTBE ¹	35500	327	0.9	72	Plant uptake and volatilisation(+), plant and microbial degradation(–) ^{44,45}
Phenol, cresols ¹	21000– 87000	0.2–0.5	1.5–2	3 × 10 ⁻² –0.3	Microbial degradation(+), sorption, plant uptake (–), volatilisation(–) ^{46–48}
Acetone ¹	Miscible	305–308	–0.2	3–4	Microbial degradation(+), phytovolatilisation(–), sorption ⁴⁹

Representative organic compounds are listed in the order of increasing water solubility. Superscript numbers associated with the organic compounds refer to the literature sources of the provided values for physicochemical properties. Superscript numbers associated with the potentially relevant processes in constructed wetlands for the mentioned compound refer to the literature concerning its treatment and/or behavior in wetlands. (+) or (–) signs associated with specific removal processes are provided if the cited literature contains explicit information or estimates on the contribution of these removal process to overall contaminant losses from constructed wetland systems. ¹Mackay et al. (2006); ²ATSDR (1995), Connecticut College Office of Environmental Health and Safety (2004), NIST (2006), Marathon Petroleum Company (2006); ³US National Library of Medicine (2007); ⁴Wishart et al. (2008); ⁵Mersmann et al. (2002); ⁶Doll (2004); ⁷Hess Corporation (2007), OMV (2005), Poulsen et al. (1992); ⁸Campanella et al. (2002); ⁹Donnelly and Fletcher (1994); ¹⁰Olson et al. (2003); ¹¹Chu et al. (2006a); ¹²Chu et al. (2006b); ¹³Moza et al. (1974); ¹⁴Machate et al. (1997); ¹⁵Giraud et al. (2001); ¹⁶Harms et al. (2003); ¹⁷Pardue et al. (1993); ¹⁸Leppich (1999); ¹⁹Jackson (1999); ²⁰Thurston (1999); ²¹Salmon et al. (1998); ²²Wright et al. (1997); ²³Kadlec (1992); ²⁴Groudeva et al. (2001); ²⁵Boopathy (2003); ²⁶Omari et al. (2003); ²⁷Gross et al. (2004); ²⁸Matamoros and Bayona (2006); ²⁹Matamoros et al. (2005); ³⁰Keefe et al. (2004); ³¹Lee et al. (2003); ³²MacLeod (1999); ³³Bedessem et al. (2007); ³⁴Wallace (2002); ³⁵Wallace and Kadlec (2005); ³⁶Williams (2002); ³⁷Wang et al. (2004); ³⁸Bankston et al. (2002); ³⁹Pardue (2002); ⁴⁰Ma and Burken (2003); ⁴¹Amon et al. (2007); ⁴²Kassenga et al. (2003); ⁴³Kassenga et al. (2004); ⁴⁴Hong et al. (2001); ⁴⁵Winnike-McMillan et al. (2003); ⁴⁶Polprasert et al. (1996); ⁴⁷Abira et al. (2005); ⁴⁸Wood et al. (2000); ⁴⁹Grove and Stein (2005).

^a Water solubility.

^b Vapour pressure.

^c Octanol–water partition coefficient.

^d Henry's law constant.

^e Calculated.

n.a.: not available.

compounds. For example, PCBs with more than two chlorine substituents and DDT have been shown to accumulate in rice plants, and could still be found in different plant compartments 60 days after incubation (Chu et al., 2006a).

2.1.3. Sorption and sedimentation

Sorption of a chemical to soil or sediment may result from the physical or chemical adhesion of molecules to the surfaces of solid bodies, or from partitioning of dissolved molecules between the aqueous phase and soil organic matter. To evaluate the sorption behavior of organic compounds in soils and sediments, the organic carbon partition coefficient (K_{oc}) is a reasonable parameter to use, and is defined as the ratio of contaminant mass adsorbed per unit weight of organic carbon in the soil to the concentration in solution. It can be roughly estimated from the K_{ow} using empirical equations and alternatively from the water solubility of the compound (Karickhoff, 1981). However, variations in K_{oc} for a given compound are affected by the sorption properties of soil organic matter. Grathwohl (1990) suggests that an empirical relationship

exists between the K_{oc} and the atomic hydrogen/oxygen ratio in natural organic matter. Thus, the extent of sorption depends on the compound's hydrophobic characteristics as well as on the organic carbon content, the chemical structure and composition of soil organic matter.

During the early stages of constructed wetland operation, sorption onto soil substrate will naturally be higher due to the high adsorption capacity of previously unexposed material (e.g. Omari et al., 2003). As long as no sorption–desorption equilibrium is reached, the system acts as a sink for the contaminant. After reaching steady-state conditions, contaminants will still be retained by reversible sorption processes, but further net loss of contaminants will not occur. This retention may increase contaminant residence time within the constructed wetland and support bioremediation by increasing exposure to degrading microorganisms (Pardue, 2002). However, sorptive processes may also negatively affect the bioavailability of contaminants. The bioavailability has currently been defined as the fraction of the compound in soil that can be taken up or transformed by living organisms at any time

(Semple et al., 2007; Wick et al., 2007). Limited bioavailability of contaminants is one of the central attributes governing the recalcitrance of chemicals in soil–sediment systems. Biodegradation may be limited due to slow desorption kinetics especially when dealing with aged sediments (Lee et al., 2003). Aging results from chemical reactions sequestering contaminants into organic matter, diffusion into very small pores or inclusion of non-aqueous phase liquids into semi-rigid films (Bosma et al., 1997; Alexander, 2000). Different fractions of the contaminant pool may display very low desorption rates, as observed for dichlorobenzenes by Lee et al. (2003). Deposition of contaminants sorbed to the solid phase can also lead to long-term sources of contamination in soils and sediments.

Mineral fractions in soils also affect sorptive interactions with solvated organic compounds in aqueous systems. However, it is generally believed that, in saturated soils, clay mineral adsorption sites for organic compounds are effectively blocked by water molecules (Teppen et al., 1998). At least for non-polar compounds like chlorinated solvents, sorption is almost completely due to partitioning into soil organic matter (Breus and Mishchenko, 2006).

Most organic chemicals can be potentially affected by sorption at least to a certain extent. Highly hydrophobic persistent organic pollutants (POP) like PCBs, PCDDs (Campanella et al., 2002), PAHs (Cottin and Merlin, 2007) and highly chlorinated benzenes (Pardue et al., 1993) are strongly affected by sorption and therefore accumulate in sediments of constructed wetlands. It is also generally believed that pharmaceuticals such as Carbamazepin are removed from the water phase by sorptive effects due to their hydrophobicity (Matamoros et al., 2005). Significant sorptive effects have also been observed for fuel hydrocarbons in wetland soils and sediments (Thurston, 1999; Omari et al., 2003), as well as lower chlorinated benzenes (MacLeod, 1999; Lee et al., 2003) and chlorinated ethenes (Lorah and Olsen, 1999; Kassenga et al., 2003). Due to their high water solubility and low hydrophobicity, sorption of polar compounds such as MTBE and acetone should be of minor importance in constructed wetland systems.

In addition, sedimentation occurs when contaminant molecules are associated with particulate organic matter (POM) that settles, or is mechanically retained, within the constructed wetland. In contaminated waters containing high amounts of POM, mechanical filtration may be the most viable approach for the attenuation of organic compounds sorbed to particles, as demonstrated for petroleum hydrocarbons (Thurston, 1999) and hexachlorobenzene (Pardue et al., 1993).

2.2. Destructive processes

2.2.1. Phytodegradation

The term phytodegradation is defined in this context as the metabolic degradation or breakdown of organic contaminants by plant enzymes or enzyme cofactors (Susarla et al., 2002). Metabolic transformations of different organic chemicals have been shown to occur in a variety of plants (Newman and Reynolds, 2004), including typical constructed wetland plants like the common reed (*Phragmites australis*), the broad-leaved cattail (*Typha latifolia*) and some poplar species (*Populus* sp.) (Bankston et al., 2002; Wang et al., 2004). The extent to which plants can degrade organic chemicals mainly depends on the specific compound of interest. For example, *P. australis* has been shown to possess enzymes degrading PCB with up to three chlorine atoms, whereas higher chlorinated PCBs were not transformed (Chu et al., 2006a,b). A well-known example of plant metabolic transformation of organic chemicals in constructed wetland research is the degradation of chlorinated solvents by hybrid poplar trees (*P. trichocarpa* × *P. deltoides*) (Newman et al., 1997, 1999; Wang et al., 2004) and other wetland plants (Bankston et al., 2002). Plant metabolic degradation can be very effective for this class of contaminants. For example, Wang et al.

(2004) demonstrated that phytodegradation was the dominant removal process in a poplar treatment of carbon tetrachloride contaminated water.

2.2.2. Microbial degradation

The nature and extent of microbial degradation of organic chemicals within a constructed wetland is also expected to strongly depend on the physico-chemical properties of the contaminant. Indeed, the biological degradability or recalcitrance of organic compounds may often be explained by its chemical structure, for instance the presence of secondary, tertiary or quaternary carbon atoms as well as functional groups. It is designative that all compounds classified as POP in the Stockholm convention carry chlorine substituents (Ritter et al., 1995); thus cleavage of carbon chlorine bonds is of particular interest for bioremediation applications in constructed wetlands.

Reddy and D'Angelo (1997) have discussed pathways and indicators for toxic organic compound removal in constructed wetlands. According to these authors, removal of toxic organics is largely a microbially mediated process, and can be subdivided into aerobic and anaerobic microbial degradation processes. Several authors have reported investigations of organic chemical removal in constructed wetlands where at least part of the contaminant elimination was assigned to microbial degradation. In the following sections, an overview of important contaminant groups will be presented. Overall, experimental evidence that allows identifying microbial degradation pathways and quantifying organic chemical degradation potentials in constructed wetlands is scarce to date. However, indirect approaches like quantification of alternative elimination processes (sorption, volatilization) and simple gaps in mass balances without process identification are often applied to assess microbial degradation.

2.2.2.1. Highly chlorinated compounds and PAHs. An important factor limiting the degradation of highly chlorinated compounds with very low water solubility, such as PCB or PCDD/Fs, is the low bioavailability of these compounds, resulting from binding to the soil or sediment matrix (Campanella et al., 2002; Leigh et al., 2006). The compounds become more soluble after some initial reductive dechlorination steps, and thus more bioavailable. For example, Leigh et al. (2006) demonstrated that willow and pine trees are associated with enhanced rhizospheric abundances of PCB degraders at a contaminated site. However, it should be clear that efficient microbial degradation takes time, due to the slow rates of dechlorination under anaerobic conditions, and the requirement for subsequent aerobic degradation steps breaking down the remaining carbon skeleton. Similar to the PCDD/Fs and PCBs, most PAHs display low bioavailabilities in soils (Manilal and Alexander, 1991). But in contrast to the chlorinated compounds discussed above, the non-chlorinated PAHs readily undergo aerobic degradation (Reddy and D'Angelo, 1997). For example, the elimination of phenanthrene in a vertical flow filter was shown to be greater than 99.9%, which corroborated with enhanced numbers of phenanthrene degraders and the formation of a known phenanthrene metabolite (Machate et al., 1997). Moreover, Giraud et al. (2001) demonstrated the degradation of fluoranthene and anthracene by several fungal isolates from a constructed wetland.

