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Benzene oxidation under sulfate-reducing conditions in columns simulating in situ conditions

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Abstract The oxidation of benzene under sulfate-reducing conditions was examined in column and batch experiments under close to in situ conditions. Mass balances and degradation rates for benzene oxidation were determined in four sand and four lava granules filled columns percolated with groundwater from an anoxic benzene-contaminated aquifer. The stoichiometry of oxidized benzene, produced hydrogen carbonate and reduced sulfate correlated well with the theoretical equation for mineralization of benzene with sulfate as electron acceptor. Mean retention times of water in four columns were determined using radon (^{222}Rn) as tracer. The retention times were used to calculate average benzene oxidation rates of 8–36 μM benzene day^{-1}. Benzene-degrading, sulfide-producing microcosms were successfully established from sand material of all sand filled columns, strongly indicating that the columns were colonized by anoxic benzene-degrading microorganisms. In general, these data indicate a high potential for Natural Attenuation of benzene under sulfate-reducing conditions at the field site Zeitz. In spite of this existing potential to degrade benzene with sulfate as electron acceptor, the benzene plume at the field site is much longer than expected if benzene would be degraded at the rates observed in the column experiment, indicating that benzene oxidation under sulfate-reducing conditions is limited in situ.

Keywords Benzene · Degradation · Natural attenuation · Sulfate-reducing conditions

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Introduction

Petroleum hydrocarbons are frequent soil and groundwater contaminants worldwide, mostly caused by leaking pipes and underground fuel tanks. Aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX), which make up a significant percentage of gasoline, are toxic (Dean 1985) and also mobile in the saturated and vadose zone of an aquifer, due to their relatively high water solubility and volatility, thus creating a potential health risk. Among the BTEX compounds, benzene is the most mobile, most toxic and has a carcinogenic potential (Aksoy 1985). Because of their environmental relevance, the fate of petroleum contaminated plumes has been extensively investigated in the past decade. There is evidence that petroleum hydrocarbon plumes are often short and stable (Wiedemeier et al. 1999); biodegradation is regarded as the main reason for the in situ disappearance of petroleum hydrocarbons. It has been known for a long time that all BTEX compounds are easily biodegraded under aerobic conditions by ubiquitous bacteria (for review see Van Agteren et al. 1998). However, due to the low water solubility and the rapid microbial consumption of oxygen, contaminant plumes can quickly become anoxic. As of 25 years ago, the anaerobic degradation of BTEX was considered as negligible (Atlas 1981). Now, it is widely accepted that all BTEX compounds can be mineralized without oxygen, under various electron acceptor conditions (Widdel and Rabus 2001; Chakraborty and Coates 2004). Benzene degradation was observed in laboratory enrichment cultures under methanogenic, nitrate-reducing, iron-reducing and sulfate-reducing conditions (Anderson and Lovley 2000; Burland and Edwards 1999; Edwards and Grbic-Galic 1992; Grbic-Galic and Vogel 1987; Kazumi et al. 1997; Lovley et al. 1995; Nales et al. 1998; Weiner et al. 1998). Even so, only two bacterial strains have been isolated so far which might be capable of metabolizing benzene without oxygen, using nitrate as electron acceptor (Coates et al. 2001), and the biochemical pathway of anaerobic benzene degradation remains to be fully elucidated (Caldwell and Suflita 2000; Chakraborty and Coates 2005; Ulrich et al. 2005). Furthermore, the experimental basis for understanding biodegradation kinetics of benzene in anoxic parts of a contaminated aquifer is still very limited. Benzene is considered as the most recalcitrant of all BTEX compounds under anoxic conditions: the majority of laboratory and field studies failed to demonstrate anaerobic benzene degradation (Aronson and Howard 1997; Johnson et al. 2003). The reasons for the resistance of benzene under anoxic conditions are largely unknown. Results from microcosm studies suggest that anaerobic benzene degraders are not ubiquitous in subsurface sediments (Kazumi et al. 1997; Nales et al. 1998; Phelps and Young 1999; Weiner and Lovley 1998). Due to the toxicity and mobility of benzene, a better understanding of the process of anaerobic benzene degradation, is crucial for evaluating Natural Attenuation (NA) or Enhanced Natural Attenuation (ENA) approaches as remediation strategies, given that these strategies are mainly based on indigenous bacteria transforming or mineralizing the contaminants.

Here, results are presented from a column experiment concerning benzene oxidation with sulfate as terminal electron acceptor. The contaminated site is located near Zeitz, Saxonia-Anhalt, Germany. The principal contaminants are BTEX, dominated by high benzene concentrations of up to 13 mM (Schirmer et al. 2006). The aquifer is anoxic within large areas of the plume, and sulfate is the main electron acceptor for microbiological oxidation reactions. Recently, evidence for in situ degradation of benzene and toluene was qualitatively demonstrated in Zeitz by means of stable carbon isotope fractionation (Vieth et al. 2005). The main objective of our study was to demonstrate and characterize anaerobic benzene degradation quantitatively under close to in situ conditions.

