

Auxiliary Substrates for Elimination of Trichloroethene, Monochlorobenzene, and Benzene in a Sequential Anaerobic–Aerobic GAC Biobarrier

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Sequential anaerobic-aerobic barrier is a novel concept for groundwater bioremediation. Trichloroethene (TCE), monochlorobenzene (MCB), and benzene (BZ) were used as model contaminants representing contaminant cocktails frequently found in the contaminated subsurface. The autochthonous microflora from a contaminated field was inoculated to eliminate model contaminants in a set of sequential anaerobic–aerobic granulated activated carbon (GAC) columns and batch studies. In the anaerobic column, the TCE was reductively dechlorinated through *cis*-dichloroethene (*cis*-DCE), vinyl chloride (VC), and ethene (ETH). Ethanol and sucrose as auxiliary substrates were added to donate electrons. In the second stage, MCB, BZ, and the lower chlorinated metabolites of TCE degradation, i.e. *cis*-Dichloroethene (*cis*DCE) and vinyl chloride (VC), were oxidatively degraded with addition of hydrogen peroxide and nitrate. This paper examines the influence of auxiliary substrates on the biological degradation of model pollutants. In the anaerobic barrier, the auxiliary substrates supply should be maintained low but stoichiometrically adequate for supporting reductive dechlorination. Supplying higher amount of auxiliary substrates provoked competitive reactions in anaerobic conditions, such as sulfate reduction and methanogenesis. If the auxiliary substrates are not utilized completely in the anaerobic phase, the remaining compounds flow into the aerobic phase. This led to unwanted conditions, i.e. oxidation of auxiliary substrates instead of pollutant elimination, and a higher consumption of electron acceptors. In the aerobic barrier, in particular, ethene proved to be a suitable auxiliary substrate for cometabolic degradation of *cis*DCE.

Keywords: Auxiliary substrates, electron acceptors, dechlorination, biobarrier, bioregeneration

INTRODUCTION

Trichloroethene (TCE), monochlorobenzene (MCB), and benzene (BZ) represent the most common contaminant cocktails found in contaminated subsurface water in various places (Böckle 1999, Nakano et al. 2000).

TCE is a nonreactive and widely used industrial solvent as a dry cleaning and metal-degreasing agent. TCE is probably the most common example of DNAPL, besides its parent compound perchloroethene (PCE) (Blatchley and Thompson 1999).

Monochlorobenzene is a colourless, flammable liquid with an aromatic, almond-like odor. MCB does not occur naturally in the environment and is widely used as a solvent for some pesticide formulations, as a degreaser, and as material for other chemical production. High levels of monochlorobenzene can damage the liver and kidneys and affect the brain (WHO 1993).

Benzene is used in the chemical industry for the production of styrene/ethylbenzene, cumene/phenol, and cyclohexane. Benzene is carcinogenic following both inhalation and ingestion. It causes leukemia and in high concentrations acute exposure primarily affects the central nervous system (WHO, 1993).

Reductive dechlorination of TCE has been reported to occur in anaerobic groundwater systems (DiStefano et al. 2001, Bradley 2003). During the reductive dechlorination process, TCE receives electron from hydrogen. Further dechlorination follows similar reaction to vinyl chloride and then to ethene. Molecular H₂ was demonstrated in the labs as well as in natural systems, including contaminated aquifers, to be the direct and initial electron donor for reductive dechlorination that was observed under sulfidogenic, methanogenic, or fermentative conditions (Freedman and Gossett 1989, Liss and Baker 1994, Maymó-Gatell et al. 1995, Middeldorp et al. 1999, Fennel, Gossett, and Zinder 1997). The supply of electron donor by various auxiliary substrates are listed in Table 1. Ethanol and sucrose were used in this research as AUXS due to economic and technical consideration. Freedman and Gossett (1989) showed the complete dechlorination to ethene under anaerobic conditions. The rate of dechlorination decrease with decreasing number of chlorine (Haston and McCarty 1999).

Besides the preferred reactions, other reactions might take place under anaerobic conditions due to the diversity of microorganisms and conditions that favor their growth. Hydrogenotrophic dechlorinators have to compete with other microbial groups that

Table 1. Auxiliary Substrates for Reductive Dechlorination Reactions

| Auxiliary Substrates | Target | Reference |
|---|---------------------------|------------------------------|
| Methane | Chloroethenes | Chang and Alvarez-Cohen 1996 |
| Methanol, ethanol, formate, lactate, acetate | TCE | Schöllhorn et al. 1997 |
| Toluene | <i>Cis</i> -DCE, VC | Schäfer and Bouwer 2000 |
| Methane | TCE | Eguchi et al. 2001 |
| Acetate | Chloroethenes | He et al. 2002 |
| Complex Donor: sugar, flour, molasses, milk, etc. | PCE, TCE, <i>cis</i> -DCE | DiStefano et al. 2001 |

utilize H_2 as their terminal electron acceptors, such as sulfate reduction. Other essential respirative pathways found in groundwater environment are methanogenic respiration and acetogenic respiration. Two steps proceed during methanogenesis, where organic electron donors and hydrogen are used as metabolic substrates to produce acetate, methane, carbon dioxide, and water. The first step in methanogenesis involve fermentation of an organic compound to form hydrogen and acetate (*acetogenesis*). The hydrogen and some of the produced acetate (CH_3COOH) are then used as electron donors for the formation of CH_4 (Wiedemeier 1999).

Metabolites of TCE, i.e. *cis*-DCE and VC, are more toxic than TCE itself. Therefore, complete dechlorination to ethene is considered necessary. Or, aerobic degradation need to be applied to further eliminate the metabolites, i.e. *cis*-DCE and VC. Under aerobic conditions, methanotrophic bacteria with nonspecific oxygenase could oxidize TCE to CO_2 without accumulation of toxic intermediates (papers cited in Bradley 2003). The process is characterized as aerobic cometabolisms (McCarty and Semprini 1994). The lower chlorinated metabolites, *cis*-DCE and VC, are also biodegradable under aerobic conditions. Cometabolic oxidation of *cis*-DCE and VC is catalyzed by several mono- and dioxygenase systems by supplying an alternate primary substrate. Glucose (Gao and Skeen 1999); methane, ethane, and ethene (Dolan and McCarty 1995; Koziollek, Bryniok, and Knackmass 1999; Freedman and Gossett 2001); propane, acetate, and yeast extract (Phelps *et al.*, 1991) have been used as auxiliary substrates for elimination of *cis*-DCE and VC under aerobic conditions.

