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Bioremediation of a diesel fuel contaminated aquifer: simulation studies in laboratory aquifer columns

A. Hess, P. Höhener *, D. Hunkeler, J. Zeyer

Swiss Federal Institute of Technology (ETH), Institute of Terrestrial Ecology, Soil Biology, Grabenstr. 3, CH-8952 Schlieren, Switzerland

Abstract

The in situ bioremediation of aquifers contaminated with petroleum hydrocarbons is commonly based on the infiltration of groundwater supplemented with oxidants (e.g., O_2, NO_3^-) and nutrients (e.g., NH_4^+, PO_4^{3-}). These additions stimulate the microbial activity in the aquifer and several field studies describing the resulting processes have been published. However, due to the heterogeneity of the subsurface and due to the limited number of observation wells usually available, these field data do not offer a sufficient spatial and temporal resolution. In this study, flow-through columns of 47-cm length equipped with 17 sampling ports were filled with homogeneously contaminated aquifer material from a diesel fuel contaminated in situ bioremediation site. The columns were operated over 96 days at 12°C with artificial groundwater supplemented with O_2, NO_3^- and PO_4^{3-}. Concentration profiles of O_2, NO_3^-, NO_2^-, dissolved inorganic and organic carbon (DIC and DOC, respectively), protein, microbial cells and total residual hydrocarbons were measured. Within the first 12 cm, corresponding to a mean groundwater residence time of < 3.6 h, a steep O_2 decrease from 4.6 to < 0.3 mg l^{-1}, denitrification, a production of DIC and DOC, high microbial cell numbers and a high removal of hydrocarbons were observed. Within a distance of 24 to 40.5 cm from the infiltration, O_2 was below 0.1 mg l^{-1} and a denitrifying activity was found. In the presence and in the absence of O_2, *n*-alkanes were preferentially degraded compared to branched alkanes. The results demonstrate that: (1) infiltration of aerobic groundwater into columns filled with aquifer material contaminated with hydrocarbons leads to a rapid depletion of O_2; (2) O_2 and NO_3^- can serve as oxidants for the mineralization of hydrocarbons; and (3) the modelling of redox processes in aquifers has to consider denitrifying activity in presence of O_2.

* Corresponding author.

1. Introduction

The contamination of soils and aquifers with petroleum hydrocarbons is a major environmental problem. Spilled hydrocarbons are transported by gravity through the unsaturated zone and are usually floating on groundwater, from where they disperse horizontally along the groundwater gradient and vertically within the capillary fringe. Removal of floating hydrocarbons by pumping is always incomplete. Due to capillary forces and sorption processes, residual hydrocarbons are trapped in pores and act as a continuing source of potentially toxic organic contaminants.

In situ bioremediation has been increasingly considered as a novel technology to clean up residual hydrocarbon contaminations in soils and aquifers. In a number of field studies, indigenous microbial populations were stimulated by infiltrating groundwater supplemented with O_2, NO_3^- and selected nutrients (Battermann, 1983; Hutchins and Wilson, 1991; Gersberg et al., 1993; Kämpfer et al., 1993; Downs et al., 1994; Nelson et al., 1994; Hunkeler et al., 1995). To mineralize the hydrocarbons, microorganisms require electron acceptors (e.g., O_2, NO_3^-, $Mn^{(III, IV)}$, $Fe^{(III)}$, SO_4^{2-}, CO_2). For thermodynamic reasons, O_2 is preferentially consumed, and since the rate of O_2 consumption often exceeds the rate of O_2 supply, the subsurface turns anoxic. Attempts to increase the supply rate of O_2 are hampered by the poor aqueous solubility of O_2. A supply of the highly soluble H_2O_2 to produce O_2 by catalytic decomposition can lead to toxic effects, to an oversaturation with O_2 and to a clogging of the aquifer by O_2 bubbles (Spain et al., 1989; Hinchee et al., 1991; Pardieck et al., 1992). Although petroleum hydrocarbons are most readily degraded under aerobic conditions (Atlas and Bartha, 1992), it has been demonstrated that a complete mineralization of a number of hydrocarbons also occurs under denitrifying (Zeyer et al., 1986), iron-reducing (Lovley and Lonergan, 1990), sulfate-reducing (Aeckersberg et al., 1991) and methanogenic conditions (Edwards and Grbic-Galic, 1994). This opens the attractive opportunity to supply, for example, NO_3^- to the contaminated zone. This oxidant has a similar redox potential as molecular oxygen (Stumm and Morgan, 1981), it has a high solubility, it is not adsorbed by the solid matrix of the aquifer, it can be converted by many microorganisms, it can simultaneously serve as oxidant and nitrogen source and it is cheap. Infiltration of NO_3^-, however, may not be tolerated by the authorities since NO_3^- is a groundwater contaminant itself and may lead to the production of other groundwater contaminants like NO_2^-, N_2O and NH_4^+.

Due to the limited access to the subsurface, studies on bioremediation processes are difficult in field systems. Therefore, a number of authors tried to simulate the processes in laboratory aquifer columns. However, previously published laboratory aquifer column studies did not deal with residual hydrocarbons, but with easily degradable substrates such as lactate or acetate as carbon and energy source for microbial growth (Cobb and Bouwer, 1991; von Gunten and Zobrist, 1992, 1993). Moreover, localization of aerobic and denitrifying processes were not reported in studies where aromatic hydrocarbons were supplied (Kuhn et al., 1985; Kuhn et al., 1988; Chen et al., 1992).