Materials and methods

Description of the field site

The contaminated site examined is located in the area of a former coal hydrogenation and benzene production plant near Zeitz (Saxonia-Anhalt, Germany). The contamination was caused by an

air raid in the Second World War and by several leakages, damages and accidents during the operation of the plant in the period between 1960 and 1990. Two aquifers are present, separated by a lignite and clay layer. Upper and lower aquifers are heterogeneous and hydrogeologically connected to some extent, due to discontinuities of the lignite–clay layer. Both aquifers are composed of river gravel and sand sediments, which contain more than 95% quartz. The general groundwater flow direction is to the North East. The groundwater of both upper and lower aquifer is heavily contaminated: benzene concentrations in the source zone were found up to 13 mM in the upper aquifer and up to 1.9 mM in the lower aquifer. Both aquifers are characterized by anoxic conditions. Sulfate, occurring in concentrations as high as 10 mM caused mainly by former lignite mining, is the main electron acceptor at the site (Schirmer et al. 2006). Sulfate concentrations in the lower aquifer downstream of the benzene source zone are in general 2.6 mM or higher (Gödeke et al. 2006). The geology and hydrogeology of the site are described in more detail elsewhere (Schirmer et al. 2006; Vieth et al. 2005). The site has been intensively investigated during recent years with respect to monitoring of NA processes (Gödeke et al. 2006; Schirmer et al. 2006).

Description of the column system

To investigate the bioremediation potential of the lower aquifer, an experimental plant was constructed by the UFZ in 2002, in which a total of eight columns can be percolated with anoxic groundwater from the site. The groundwater was extracted from the lower aquifer at a depth between 22 and 30 m below ground surface, by means of two extraction wells (EB1 and EB2) located down gradient the source of contamination, and injected directly into the columns. The columns are made of stainless steel; each column is 6 m long and 25 cm in diameter. All the columns were installed horizontally for technical reasons. Four columns were filled with sand, and four columns were filled with lava granules. The sand was taken from a nearby aerobic sand pit, showing a similar lithology as the sandy layers of the Zeitz aquifer sediment from where the groundwater was extracted (Gödeke et al. 2006), but sieved before use. It resulted a grain size between 2 and 3.15 mm, leading to an effective porosity of 0.43. The lava is a volcanic rock from the Eifel under-saturated with silicon oxide (SiO_2), having an effective porosity of 0.5. Porosities of sand and lava granules were measured in hydraulic porosity experiments. Assuming to have a greater specific surface area than the sand particles, the lava granules were expected to support the development of more bacteria per unit volume of the columns. The sand and lava filled columns are each connected in series, respectively, interconnected by 1-in. pipelines. Figure 1 shows a schematic picture of the flow system. Before starting the experiment described in this paper, the four columns filled with lava granules (columns 5–8) were equilibrated for 18 months with anoxic groundwater from the site at a flow rate of 3.5 m^3 h^{-1}. Columns 1–4 were filled with sand in autumn 2003. The flow rate was changed to 1 L h^{-1} in November 2003, marking the start of the experiment. The first samples for analytical parameter from the sand filled columns were taken 169 days after start. For comparison, data from the lava granules filled columns are shown for the same time period. The flow rate was controlled by rotary piston flow pumps (Ismatec, Switzerland). The columns were held at temperatures between 12 and 20°C (mean temperature 15°C), and the temperature of the pumped groundwater was about 13°C during the whole experimental time. The groundwater contained on average 300 μM benzene, ethylbenzene and xylenes in trace amounts (<1 μM), 4 mM sulfate, 300 μM sulfide, 120 μM ammonium, 5 μM *ortho*-phosphate, 150 μM potassium, 2.3 mM magnesium, 2.2 mM sodium and 6.1 mM calcium. The amount of sulfate in the groundwater was more than three times higher than necessary for the mineralization of benzene with sulfate as electron acceptor (see Eq. 1, in Sect. 'Results and discussion').

Determination of mean retention times in columns

Mean retention times of the water in four columns (columns 2, 4, 5 and 7; see Fig. 1) were determined

separately using radon (^{222}Rn) as tracer, at the following operation days: column 7, between operation days 371–373; column 5, between operation days 510–524; column 4, between operation days 535–536; column 2, between operation days 577–578. An analytical method developed at the UFZ (Freyer et al. 2003) allows the determination of the concentration of radon in water online with a time resolution of 1 min. Anoxic sulfidic groundwater of the Zeitz test field was filled to the rim in a 20-L canister under nitrogen atmosphere and spiked by injection syringe with a defined volume of ^{222}Rn, which had previously been emanated by ^{226}Ra in a closed, nitrogen filled vessel. The ^{222}Rn-labeled groundwater was subsequently discharged into a single column via a three-way valve and a dosage pump, using a dosage rate of 1 L h^{-1}. Six litres ^{222}Rn-labeled groundwater were applied in each column. At the column outlet, the ^{222}Rn-labeled groundwater was channeled via a three-way valve into a diffusion cell, where the ^{222}Rn was stripped out of the water into a nitrogen gas stream and analyzed online (Freyer et al. 2003). The mean retention time for each column was calculated using the time needed for half of the tracer mass to break through the column.