Benzene is characterized by large negative resonance energy resulting in thermodynamic stability. Microbial degradation of BZ and related compounds require specific enzymatic systems to cleave the aromatic ring, which is necessary for the mineralization of the carbon. Figure 1 illustrates the common initial degradation pathway of BZ prior to further degradation, which

is also experienced by other aromatics (Baker and Herson 1994). Benzene is rapidly degraded in the presence of molecular oxygen (Nishino *et al.* 1992, Alvarez and Vogel 1995). Benzene can be degraded without auxiliary substrates.

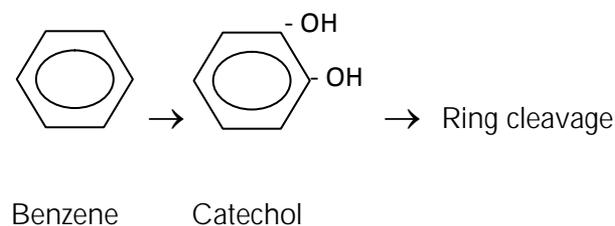


Figure 1. Common Preliminary Degradation Reactions of Benzene

Degradation of monochlorobenzene proceeds through the pathways for degradation of benzene as illustrated in Figure 1. MCB is first converted to the analogous chlorocatechol, followed by breaking the aromatic nucleus. The dechlorination of ring cleavage product comes subsequently (Baker and Herson 1994). MCB is degraded without auxiliary substrates. MCB is more rapidly degraded in the presence of molecular oxygen (Nishino *et al.* 1992, Alvarez and Vogel 1995). Terminal electron acceptors (TEA), such as oxygen and nitrate, are required to oxidize and transform the entering substrates into innocuous compounds. The use of nitrate as an alternative electron acceptor may offer advantages over oxygen in situations where bioremediation is to be promoted under denitrifying conditions, particularly for aromatic hydrocarbons (Liss and Baker 1994; some studies cited by Durant *et al.*, 1999). The solubility of nitrate is higher than of oxygen and thus will not be retarded and will diffuse better in the aqueous phase of the system. If one would minimize the use of oxygen, which means reducing the cost of oxygen pumping or hydrogen peroxide supply, denitrifying condition should be supported.

This paper examines the influence of controlled variables, i.e. auxiliary substrates and electron acceptors, on biological degradation of TCE, MCB, and BZ on sequential anaerobic-aerobic GAC barrier system.

MATERIALS AND METHODS

All the granular activated carbon used in the experiments were from Chemviron Carbon, Neu-Isenburg, Germany (FILTRASORB TL 8-30).

ENRICHMENT

Inoculums were obtained by means of membrane filtration (Polyethersulfonfilter, 0,45 μm) of contaminated groundwater area from Bitterfeld, Leipzig-Germany. The cake was put into three 500 ml Erlenmeyer flasks containing chloride-free medium [K_2HPO_4 , KH_2PO_4 , NH_4NO_3 , NaNO_3 , MgSO_4 , CaSO_4 , $\text{Fe}_2(\text{SO}_4)_3$, and trace elements]. A small volume of TCE in an open-disk was put into a closed chamber in such a way that the volatilized TCE will diffuse into the medium and act as the only carbon source. A similar procedure was applied for the MCB and Benzene in separate chambers. Measurement of Cl^- verified the activity of autochthonous bacteria.

Continuous Column Experiments

A laboratory scale (Figure 2) column set, with 2 vertical 0.164L columns (diameter 3.5cm and height 17cm) was filled with Filtrasorb GAC TL 830 (Chemviron Carbon, Belgium). The GAC was washed in demineralized water to remove fines, followed by drying at 105°C. A reference set was operated under sterile conditions. After the preloading period, bacterial mixed cultures capable of utilizing TCE, BZ, and MCB were injected (active column), while in the sterile column NaN_3 was added continuously to hinder the growth of microorganisms. During the loading period, the flowrate to GAC columns varied from 50 to 90 L/d. Two similar sets, but packed with pumice stone (PS), were build to examine the nonadsorptive operation. For the bioregeneration experiment, the flowrate (medium and substrate) to each GAC column was adjusted to approximately 4L per day. The pore volumes of the GAC columns were $\pm 57\%$.