Recently, we reported on a field study of an in situ bioremediation of a diesel fuel contaminated aquifer in Menziken, Switzerland (Hunkeler et al., 1995). Groundwater supplemented with O_2 (320 μM) and NO_3^- (1020 μM) as electron acceptors and

$NH_4H_2PO_4$ (75–125 μM) as nutrients was infiltrated and the oxidants were almost completely consumed at a monitoring well located 20 m downstream of the infiltration. A tracer experiment with chloride, bromide and fluorescein revealed that this distance corresponded to a mean groundwater residence time of 22 h. No wells were available in between and therefore the localization of hydrocarbon mineralization and oxidant consumption processes could not be achieved. Furthermore, accurate carbon and nitrogen mass balances and electron balances could not be established, since the field site is an open system in which certain mass fluxes (e.g. transport of soil gases) can only be estimated. The remediation processes at Menziken were simulated in laboratory aquifer columns equipped with many sampling ports and filled with aquifer material from the field site. The aim of this project was: (1) to study the localization of oxidant consumption, hydrocarbon mineralization and growth of microorganisms along the infiltration path; and (2) to establish carbon, nitrogen and electron balances of the mineralization process.

2. Materials and methods

2.1. Experimental set-up

Two laboratory aquifer columns were constructed of Plexiglas cylinders (length 47 cm, inner diameter 5 cm, wall thickness 0.5 cm). A scheme of the set-up is shown in Fig. 1A. At the bottom, a Plexiglas sieve plate covered with a nylon net (mesh 70 μm) was placed 2 cm above the column inlet. The outlet of the columns was made of stainless-steel tubing placed in a rubber stopper. Sampling ports were made of stainless-steel hypodermic needles (ID 1.5 mm, Unimed, Geneva, Switzerland) placed into GC septa (Injection Rubber Plugs Part. No. 201-35584, Shimadzu, Japan) that were fitted into 4.8 mm borings. Sampling ports were positioned starting at 1.5 cm from the sieve plate with a vertical distance of 1.5 cm (first six ports) and 3 cm, respectively (Fig. 1A). Prior to column operation, an experiment was conducted by filling the column with stagnant anoxic water, and no O_2 permeation was observed over 3 days. Aquifer material from the contaminated site at Menziken was excavated, sieved (< 4.5 mm) and analyzed as reported by Bregnard et al. (1996). Dried material consisted of $< 1\%$ silt and clay (< 0.02 mm), 13% fine sand (0.02–0.2 mm) and 86% coarse sand (0.2–4.5 mm). The material having a field capacity of $\sim 13.5\%$ water by volume contained weathered hydrocarbons (825 mg kg^{-1}). It was additionally spiked with fresh diesel fuel (Esso, Switzerland) to yield a total hydrocarbon concentration of 1100 mg kg^{-1}. The material was homogenized by vigorous stirring and filled into the columns. After allowing the material to settle within the first 2 days, a total porosity of 0.14 was calculated from the volume and the weight of the column fillings. The columns were operated with an artificial groundwater at 12 ± 0.5°C (in situ temperature) in upright position under upflow conditions (flow rates of 8.8 ± 0.5 ml $h^{-1)}$. The artificial groundwater consisted of 6000 μM $NaHCO_3$, 1610 μM $NaNO_3$, 100 μM $MgSO_4 \cdot 7H_2O$ in distilled water and it was autoclaved at 120°C. Sterile solutions of $CaCl_2 \cdot 2H_2O$ (250 μM final conc.), HCl (1000 μM) and KH_2PO_4 (10 μM) were added after