Chemical analyses

Samples for analyses of BTEX, sulfate, sulfide and bicarbonate were taken once per week at each column outflow after flow lengths of 6, 12, 18 and 24 m (Fig. 1). The chemical analyses were carried out following German standard analytical procedures [according to 'Deutsche Industrienorm' ('DIN'), German industrial standard]. BTEX concentrations were determined by headspace gas chromatography, following DIN 38407—F 9-1. *m*-Xylene and *p*-xylene could not be separated by the method used, and the concentrations were reported as a sum of both isomers. The detection limits were as follows: 0.009 μM for *m*-xylene/*p*-xylene and ethylbenzene, 0.011 μM for *o*-xylene and 0.014 μM for benzene and toluene. BTEX samples were taken and fixed by completely filling 100-mL glass flasks, previously prepared with 1 mL H_2SO_4 (99.5%), and subsequently plugged using glass stoppers. Sulfide concentrations were determined

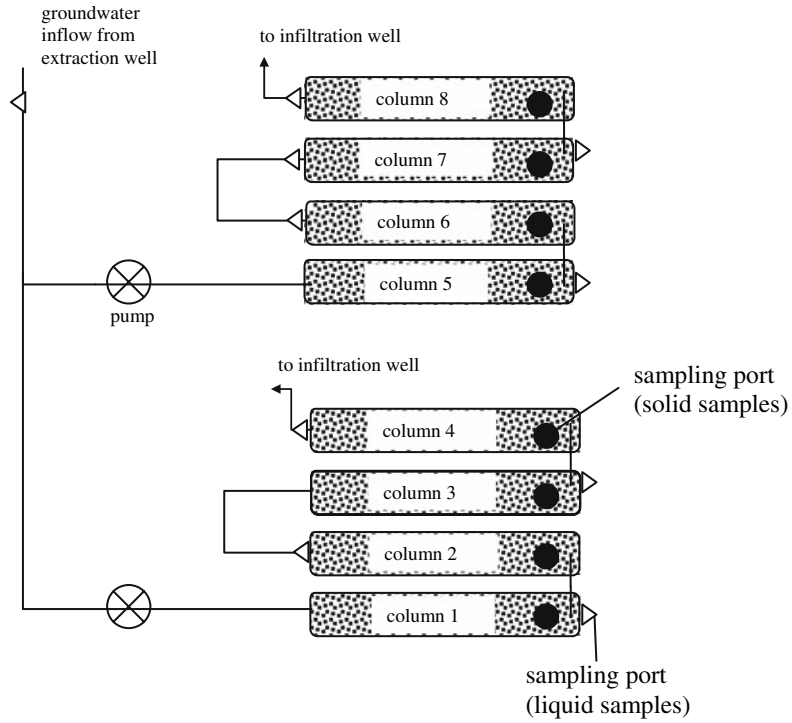


Fig. 1 Design of the column system used for determining stoichiometries and degradation rates for benzene degradation under sulfate-reducing conditions. Each column is 6 m long and 25 cm in diameter. Columns 1–4 were filled with sand and columns 5–8 with lava granules

according to DIN 38405 part 26. Samples for sulfide were taken in 100-mL graduate flasks previously filled with 10 mL zinc acetate dehydrate solution (20 g L^{-1}) for fixing sulfide immediately after sampling. The detection limit was 0.3 μM sulfide. Sulfate was analyzed according to DIN EN ISO 10304 part 1/2 (D 19/20). Samples for sulfate were taken in completely filled 250-mL polyethylene screw cap bottles prior to analyses. The detection limit was 5 μM sulfate. Bicarbonate was analyzed by titration using hydrochloric acid according to DIN 38409 H7. Samples for bicarbonate were taken in completely filled 250-mL polyethylene screw cap bottles prior to analyses. Ammonium was analyzed photometrically according to DIN 38406 part 5 (E 5). *Ortho*-phosphate was analyzed photometrically according to DIN EN 1189 (D 11). Nitrate and chloride were analyzed by ion chromatography according to DIN EN ISO 10304 part 1/2 (D 19/20). Dissolved oxygen, pH, redox and temperature were measured using appropriate electrodes (WTW, Weilheim, Germany).