2.2.2.2. Petroleum hydrocarbons. The large and diverse group of petroleum hydrocarbons mainly consists of paraffines, naphthenes, and, to a lesser extent – aromatic, as well as polar hydrocarbons in variable portions. In constructed wetland research, they are often monitored as composite parameters like total hydrocarbons (THC), total petroleum hydrocarbons (THP) or diesel/gasoline range organics (D/GRO). In contrast to the high molecular weight compounds associated with wax and tar fractions, most petroleum

hydrocarbons found in fuels are considerably more water soluble than PCDD/Fs, PCBs and PAHs. This class of contaminant displays a significant sorption potential, but is generally more easily degraded and readily mineralized under aerobic conditions. Several authors have previously reported significant petroleum hydrocarbons removal rates in constructed wetlands (Ji et al., 2002; Omari et al., 2003; Gessner et al., 2005). Groudeva et al. (2001) assessed the relationship between crude oil removal and the associated indigenous bacterial and fungal microflora in a constructed wetland. Salmon et al. (1998) demonstrated hydrocarbon removal rates of up to 90% in a constructed wetland with a porous mineral substrate matrix. For instance, 10% of the removal was assigned to sorption processes, volatilization was estimated as <25%, and microbial degradation and eventual plant uptake were assumed to account for 60% of the observed losses.

2.2.2.3. Volatile organic compounds. Removal of chlorinated VOCs like chlorinated ethenes and chlorobenzene (CB) from constructed wetlands has been increasingly studied in recent years (Williams, 2002; Haberl et al., 2003; Keefe et al., 2004). Some microbial degradation pathways for these compound classes are well known, and chiefly include reductive dechlorination and aerobic oxidation. However, the most likely removal process that significantly competes with microbial degradation of these compounds is volatilization. Reductive dechlorination of tetrachloroethene (PCE) to trichloroethene (TCE), dichloroethenes (DCEs) and vinyl chloride has recently been investigated in an upward-flowing vertical flow constructed wetland (Amon et al., 2007). Bankston et al. (2002) could only assign 5% of labelled ^{14}C -TCE removal to microbial mineralization in wetland microcosms planted with broad-leaved cattail and eastern cottonwood, and pointed out that volatilization was the dominant removal process in their system (>50%). Kassenga et al., 2003, 2004) studied reductive dechlorination of *cis*-DCE in upflow wetland mesocosms and anaerobic microcosms derived from the former systems. After an operation time of twelve weeks, over 90% of the *cis*-DCE was degraded to vinyl chloride within the wetland mesocosms. It was concluded that reductive dechlorination can actively proceed in anaerobic zones adjacent to aerobic zones in the wetland rhizosphere (Kassenga et al., 2004). CB is preferentially degraded under aerobic conditions via a dioxygenase catalysed pathway (Reineke and Knackmuss, 1988). CB may also be degraded via reductive dechlorination (Nowak et al., 1996; Jackson, 1999) or mineralized by other anaerobic processes (Nijenhuis et al., 2007). According to MacLeod (1999), mineralization of CB in a reed bed system accounted for approximately 25% of the observed losses after 47 days. Braeckvelt et al. (2007) found evidence that reductive dechlorination of CB or other anaerobic degradation pathways may simultaneously occur with aerobic degradation pathways in a constructed wetland ecosystem.

Similar to chlorinated solvents, BTEX compounds are relatively water soluble and significantly volatile. They may be microbially degraded under aerobic as well as anaerobic conditions (Wilson and Bouwer, 1997; Phelps and Young, 1999). Removal efficiencies ranging from 88% to 100% have been reported in BTEX-treating constructed wetlands with inflow concentrations below 2 mg L^{-1} (Bedessem et al., 2007). Primary removal was assumed to be microbially mediated, whereas other authors assigned a significant role to volatilization, especially in a SF system (Machate et al., 1999) and a system with forced bed aeration, which probably enhanced emissions of BTEX compounds (Wallace, 2002).

The gasoline additive MTBE is characterized by a high water solubility and high Henry coefficient, resulting in its ubiquitous occurrence in the aqueous environment (Hong et al., 2001), and its great volatilization potential in constructed wetlands. Additionally, MTBE displays especially low microbial degradation rates

under anaerobic conditions, even though degradation pathways under varying environmental conditions have been described (Somsamak et al., 2006; Haggblom et al., 2007). Hong et al. (2001) reported that in laboratory systems with poplar cuttings, the main ^{14}C MTBE removal mechanism was sequestration and volatilization by the poplar plants after approximately 10 d. In a similar experiment, Winnike-McMillan et al. (2003) found that approximately 3.5% of the MTBE label was recovered as $^{14}\text{CO}_2$, indicating a minor MTBE mineralization by microorganisms or poplar plants.

3. Metabolic potentials of constructed wetlands

Constructed wetlands may support a large spectrum of biogeochemical reactions and various environmental conditions at the wetland system scale. This function is essential for organic contaminant transformation. Indeed, the prevailing conditions generally determine both the thermodynamic feasibility of chemical reactions and the activity of indigenous microbial guilds harboring the enzymatic capacity to achieve the target biochemical reactions. In several respects, constructed wetlands are complex bioreactors characterized by considerable fluxes of material and energy governing chemical reactions over spatial and temporal gradients. Those fluxes are particularly pronounced in certain zones, such as the rhizosphere. These fluxes permit the maintenance of thermodynamic non-equilibrium conditions, and enable various reactions with exergonic free energy changes to occur (Hanselmann, 1991). In constructed wetlands, the biogeochemical reactions affecting contaminant removal mainly depend on two types of processes simultaneously occurring at different scales: (1) the various and co-existing redox processes at the wetland system scale, and (2) the processes occurring at the rhizosphere scale.

3.1. Redox processes at the constructed wetland system scale

3.1.1. Oxic–anoxic interfaces

Oxic–anoxic interfaces are dynamically established in wetlands as a result of water table fluctuations, oxygen diffusion/advection through the water column and soil, and active oxygen transport throughout the rhizosphere via plant tissues (D'Angelo, 2002) (Fig. 1). First, the progressive constitution of sharp redox and dissolved oxygen gradients leads to the creation of sequentially adjacent anaerobic and aerobic zones (Bezbaruah and Zhang, 2004; Wiessner et al., 2005). These interfaces are then mechanically and chemically sustained and shaped via biogeochemical activities (Burken and Schnoor, 1998). At the soil–water interface of mineral soil wetlands, a thin layer of oxidized soil matrix (evident from reddish-brown iron oxide precipitates) is usually formed (Chen et al., 1980). Conversely, the deeper sediments generally remain anoxic, a state reflected by the presence of the reduced forms of redox sensitive species (Reddy and D'Angelo, 1997; Diakova et al., 2006). Organic chemicals supplied to a constructed wetland undergo removal processes analogous to naturally occurring organic matter. D'Angelo and Reddy (1999) showed that, amongst the relevant soil factors regulating potential rates and modes of organic carbon mineralization in wetland soils, electron donor and acceptor availability appear to be essential. For instance, redox potentials were the main variables governing the observed differences in the removal patterns, and efficiently explained variation in the treatment of linear alkyl-benzene sulfonates (Huang et al., 2004) and pharmaceuticals (Matamoros and Bayona, 2006; Matamoros et al., 2005, 2007). Similarly, Meade and D'Angelo (2005) showed that redox interfaces significantly influenced pentachlorophenol mineralization rates within rice plant rhizosphere. However, degradation was significantly faster under strictly anaerobic conditions, which lack redox interfaces, when compared to treatments

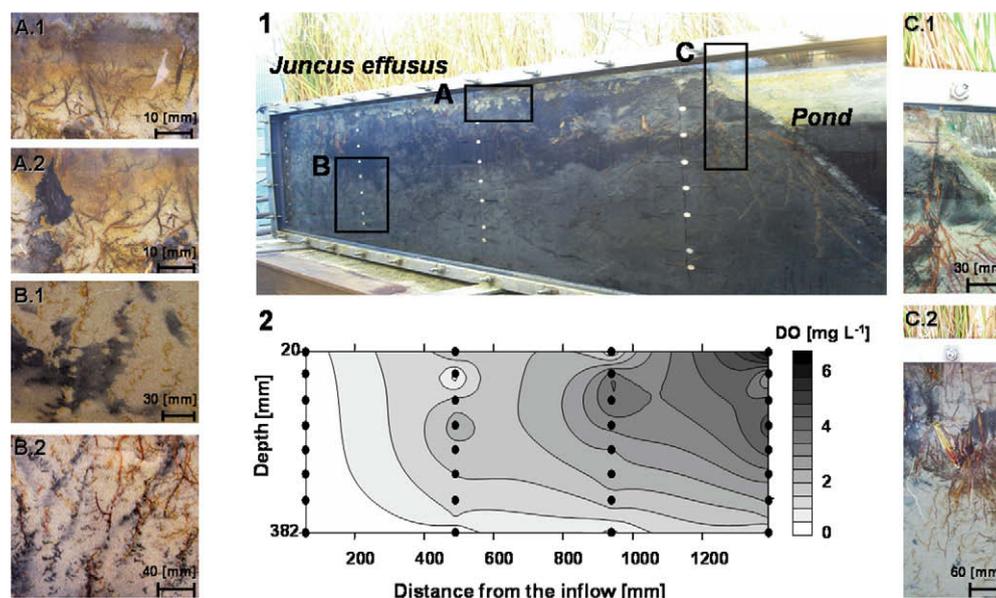


Fig. 1. Spatial patterns of visible biogeochemical processes in a cross section of a model subsurface horizontal-flow constructed wetland treating *cis*- and *trans*-1,2-dichloroethene (DCE) contaminated suboxic groundwater. A complex coloration pattern of the filling sand matrix is observed at the wetland system scale through a glass board (1) after 350 days of contaminated groundwater supply, reflecting zones of iron sulphide mineral precipitation. Three zones of enlargement are depicted by rectangles and correspond to the soil–water interface in the unsaturated layer of the wetland (A), the saturated rooted zone (B) and the wetland sand matrix–polishing pond interface (C). A conceptual mapping of dissolved oxygen concentration values corresponding to 150 days of contaminated water supply (2) was obtained by planar oxygen sensor spots measurements deployed across the system (represented by the black spots), and emphasizes the occurrence of both horizontal and vertical dissolved oxygen gradients. In the unsaturated zone, the formation of reddish-brown iron oxide precipitates at the soil–water–atmosphere interface (A.1) may also occur in close vicinity to zones of black iron sulphide formation (A.2). The precipitates of iron sulphide in the saturated zone may appear as distinct and homogeneously sized black patches associated with the rootlets (B.1) or root (C.2). The precipitates of iron sulphide in the saturated zone may appear as distinct and homogeneously sized black patches associated with the rootlets (B.1) or root (C.2). The precipitates may also form biogeochemically reactive meso-compartments, such as speckled spots associated with the rootlets along a vertical axis (B.2). Further information on this model wetland can be found in Imfeld et al. (in press).

displaying extensive interfacing within the rhizosphere and soil matrices. While aerobic transformation is generally faster than anaerobic transformation for low-chlorinated compounds, the aerobic degradation rate is relatively slower for highly chlorinated compounds, which are more efficiently transformed via reductive dechlorination (Amon et al., 2007). This example highlights the potential interest in coupling reductive and oxidative processes in wetland systems to reach an efficient transformation for certain contaminants and their metabolic endproducts (Armenante et al., 1992; Master et al., 2002).