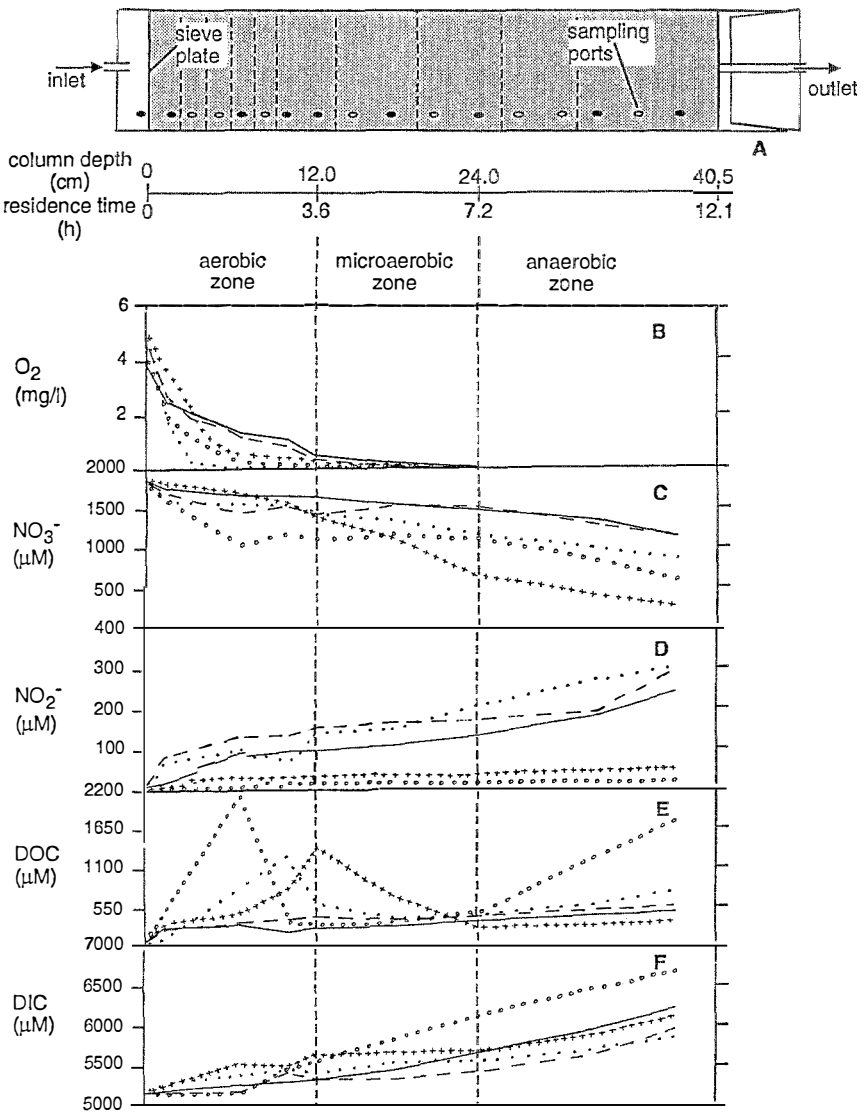

Fig. 1. Schematic of column set-up and sampling ports (A). *Solid circles* indicate sampling ports used for chemical measurements. O_2 was measured in all of the first 8 ports. Changes over time of concentration profiles of O_2 (B), NO_3^- (C), NO_2^- (D), DOC (E) and DIC (F) along the column. The profiles shown are a representative selection out of 8–11 profiles measured over 96 days. The profiles were measured on the following days: (B) · · · · · = day 12, ∘ ∘ ∘ = day 18, + + + + + = day 29, — — — = day 60, ———— = day 82; (C)–(F) · · · · · · = day 15, ∘ ∘ ∘ = day 19, + + + + + = day 27, — — — = day 62, ———— = day 90.

autoclaving. The artificial groundwater contained 4.4 ± 0.6 mg l^{-1} O_2 at the sampling port located in front of the bottom sieve plate of the columns (Fig. 1A). Two identical columns were operated over 96 days. During that time, a total of 8–11 concentration

profiles were measured in intervals of 4–14 days, and comparable concentration profiles were observed in both columns. Transport parameters were characterized by adding 1 m*M* NaBr to the artificial groundwater at day 22 and 55 of operation. Breakthrough curves were obtained by measuring Br^- and analyzed according to Brenner (1962). They yielded effective porosities of 0.080 ± 0.002, average groundwater residence times of 12.1 ± 0.2 h in the columns and longitudinal dispersivities of 4 ± 1.3 cm. At day 96, the aquifer material was extruded from one column and sliced into 10 samples, as shown in Fig. 1A (dashed lines). All data including profiles of dissolved parameters shown in the results section were obtained from this column.

2.2. Chemicals and analytical methods

All chemicals were purchased from Fluka (Buchs, Switzerland) or Merck ABS (Dietikon, Switzerland) at the highest purity available and were used as received. Filtrations were done using 0.2 μm Millipore PTFE filters (Millipore, Volketswil, Switzerland). Microbial cells were removed from aquifer solids by vortexing 1 g of aquifer material in 10 ml filtered artificial groundwater containing 0.1% $Na_4P_2O_7$ (Balkwill et al., 1988). The aquifer solids were allowed to settle down for 2 min, and then, the cells in the supernatant were fixed, stained with DAPI and directly counted with a Zeiss Axioplan epifluorescence microscope (Zeiss, Oberkochen, Germany) as reported by Hahn et al. (1992). Protein concentrations were determined by the Bradford method (Sedmak and Grossberg, 1977) with bovine serum albumin as standard. Unless stated otherwise, groundwater samples from the columns were filtered. For the measurement of Ca^{2+}, dissolved iron, dissolved manganese and dissolved organic carbon (DOC), samples were acidified with 0.1% distilled HNO_3. O_2 concentrations were measured in unfiltered samples by the azide modification of the Winkler method (APHA, 1989) adapted to sample volumes of 4 ml. All procedures for O_2 measurement, including the transfer from the sampling ports of the columns into vials, were carried out under a stream of N_2 gas preventing contamination with O_2 from air. Concentrations of NO_3^-, SO_4^{2-}, Cl^- and Br^- were determined by a Dionex DX-100 ion chromatograph equipped with an IonPac AS4A-SC column using an eluent of 1.8 m*M* Na_2CO_3 and 1.7 m*M* $NaHCO_3$. Ca^{2+} was determined by a Dionex DX-100 ion chromatograph equipped with an IonPac AS12A-SC column using an eluent of 20 m*M* CH_4SO_3. NH_4^+, NO_2^- and HS^- were measured colorimetrically (APHA, 1989) and dissolved iron and manganese were quantified by atomic absorption spectroscopy according to standard methods (APHA, 1989). DOC was measured in a Horiba TOC analyzer by infrared detection of CO_2 after catalytic oxidation at 900°C. Before measurement, the acidified samples were vigorously bubbled with N_2 to ensure complete stripping of CO_2. Dissolved N_2O and CH_4 were quantified using the head space technique described by Bossard et al. (1981). The partial pressures of the gases were determined by gas chromatography (Carlo Erba Model 8000, Rodano, Italy) on a HayeSep D column (length 1.8 m, inner diameter 3.2 mm; Alltech Inc., Deerfield, Illinois, U.S.A.) at 70°C with N_2 as carrier (flow 19 ml min^{-1}) and a Carlo Erba thermal conductivity detector (220°C). Concentrations of dissolved gases were calculated from Henry's law and the following Henry constants corrected for 12°C: N_2O: 0.0374 *M* atm^{-1} (Weiss and Price,

1980) and CH_4: 0.00177 M atm^{-1} (Yamamoto et al., 1976). Alkalinity was measured by potentiometric titration using Gran plots for graphical determination of the end point (Almgren et al., 1983). pH was measured in unfiltered and unstirred samples with a pH electrode. Dissolved inorganic carbon (DIC = $H_2CO_3 + HCO_3^- + CO_3^{2-}$) was calculated from alkalinity and pH with equations and constants taken from Stumm and Morgan (1981). For stable carbon isotope analysis, 150 ml of sample were collected under N_2 in a flask containing 5 ml of a CO_2-free solution of NaOH (1.5 M) and DIC was precipitated by adding 2 g $BaCl_2$, filtered and dried. The $BaCO_3$ was converted to CO_2 on a vacuum line by adding H_3PO_4. The $^{13}C/^{12}C$ ratios were measured with an isotope-ratio mass spectrometer and are reported in the usual delta ($\delta^{13}C$) notation referenced to the PDB standard (Belemnite from Pee Dee formation; Boutton, 1991). Hydrocarbons were extracted in a Soxhlet apparatus using dichloromethane (15 h at 55°C) and then analyzed by capillary gas chromatography. Total hydrocarbons were quantified by infrared spectroscopy (IR) or by integrating GC chromatograms. These methods were described in detail by Bregnard et al. (1996).