Microbiological analyses

Sand material was removed from the top layer of each column using a sterile spoon, after opening a sample port (Fig. 1). The material was immediately transferred into sterile 250-mL bottles which were completely filled with anoxic sulfidic groundwater derived from the column inflow and closed with air-tight screw caps. The material was processed in the laboratory on the same day. All steps for preparing enrichment cultures were done in an anaerobic glove box [gas atmosphere—N_2:H_2 (95:5); Coy Laboratory Products Inc., USA]. Approximately 50 g sand material from a single column was redistributed into four 120-mL serum bottles (Glasgerätebau Ochs, Bovenden-Lenglern, Germany). Two bottles were filled with anoxic groundwater (column inflow) or anoxic mineral salt medium, respectively, leaving a headspace of 5 mL. The mineral salt medium contained the following stock ingredients (in g L^{-1}): $MgCl_2$, 0.2; KH_2PO_4, 0.5; NH_4Cl, 0.4; KCl, 0.4; and $CaCl_2$, 0.1. The medium was completed by adding the following amounts of stock solutions (L^{-1}): 30 mL $NaHCO_3$ (84 g L^{-1}, CO_2-saturated); 1 mL SL–10 trace element solution (Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ, medium number 320) chelated with EDTA (5.2 mg L^{-1} stock solution); 5 mL vitamin solution [containing, in mg L^{-1}: 4-aminobenzoate, 8; D(+)-biotine, 2; nicotinic acid, 20; Ca-D(+)-pantothenate, 10; pyridoxamine hydrochloride, 30; thiamine dichloride, 20]; 1 mL selenite-tungstate solution (containing, in mg L^{-1}: NaOH, 500; $Na_2SeO_3 \cdot 5H_2O$, 3; $Na_2WO_4 \cdot 2H_2O$, 4); and 20 mL Na_2SO_4 (142 g L^{-1}), yielding a final sulfate concentration of 20 mM. Finally, the medium was reduced with a few grains of sodium dithionite. All mentioned solutions were sterilized by filtration or autoclaving and flushed with N_2 to remove oxygen before use. The pH was adjusted to 7 with HCl (1 M) or NaOH (1 M). The sand material suspended in mineral salt medium was spiked with benzene from an anoxically prepared benzene stock solution (5 mM), for final concentrations of approximately 150 μM benzene. The bottles were closed with aluminum crimped Teflon-coated butyl septa (ESWE Analysentechnik, Gera, Germany). The resulting 16 enrichment cultures were incubated statically at room temperature in the dark and analyzed for benzene and sulfide concentration at regular intervals. The cultures were sampled inside the glove-box, by briefly opening the bottles and withdrawing liquid aliquots. No significant amounts of sulfide and benzene escaped out of the bottles by sampling this way, as verified by sterile controls. In the following, consumed benzene was replenished by adding 1–2 μL of pure benzene into the cultures, using sterile syringes (Hamilton, Reno, USA). Benzene-free biotic controls produced slight amounts of sulfide (<0.3 mM), probably due to consumption of hydrogen introduced into the microcosms from the atmosphere of the glove-box.

Results and discussion

Benzene degradation pattern in sand filled columns

Benzene was degraded in all four sand filled columns during an experimental time of 430 days, as shown in Fig. 2a. On an average, 120 μM