3.1.2. Reduction and oxidation processes

As hydrologic and geochemical conditions in a constructed wetland change over space and time, the dominant terminal electron-accepting processes (TEAPs) undergo a concomitant shift, resulting in different rates of degradation for the organic chemical. Eco-thermodynamic considerations may also be important when calculating the probability of occurrence and the direction of a biochemical transformation in constructed wetland systems (e.g. low vs. high theoretical bio-energetic capacity for a given compound, under a given condition) (Hanselmann, 1991; Dolfing and Janssen, 1994). Furthermore, the occurrence and relative contribution of a particular pathway is strongly related to oxygen and alternative electron acceptor concentrations, availability and spatiotemporal distributions. In anaerobic zones, reductive biotic and abiotic processes may directly compete for the consumption of electron equivalents with reductive degradation processes of organic contaminants. The reduction of alternative electron acceptors (NO_3^- , SO_4^{2-} , HCO_3^- and possibly FeOOH and MnO_2) in wetlands mainly depends on the soil type, and the electron donor to acceptor ratios of the influent water, but also may depend on the presence or absence of other electron accepting species (Burgoon et al., 1995; Reddy and D'Angelo, 1997). The electron acceptor and donor reactions catalyzed by metal species within the iron cycle may be relevant for biogeochemical reaction in the vicinity of

the capillary fringe separating water saturated and unsaturated soil zones. Fe(II) may be oxidized by oxygen penetrating from the unsaturated zone to form Fe(III) , which may in turn act as electron acceptor for oxidation reactions. These processes may be important in case of water table fluctuation leading to formation of Fe(III) oxides at the capillary fringe, which may become electron donors if the water table rises again and anoxic conditions are prevailing.

However, in more aerobic zones of the water column, degradation is coupled to oxygen. Biochemically relevant oxygen transfers in wetland sediments often permit an important theoretical bio-energetic capacity ($\Delta G \ll 0$, expressing a high potential for an exergonic reaction) with respect to a given contaminant transformation. Oxygen can be transferred via three main pathways: (1) with the influent water, (2) physical transfer from the atmosphere into the water column and (3) transport via the plant tissues into the water column through root oxygen release within the rhizosphere (Tanner et al., 2002). However, the physical and phytological oxygen transport into water and sediments is generally of relatively low significance at the system scale (Rousseau, 2007), and the coupling of respiration processes leads to microaerophilic sediments in most parts of the systems. The concurrent oxygen consumption during microbial degradation of naturally-occurring organic matter outside of the contaminant pool, and the parallel oxidation of common redox-sensitive species may result in a limited supply of electron acceptors and further hinder complete oxidative contaminant transformation. Hence, the relevance of anaerobic degradation pathways is generally expected to be high at the system scale. This underlines an important point, that the significance of oxidative transformation processes may be bound to the rhizosphere and surface water zones. Some of these aspects are illustrated in Fig. 1, showing the spatial patterns of several biogeochemical processes distributed in a cross section of a model wetland treating *cis*- and *trans*-1,2-DCE contaminated groundwater (Imfeld et al., in press).

3.2. Processes at the rhizosphere scale

Constructed wetlands are considered to be particularly valuable in cases where contaminations are initially inaccessible by the rhizosphere, and may be of special relevance when contaminated groundwater is conveyed towards the surface (Newman et al., 1998; Newman and Reynolds, 2004). Indeed, the rhizosphere represents an interface of intense soil–plant–microbe–aqueous phase interactions, enabling spatial and temporal variations of redox conditions at the root system scale. The zone is characterized by concentration gradients occurring both in a radial and longitudinal axis along an individual roots and rootlets of mineral nutrients, pH, redox potential, rhizodeposition and microbial activities. In the case of several hydrophytes, the simultaneous release of oxygen and organic carbon in their rhizosphere may result in redox gradients displaying spatial and temporal variations (Liesack et al., 2000; Wei et al., 2003). These properties may in turn enhance the degradation of organic compounds in the rhizosphere as a result of the higher densities and greater activities of microorganisms when compared to the surrounding soil (Cunningham et al., 1996; McCutcheon and Schnoor, 2003). A correlation between microbial activity at the rhizosphere level and enhanced total petroleum hydrocarbon degradation could be observed, while the microbial metabolic diversity appeared to vary between vegetated and unvegetated contaminated soils (Banks et al., 2003a,b). Similarly, a conceptual-based model simulation documented the relationship between simultaneous growth of roots and associated microorganisms and increased biodegradation of crude oil contaminants (Thoma et al., 2003a,b).

While some concepts tend to neglect plant mediated oxygen transfer (Wu et al., 2000; Nivala et al., 2007), spatial and temporal redox gradients of putatively enabled oxidative processes are generally expected to decrease with increased distance from the roots. Besides the plant related factors, temporal aspects of the redox variability in the rhizosphere (Wiessner et al., 2005), as well as several environmental factors such as light intensity, and water temperature (Soda et al., 2007), redox conditions and microbial oxygen demand in the sediment (Laskov et al., 2006), have to be considered when employing constructed wetlands for the treatment of organic chemicals.

Rhizodeposition products, composed of exudates, lysates, mucilage, secretions and decaying plant material, significantly contribute to the flow of organic matter across the boundaries of the rhizosphere zone. Rhizodeposition products represent an important class of diverse compounds potentially relevant to the context of contaminant removal (Rentz et al., 2005), and may differ qualitatively from the organic substrates originating in the influent water. They can be used as carbon and energy sources by the microorganisms (Jones et al., 2004), and may stimulate co-metabolic degradation of xenobiotics (Donnelly and Fletcher, 1994; Horswell et al., 1997; Moormann et al., 2002). For instance, Chung et al. (2007) recently observed a substantial organic acid release during the operation of treatment systems with bacterial biomass. This increased acid load may favor the mineralization rate of phenanthrene under waterlogged conditions. In contrast to these findings, (Rentz et al., 2004, 2005) showed that the phenanthrene-degrading activity of *Pseudomonas putida* ATCC 17484 was repressed when exposed to different types of root exudates.

4. Investigation of processes in constructed wetland systems

Due to the complex fate of organic chemicals in constructed wetland systems, sole mass balance and budget calculations between the influent and the effluent water are often inefficient when characterizing relevant processes within the system. An appropriate monitoring strategy would ideally enable (1) assessing

the status and contribution of the different removal processes occurring in the system, and (2) evaluating the long-term maintenance of functionality in regard to mobilization and/or transformation of organics. Integrative experimental designs based on both *in situ* hydrogeochemical and microbiological indicators may garner complementary information and create a stronger basis for evaluating the *in situ* biogeochemical processes in constructed wetlands. Concepts and methods used in Monitored Natural Attenuation (MNA) approaches are currently being applied to provide quantitative and/or qualitative information about the reactive transport processes of contaminants in groundwater systems (e.g. Haack and Bekins, 2000; Grandel and Dahmke, 2004; Meckenstock et al., 2004; Rugner et al., 2006), and could be efficiently used for constructed wetland studies. Fig. 2 summarizes the possible integration of several complementary experimental approaches for assessing organic contaminant removal processes in constructed wetlands.

4.1. Sampling design and techniques

Sampling designs must balance the costs associated with acquiring the necessary background information with the risks of developing an interpretation and decision based upon insufficient information. Stratified, clustered and systematic sampling strategies (Cochran, 1963; Christman, 2000) are particularly relevant, and may be used to assess *in situ* removal processes in constructed wetlands (Moustafa and Havens, 2001). For example, Amon et al. (2007) used a systematic sampling approach (66 piezometer nests regularly spaced over the investigated wetland) to assess the fate of chlorinated ethenes. Furthermore, choosing the appropriate sampling scale for the research question is crucial when investigating biogeochemical reactions and microbial communities. As the distribution of terminal electron-accepting processes in constructed wetlands may vary over small spatial and temporal scales, the chemical heterogeneity should be characterized to avoid erroneous interpretations. For instance, Hunt et al. (1997) assessed the hydrogeochemical heterogeneity in constructed wetlands using several sampling scales, and illustrated the diverging interpretations of the occurring processes. Moreover, even the choice of the sampled wetland compartment has to be considered when discerning a research question and evaluating contaminant properties. In most of the studies, pore water samples were retrieved. The collection of core soil or sediment samples from constructed wetlands allows effective sampling, but the invasive nature of the collection could bias the information regarding autochthonous microbial degraders dynamics (White et al., 2006), or specific processes such as sorption of hydrophobic contaminants (Cottin and Merlin, 2007).

However, transformations at aerobic–anaerobic interfaces are still rather difficult to measure. Current efforts are specifically focused on developing the capability to sample integratively over spatial and temporal scales (Alvarez et al., 2004; Petty et al., 2004). In parallel to refinements of sampling devices, reliable and accurate analytical methods are under development. For example, Huang et al. (2005) used headspace solid-phase microextraction (HS-SPME) as an on-site sampling technique (Ouyang and Pawliszyn, 2006) for evaluating the behavior of volatile fatty acids and volatile alkylsulfides in a constructed wetland.

4.2. Monitoring methods

4.2.1. Hydrogeochemistry

Understanding of contaminant behavior in a constructed wetland will require a combined evaluation of footprints of biogeochemical reactions along with variations in contaminant and metabolic intermediates concentrations. The chemical framework