3. Results

3.1. Consumption of oxidants

Changes over time of oxidant concentration profiles (O_2, NO_3^-) are shown in Fig. 1B and C. At the infiltrating end of the column, the O_2 gradient was steep during early column operation and then gradually got more gentle towards the end of the experiment (Fig. 1B). At 12 cm column depth, which corresponds to a mean residence time of the groundwater of 3.6 ± 0.1 h, concentrations of O_2 never exceeded 0.3 mg l^{-1}. In this study, the zone from 0 to 12 cm is designated as aerobic zone. Between 12 and 24 cm column depth, O_2 concentrations were between 0.1 and 0.3 mg l^{-1}, and therefore, this zone is designated as microaerobic zone. At sampling ports beyond 24 cm column depth, O_2 concentrations were always below detection limit (< 0.1 mg l^{-1}), and therefore, this zone is designated as anaerobic zone. NO_3^- profiles showed distinct fluctuations within the first 27 days of column operation, with the highest consumption rates on day 27 (Fig. 1C). From day 41 to 90, NO_3^- profiles became more and more stable (Fig. 1C). Arithmetic means of oxidant concentration profiles (O_2, NO_3^-) are shown in Fig. 2A. The mean NO_3^- consumption rate over the entire experiment was higher in the aerobic and anaerobic zones than in the microaerobic zone (Fig. 2A).

3.2. Production of reduced inorganic compounds

NO_2^- concentrations increased in all zones of the column (Fig. 1D and 2A). High NO_2^- concentrations were found at day 15 and from day 62 to 90 (Fig. 1D). Low NO_2^- concentrations were observed between day 19 and 27. N_2O concentrations measured in the column effluent ranged from 11 to 12 μM between day 5 to 27 and day 62 to 90 and were below 2 μM in between (data not shown). Mean NH_4^+ concentrations increased from 2 ± 2 μM in the infiltrating water to 7 ± 4 μM in the column effluent.

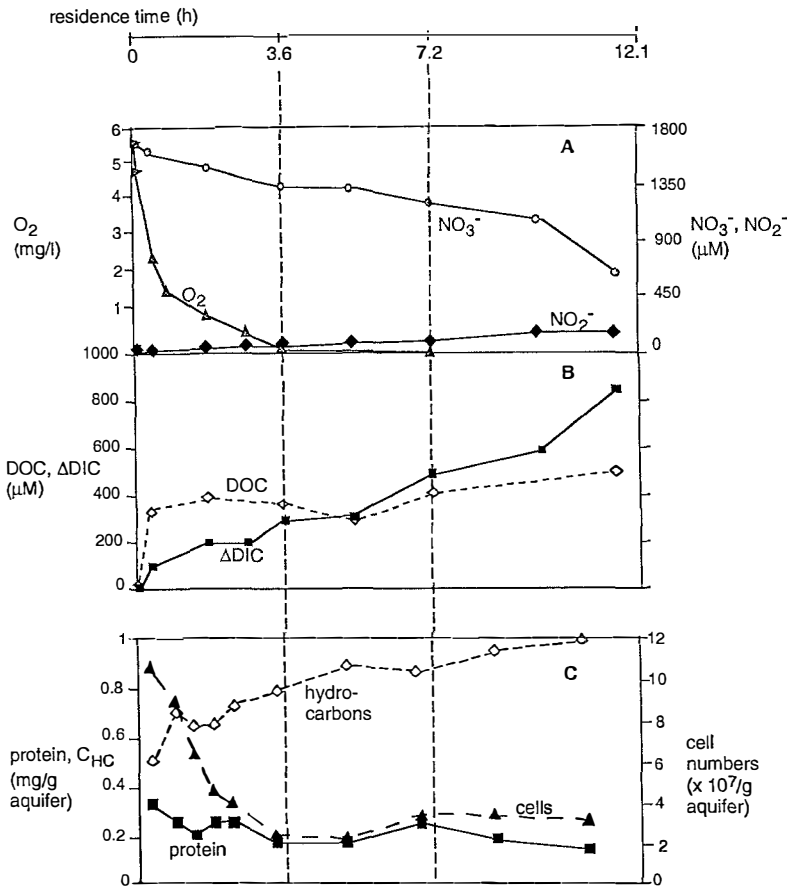

Fig. 2. Arithmetic means of 8–11 concentration profiles of O_2, NO_3^- and NO_2^- (A) and ΔDIC (= increase of DIC relative to concentration in inlet) and DOC (B). The final profiles (after an incubation of 96 days) of cell numbers, proteins and total hydrocarbons (C_{HC}, measured by IR) in the aquifer material are shown in (C).

Concentrations of dissolved iron and manganese measured on day 62 were below detection limit (5 μM) on all sampling points. H_2S and CH_4 could not be detected in the effluent (detection limits 1 and 2 μM, respectively). During the 96 days of column operation, a formation of gas bubbles in the aquifer material in the column was visible. Most bubbles were located in the anaerobic zone. In the effluent of the column, partial pressures of O_2, CH_4, CO_2 and N_2O were < 0.003 atm. We concluded that the gas formed in the column consisted mainly of N_2.