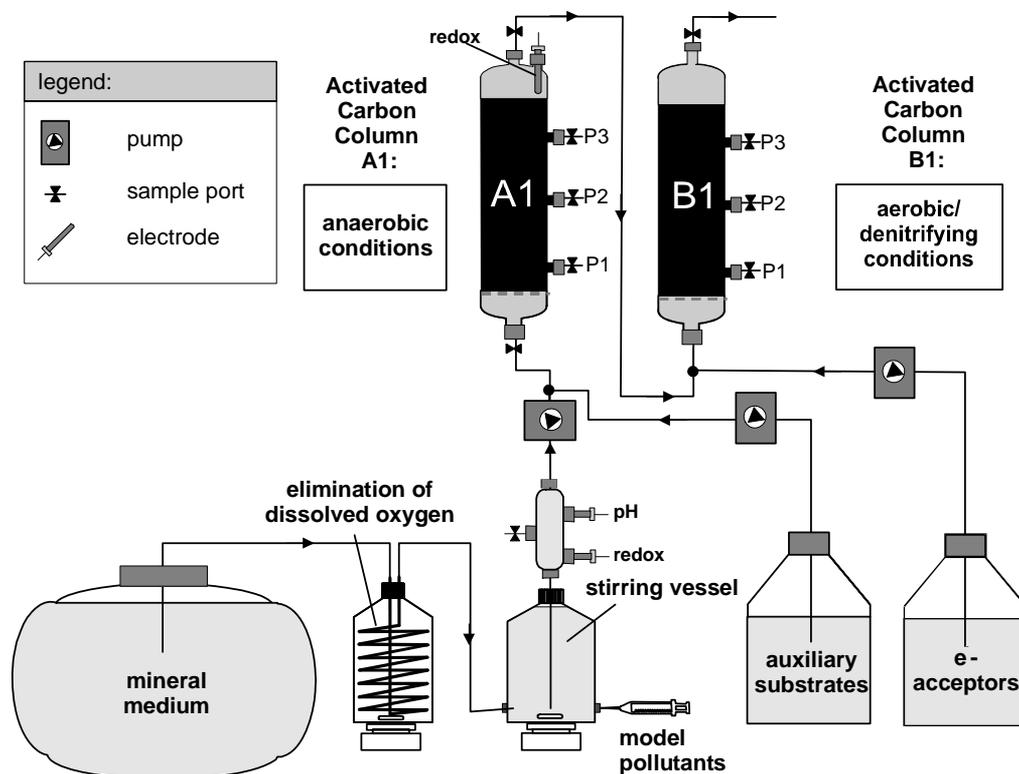


Figure 2. Sequential Anaerobic-Aerobic GAC Column Setup

ANALYTICAL METHOD

Analysis of hydrocarbons was performed by GC from Hewlett Packard (GC Series II 5890) equipped with a head-space sampler, flame ionization detector (FID), and electron capture detector (ECD). Separation was accomplished in a 50-m capillary column (PONA id 0.21 mm, methyl silicon film 0.5 μm thickness, Hewlett Packard). Sulphate and chloride ionic concentrations were determined with ion chromatograph (IC 2010I Dionex) with suppression system and conductivity detector. Measurement of redox potential and pH was done regularly with a multimeter (pH 91 WTW). Determination of O_2 was carried out with a dissolved O_2 meter (Oxi 330-WTW). H_2O_2 level was monitored with peroxide test strip (Merck).

RESULTS

ANAEROBIC PHASE

Dechlorination activity started after day 30 as chloride was detected in the effluent. Transformation of TCE into *cis*-DCE, VC and Ethene are shown in Figure 3. Formation of *cis*-DCE exceeded significantly the TCE influent concentration for about 40 days. Then the mixed cultures enriched from contaminated area transformed TCE into *cis*-DCE stoichiometrically. The chloride peak preceded the *cis*-DCE peak due to a moderate retention of *cis*-DCE on activated carbon (Figure 3A). This effect occurred again when the contaminant influent concentration was gradually reduced (between 600 and 800

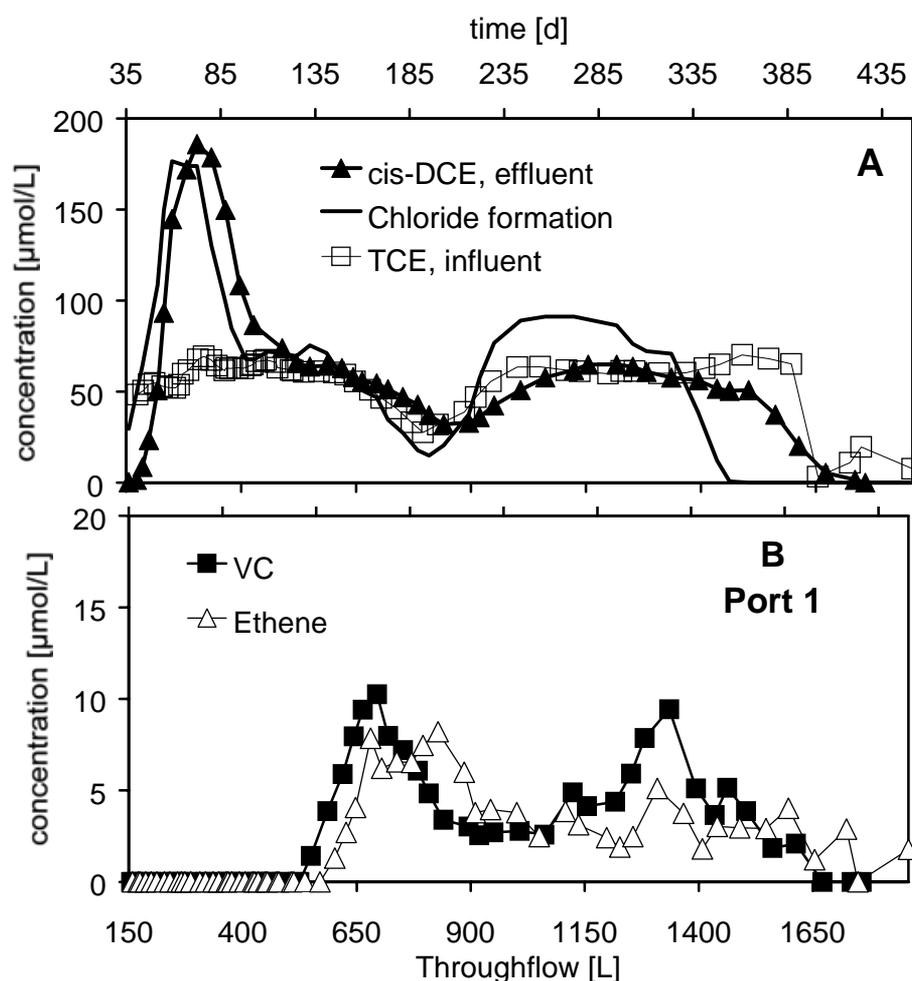


Figure 3. (A) TCE Influent, Formation of *cis*-DCE and Chloride, (B) VC and ETH in Port 1.

L throughflow) or increased (between 800 and 960 L throughflow), and when reductive dechlorination decreased due to a lack of auxiliary substrates (after 1340 L throughflow)

After throughflow 550 L further dechlorination to VC was observed in all port samples. About a half of the VC was transformed further into ETH in the Port 1 (Figure 3B). The low VC concentration compared to the *cis*-DCE shows that the biotransformation rate to VC is lower than that to *cis*-DCE. Transformation of *cis*-DCE to VE and of VC to ETH was observed at 5 to 50 % yield (product/reactant) especially as the AUXS was highly available.

Biodegradation rate curves of TCE and *cis*-DCE (figures 4A and B) were made based on concentration of reaction products. Volatile products could cause losses in the sampling steps. In overall anaerobic column the rates of TCE degradation (or *cis*-DCE production) fell into three zones, i.e. 1100, 300, and 125 $\mu\text{mol/d}$. The rate alternation was due to changes in both auxiliary supply and contaminant concentration. Although there were some probable losses during sampling and imbalance due to further

mineralization of ETH, the production of VC, or *cis*-DCE degradation, was observed at slower rate, i.e. at ca. 75 $\mu\text{mol/d}$, than the rate of TCE dechlorination to *cis*-DCE.