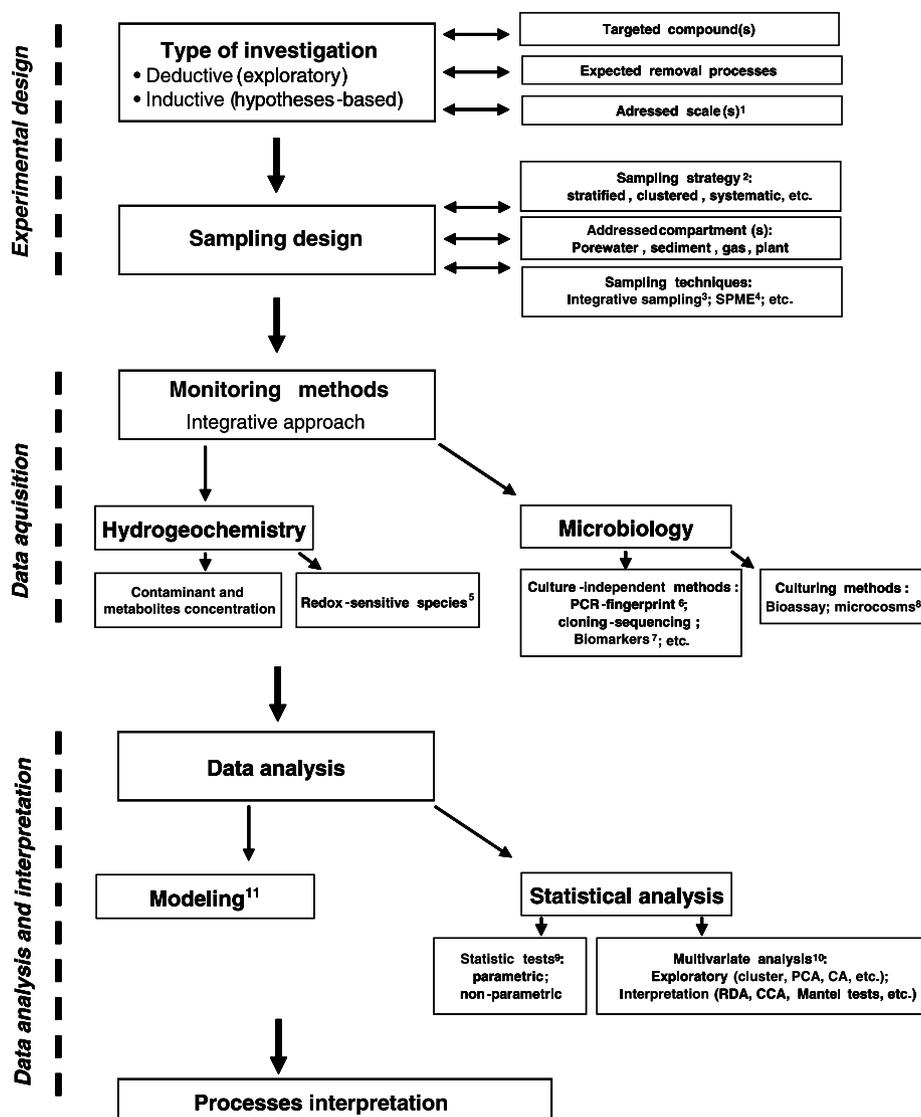


Fig. 2. Flowchart of a possible integrative monitoring approach for processes investigation in constructed wetland systems. ¹Hunt et al. (1997). ²CoChran (1963), Christman (2000) and Moustafa and Havens (2001). ³Petty et al. (2004) and Alvarez et al. (2004). ⁴Huang et al. (2005). ⁵Reddy and D'Angelo (1997), Christensen et al. (2000) and Kassenga et al. (2004). ⁶Pace et al. (1986) and Nocker et al. (2007). ⁷Jin and Kelley (2007) and Ibekwe et al. (2007). ⁸Kassenga et al. (2003), Grove and Stein (2005) and Lorah and Voytek (2004). ⁹Berryman et al. (1988), Dixon and Florian (1993) and Legendre and Legendre (1998). ¹⁰Legendre and Legendre (1998), Wackernagel (2003) and Ramette (2007) and Kitanidis (1997). ¹¹Wynn and Liehr (2001), Langergraber (2007), Keefe et al. (2004) and Tomenko et al. (2007).

potentially affecting the transformation of the targeted contaminant(s) needs to be evaluated for interpreting system observation with respect to natural attenuation potential (Reddy and D'Angelo, 1997). For example, the capacity of a system to support biological reductive dechlorination of chlorinated solvents can be assessed by monitoring concentrations of potential electron donors, typically evaluated as total organic carbon (Wiedemeier et al., 1998). Moreover, the availability of alternative electron acceptors and the distribution of microbially mediated redox reactions can be evaluated in porewater or in reliable solid fraction analyses based on redox-sensitive constituents that are characteristic of particular processes (e.g. dissolved oxygen, iron, and manganese). A valuable review on methods and concepts for characterizing hydrogeochemistry and electron donor-acceptor interactions in ground water systems is provided by Christensen et al. (2000), which could be also applied to characterize wetlands.

It is likely that the relative pore water composition with respect to redox-sensitive species will reflect the dominating redox processes at the investigated zones (Christensen et al., 2000). The

analysis of this information along with contaminant mass variation may allow mass and further electron budgeting to evaluate the efficacy of intrinsic bioremediation and/or exploratory statistical analyses to depict trends in the variations (see Section 4.3.1). Indeed, a mass balance for the terminal electron acceptors (TEA) required for biochemical oxidation of a particular contaminant and the total TEA available from both the pore water and wetland sediments, permits a researcher to calculate the supply of TEA needed to sustain the targeted bioattenuation rates. However, the balance is generally rendered difficult due to sampling biases, quality variability and high background levels for some redox-sensitive species. Diakova et al. (2006) used the concentration ratio of iron oxidation states in individual zones of a constructed wetland as a sensitive indicator for redox properties. Braeckvelt et al. (2007) correlated the mobilization of ferrous iron with monochlorobenzene removal and detected biodegradation. Furthermore, the redox process interpretation can be improved by measuring the concentrations of expected metabolites and fermentative end-products (e.g. CO₂, methane, hydrogen, acetate, ethene). This approach has

been applied successfully by Kassenga et al. (2003) while assessing the potential for dechlorination by demonstrating the co-existence of methanogenic conditions and ethene production in wetland sediment core samples. In particular, H₂ measurements may represent a powerful tool for analyzing the energetics of microbial processes (Hoehler et al., 1998) and might be relevant for evaluating the stability of *in situ* conditions with respect to bioremediation by providing an indication of the redox level dynamics (Christensen et al., 2000). For instance, Kassenga et al. (2004) studied hydrogen concentration dynamics during dechlorination of *cis*-1,2-DCE and *cis*-1,2-dichloroethane in microcosms derived from a constructed wetland, and found H₂ concentration to be a key parameter for characterizing biodegradation potentials. Moreover, this study illustrated the effectiveness of coupling measurements of microbial activity with the observation of biogeochemical processes occurring in a wetland system.

4.2.2. Microbiology

The remarkable development of molecular approaches may efficiently complement both hydrogeochemical investigations and culture-based techniques. These techniques offer the potential for assessing the structure and function of microbial populations involved in the bioremediation of organic chemicals in complex wetland systems. In particular, the application of molecular tools may enable a better mechanistic understanding of the relationship between quantitative and qualitative aspects of species diversity and environmental factors, such as contaminant load, specific nutrient requirements or prevailing TEAPs. Knowledge about these interactions may in turn contribute to enhanced organic chemical treatment effectiveness in constructed wetlands by identifying factors that need to be considered during the development of more reliable and efficient systems.

4.2.2.1. Microcosms and culture-independent techniques. Microcosms based on inoculants originating from constructed wetlands have been used to assess the occurrence and kinetics of biodegradation of *cis*-1,2-DCE (Kassenga et al., 2003) or polar organic solvents (Grove and Stein, 2005). Such investigations also contributed to the identification of anaerobic biodegradation of 1,1,2,2-tetrachloroethane (TeCA) and 1,1,2-trichloroethane (TCA), as well as associated geochemical conditions and microbial consortia identifications (Lorah and Voytek, 2004). However, their use remains limited mainly due to the difficulty associated with scaling the processes to relevant field parameters (Amann et al., 1995). Additionally, several *in situ* biotic degradation pathways are possible for most of the contaminant groups, and involve different microbial guilds (Reineke and Knackmuss, 1988; Aislabie et al., 1997; Juhasz and Naidu, 2000; Williams, 2002). Therefore, studying the phylogenetic diversity, composition and/or structure of indigenous microbial communities using culture independent molecular approaches (Nocker et al., 2007) may provide key information on the functioning of wetland systems during the treatment of organic chemicals. Molecular DNA-based approaches are generally based on amplification of genetic markers by polymerase chain reaction (PCR) using universal or specific primers. Some genetic markers, such as the 16S rRNA genes (Pace et al., 1986), permit the assessment of microbial diversity, whereas functional genes are preferentially targeted for investigating relevant metabolic capabilities. In a recent contribution, Jin and Kelley (2007) combined total phospholipid fatty acids (PLFA) identification of eukaryotes and prokaryotes with PCR-denaturing gradient gel electrophoresis (DGGE) to assess the microbial community diversity and composition in different types of constructed wetlands. Similarly, Ibekwe et al. (2007) used a PCR-DGGE approach followed by bacterial sequence retrieval to correlate the water quality changes with the microbial community diversity and composition

of sediment, water and rhizosphere samples of a constructed wetland. Lorah and Voytek (2004) characterized the microbial community in microcosms consisting of sediment originating from wetlands treating 1,1,2,2-TeCA and 1,1,2-TCA using terminal-restriction fragment length polymorphism (T-RFLP) and focused on a particular group of dehalogenating bacteria using a taxon-specific PCR approach.

Thus, 16S rRNA-targeted techniques may yield significant information regarding the structure of *a priori* unknown degrading microbial communities directly from wetland sediment or pore water samples. However, targeted populations in constructed wetlands could include bacteria, but also fungi, protists, nematodes or macrophytes that are capable of bioremediation processes. For instance, the development of the ability of wetland prokaryotic or eukaryotic populations capable of adapting to and transforming contaminants of concern is a critical issue that remains difficult to holistically tackle. High-throughput technologies, such as DNA chips or gene expression arrays (Freeman et al., 2000), may provide highly resolved information about the genetic diversity of complex contaminated systems subjected to recurrent, small scale variations in geochemical conditions. Moreover, these emerging techniques may enable researchers to gather knowledge about the relationship between structure and function of these various wetland communities. In turn, an increased understanding of the intrinsic contaminant transformation processes would help supporting the development of organic contaminant stress indicators and facilitate the assessment of spatiotemporal evolution.

4.2.2.2. Emerging techniques. Emerging techniques are being developed to provide more information about the relationship between structure and function of target communities in contaminated aquatic systems and have been recently reviewed (Weiss and Cozzarelli, 2008). Radioactive (¹⁴C) or stable isotope (¹³C) tracers may be used to track the partitioning, transformation and mineralization of organic contaminants. The use of ¹⁴C labelled contaminants has been effective in several wetland studies for pentachlorophenol (Meade and D'Angelo, 2005), TCE (Bankston et al., 2002; Amon et al., 2007) and CB (MacLeod, 1999), however tracing of radioactive compounds is regulated and mostly not allowed in open systems. In addition, combined compound-specific stable isotope approaches have been used to characterize the *in situ* biodegradation of CB in a constructed wetland. *In situ* tracer experiments based on ¹³C-labelled CB have been accomplished to characterize microbial degradation by means of transformation of labeled carbon in to microbial lipids upon metabolism of CB. In parallel, compound specific isotope fractionation analysis (CSIA) of CB was employed to monitor the degradation of CB along the water flow path in a constructed wetland system (Braeckvelt et al., 2007). CSIA represents a powerful tool for assessing biodegradation of organic chemicals in field studies, as the extent of isotope fractionation mainly depends on the biochemical reaction mechanism involved (Meckenstock et al., 2004; Fischer et al., 2007; Rosell et al., 2007). This technique may also help documenting and characterizing biodegradation and pathways during longitudinal surveys in complex constructed wetland systems. Recently, Imfeld et al. (in press) traced the temporal and spatial changes of the dominant degradation mechanism of *cis*- and *trans*-dichloroethenes (DCE) in a model wetland. This was rendered possible because the mechanisms of DCE oxidation and reductive dechlorination result in different characteristic isotope effects that could be measured by means of CSIA.

Furthermore, the use of stable isotope probing techniques (DNA- or RNA-SIP) would permit the identification of microbial community members that are actively involved in the metabolic cycling of organic chemicals in wetland porewater or sediments (Kreuzer-Martin, 2007; Neufeld et al., 2007) at the system scale

or at discrete depth intervals. Moreover, the study of RNA transcripts would provide insights into the potential metabolic activity (e.g. mRNA of functional genes), whereas proteome and metabolome analysis hold promise for gathering knowledge about the status of protein expression and metabolic activity in constructed wetlands treating organic chemicals.