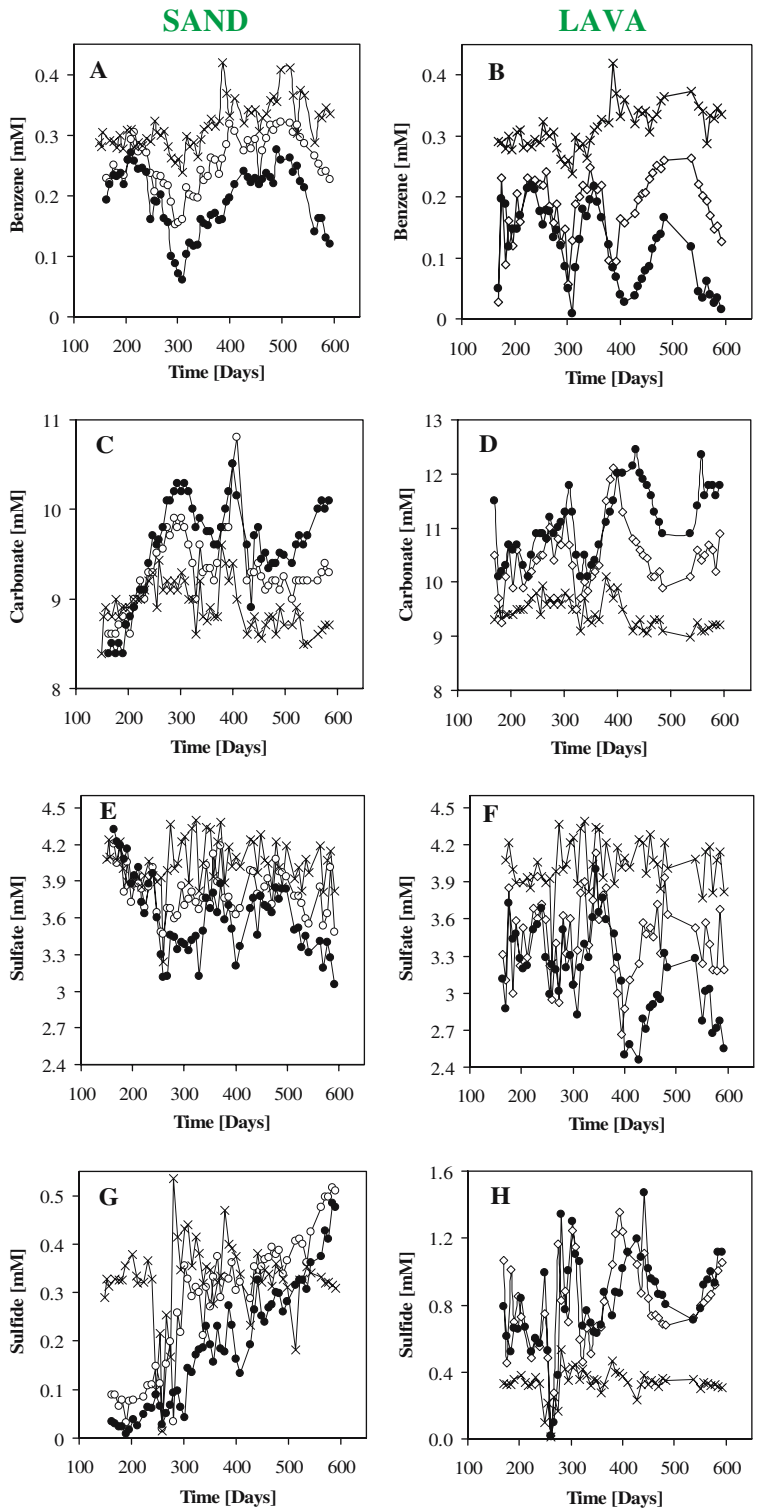


Fig. 2 Concentrations of benzene (a, b), carbonate (c, d), sulfate (e, f) and sulfide (g, h) monitored during the column experiment. Data in a, c, e and g (*left-sided diagrams*) show compound concentrations in the inflow and outlets of the sand filled columns 2 (after 12 m flow path) and 4 (after 24 m flow path), respectively. Data in b, d, f and h (*right-sided diagrams*) show compound concentrations in the inflow and outlets of lava granules filled columns 5 (after 6 m flow path) and 8 (after 24 m flow path). *Cross symbol* inflowing groundwater; *open diamond* after 6 m at outlet of column 5; *open circle* after 12 m at outlet of column 2; *filled circle* after 24 m at outlets of columns 4 and 8

benzene was degraded in total in 24 m flow path; the degradation capacity was not stable but fluctuated between 25 and 259 μM benzene (Fig. 2a). The average temperature inside the columns varied between 12°C during winter and up to 20°C during summer, but data on benzene degradation and temperature did not correlate (data not shown), thus ruling out an influence of the in situ temperature on the degradation process. Benzene degradation was accompanied by sulfate consumption (Fig. 2e) and carbonate production (Fig. 2c) as from 250 operation days. Sulfide disappeared to more than 90% yet after 200 operation days (Fig. 2g). A blackening of the sand material was observed in all columns, indicating that sulfide was mostly precipitated, perhaps as iron sulfides, after reaction with metal ions of the sand material. Sulfide concentrations began to increase slowly in all four sand filled columns during the course of the experiment (Fig. 2g), suggesting that the precipitation capacity of the sand material was decreasing with time.

Benzene degradation pattern in lava granules filled columns

Benzene degradation patterns in the column system filled with lava granules are shown in Fig. 2b. Between operation days 169–380, benzene was degraded almost solely during passage of the first 6 m (column 5), accompanied by carbonate production (Fig. 2d) and sulfate consumption (Fig. 2f). As from 380 operation days until 599 operation days, these degradation pattern were clearly observed in the remaining columns, too (Fig. 2b, d, f, h; for clarity only shown for effluent concentrations at the end of the column system). This could be due to changing flow conditions in the whole column system, or due to increasing microbial degradation in the last three columns. The degradation capacity for benzene of the whole lava granules filled column system fluctuated between 62 and 241 μM benzene (Fig. 2b), similar to the sand filled columns. Sulfide was produced mainly in the first lava granules filled column, in concentrations of up to 1.2 mM sulfide (Fig. 2h).