3.3. Dissolved organic and inorganic carbon

An increase in DOC concentrations was usually observed in the aerobic and anaerobic zone (Fig. 1E), whereas a slight decrease was often observed in the microaer-

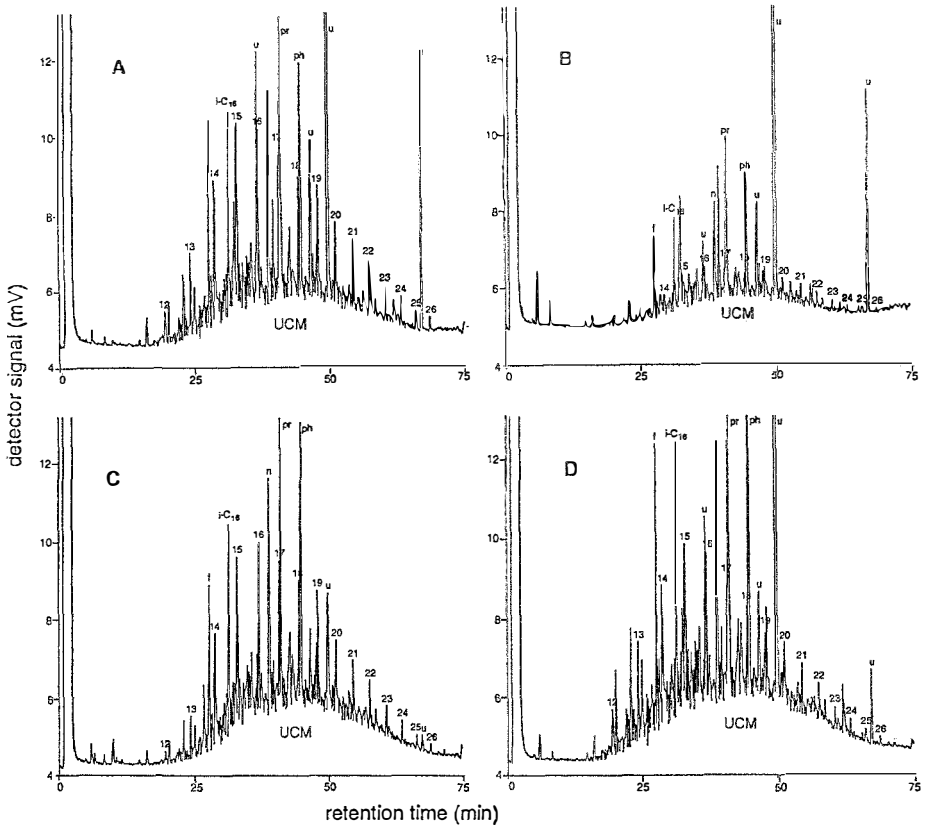

Fig. 3. GC analysis of the aquifer material at the start of the experiment (A) and after 96 days in slice 0–2.25 cm (B), slice 13.5–19.5 cm (C) and slice 31.5–40.5 cm (D). *UCM* = unresolved complex mixture; *11–26* = carbon numbers of *n*-alkanes, *f* = farnesane (*2,6,10*-trimethyldodecane); *i*-C_{16} = C_{16}-isoprenoid alkane (*2,6,10*-trimethyltridecane); *n* = norpristane (*2,6,10*-trimethylpentadecane); *pr* = pristane (*2,6,10,14*-tetramethylpentadecane); *ph* = phytane (*2,6,10,14*-tetramethylhexadecane); *u* = unknown impurities (partially identified as phthalates).

obic zone. However, in the first 27 days of the experiment, DOC concentration peaks sometimes appeared in all zones (Fig. 1E); later on, concentration profiles stabilized. DIC concentrations increased slightly more in the aerobic and the anaerobic zone than in the microaerobic zone (Fig. 1F and 2B).

3.4. Removal of hydrocarbons

Concentrations of total hydrocarbons (measured by IR) in 10 slices of aquifer material after 96 days of column operation are shown in Fig. 2C. GC chromatograms of aquifer material samples before and after 96 days of column operation are shown in Fig. 3A–D. The concentrations of total hydrocarbons and individual components are given in Table 1. Highest removal of hydrocarbons was observed in the sample taken nearest

Table 1
Quantification of diesel fuel components by GC chromatograms

Diesel fuel components	Initial concentration ($mg\ kg^{-1}$)	Residues after 96 days ($mg\ kg^{-1}$)		
		slice 0–2.25 cm	slice 13.5–19.5 cm	slice 31.5–40.5 cm
Total hydrocarbons	1200	367	795	958
Total *n*-alkanes (*n*-C_{11} to *n*-C_{26})	58	8	26	27
Total *i*-alkanes (f, *i*-C_{16}, n, pr, ph)	60	17	41	51
Unidentified peaks (including u)	108	98	78	152
Unresolved complex mixture UCM	974	244	650	728
Ratio of *n*-alkanes to *i*-alkanes	0.97	0.47	0.63	0.53
Ratio of UCM to total hydrocarbons	0.81	0.66	0.82	0.76

from the column inlet. In that sample, concentrations of *n*-alkanes and *i*-alkanes decreased to 14% and 28% of initial concentrations, respectively. The ratio of *n*-alkanes to *i*-alkanes decreased from 0.97 to 0.47 (Table 1). In the anaerobic zone, concentrations of *n*-alkanes and *i*-alkanes decreased to 47% and 85% of initial concentrations, respectively (Fig. 3; Table 1), and the ratio of *n*-alkanes to *i*-alkanes decreased also significantly. A distinct removal of UCM (Unresolved Complex Mixture) compounds was observed in the aerobic zone (Fig. 3; Table 1), whereas no preferential removal of UCM compounds relative to total hydrocarbons was detectable in the microaerobic and anaerobic zone (Table 1).