4.3. Data treatment

Detailed hydrogeological and microbiological investigations of complex and heterogeneous systems, such as constructed wetlands, generally results in large data sets that must be further processed in order to facilitate the interpretation of on-going biogeochemical processes (Fig. 2).

4.3.1. Statistical analysis

Water quality data rarely meet the statistical assumption of normality. Therefore, non-parametric statistics are generally recommended when evaluating the significance of these trends over space and/or time. Furthermore, assessment of trends over sampling points and/or treatments (e.g. soil strata; reaches over the flowpath) can be achieved with Mann-Whitney/Wilcoxon rank sum tests or non-parametric χ^2 statistics, accordingly, whereas temporal trends in water quality data can be investigated with Mann-Whitney, Spearman and Kendall temporal tests (e.g. Berryman et al., 1988; Dixon and Chiswell, 1996; Legendre and Legendre, 1998). Multivariate analyses will be of particular interest when assessing causal processes in wetlands by evaluating trends over several variables (Legendre and Legendre, 1998). The application of these tools in several disciplinary fields of relevance for constructed wetland research has been intensively reviewed: geology and geochemistry (Wackernagel, 2003), microbial ecology (Ramette, 2007), and hydrogeology (Kitanidis, 1997). Briefly, after transformation, normalization or standardization, the data sets are separately or complementarily subjected to exploratory (e.g. cluster analyses; principal component analysis, PCA; correspondence analysis, CA; principal coordinate analysis, PCoA; and nonmetric multidimensional scaling, NMDS) or interpretation analyses (e.g. indirect gradient analyses; redundancy analysis, RDA; canonical correspondence analysis, CCA; variation partitioning, Mantel test). Exploratory multivariate analyses are used to reveal patterns in large data sets (e.g. existence of clusters or groups of objects), but do not directly explain their occurrence. For example, Imfeld et al. (in press) used PCA to enhance the interpretation of the development of hydrogeochemical processes in a model wetland. In contrast, interpretative analyses allow the researcher to address the relationship between species patterns and environmental variables (Legendre and Legendre, 1998).

4.3.2. Modeling

Several groups of simple (Tanner et al., 1995; Kadlec, 2000), Monod-type (Mitchell and McNevin, 2001) and mechanistic (Wynn and Liehr, 2001) models, as well as multi-component reactive transport models focusing on the characteristics of internal processes (Langergraber, 2007) have been developed. Keefe et al. (2004) successfully used stream modeling techniques (one-dimensional transport model with inflow and storage) for the investigation of VOCs behavior in a constructed wetland. Recently, Tomenko et al. (2007) compared the predicted efficiency of constructed wetland treatments using multiple regression analysis and artificial neural networks. Though some numerical analyses may substantially contribute to the investigation of reactive transport and support quantification of physicochemical processes in constructed wetlands, their applicability for evaluating the fate of organic chemicals in constructed wetlands remains challenging. Indeed, the numerical analysis of explicit

design equations is often limited in describing the complexity and interconnectivity of processes in constructed wetlands. Moreover, linking wetland hydraulics to removal pathways is particularly difficult, due to non-uniform hydraulic flow, heterogeneity of retardation factors and complexity of degradation processes. Nevertheless, values retrieved from a combined hydrogeochemical and microbiological assessment can be used to generate conceptual models of both microbially mediated TEAPs and transformation reactions to better elucidate the processes associated with natural attenuation of organic chemicals in constructed wetlands.

5. Conclusions

Constructed wetland technology for the treatment of organic chemicals is an emerging field (EPA, 2000; Schröder et al., 2007). In this context, a better understanding of the processes governing the removal of organic chemicals is crucial for further system optimization. Valuable information regarding the fate of organic contaminants in constructed wetlands can be retrieved based on their physico-chemical properties and eco-thermodynamic considerations. This information may in turn support improved testing and better optimization of system designs and operational modes. Additionally, a comprehensive follow-up study investigating the longitudinal effect of hydrogeochemical and microbial indicators of degradation is required for characterizing system-intrinsic processes and reactions controlling the fate of organic compounds. Ultimately, an integrative approach will better document wetland system efficiency and reliability with respect to organic contaminant removal. In this context, monitoring bioremediation would involve correlating specific groups of organisms with their observed degradation activities and functions in constructed wetlands. Gathering knowledge about the relationship between the quantitative and qualitative development of these organisms and key environmental factors, such as characteristic patterns of redox gradients at various scales, is relevant for improving the contaminant attenuation functions of constructed wetland ecosystems. The application of approaches and techniques recently developed in related fields, such as contaminant hydrology, environmental microbiology and biotechnology, phytoremediation, statistics and environmental modelling, will tremendously enhance these investigations and open new possibilities for process characterization and interpretation in constructed wetlands.

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A.2 Pilot-scale constructed wetland systems



Figure A2-1: Pilot-scale constructed wetland and unplanted reference systems in summer 2005.



Figure A2-2: Pilot-scale constructed wetland with dormant plants and unplanted reference systems in winter 2006.



Figure A2-3: Pilot-scale constructed wetland and unplanted reference systems in summer 2009; strong plant growth after fertilization.

A.3 Model rhizosphere reactors

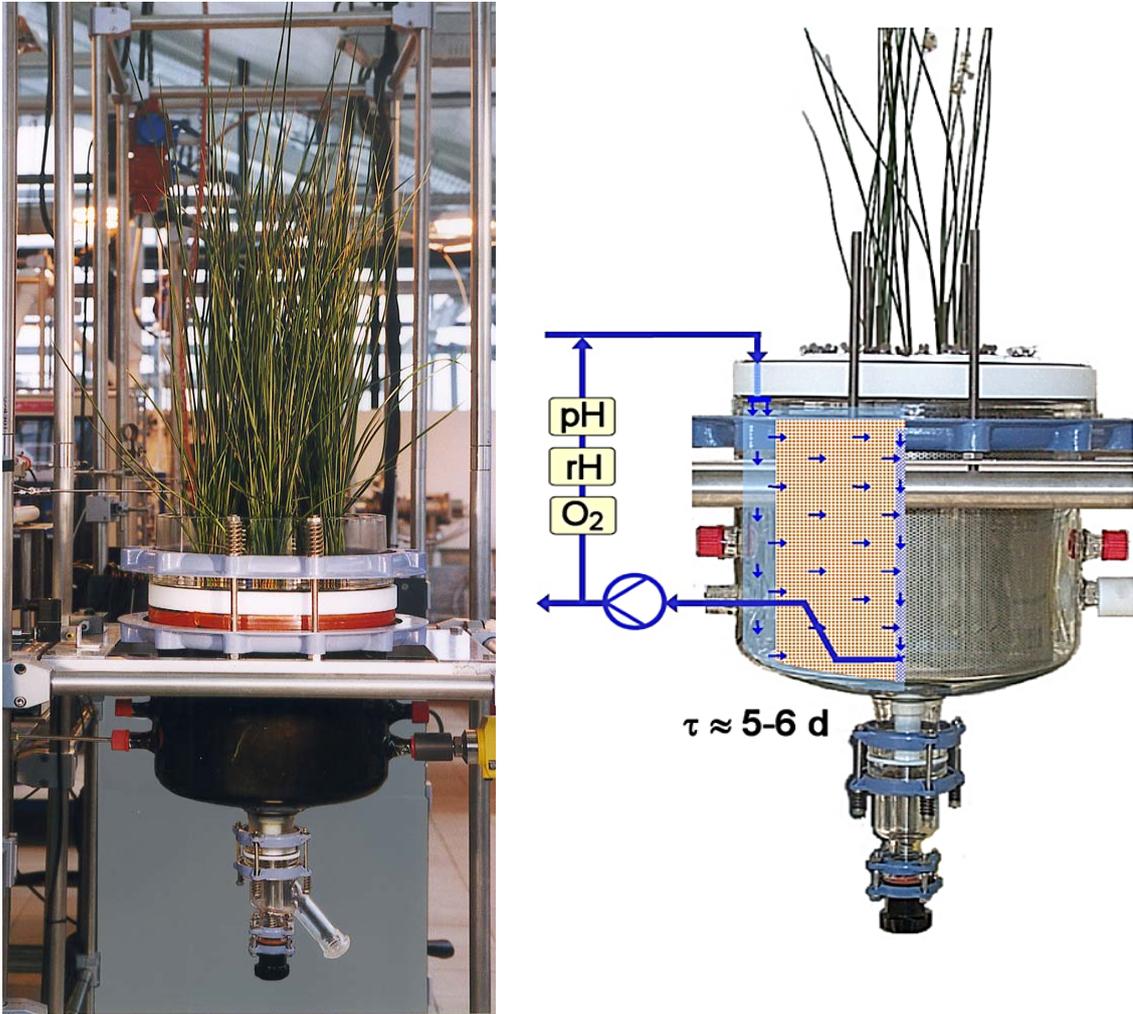


Figure A3-1: Model rhizosphere reactors. Left: Lateral view; right: Operation scheme.



Figure A3-2: Model rhizosphere reactors: Detail of the PTFE lid and plant openings containing Juncus effusus.

Erklärung

Hiermit versichere ich, dass die vorliegende Arbeit ohne unzulässige Hilfe und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt wurde und dass die aus fremden Quellen direkt oder indirekt übernommenen Gedanken in der Arbeit als solche kenntlich gemacht worden sind.

Bei der Auswahl und Auswertung des Materials sowie bei der Erstellung der Publikationen habe ich von den folgenden Personen Unterstützungsleistungen erhalten (in alphabetischer Reihenfolge):

G. Imfeld (2.3, Appendix A.1)

M. Kästner (Chapter 2, 3, 4)

P. Kusch (Chapter 2, 3, 4, Appendix A.1)

N. Reiche (Chapter 2.1, 2.2)

H.-H. Richnow (Chapter 2.3, Appendix A.1)

H. Rokadia (Chapter 2.3)

M. Rückert (Chapter 2.1)

N. Stelzer (Chapter 2.3)

S. Trapp (Chapter 2.2)

S. Weber (Chapter 2.3)

A. Wiessner (Chapter 2.1, 2.2)

Ich versichere, dass außer den genannten keine weiteren Personen bei der geistigen Herstellung der vorliegenden Arbeit beteiligt waren, insbesondere auch nicht die Hilfe eines Promotionsberaters in Anspruch genommen wurde, und dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die vorgelegte Arbeit wurde weder im Inland noch im Ausland in gleicher oder in ähnlicher Form einer anderen Prüfungsbehörde zum Zwecke einer Promotion oder eines

anderen Prüfungsverfahrens vorgelegt. Frühere Promotionsversuche haben nicht stattgefunden.

Mein Anteil an der wissenschaftlichen Leistung der vorgelegten Publikationen ist im Folgenden erklärt. Als meine fachlichen Betreuer haben P. Kuschk und M. Kästner an allen Veröffentlichungen vor allem bei der Planung der Versuche und Konzeption der Artikel mitgewirkt.

Chapter 2.1: Braeckevelt, M., Mirschel, G., Wiessner, A., Rueckert, M., Reiche, N., Vogt, C., Schultz, A., Paschke, H., Kuschk, P., Kaestner, M. (2008). Treatment of chlorobenzene contaminated groundwater in a pilot-scale constructed wetland. *Ecological Engineering* 33: 45–53.