The redox potential in the effluent of anaerobic column altered considerably due to various reactions that took place in the column. The theoretical sulfate demand is 6 mol SO_4 /mol sucrose (4 mg SO_4 /mg $\text{DOC}_{\text{SUCR.}}$) and 1.5 mol SO_4 /mol ethanol (6 mg SO_4 /mg DOC_{EtOH}). Because the sucrose and EtOH were dosed in the same mass portion, the mean theoretical sulfate demand was ca. 5 mg SO_4 /mg $\text{DOC}_{\text{AUX.}}$. Stepwise auxiliary substrate reduction to around 16 mg/L and 9 mg/L DOC after 125 L and 425 L throughflow, respectively, rendered a reduction of sulfate utilization to around 7 mg/L. The reduction of auxiliary substrate to 5 mg/L DOC (between 700 and 800 L) as well as from 1050 L led again to declination of sulfate utilization. Finally, the sulfate reduction parallel went along the dechlorination activity with further reduction to 1 mg/L DOC after 1300 L to termination of sulfate reducing activity (Figure 5).

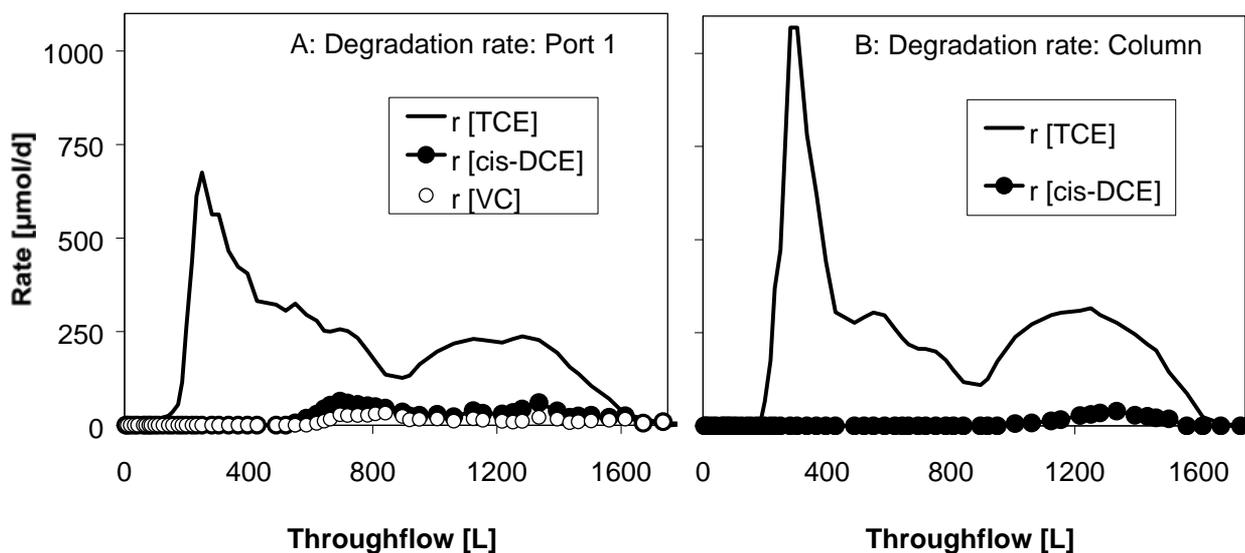


Figure 4. Biodegradation rate of TCE and *cis*-DCE in (A) Port 1 and (B) overall anaerobic column experiments.

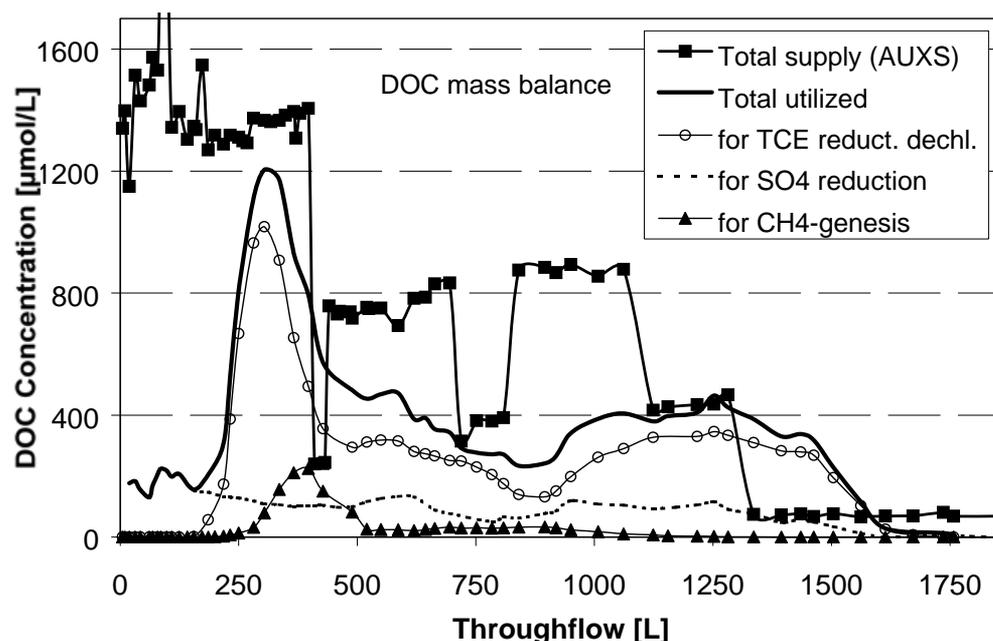


Figure 5. Mass balance of various reactions in anaerobic GAC column in terms of AUXS demands. AUXS supplied the DOC for those reactions

The methanogenesis activity significantly declined after 425L throughflow and completely ceased after 1100L throughflow. Notably, the reductive dechlorination processes were stable although the microbial community varied significantly.

Figure 5 displays various reactions in terms of their DOC needs during the experiments. Generally, the DOC was utilised mainly for TCE reductive dechlorination. Methanogenesis and sulfate reduction reactions increased when the AUXS was supplied excessively. The AUXS alteration was not instantly affecting the TCE reductive dechlorination, sulfate reduction or methanogenesis.