3.5. Growth of microorganisms

At the beginning of column operation, the aquifer material contained $(2.5 \pm 1.7) \cdot 10^7$ indigenous microorganisms per gram, as counted directly using the DAPI staining technique. After 96 days of operation, highest cell numbers $(10.5 \pm 2.5) \cdot 10^7$ cells g^{-1} were found in the sample closest to the column inlet (Fig. 2C). Cell numbers decreased with increasing column depth and reached a plateau beyond 8 cm. The protein concentrations increased from initially 0.1 mg protein g^{-1} up to 0.33 mg g^{-1} near the column inlet (Fig. 2C). The ratio of protein to cells had an almost constant value of $(1.7 \pm 0.3) \cdot 10^{-12}$ g protein $cell^{-1}$ beyond 8 cm column depth, but was considerably higher within the first 8 cm of the column.

3.6. Carbon and nitrogen mass balances

After 96 days of column operation, carbon and nitrogen mass balances were calculated as follows: Total hydrocarbons (measured by IR) and proteins were analysed in all 10 slices of aquifer material in the column (Fig. 1A), and weighted averages for the aerobic, microaerobic and anaerobic zone were calculated. The elemental composition of hydrocarbons was considered as 86% (w/w) carbon and 14% (w/w) hydrogen ($= \langle CH_2 \rangle$, see Table 2). Proteins were considered to consist of 51% (w/w) carbon and 20% (w/w) nitrogen (Balkwill et al., 1988). Production or consumption of dissolved

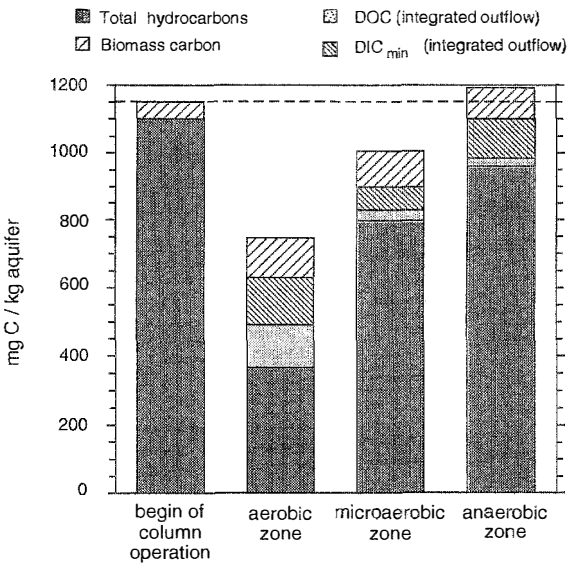

Fig. 4. Overall carbon balance within 96 days of column operation.

species were quantified by multiplying concentration changes of the averaged concentration profiles (Fig. 2A and B) across aerobic, microaerobic and anaerobic zones times the total volume of groundwater flowing through the column in 96 days. The results were termed total production or total consumption of species. Total DIC production was separated in DIC originating from mineralization (DIC_{min}) and DIC originating from carbonates ($DIC_{carbonate}$) as shown in the section ^{13}C balance, Eq. (1).

In the carbon mass balance, 94% of the organic carbon that was initially present (total hydrocarbons and biomass carbon) could be recovered (Fig. 4), when 4 fractions were quantified: Total hydrocarbons, biomass carbon, integrated production of DOC and of DIC_{min}. The loss of 6% of the organic carbon may be attributed to the wash out of dissolved, volatile hydrocarbons, which were not quantified by the DOC analysis. In the aerobic and microaerobic zone, the decrease of total hydrocarbons was not balanced by the increase of products (biomass, DOC and DIC_{min}; Fig. 4). In the anaerobic zone, the increase of products exceeded the decrease in hydrocarbons.

In the nitrogen balance, only 36.7% of the total consumption of NO_3^--N (22.3 mmol) could be analytically recovered: 22.7% was washed out as NO_2^--N, 1.1% as N_2O-N, and $< 0.5\%$ as NH_4^+-N. At the end, 12.4% of the NO_3-N was found in proteins.

3.7. Electron balance

Based on the analytical data, a scheme of microbially mediated processes was established that should allow to couple the oxidation of hydrocarbons to the consumption of oxidants. Two electron donating processes (oxidation of hydrocarbons to DIC_{min} and protein carbon, respectively) are balanced with 5 electron accepting processes for

Table 2
Scheme of processes used for the quantification of electron balances

Microbially mediated electron donating processes:			
Production of DIC_{min}	$\langle CH_2 \rangle^{b} + 2H_2O$	→	$\mathbf{DIC_{min}'}^{a} + 6e^- + 6H^+$
Production of biomass	$\langle CH_2 \rangle^{b} + H_2O$	→	$\mathbf{biomass\text{-}C}^{c,d} + 2e^- + 2H^+$
Microbially mediated electron accepting processes:			
Reduction of O_2	$\mathbf{O_2}^{a} + 4e^- + 4H^+$	→	$2H_2O$
Reduction of NO_3^- to NO_2^-	$NO_3^- + 2e^- + 2H^+$	→	$\mathbf{NO_2^-}^{a} + H_2O$
Reduction of NO_3^- to N_2O	$NO_3^- + 4e^- + 5H^+$	→	$\frac{1}{2}\mathbf{N_2O}^{a} + 2\frac{1}{2}H_2O$
Reduction of NO_3^- to N_2	$NO_3^- + 5e^- + 6H^+$	→	$\frac{1}{2}N_2 + 3H_2O$
Production of proteins	$NO_3^- + 8e^- + 10H^+$	→	$\mathbf{protein\text{-}N}^{c,e} + 3H_2O + 4H^+$

[a] Quantified as total production or consumption over 96 days.
[b] $\langle CH_2 \rangle$ = hydrocarbons.
[c] Quantified as weighted average of sliced samples analyzed after 96 days.
[d] The redox state of biomass was assumed to be 0.
[e] The redox state of nitrogen in proteins was assumed to be −III.