Diese Publikation habe ich hauptverantwortlich konzipiert und eigenständig verfasst; bei der Auswertung des Materials haben mich A. Wiessner und M. Rückert unterstützt. Die zugrunde liegenden Versuche wurden von mir eigenständig durchgeführt; bei der Probenahme und Analyse unterstützten mich G. Mirschel und H. Paschke. Die mikrobiologischen Versuche wurden von C. Vogt und A. Schultz durchgeführt.

Chapter 2.2: Braeckevelt, M., Reiche, N., Trapp, S., Wiessner, A., Paschke, H., Kuschk, P., Kaestner, M. (2011). Chlorobenzenes removal efficiencies and removal processes in a pilot-scale constructed wetland treating contaminated groundwater. *Ecological Engineering*, accepted for publication.

Dieser Artikel wurde von mir hauptverantwortlich konzipiert und verfasst. Die zugrunde liegenden Versuche habe ich selbst durchgeführt, mit Ausnahme der Emissionsmessungen, die von N. Reiche ausgeführt wurden. Bei der Analytik der Wasserproben unterstützte mich H. Paschke und bei der Auswertung der Daten A. Wiessner. Die Modellierung verschiedener Eliminierungsprozesse wurde von S. Trapp konzipiert und angeleitet; die Berechnungen und das Verfassen des entsprechenden Kapitels wurden von S. Trapp und mir in Zusammenarbeit realisiert.

Chapter 2.3: Braeckevelt, M., Rokadia, H., Imfeld, G., Stelzer, N., Paschke, H., Kuschk, P., Kästner, M., Richnow, H.-H., Weber, S. (2007). Assessment of *in situ* biodegradation of monochlorobenzene in contaminated groundwater treated in a constructed wetland. *Environmental Pollution* 148: 428-437.

Diese Publikation wurde von mir und G. Imfeld verfasst, wobei G. Imfeld vor allem an der Konzeption des Artikels und an der statistischen Auswertung und Interpretation der erhaltenen Ergebnisse mitwirkte. Die zugrunde liegenden physikochemischen Daten wurden von mir hauptverantwortlich erhoben, unterstützt durch H. Paschke bei der Analytik der organischen Kontaminaten. Die Isotopenfraktionierungs- und BACTRAP-Versuche haben H. Rokadia und ich in Kooperation ausgeführt, wobei uns N. Stelzer und S. Weber bei der Planung und Auswertung behilflich waren.

Chapter 3: Braeckevelt, M., Seeger, E.M., Paschke, H., Kusch, P., Kaestner, M. (2011). Adaptation of a constructed wetland to simultaneous treatment of monochlorobenzene and perchloroethene. *International Journal of Phytoremediation*, accepted for publication.

Dieser Artikel wurde von mir selbstständig und ohne Unterstützung durch andere verfasst. Die zugrunde liegenden Daten wurden von mir selbst (2007 und 2008) und von E.M. Seeger (2006) erhoben, H. Paschke unterstützte uns bei der Analytik der organischen Kontaminanten.

Chapter 4: Braeckevelt, M., Kaestner, M., Kusch, P. Removal of monochlorobenzene and perchloroethene in wetland rhizosphere model systems. *Engineering in Life Sciences*, submitted.

Diesen Artikel habe ich selbstständig und hauptverantwortlich verfasst und die zugrunde liegenden Versuche wurden von mir eigenständig durchgeführt.

Appendix A.1: Imfeld, G., Braeckevelt, M., Kusch, P., Richnow, H.-H. (2009). Review: Monitoring and assessing processes of organic chemicals removal in constructed wetlands. *Chemosphere* 74: 349–362.

Dieser Review-Artikel wurde von G. Imfeld und mir zusammen konzipiert und verfasst. Für das Kapitel 2 „Removal processes in constructed wetlands“ wurde die zugrunde liegende Literaturrecherche von mir durchgeführt, das Material ausgewertet und der Text und die Tabelle hauptverantwortlich und eigenständig erstellt. An den anderen Kapiteln habe ich vor allem konzeptionell mitgearbeitet. H.-H. Richnow unterstützte uns bei der Konzeption des Artikels.

Leipzig, den 01.02.2011

Mareike Braeckevelt

Zusammenfassung der wissenschaftlichen Ergebnisse
zur Dissertation

Constructed wetland treatment of groundwater contaminated by chlorinated volatile organic compounds

Der Fakultät für Chemie und Mineralogie der Universität Leipzig
vorgelegt von

Diplom-Chemikerin Mareike Ursula Braeckevelt
im Februar 2011

Angefertigt am Helmholtz-Zentrum für Umweltforschung - UFZ
in Koordination mit dem Institut für Technische Chemie

1. Introduction

Chlorinated volatile organic compounds (VOC) such as monochlorobenzene (MCB), dichlorobenzenes (DCBs) and perchloroethene (PCE) are among the most widespread groundwater pollutants worldwide. Chlorinated VOC contaminated groundwater causes environmental hazards particularly when it discharges into rivers and river floodplains. Constructed wetlands (CW) represent a promising cost-efficient, near-natural technology to prevent the contamination of surface waters. The overall goal of the thesis was to contribute to evaluation of the emerging technology of CW treatment of chlorinated VOC and provide knowledge on contaminant removal processes and efficiencies, as a basis for optimization of the technology.

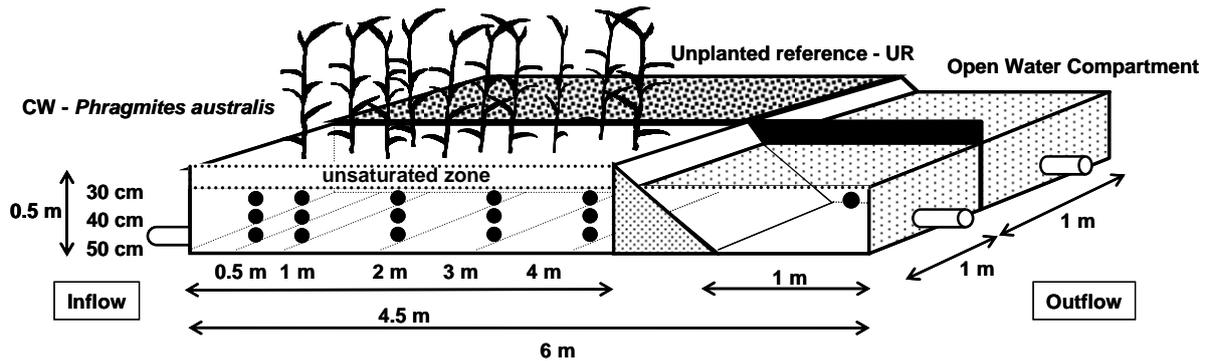
The study consisted of three main parts:

- The removal efficiency and the seasonal variability of removal, the relevance of different removal pathways and the influence of planting were determined for treatment of chlorinated benzenes contaminated groundwater in horizontal subsurface flow (HSSF) CW in the temperate climate zone.
- The adaptation of a HSSF CW to removal of MCB and PCE was investigated to determine potentials and constraints of simultaneous treatment of oxidizable and reducible chlorinated VOC.

- The potential of technical optimizations, especially optimization of oxygen and carbon source availability, was investigated in rhizosphere model reactors treating MCB and PCE contaminated water. Removal efficiency data were determined based on well-defined specific treatment conditions.

2. Experimental systems

2.1. Pilot-scale HSSF constructed wetland

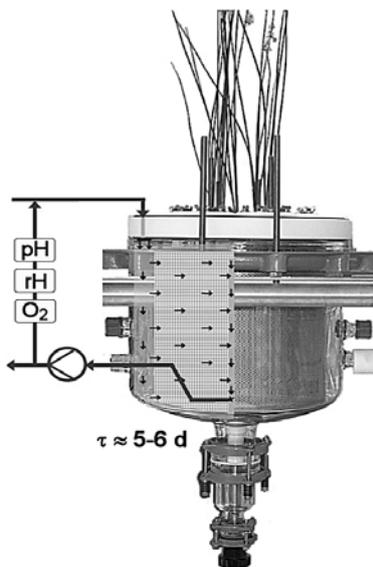


- sampling points

Figure 1: Pilot-scale horizontal sub-surface flow constructed wetland in Bitterfeld.

HSSF CW systems have been chosen for the treatment of chlorinated VOC contaminated groundwater due to their inherent gradient characteristics, making them suitable for supporting both oxidative and reductive processes, and their expected low volatilization potential.

2.2. Model rhizosphere reactors



- Cylindrical glass vessel containing a metal basket (Ø 28 cm, height 30 cm, $V=18.5$ L)
- Filter material: 21 kg gravel (2-4 mm)
- Pore volume: 10-11 L
- Inflow rate: 1.7 L d^{-1}
- Internal recirculation flow rate 69 L d^{-1}
- Hydraulic retention time 5-7 days
- PTFE lid with 5 plant openings
- Planting: *Juncus effusus*
- Redox, oxygen and pH electrodes

Figure 2: Model rhizosphere reactor operation scheme and specifications (developed by U. Kappelmeyer).

The macro-gradient free operation of the rhizosphere model reactors allows precise determination and regulation of overall rhizosphere conditions (redox potential, oxygen concentration, pH, organic carbon content).

3. Results

3.1. Pilot-scale study: Treatment of chlorobenzene contaminated groundwater

Load removal efficiency, annual means and seasonal variations. Treatment of MCB and 1,4-DCB was efficient, in particular in the upper filter layer, while 1,2-DCB removal was less pronounced. Load removal efficiencies amounted to 59-65% ($2327\text{-}3179 \mu\text{mol m}^{-2} \text{d}^{-1}$) for MCB and 59-69% ($27\text{-}35 \mu\text{mol m}^{-2} \text{d}^{-1}$) for 1,4-DCB in the summer period. The annual mean removal of chlorobenzenes was significantly enhanced by the presence of plants (*Phragmites australis*) (Figure 3). Seasonal variations in removal efficiency were evident (Figure 4). Less pronounced concentration decrease in the summer season was likely due to a higher organic carbon input by the plants during the growing season, leading to oxygen limitation.

Table 1: Potential MCB removal from the CW and unplanted reference system through the various pathways and comparison to measured MCB load removal in the summer period.

	MCB [$\text{mg m}^{-2} \text{d}^{-1}$]	
	Constructed wetland	Unplanted reference
Oxidation of MCB	5 525 - 11 025	825
Translocation by plants	130 - 525	-
Escape via aerenchym	10 - 120	-
Emission from filter surface	6 - 14	2 - 70
Sum removal	5671 - 11 684	827 - 895
Measured removal	262 - 358	34 - 246

Modeling of MCB removal processes. Modeling demonstrated that plants potentially enhance removal as a result of root oxygen release, intensification of water table fluctuations and direct uptake (Table 1). The model calculations also confirmed the high potential for oxidative microbial MCB degradation in the system. The observed overall MCB removal was much smaller than the potential removal, presumably resulting partly from high additional oxygen demand in the CW.