AEROBIC COLUMN

Aerobic column experiment in the sequential anaerobic–aerobic biobarrier was aimed to eliminate the contaminants that are persistent under anaerobic conditions, as well as to further decompose the metabolites from anaerobic processes. In these lab-scale

experiments, the pertinent compounds were the monochlorobenzene (MCB), benzene (BZ), and metabolites from TCE degradation. To favor the aerobic microorganism activities H_2O_2 and nitrate were added as electron acceptors. The emphasis of the aerobic column experiments was to examine the role of electron acceptors in the system, i.e. how the oxygen and nitrate control the biodegradation of both MCB and BZ. The auxiliary substrates role was also weight up on the overall sequential anaerobic-aerobic system.

Biodegradation rate of MCB is depicted in Figure 6. The rates fell into three zones, i.e. 750 (high), 400 (middle) and 100 (low) $\mu\text{mol/d}$. This alternation was due to both auxiliary substrate and peroxide supply changes.

No more BZ was detected in the effluent of active column at the end of the experiments. Metabolites from anaerobic column were *cis*-DCE, VC and ETH. These metabolites flowed into the second column, i.e aerobic phase. The same amount of *cis*-DCE and VC existed in the influent and effluent of aerobic column. It means that there was no metabolites elimination.

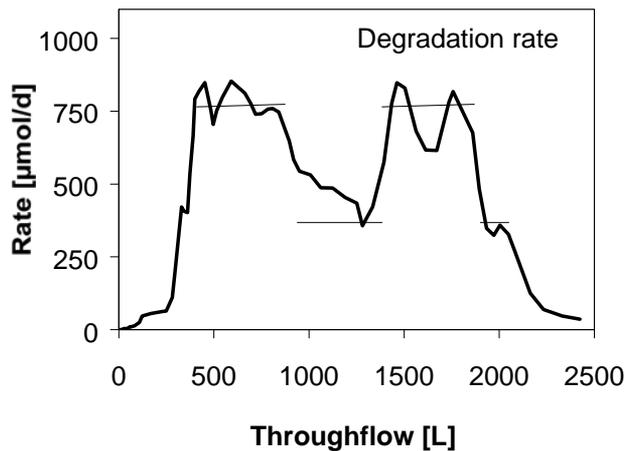


Figure 6. Biodegradation rate of monochlorobenzene

However, one could observe a slightly retardation of *cis*-DCE by GAC (data not shown). Oxidation reactions of *cis*-DCE and VC did not happen in the experiments although electron acceptors were sufficiently available.

In order to avoid toxic effects, the concentration of H_2O_2 was increased gradually from 10 mg/L up to 100 mg/L, or in the form of oxygen from 5 to 50 mg O_2 /L. Figure 8 shows that at low concentration of O_2 no biodegradation of MCB, *cis*-DCE, VC, nor BZ was observed.

Aerobic dechlorination started after increasing the O_2 level to 30 mg/L. Further increments to 50 mg/L H_2O_2 clearly improved dechlorination. The short decrease of hydrogen peroxide after 600 L throughflow resulted in an immediate drop of chloride formation (Figure 8). On the contrary, a rapid and temporary rise on H_2O_2 supply (140 mg/L) significantly enhanced the chloride release, indicating higher activity of MCB-degrading microorganisms.

Most of the oxygen was consumed in the first part of aerobic column and no hydrogen peroxide left in the effluent until about 480 days. But then gradually the oxidation zone rose up. As more oxygen available in port 2 more chloride formation was observed. The results then were confirmed with the extraction test.

Each mol DOC needs 1 mol oxygen to form CO_2 . Obviously, the remaining DOC value was much higher than the available oxygen in the initial phase. In that phase the need for electron acceptor was fulfilled through nitrate denitrification. Along with the AUXS stepwise reduction, the oxygen supply was gradually increased. Therefore, the oxygen became stoichiometrically sufficient for AUXS biodegradation for the rest time of the experiments.

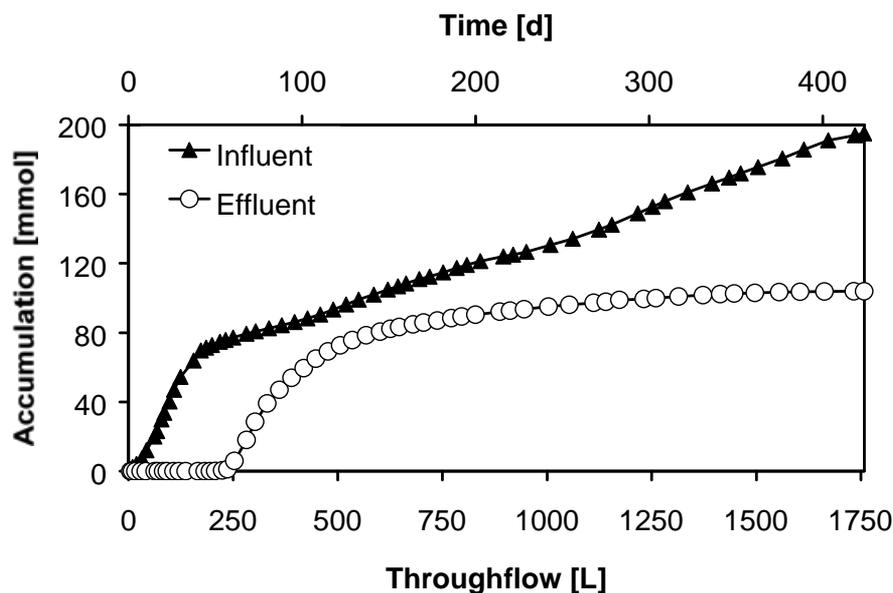


Figure 7. Elimination of benzene under aerobic column (B1) and (B2).