Calculation of electron balances

Benzene was the predominant carbon and electron source in the inflowing groundwater, thus allowing calculating stoichiometries for both lava granules and sand filled columns on the basis of the amounts of benzene oxidized, sulfate consumed and carbonate produced (Table 1). Sulfide data were not used due to the above mentioned precipitation of sulfide particularly inside the sand filled columns (Fig. 2g). For the sand filled columns, data beginning with operation day 248 were taken, since no correlation between benzene degradation and sulfate reduction or carbonate production was observed between 169 and around 250 operation days (Fig. 2a, c, e), indicating that benzene was at first degraded, but not mineralized with sulfate as terminal electron acceptor. For calculating electron and mass balances, the following theoretical equation for complete mineralization of benzene was taken:

$$C_6H_6 + 3\,H_2O + 3.75\,SO4^{2-} \rightarrow 6\,HCO_3^- + 1.87\,HS^- + 1.88\,H_2S + 0.37\,H^+ \quad (1)$$

In the sand filled columns, for the whole distance (24 m) and in the columns 3 and 4 (12–24 m flow distance), the amounts of reduced sulfate were in the same range as expected by stoichiometric degradation of benzene with sulfate as electron acceptor (Table 1). In columns 1 and 4, slightly more carbonate was produced than theoretically expected, in column 3 slightly less (Table 1), further indicating a mineralization of benzene in these columns. In the first column, however, less than half of the amount of needed sulfate for benzene mineralization was reduced; here, other electron acceptors, e.g., ferric iron provided by iron coatings on sand particles, might have taken up electrons. In comparison, in the second column twice the needed amount of sulfate for benzene mineralization was reduced, which can be explained by the oxidation of additional carbon compounds contaminating the sand material of column 2, indicated by the highest carbonate production of all sand filled columns (Table 1). Spatial heterogeneities, leading to slightly patchy mixing, might be an alternative reason for the

Table 1 Stoichiometries of oxidized benzene, reduced sulfate and produced carbonate in sand and lava granules filled columns, based on data recorded from operation days 169–599 (lava granules filled columns) or 248–599 (sand filled columns)

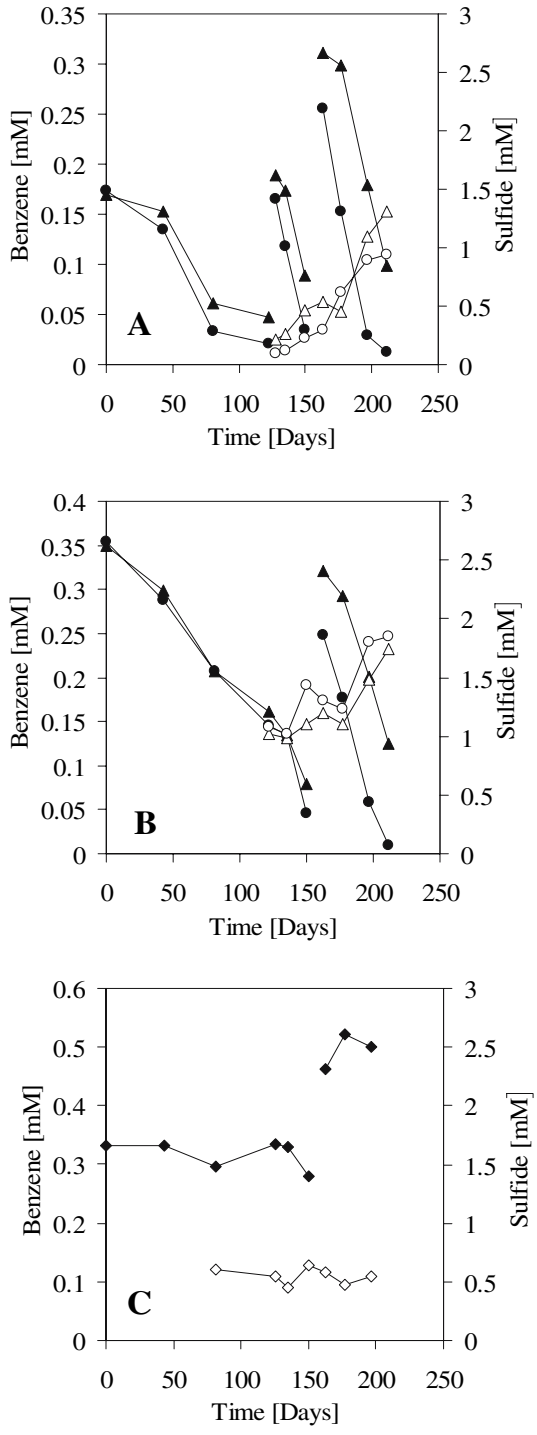
Column	Benzene oxidized (μM)	Sulfate reduced (μM)	Expected sulfate reduction (%)[a]	Hydrogen carbonate produced (μM)	Expected hydrogen carbonate production (%)[a]
Sand filled columns					
0–6 m (column 1)	32.5	43.3	35.5	266.6	136.7
6–12 m (column 2)	19	158.4	222.2	189.4	166.1
12–18 m (column 3)	35.6	112.4	84.2	188.9	88.4
18–24 m (column 4)	33.2	138.4	111.2	225.5	113.2
0–24 m (columns 1–4)	120.3	452.5	100.3	870.1	120.5
Lava filled columns					
0–6 m (column 5)	107.1	525.9	131	1,077.5	167.7
6–12 m (column 6)	37.2	150.8	108.1	368.2	165
12–18 m (column 7)	32.9	111	90	270.5	137
18–24 m (column 8)	27.5	145.8	141.4	246.6	149.5
0–24 m (columns 5–8)	204.7	933.5	121.6	1,962.8	159.8