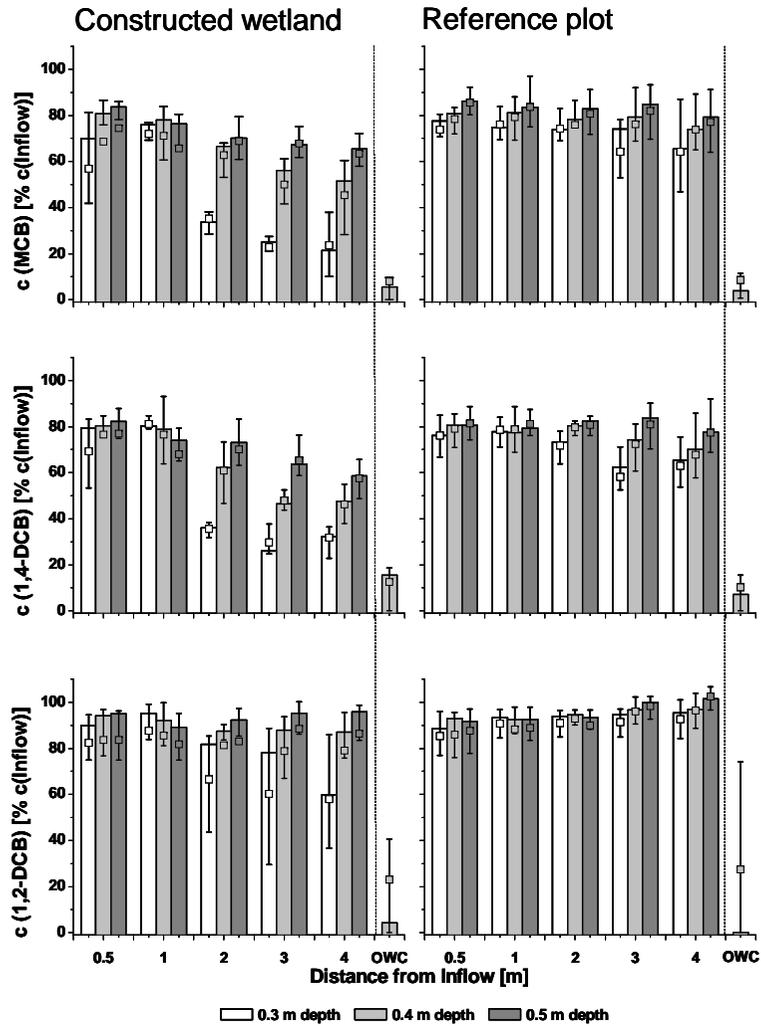


Figure 3: Annual median and mean MCB, 1,2-DCB and 1,4-DCB concentrations (in % of inflow concentrations) in the CW and unplanted reference ($n_{CW} = 14$, $n_{UR} = 15$). Columns represent the medians, error bars the 25th and 75th percentiles, and squares the means of the annual data sets.

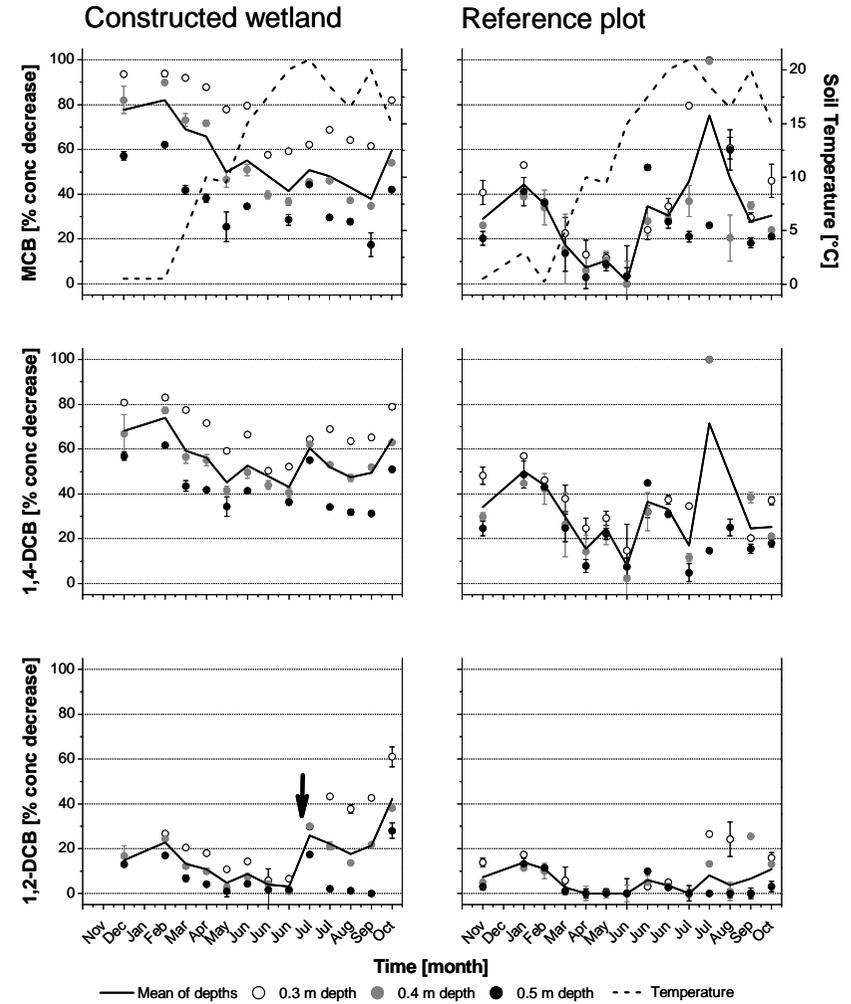


Figure 4: Seasonal variations of MCB, 1,4-DCB and 1,2-DCB concentration decrease between the inflow and 4 m of the flow path and soil temperature in the CW and unplanted reference. Error bars denote standard deviations. The arrow indicates a decrease in 1,2-DCB inflow concentrations.

Biodegradation of MCB. Biodegradation of MCB was assessed in the pilot scale HSSF CW applying an innovative integrated approach. Stable carbon isotope fractionation analysis revealed a correlation between decreasing MCB concentrations and enrichment of the heavier carbon isotope ^{13}C at increasing distance from the inflow, indicating biodegradation of MCB (Figure 5-1).

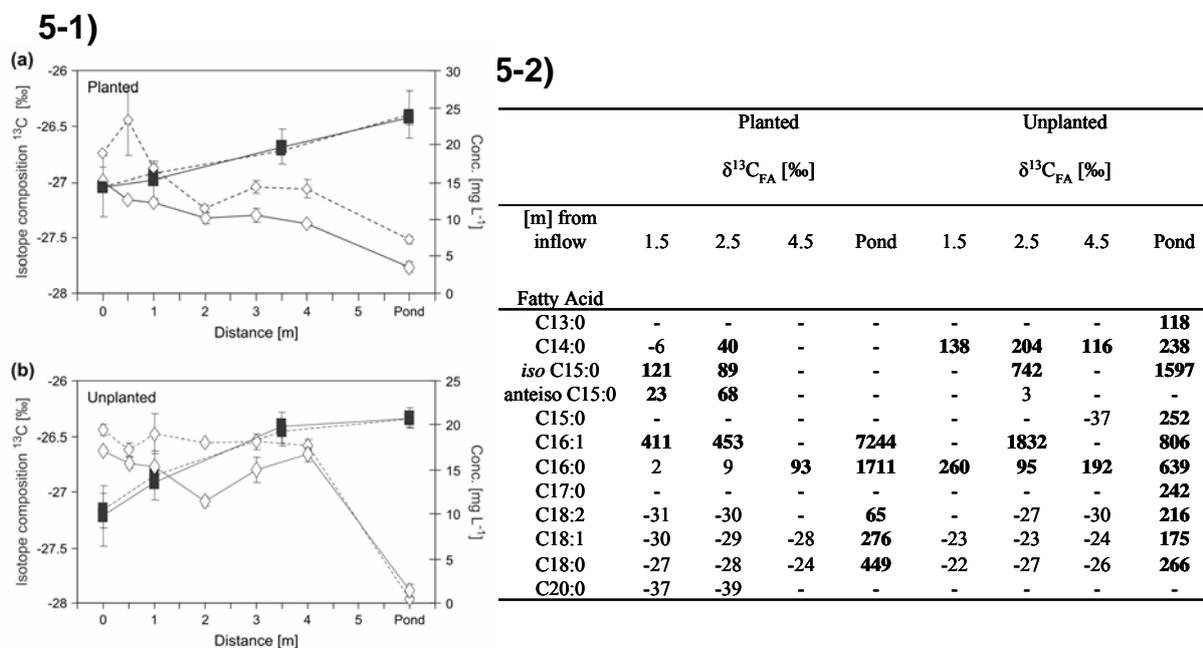


Figure 5: 5-1) Concentration (diamonds) and isotopic composition (squares) of MCB in the planted CW (a) and unplanted reference system (b) (line, 14/04/2005; dashed, 09/05/2005). Error bars show the standard deviation. 5-2) Carbon isotope composition of fatty acids extracted from *in situ* microcosms incubated for 6 weeks with $^{13}\text{C}_6$ labeled monochlorobenzene at 1.5, 2.5 and 4.5 m from the inflow point as well as in the ponds of the planted CW and unplanted reference system.

In addition, tracer experiments using *in situ* microcosms (BACTRAPs) loaded with ^{13}C labeled MCB were carried out in the CW and unplanted reference plot. Incorporation of ^{13}C derived from labeled MCB into bacterial fatty acids proved active microbial MCB degradation (Figure 5-2). The detection of ^{13}C -labeled benzene as a metabolite indicated anaerobic reductive dechlorination of MCB. The high potential of aerobic microbial MCB degradation could be confirmed applying conventional microbiological methods (MPN counts, aerobic microcosm degradation experiments).

Emissions from the filter surface. Emission measurements indicated that surface volatilization was a minor removal process, accounting for only 2-4% of the total MCB removed¹. The high temporal variability of MCB volatilization fluxes was related to the corresponding weather conditions (Figure 6). In general, contaminant volatilization from the

¹ Emission measurements were carried out by Dr. Nils Reiche

CW was more consistent than from the unplanted reference, probably due to buffering of environmental impacts (temperature, wind etc.).

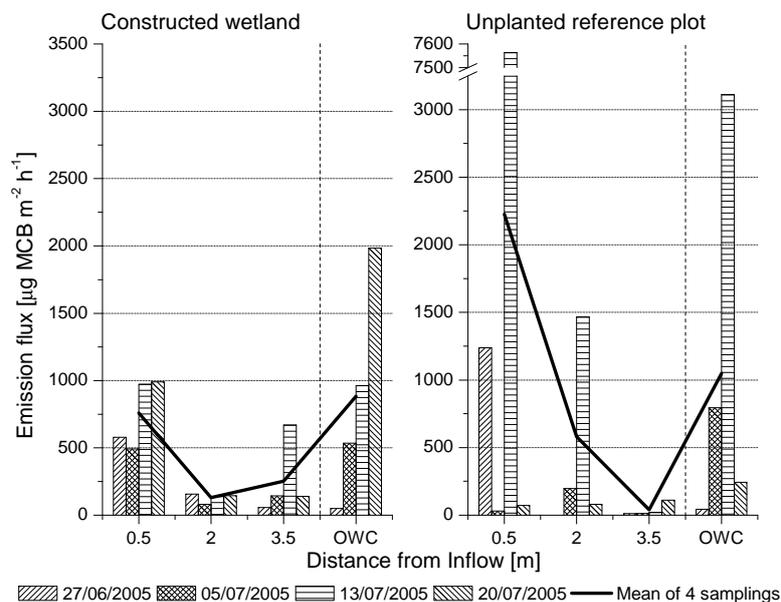


Figure 6: MCB volatilization fluxes from the CW and UR filters, plotted against the distance from the inflow, as well as from the open water compartments (OWC) (4 sampling data sets).