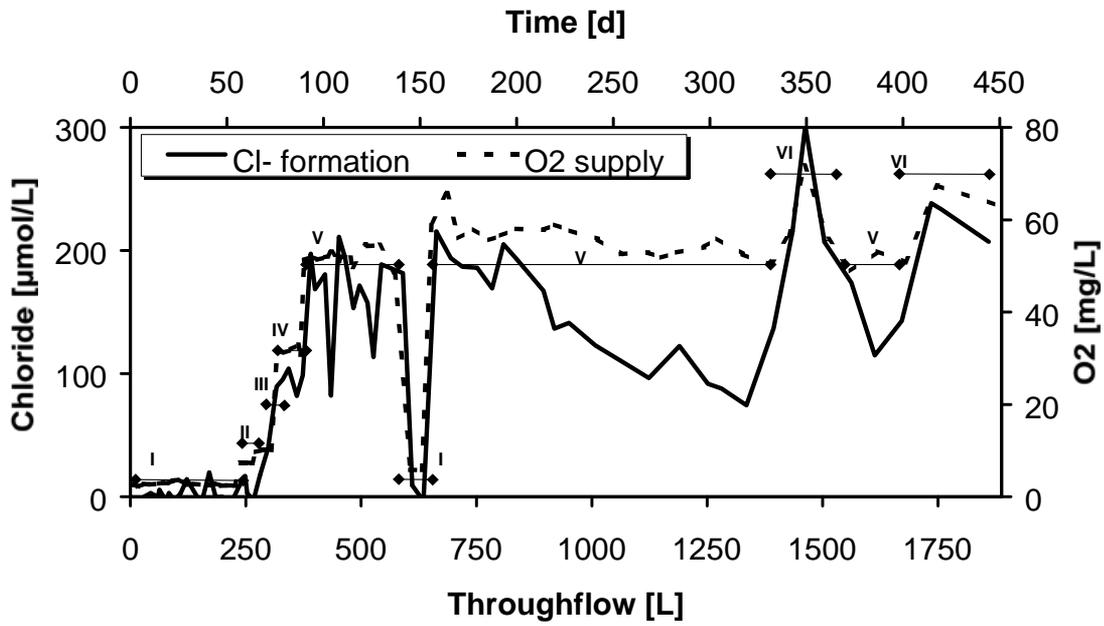


Figure 8. Oxygen supply and chloride formation on biodegradation of MCB in the activated carbon column. The notations I, II, III, IV, V, and VI indicate the level of 5, 15, 20, 30, 50, and 70 mg O₂/L.).

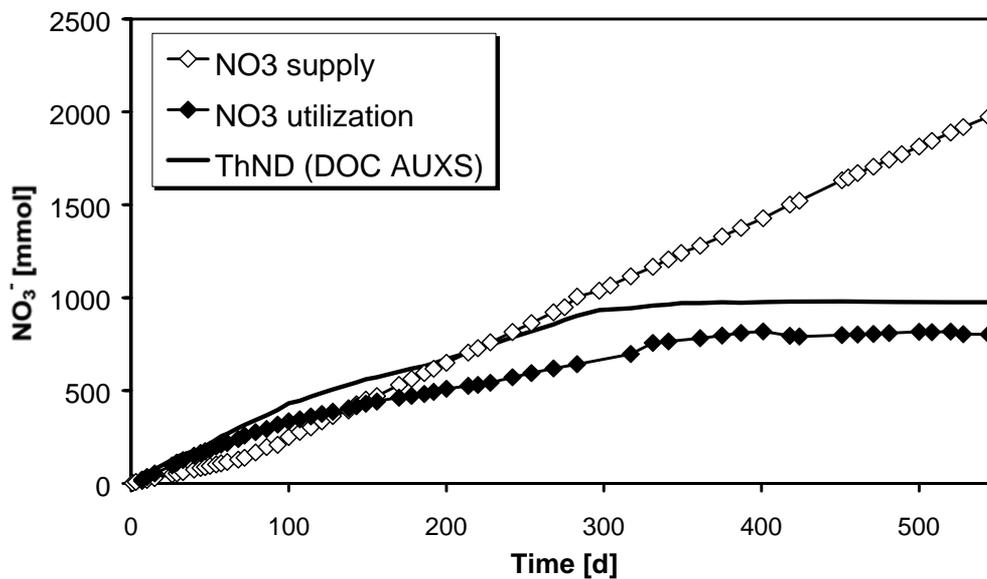


Figure 9. Nitrate balance (supply and utilization) and theoretical nitrate reduction demand for the DOC measured in the influent of aerobic column.

Nitrate has been delivered constantly through the experiments at concentration of 435 $\mu\text{mol/L}$ or 37 mg/L . Every mol of EtOH needs 2.4 mol nitrate (3.1 $\text{mg NO}_3/\text{mg DOC}_{\text{EtOH}}$) and every mol of sucrose needs 9.6 mol nitrate (41.3 $\text{mg NO}_3/\text{mg DOC}_{\text{Sucr.}}$). Thus, the correlation between nitrate reduction and AUXS supply could be represented as 22.2 $\text{mg NO}_3/\text{mg DOC}_{\text{AUXS}}$.

The theoretical nitrate demand (ThND) was calculated based on available DOC in the influent of In the initial phase of column experiments, the oxygen level was inadequate for the oxidation reactions of available organic compounds. Therefore, the denitrifying conditions played an important role in that phase of experiments. However, the nitrate supply showed no significant stimulation of the reduction of MCB.

Figure 9 shows that initially nitrate was transformed completely due to O_2 -limited conditions. With the increasing O_2 supply the nitrate demand decreased again. Thus, supply of nitrate was not completely utilized. On the other hand, although nitrate was supplied at high rate, oxygen was completely utilized. The level of O_2 rose up in the end of experiments as the influent of MCB and AUXS were low.

Through the step-by-step AUXS supply reduction and in the mean time increases in the oxygen supply, the dechlorination was accelerated again (1300 – 1870 L throughflow in Figure 8, shown by the higher Cl⁻ formation).

Additionally, the termination of pollutant supply and uncoupling of the aerobic from the anaerobic phase at 1870 L throughflow contributed to a further dechlorination of MCB. The upper part of the GAC column was regenerated too in this phase (A). Oxygen for dechlorination of MCB was inadequately available before. Finally, dechlorination completely bio-regenerated the aerobic GAC column.