[a] For a complete mineralization: $C_6H_6 + 3\ H_2O + 3.75\ SO_4^{2-} \rightarrow 6\ HCO_3^- + 1.87\ HS^- + 1.88\ H_2S + 0.37\ H^+$

non-stoichiometric sulfate consumption in columns 1 and 2. In the lava granules filled columns 6 and 7, the amount of sulfate reduced came close to the theoretical amount according to Eq. 1; in columns 5 and 8, more sulfate was reduced (Table 1). Carbonate production was generally higher than theoretically necessary for benzene mineralization. These data indicate that besides benzene, other carbon compounds, which possibly stem from the lava material itself, were oxidized with sulfate or other, not identified electron acceptors. Summarizing, the data shown in Table 1 strongly indicate that benzene was mineralized with sulfate as electron acceptor in both sand and lava granules filled columns.

Benzene degradation in microcosms

In order to verify that the columns were colonized by anaerobic benzene-degrading microorganisms, microcosms were prepared from sand material of all four sand filled columns and incubated under sulfate-reducing conditions with benzene as sole source of carbon and energy. Two different sets were prepared: one set using inflowing groundwater as liquid phase, and one set using anoxic mineral salt medium as liquid phase. In all microcosms, benzene was repeatedly degraded, and sulfide concentrations increased to concentrations of up to 2 mM. Figure 3 shows a high degree of benzene degradation and sulfide production in microcosm prepared from sand material of column 1. Benzene degradation and sulfide production patterns in microcosms prepared from sand material of the remaining columns were similar. Initial benzene degradation rates in the microcosms ranged between 1.1 and 2.2 μM day^{-1}, indicating that the four columns were colonized by benzene-degrading bacterial communities. Re-spiking with benzene led to increasing benzene degradation rates in the microcosms (2.4–14.1 μM day^{-1}). Rates did not differ between microcosms prepared with anoxic mineral salt medium or groundwater as liquid phase (Fig. 3), indicating that the groundwater/sand system provided the microorganisms with all necessary micro- and macro-nutrients for benzene metabolization. The rates in our study were comparable to initial benzene degradation rates under sulfate-reducing conditions in microcosms reported in the literature (Edwards and Grbic-Galic 1992; Nales et al. 1998), which showed a range between 0.4 and 3.2 μM benzene day^{-1}. No benzene was degraded in microcosms containing groundwater without sand material, neither in bottles prepared in parallel to the sand-containing microcosms (Fig. 3), nor in previously prepared bottles incubated for 929 days at 14°C (data not shown). We



◀**Fig. 3** Benzene degradation and sulfide production in microcosms prepared from sand material of column 1, suspended in mineral salt medium (a) or suspended in groundwater (b; inflow of the column system). c Groundwater (inflow of the column system) without sand material. Addition of benzene is indicated by *interrupted lines*. *Filled triangle* benzene (bottle 1); *filled circle* benzene (bottle 2); *filled diamond* benzene (groundwater without sand); *open triangle* sulfide (bottle 1); *open circle* sulfide (bottle 2); *open diamond* sulfide (groundwater without sand)

assume that the benzene-degrading microorganisms were closely associated to the sand particles, underlining the importance of attachment of bacterial communities responsible for the degradation of contaminants in subsurface systems. It has previously been shown that attached bacteria contribute 90–99.99% of the total bacteria community or biomass in pristine and contaminated aquifers (Alfreider et al. 1997; Griebler et al. 2002; Hazen et al. 1991; Kölbel-Boelke et al. 1988). The phylogenetical and physiological diversity of the anaerobic benzene-degrading bacterial community attached to the sand particles will be investigated in near future.