3.2. Simultaneous treatment of MCB and PCE in CW

A pilot scale HSSF CW able to treat MCB contaminated groundwater was adapted to a mixture of MCB and PCE, representing lowly and highly chlorinated model VOC.

Adaptation. Removal of MCB was temporarily impaired by PCE addition, probably resulting from toxic impacts on the previously unadapted microbial communities, and displacement of sorbed MCB by adsorption of the new contaminant. Simultaneous treatment of MCB and PCE required a long adaptation period of 2.5 years, as was evident from the development of PCE and metabolite concentration patterns (Figure 7). Thus, long adaptation times have to be considered in planning and installation of real-scale systems.

Removal performance. After adaptation, a MCB concentration decrease of up to 64% (55 μM) was observed. Complete removal of 10-15 μM PCE was achieved, and outflow concentrations of chlorinated metabolites were negligible.

Complete PCE dechlorination. Ethene production and detection of *Dehalococcoides sp.*, the only known microbial species able to dechlorinate PCE to ethene, indicated complete PCE dechlorination, described for the first time in a CW. Up to 100% of the PCE inflow load (1250 \pm 260 $\mu\text{mol d}^{-1}$) were dechlorinated on a filter passage of just 1 m, and a maximum of 30% was recovered as ethene.

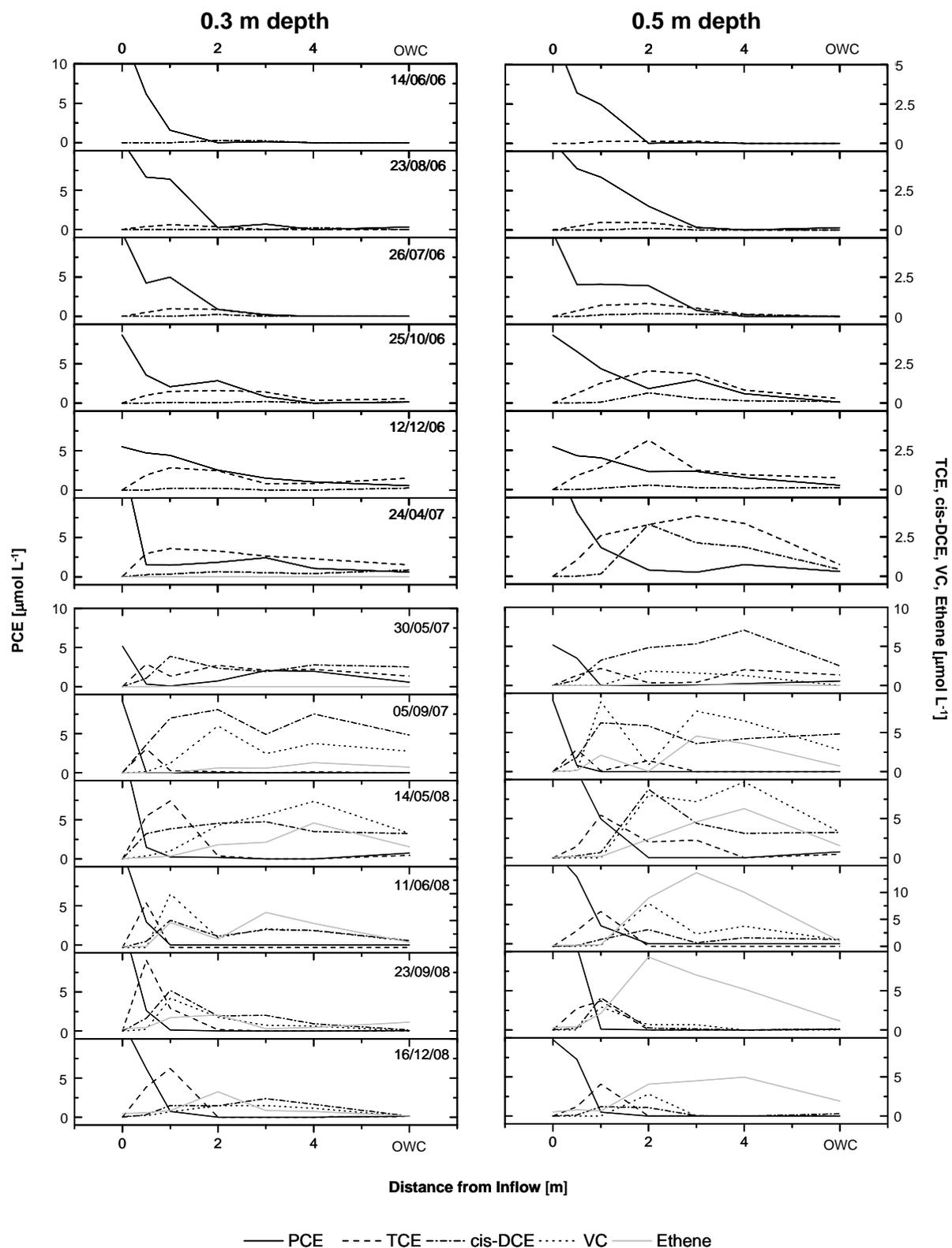


Figure 7: PCE removal and formation of metabolites over the course of the CW flow path in 0.3 and 0.5 m depth, June 2006 – December 2008 (PCE inflow concentrations were 10-15 μM) (OWC=open water compartment).

3.3. Engineering approaches to optimization of MCB and PCE removal

Technical optimization of MCB and PCE treatment in CW by application of different treatment conditions was tested in macro-gradient free rhizosphere model reactors (Figure 2).

Reduced wetland rhizosphere conditions and high organic carbon availability support reductive dechlorination, and auxiliary carbon addition may be necessary for treatment of groundwater containing reducible contaminants. However, high organic carbon loads can impair the removal of oxidizable compounds due to competition for electron acceptors. With regard to CW treatment of mixed groundwater contaminations, the impact of various organic carbon loads and sulphate reduction (Table 2) on removal of highly and lowly chlorinated VOC was assessed.

MCB removal. MCB removal was very efficient under low carbon load (overall oxic) and moderate carbon load (overall reducing) conditions (Table 2). Higher loads of easily degradable carbon (300 mg L^{-1} acetate) resulted in a lower mean removal efficiency of 57%. Sulphate reduction and associated sulphide toxicity constitute a hazard for CW treatment of MCB contaminated groundwater containing sulphate, causing a decrease in removal efficiency by 50% at the end of experiment 2.

Table 2: Mean load removal efficiencies (in %) for MCB and PCE in the model rhizosphere reactors under different experimental conditions. Experiment 1: low organic carbon loads. Experiment 2: low organic carbon loads and sulphate reducing conditions. Experiment 3: high loads of easily degradable organic carbon (300 mg L^{-1} acetate). Experiment 4: moderate fluctuating loads of easily degradable organic carbon ($0\text{-}150 \text{ mg L}^{-1}$ acetate).

Experiment	1	2	3	4
	MCB			
Number of data sets	12	10	11	12
Mean removal [%]	99.8±0.3	90.9±15.6	57.2±21.6	94.6±4.9
	PCE			
Number of data sets	13	11	8	12
Mean removal [%]	87.2±2.7	79.4±10	80.2±7.9	88.4±5.5

PCE removal. Under low carbon load and high carbon load conditions, 79-87% of PCE loads were eliminated, but no microbial reductive dechlorination activity was detected. Thus, plant uptake and phytovolatilization are considered to be important removal processes for PCE. Under fluctuating moderate carbon load conditions, reductive dechlorination was initiated, leading to a mean PCE removal of >88%. According to PCE inflow loads of $31 \mu\text{mol d}^{-1}$, and outflow loads of $1.8 \mu\text{mol trichloroethene d}^{-1}$ and $1.2 \mu\text{mol cis-dichloroethene d}^{-1}$, about 10% of the added PCE were proven to be dechlorinated. However, the initiation and maintenance of efficient PCE dechlorination requires further optimization.

4. Conclusions and Outlook

The use of HSSF CW for treatment of chlorinated VOC contaminated groundwater showed to be a promising cost-efficient alternative to conventional pump-and-treat technologies, particularly for surface-near groundwater discharging into river floodplains or wetlands. The technology is suitable also for the treatment of groundwater containing both highly and lowly chlorinated (microbially oxidizable and reducible) VOC. Adaptation of oxygen and carbon availability confirmed the potential of biostimulation for optimization of CW treatment performance. Aerobic and anaerobic microbial degradation were shown to be important removal processes for chlorinated VOC in CW, and different degradation pathways were simultaneously active in the gradient dominated CW filter. In contrast, volatilization from the filter surface was shown to be negligible.

Limitations. Limitations of the HSSF CW technology for chlorinated VOC elimination became evident in the presented study, mainly connected to spatial inhomogeneities in removal efficiency (low chlorobenzenes removal efficiency in deeper filter layers, and on the second part of the CW flow path), and generally insufficient oxygen availability for treatment of oxidizable contaminants.

Potential solutions. To achieve complete removal of oxidizable VOC, more oxic systems are recommended, e.g. shallower systems or systems combined with a long, shallow pond, as well as operation with enhanced water table fluctuations (fill-drain mode) or with vertical down-flow mode. It may also be advantageous to use wetlands with a length-to-width ratio smaller than 1, as removal efficiency per m^2 was considerably greater close to the inflow in the pilot-scale study system.

Further research needs. Plant uptake and phytovolatilization as potentially relevant removal processes should be addressed in future studies. Further knowledge on microbial degradation pathways for chlorinated VOC, and quantification of their contribution to contaminant removal in CW, is another challenging task. In addition, elucidation of the role of sorption capacity and sorption-desorption-kinetics of CW filter materials containing high organic carbon contents should be attempted. For real-scale application of the CW technology, estimations of area demand and loading capacity are necessary, based on empirically determined loading specifications or exponential decline modeling. However, a large number of systems have to be considered to provide a sound basis for loading criteria and removal rate constants.

5. Publications

Braeckevelt, M., Rokadia, H., Imfeld, G., Stelzer, N., Paschke, H., Kuschk, P., Kästner, M., Richnow, H.-H., Weber, S. (2007). Assessment of *in situ* biodegradation of monochlorobenzene in contaminated groundwater treated in a constructed wetland. *Environmental Pollution* 148: 428-437.

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Braeckevelt, M., Reiche, N., Trapp, S., Wiessner, A., Paschke, H., Kuschk, P., Kaestner, M. (2011). Chlorobenzenes removal efficiencies and removal processes in a pilot scale constructed wetland treating contaminated groundwater. *Ecological Engineering* 37: 903–913.

Braeckevelt, M., Kaestner, M., Kuschk, P. (2011). Removal of monochlorobenzene and perchloroethene in wetland rhizosphere model systems. *Engineering in Life Sciences* 11: 298-308.