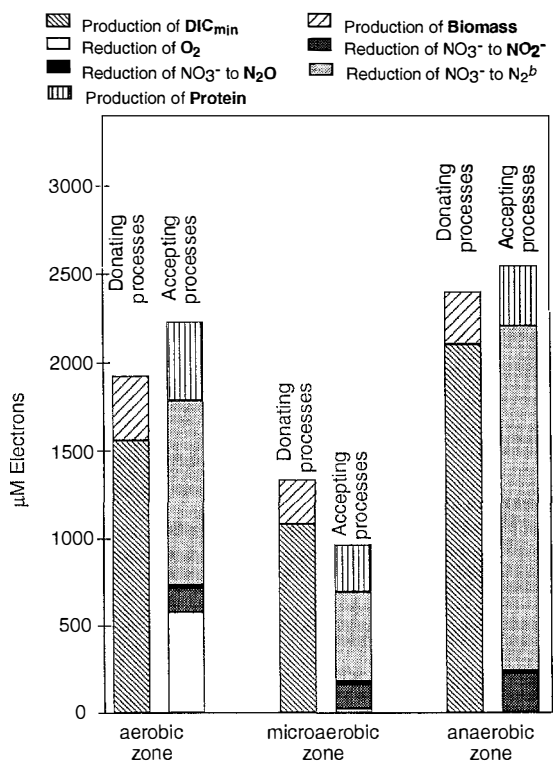

Fig. 5. Summary of processes considered for electron balances.

each zone of the column (Table 2; Fig. 5). The production of DOC was assumed not to be coupled to electron transfer reactions. Furthermore, it was assumed that production of N_2 by denitrification accounted for the missing nitrogen in the nitrogen balance. Overall, a reasonable balance between electron donating and accepting processes was found (Fig. 5). Note that more electrons were consumed by reduction of NO_3^- than by reduction of O_2. This was observed even in the aerobic zone.

3.8. ^{13}C balance

The origin of inorganic carbon produced in the column was traced by measuring $^{13}C/^{12}C$ ratios in substrates and products and by using the balance Eq. (1) and (2):

$$DIC_{in} + DIC_{min} + DIC_{carbonate} = DIC_{out} \quad (1)$$

and

$$\delta^{13}C_{in} \cdot DIC_{in} + \delta^{13}C_{min} \cdot DIC_{min} + \delta^{13}C_{carbonate} \cdot DIC_{carbonate} = \delta^{13}C_{out} \cdot DIC_{out} \quad (2)$$

where DIC_{in} = concentration of DIC in column inlet (5.24 m*M*, experimentally determined); DIC_{min} = unknown concentration of DIC originating from mineralization; $DIC_{carbonate}$ = unknown concentration of DIC originating from carbonates; DIC_{out} = concentration of DIC in column outlet (6.15 m*M*, experimentally determined); $\delta^{13}C_{in}$ = isotope ratio of DIC in column inlet (−4.64‰, experimentally determined); $\delta^{13}C_{min}$ = isotope ratio of carbon in petroleum hydrocarbons (−28‰; Boutton, 1991); $\delta^{13}C_{carbonate}$ = isotope ratio of DIC of carbonates from the column (+0.7‰, experimentally determined); and $\delta^{13}C_{out}$ = isotope ratio of DIC in outlet (−7.82‰, experimentally determined).

Solving Eq. (1) and Eq. (2) yields the two unknown concentrations of DIC_{min} (0.84 m*M*) and of $DIC_{carbonate}$ (0.07 m*M*). The concentration of $DIC_{carbonate}$ calculated in this way corresponded well with the increase of the concentration of Ca^{2+} in the column (0.067 m*M*). In this study, the inflowing and outflowing water was always undersaturated with respect to calcite [calculations with stability constants given in Stumm and Morgan (1981)]. Ca^{2+} liberated from carbonates may not be quantitatively washed out of columns, but be adsorbed to the aquifer matrix or undergo ion-exchange processes (Bjerg and Christensen, 1993). However, the good agreement of the carbonate dissolution calculated on the basis of Ca^{2+} concentrations with the ^{13}C isotopic balance suggests that after 60 days of column operation, the sorption and ion-exchange processes of Ca^{2+} had reached steady state, and that the quantification of $DIC_{carbonate}$ was correct.

4. Discussion

4.1. Localization of O_2- and NO_3^--consuming processes

The results demonstrate that O_2 concentrations in the infiltrating artificial groundwater decreased to < 0.1 mg l^{-1} within mean residence times of 7.2 h, and that no

breakthrough of O_2 occurred within 96 days. In aquifers, mean groundwater flow velocities are often below 10 m day^{-1} (de Marsily, 1986). According to the results of this study, O_2 would be consumed within a distance of < 3 m from an infiltration well in petroleum hydrocarbon contaminated aquifers. This is consistent with many findings at field sites (Battermann, 1983; Hutchins and Wilson, 1991; Gersberg et al., 1993; Kämpfer et al., 1993; Downs et al., 1994; Nelson et al., 1994; Hunkeler et al., 1995) and illustrates the difficulties to supply O_2 beyond a certain distance from an infiltration well. The results demonstrate furthermore that NO_3^- concentrations decreased on the average from 1610 to 660 μM, and that a decrease of NO_3^- concentrations was observed in all zones of the column irrespective of the O_2 concentrations of the bulk solution. 36.7% of the NO_3^- consumed in the columns was found in NO_2^-, N_2O, NH_4^+ and proteins. The low NH_4^+ concentrations in the column (< 7 μM) suggest that obligate anaerobic microorganisms known to use dissimilatory reduction of NO_3^- to NH_4^+ for respiration were of little importance. The electron balance (Fig. 5) supports the assumption that the rest of the NO_3^- consumed in the column was converted to N_2. In summary, this means that denitrifying mineralization was the major NO_3^- consuming process. The electron balance (Fig. 5) illustrates that already in the aerobic zone, the main consumption process of NO_3^- was reduction of NO_3^- to N_2 and NO_2^-. Thus, denitrification occurred in this zone in spite of the presence of O_2 in bulk solution.