Determination of benzene degradation rates in columns

Single column retention times were determined for the sand filled columns 2 and 4, and for the lava granules filled columns 5 and 7 (see Fig. 1), using a radon tracer method (Freyer et al. 2003). The retention times in both sand filled columns were similar (around 24 h), resulting in groundwater flow velocities of approximately 6 m day^{-1}, a higher value compared flow velocities measured in the lower aquifer, which range between 0.33 and 3.1 m day^{-1} (Gödeke et al. 2006). On the basis of the retention times, average benzene degradation rates between 26.1 and 35.8 μM day^{-1} were calculated for the two sand filled columns, respectively (Table 2). Considering the similar amounts of oxidized benzene in all four sand filled columns (Fig. 2a; Table 1), the data suggest that the flow conditions in the sand filled columns were constant and comparable during the whole experimental time. In contrast, the retention times determined for the lava granules filled columns were higher than observed for the sand filled columns, and differed by a factor of 7 among themselves (Table 2), indicating rather inhomogeneous flow conditions in the lava

granules filled column system. Column 5 showed an extremely high retention time of 312 h, indicating that the pore volume inside column 5 was much higher compared to the other columns. Average benzene oxidation rates were lower in both lava granules filled columns compared to the sand filled columns (Table 2). The degradation rates determined in the sand filled columns are comparable to the highest benzene degradation rates observed under sulfate-reducing conditions in microcosms repeatedly fed with benzene (52 μM benzene day^{-1}, incubated at room temperature, Nales et al. 1998); to our knowledge, rates for benzene oxidation under sulfate-reducing conditions have yet not been published for a column system. Because temperature and also groundwater biogeochemistry and sediment lithology are very similar to the field conditions and the columns were running for almost 2 years, the observed average degradation rates for the sand filled columns reflect rates by which benzene might be degraded in situ. Indeed, the benzene plume of the lower aquifer at the Zeitz site has a minimum length of 1,000 m, as groundwater monitoring demonstrated; 800 m downstream from the source zone, which contains up to 2 mM benzene, benzene concentrations are as high as 150 μM (Schirmer et al. 2006; Gödeke et al. 2006). If benzene was degraded in situ in similar rates as observed in the column experiment, the plume would be considerably shorter; hence, the results indicate that in situ benzene degradation is limited. It was sometimes observed that anaerobic benzene degradation was inhibited in the presence of other organic compounds, e.g., toluene (Da Silva and Alvarez 2004; Cunningham et al. 2001) or ethanol (Ruiz-Aguilar et al. 2003). In our column study, the groundwater used contained benzene as major carbon and energy source, thus ruling out the possible preferential degradation of other carbon compounds. In parts of the Zeitz plume, especially downstream of the source zone in the lower aquifer, other aromatic hydrocarbons besides benzene are only detectable in small amounts (Gödeke et al. 2006, unpublished data), pointing out that such potential inhibition processes may not play a significant role in situ; on the other hand, sulfate was always monitored in the lower aquifer downstream of the benzene source in concentrations of more than 2.6 mM (Gödeke et al. 2006), suggesting that an inhibition of anaerobic benzene oxidation by insufficient sulfate concentrations is not likely within the lower aquifer. Additionally, sulfide in concentrations up to 1 mM was always detected in monitoring wells of the lower aquifer, indicating prevailing sulfidic conditions. An explanation for lower degradation rates in the field could be the limited simultaneous bioavailability of sulfate and benzene for the microbial community, caused by a limited transverse mixing of benzene (electron donor) and sulfate (electron acceptor) in the field. The transport of compounds might be disturbed or slowed down by aquifer heterogeneities, which have been observed in the lower aquifer of the test site (Schirmer et al. 2006; Gödeke et al. 2006). Slow transverse mixing was found to control degradation rates in many cases (e.g., Thornton et al. 2001; Thullner et al. 2002).

Table 2 Mean retention times and derivated mean benzene oxidation rates determined for columns 2, 4, 5 and 7

	Sand filled columns		Lava granules filled columns	
	Column 2 (6–12 m)	Column 4 (18–24 m)	Column 5 (0–6 m)	Column 7 (12–18 m)
Retention time (h)[a]	23.2	21.9	312	44
Benzene oxidized (μM)[b]	19	33.2	107.1	32.9
Mean benzene oxidation rate (μM benzene day^{-1})	26.1	35.8	8.2	18

[a] Using radon as tracer (see Sect. 'Materials and methods')

[b] Mean value of the column influent and effluent benzene concentration data recorded between operation days 169–599 (for the lava granules filled columns) or 249–599 (for the sand filled columns) (see also Fig. 2; Table 1)

Conclusions

The degradation of benzene under sulfate-reducing conditions was monitored in a long-term column experiment under close to in situ conditions. Stoichiometric calculations indicate that benzene was mineralized with sulfate as electron acceptor. Coarse sand, used as column filling material, was colonized by anaerobic benzene-degrading microorganisms, as shown by an accompanying microcosms study. The results point out that active anaerobic benzene-degrading microorganisms might be attached in situ on surfaces. The study suggests a high potential for NA of benzene under sulfate-reducing conditions at the field site Zeitz. Indeed, the benzene plume at the site is much longer than expected if benzene would be degraded in rates observed in the column system, indicating that in situ degradation of benzene is limited.

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