DISCUSSION

Auxiliary substrates

Reductive dechlorination

Reductive dechlorination of TCE through *cis*-DCE to ETH requires hydrogen as electron

donor. To examine the effect of auxiliary substrate (AUXS) on reductive dechlorination, the concentrations of ethanol and sucrose were gradually modified. In the beginning of the experiments, high supply of AUXSs was introduced to the anaerobic column, i.e. 50 mg/L DOC . The reduction of AUXS supply to 16 mg/L DOC , then from 16 to 9 mg/L DOC and from 9 to 5 mg/L DOC as well as temporary decline showed no significant effects on the dechlorination processes. However, further reduction from 5 to 1 mg/L DOC after 320 days (1.300 L throughflow) lead to termination of the microbial dechlorination in the anaerobic column. To further understand the system, H_2 supply and demand estimation was made. Stoichiometric approach was made to calculate the AUXS demand for reductive dechlorination based on ethanol and sucrose sequential reactions to form H_2 . The calculation relied strongly upon assumption that the electron donor necessary for the halorespiration from TCE to ETH, i.e. H_2 , generated from biodegradation of AUXS to formate.

Theoretical H_2 production was 0.065 mg/mg sucrose and 0.174 mg/mg ethanol . These number correspond to approximately 0.15 $\text{mg/mg DOC}_{\text{Sucr.}}$ and 0.06 $\text{mg/mg DOC}_{\text{EtOH}}$. Since the ethanol and sucrose were always supplied in 1:1 mass ratio, then for the reduction of sucrose and ethanol to formate a metabolite H_2 production of approximately 0.105 $\text{mg H}_2/\text{mg DOC}_{\text{AUXS}}$ were estimated.

Stoichiometrically, the H_2 demand for the conversion of TCE to ETH is 3 $\text{mol H}_2/\text{mol TCE}$ or 0.04 $\text{mg H}_2/\text{mg TCE}$. Available H_2 that was produced from auxiliary substrates was utilised completely. Thus, the theoretical AUXS demand for TCE is 0.43 $\text{mg DOC}_{\text{AUXS}}/\text{mg TCE}$ (4.7 $\text{mmol DOC}_{\text{AUXS}}/\text{mmol TCE}$). Theoretical sucrose and ethanol demand for a complete dechlorination of 7.5 mg/L TCE is 3.3 $\text{mg DOC}_{\text{AUXS}}/\text{L}$. Thus, it is clear that the actual AUXS supply (50–5 mg/L) surpassed the theoretical AUXS demand (3.3 mg/L). Further reduction of AUXS from 5 to 1 mg DOC/L was stoichiometrically resulting in insufficient electron donor for a complete dechlorination or conversion of TCE. Even for

the dechlorination of TCE to *cis*-DCE the supply was inadequate.

In absence of common electron acceptor (oxygen and nitrate) like in our anaerobic experiments, the system could favour sulfate reducing bacteria and methanogenesis besides the reductive dechlorination. These reactions are disadvantageous for reductive dechlorination system because they compete to use auxiliary substrates (AUXS) in the anaerobic phase. Sulfate-reducing bacteria normally out-compete methanogenic bacteria (Wiedemeier *et al.*, 1999). Therefore, methane is rarely detected in groundwater where sulfate exists in high concentration. However, when hydrogen availability is high, such as in our initial phase of anaerobic experiments, one could observe methane building. The activity of methanogenic started after throughflow 250 L when the AUXS availability was high.

Reductive dechlorination of TCE to ethene is more favorable thermodynamically compared to sulfate reduction and methanogenesis. Therefore, supply of H₂ in the form of AUXS should be maintained at low or stoichiometrical demand of reductive dechlorination. This is because the competing reactions respond more sensitively to the alteration of auxiliary substrate supply, compared to reductive dechlorination. The H₂ threshold concentration for a halorespiration to occur is lower than that for methanogenesis or sulfate reduction (citation of several authors

by Löffler *et al.*, 1999; He *et al.*, 2002). When auxiliary substrate is reduced, the system suppress the methanogenic bacteria. This is because the methanogenesis is favored when hydrogen concentration is more than 5 nM, or excess of auxiliary substrate (Wischnak and Muller, 2000). Our mass balance clarified that the shares in AUXS through sulfate reduction or methanogenesis were considerably low. Most part of the AUXS was available for halorespiration. Halorespiration (TCE reduction through *cis*-DCE and VC to ETH) gains greater energy than through sulfate reduction or methanogenesis. Libelo *et al.* (1998) confirmed that supplying H₂ at low AUXS concentration will further degrade TCE to ETH. Lower AUXS supply could therefore maintain the dechlorination activity and suppress sulfate reduction and methanogenesis processes.

The effect of auxiliary substrate on the anaerobic processes in the column could also be shown by the redox potential. The redox potential dropped immediately to ca. -300 mV at the beginning of anaerobic experiments due to high availability of AUXS and high activity of inoculated microorganisms. This very low redox potential favoured the growth of methanogenesis bacteria). The low redox condition existed around 40 days of operation. Reduction of AUX allowed the halorespiration microorganisms to survive. As the reaction zone was dominated by the reductive dechlorination afterward, the redox potential was relatively stable at approximately -100 mV.

Table 4. 1 Theoretical demand and the actual supply of H₂ for dechlorination of TCE to *cis*-DCE, VC and ETH.

| Reaction | Theoretical H ₂ demand [mg/L] | Actual H ₂ supply* [mg/L] | | | | |
|------------------------|---|--------------------------------------|----|----|----|----|
| | | 50 | 16 | 9 | 5 | 1 |
| TCE to <i>cis</i> -DCE | 1.1 | ++ | ++ | ++ | ++ | + |
| TCE to VC | 2.2 | + | + | + | + | - |
| TCE to ETH | 3.3 | + | + | + | + | -- |

*supply of H₂ through AUXS, + = enough supply; -- = not enough supply.

Oxidative dechlorination

In our experiments, the autochthonous micro flora of the Bitterfeld site proved to be capable of degrading VC oxidatively regardless of the auxiliary substrate provided. In all samples taken after 48 d, VC was completely degraded. Degradation of VC without the addition of auxiliary substrates (flask no. 10) indicated the use of VC as sole carbon and energy source (Davis and Carpenter, 1990; Hartmans and DeBont, 1992; Verce *et al.*, 2000). However, in our batch tests the presence of organic material added with the inoculum and serving as auxiliary substrates could not be excluded.