4.2. Coupling of oxidant consumption with hydrocarbon mineralization

The electron balance strongly indicates that the oxidants consumed in the column were mainly used for mineralization of hydrocarbons to DIC_{min}. The carbon and ^{13}C balances give further evidence that DIC originated mainly from the mineralization of hydrocarbons. Without proper balances, it often cannot be assessed whether total DIC produced in the column originated from mineralization of contaminants or, for example, from the dissolution of carbonates.

In the aerobic zone of the column, more hydrocarbons were removed than in the microaerobic and anaerobic zone (Fig. 2C, 3 and 4). This could either be due to biodegradation or due to transport. Linear and isoprenoid alkanes with neighbouring retention times in the GC chromatogram (e.g., *n*-C_{17} and pristane) have the same physico-chemical properties (e.g., solubility, octanol–water partitioning) and thus should show a similar transport behaviour. However, *i*-alkanes are less biodegradable due to steric effects (Watkinson and Morgan, 1990). The decrease of the ratio of *n*-alkanes to *i*-alkanes is therefore a strong indication for that *n*-alkanes are mineralized rather than transported (Pritchard and Costa, 1991). Microbial degradation of *n*-alkanes occurred in the aerobic as well as in the anaerobic zone of the column. Mineralization of *n*-[1-^{14}C]hexadecane to $^{14}CO_2$ under denitrifying conditions was confirmed in a microcosm study using the contaminated aquifer material from the same site (Bregnard et al., 1996).

4.3. Role of oxidants

In the aerobic degradation of hydrocarbons, O_2 plays a dual role: It is a co-substrate in initial transformation reactions by oxygenases (Watkinson and Morgan, 1990) and it

also serves as the final electron acceptor for mineralization. Facultative denitrifying microorganisms can substitute NO_3^- and even NO_2^- or N_2O for O_2 as terminal electron acceptor (Tiedje, 1988). However, a substitution for O_2 as the co-substrate of oxygenases is not possible, and yet unidentified alternative enzymes have to catalyze the initial oxidation of hydrocarbons. In the absence of O_2, denitrifying hydrocarbon degraders are known to exhibit slow growth on some hydrocarbons as sole carbon source (Häner et al., 1995; Rabus and Widdel, 1995) but frequently grow faster on partially oxidized transformation products of hydrocarbons such as alcohols or fatty acids (Schocher et al., 1991; Seyfried et al., 1994). The largest increase of DOC concentrations was observed right at the column inlet, where aerobic conditions existed. It is not known whether this DOC consisted of hydrocarbons or of transformation products from aerobic mineralization. Future studies should identify this organic carbon compounds to answer the question whether aerobic organisms generate partially oxidized transformation products that serve as substrates for denitrifying mineralization.

4.4. O_2 threshold for denitrification

For facultatively denitrifying microorganisms it has been demonstrated that the O_2 concentration is the dominant regulator for the expression of enzymes for the reduction of NO_3^-, NO_2^- and N_2O in the respiratory chain. Most of these organisms synthesize the denitrifying enzymes only when O_2 concentrations are below 0.2–0.67 mg O_2 l^{-1} (Tiedje, 1988). This concentration range is frequently regarded as an O_2 threshold for denitrification (Tiedje, 1988). In this study, denitrifying processes were observed in the presence of mean O_2 concentrations above this reported O_2 threshold.

The O_2 threshold concept has been applied in numerical models describing the aerobic and denitrifying mineralization of contaminants in aquifers (Kinzelbach et al., 1991; Schäfer et al., 1994). The problem of ''aerobic denitrification'' is accounted for in the model formulations using mobile and immobile regions (Kinzelbach et al., 1991). This concept allows aerobic mobile regions close to anaerobic immobile regions and therefore, it is possible to have anaerobic processes in cells of the model where there is still oxygen available in the mobile water. Microelectrode studies (Jørgensen and Revsbech, 1983) confirm such concepts by showing that the O_2 concentration in stagnant water or within soil aggregates can drop from 10 to < 0.1 mg l^{-1} within a distance of 0.5 mm. The data presented in our study are suited to validate the model concept proposed by Kinzelbach et al. (1991).

5. Summary and conclusions

The study demonstrates that the infiltration of aerobic, NO_3^--containing artificial groundwater into laboratory aquifer columns filled with aquifer material contaminated with hydrocarbons results in the consumption of oxidants and a transformation of hydrocarbons to dissolved inorganic and organic carbon and bacterial biomass. Although the spatial penetration of O_2 was limited, removal of hydrocarbons and production of DIC occurred within the entire column length. A preferential degradation of *n*-alkanes

compared to *i*-alkanes, but only a small removal of total hydrocarbons was observed in the anaerobic zone that prevailed at distances > 24 cm from the infiltration. Denitrification was observed not only under anaerobic conditions, but also in the presence of O_2 in the bulk solution. The results underline the beneficial role of NO_3^- addition for aquifer bioremediation and have implications for the design of appropriate transport and reaction models.

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