In contrast to VC dechlorination, significant differences in *cis*-DCE dechlorination were observed. Addition of ethene resulted in a complete dechlorination within 48 d, thus confirming the positive results reported previously (Koziollek *et al.*, 1999; Freedman *et al.*, 2001). In the presence of other auxiliary substrates, *cis*-DCE was still detectable after 138 day. The auxiliary substrates monochlorobenzene, benzene, and toluene stimulated *cis*-DCE dechlorination significantly and were completely degraded. Addition of formate, sucrose, and ethanol also improved *cis*-DCE dechlorination. Sucrose and ethanol have the potential to stimulate further dechlorination of *cis*-DCE in the presence of oxygen. The oxidation of methane or ammonium was not observed. Corresponding to the low metabolic activity in the methane and ammonium amended flasks, oxidative dechlorination of *cis*-DCE was low. However, the lowest chloride formation was observed without addition of an auxiliary substrate.

Interestingly, the end product of reductive dechlorination, i.e. ethene, as well as the aromatic compounds i.e. monochlorobenzene, benzene and toluene, are suitable auxiliary substrates for oxidative dechlorination. This suggests the benefit natural attenuation in pollutant cocktails containing aromat.

Electron acceptors

The aerobic phase clearly showed the role of oxygen as electron acceptor (H_2O_2). All of

molecular oxygen was used to oxidise model contaminants and a part of auxiliary substrates. As oxygen was depleted, then part of the auxiliary substrate used nitrate as electron acceptors. This fact showed electron acceptor preferential.

Figure 10 shows that monochlorobenzene predominate the utilisation of electron acceptor (oxygen). Biodegradation of MCB and BZ with nitrate as terminal electron acceptor could not be observed. The high DOC in the influent at the initial phase was oxidised with nitrate. AUXS was supplied to favour reductive dechlorination purpose and used by various microorganisms in the anaerobic column. However, there was remaining AUXS entering the subsequent aerobic phase. The remaining AUXS can be measured by DOC method, which did not quantify volatile chlorinated carbons. Therefore, this DOC value indicated only DOC from the remaining AUXS.

As a source of molecular oxygen, hydrogen peroxide (H_2O_2) is more manageable for groundwater application compared to air injection. The liquid form of H_2O_2 is easier to handle than the gaseous form of oxygen. The H_2O_2 also decomposes rapidly soon after headspace is available. However, this rapid decomposition poses also unfavourable effect, i.e. performing poor distribution of oxygen. Furthermore, the formation of hydroxyl free radicals associated in the decomposition of peroxide may obstruct the active microbial population through oxidation of cellular components (Liss and Baker, 1994).

High aerobic levels of oxygen (more than 7 mg/L) is required to initiate biological elimination of some aromat. However, the use of nitrate as an alternative electron acceptor may offer advantages over oxygen in situations where bioremediation is to be promoted under denitrifying conditions, particularly for aromatic hydrocarbons (Liss and Baker, 1994; some studies cited by Durant *et al.*, 1999). The solubility of nitrate is higher than of oxygen and thus will not be retarded and will diffuse better in the aqueous phase of the system. If one would minimise the use of oxygen, which means reducing the cost of oxygen pumping or hydrogen peroxide supply, denitrifying condition should be supported.

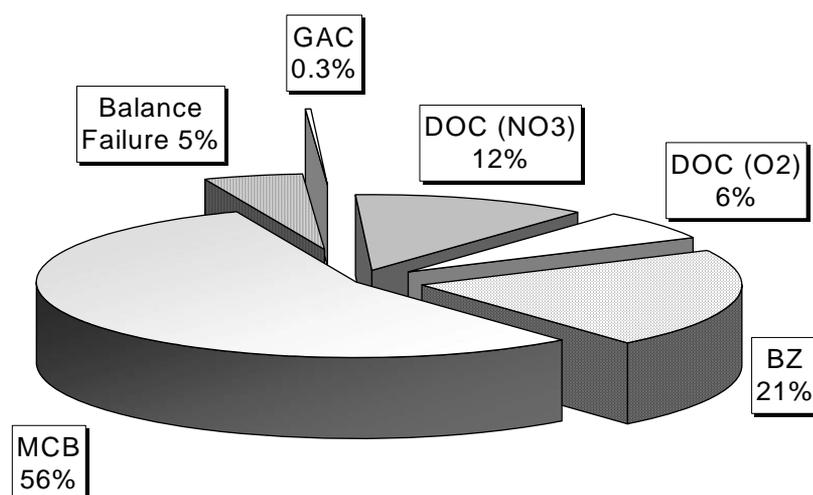


Figure 10. Electron acceptor utilisation balance in the aerobic column.

It was shown during aerobic column experiments, that initially the denitrifying condition was high when oxygen supply was in low level (below 30 mg O₂/L). Increasing O₂ level to 50 mg/L support the cometabolism of contaminants. But unfortunately, all contaminants did not use nitrate as electron acceptor preferentially. Only AUXS used the nitrate as electron acceptor. Durant et al. (1999) suggested not to exceed high oxygen saturation level (30 mg O₂/L) as this level could inhibit completely the denitrifying bacteria. Testa and Winegardner (2000) reported that at higher level of H₂O₂, i.e. more than 850 mg/L or 400 mg O₂/L, it can act as a biocide.

CONCLUSION

In the sequential anaerobic-aerobic systems for elimination of TCE, MCB and BZ one should maintain the supply of auxiliary substrates low but stoichiometrically adequate for supporting reductive dechlorination stoichiometrically. Supplying higher amount of auxiliary substrates provoked competitive reactions in anaerobic conditions, such as sulfate reduction and methanogenesis. If the auxiliary substrates do not utilised completely in the anaerobic phase, the remaining flow into the aerobic phase. This lead to unwanted conditions, ie. oxidation of auxiliary substrates instead of pollutant elimination.

One should supply electron acceptor in the aerobic phase to oxidise pollutant contaminants and metabolites from previous anaerobic phase. In our experiments molecular oxygen was still the preferred electron acceptor for the model contaminants used in this research. Molecular oxygen can be supplied with hydrogen peroxide. One should avoid supplying hydrogen peroxide in high amount to avoid the toxic effect of hydrogen peroxide to microorganisms. A maximum level of 30 mg/L did not show inhibition to denitrifying bacteria while supported the oxidation of model contaminants. More distributed electron acceptor supply along the column could be beneficial for the application in biobarrier.

ACKNOWLEDGEMENT

This study is a part of the SAFIRA research programme, funded by the German Ministry of Education and Research (BMBF No. 02WT9937/7).

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