

Petroleum hydrocarbon mineralization in anaerobic laboratory aquifer columns

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Abstract

The anaerobic biodegradation of hydrocarbons at mineral oil contaminated sites has gathered increasing interest as a naturally occurring remediation process. The aim of this study was to investigate biodegradation of hydrocarbons in laboratory aquifer columns in the absence of O₂ and NO₃⁻, and to calculate a mass balance of the anaerobic biodegradation processes. The laboratory columns contained aquifer material from a diesel fuel contaminated aquifer. They were operated at 25°C for 65 days with artificial groundwater that contained only SO₄²⁻ and CO₂ as externally supplied oxidants. After 31 days of column operation, stable concentration profiles were found for most of the measured dissolved species. Within 14 h residence time, about 0.24 mM SO₄²⁻ were consumed and dissolved Fe(II) (up to 0.012 mM), Mn(II) (up to 0.06 mM), and CH₄ (up to 0.38 mM) were produced. The alkalinity and the dissolved inorganic carbon (DIC) concentration increased and the DIC became enriched in ¹³C. In the column, *n*-alkanes were selectively removed while branched alkanes persisted, suggesting a biological degradation. Furthermore, based on changes of concentrations of aromatic compounds with similar physical-chemical properties in the effluent, it was concluded that toluene, *p*-xylene and naphthalene were degraded. A carbon mass balance revealed that 65% of the hydrocarbons removed from the column were recovered as DIC, 20% were recovered as CH₄, and 15% were eluted from the column. The calculations indicated that hydrocarbon mineralization coupled to SO₄²⁻ reduction and methanogenesis contributed in equal proportions to the hydrocarbon removal. Hydrocarbon mineralization coupled to

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Fe(III) and Mn(IV) reduction was of minor importance. DIC, alkalinity, and stable carbon isotope balances were shown to be a useful tool to verify hydrocarbon mineralization.

Keywords: Intrinsic bioremediation; Petroleum hydrocarbons; Groundwater; Stable carbon isotopes; Methanogenesis; Sulfate reduction

1. Introduction

Contamination of aquifers with petroleum hydrocarbons (designated as hydrocarbons throughout the text) is a widespread environmental problem that has led to the development of several remediation technologies. In situ bioremediation has found special attention because it ideally leads to complete mineralization of the hydrocarbons without expensive excavation of the contaminated zone (Battermann, 1983; US National Research Council, 1993). In aquifers contaminated with hydrocarbons, indigenous microbial populations that can mineralize hydrocarbons are usually established (Thomas and Ward, 1994). In the contaminated zones, anaerobic conditions often prevail because the O_2 demand exceeds the O_2 supply (Bouwer, 1992). Therefore, engineered in situ bioremediation schemes are usually based on an external supply of oxidants (O_2 , H_2O_2 , and/or NO_3^-) to increase the aerobic and denitrifying mineralization rates (Battermann, 1983; Hutchins et al., 1991; Hunkeler et al., 1995).

During the last years, however, it has been shown in laboratory studies that the mineralization of various aromatic and aliphatic hydrocarbons can also be coupled to the reduction of Fe(III), Mn(IV), SO_4^{2-} and CO_2 as summarized by Holliger and Zehnder (1996). Field studies suggest that biodegradation under these redox conditions can significantly contribute to the removal of hydrocarbons from aquifers (Baedecker et al., 1993; Borden et al., 1995; Thierrin et al., 1995). In an aquifer contaminated with crude oil, which was not treated by active measures (Essaid et al., 1995), a greater amount of hydrocarbon was degraded under anaerobic conditions (60%) than under aerobic conditions (40%) due to the limited supply of O_2 . The authors of the study estimated that about 19% of the hydrocarbon degradation was coupled to Fe reduction, 5% was coupled to Mn reduction, and 36% was coupled to methanogenesis. We reported on an aquifer at Menziken, Switzerland, which was contaminated with diesel fuel and treated by adding O_2 , NO_3^- and nutrients. Despite these additions, dissolved Fe(II), Mn(II) and CH_4 were found in the contaminated zone, and the SO_4^{2-} concentration decreased, indicating that hydrocarbons were mineralized under Fe-reducing, Mn-reducing, SO_4^{2-} -reducing, and methanogenic conditions as well (Hunkeler et al., 1995). In the field, however, it is difficult to demonstrate that the consumption of oxidants is coupled to the mineralization of hydrocarbons, and that a related amount of hydrocarbons is removed (Madsen, 1991; Hunkeler et al., 1997). Laboratory aquifer columns allow an ex situ verification of hydrocarbon mineralization, since complete carbon mass balance can be established. Hydrocarbon mineralization under aerobic and denitrifying conditions in the contaminated aquifer at Menziken was verified previously using laboratory aquifer columns filled with contaminated aquifer material from the site (Hess et al., 1996).

In this study, hydrocarbon mineralization in the absence of O_2 and NO_3^- was investigated using laboratory aquifer columns. The objectives of the investigations were (1) to study the evolution of Fe-reducing, Mn-reducing, SO_4^{2-} -reducing, and methanogenic conditions; (2) to relate the consumption of oxidants and the production of reduced species to the production of dissolved inorganic carbon (DIC) based on DIC, alkalinity, and stable carbon isotope balances; (3) to evaluate the contribution of the various redox processes to hydrocarbon mineralization; (4) to perform a carbon mass balance; and (5) to determine which hydrocarbons were degraded.

2. Materials and methods

2.1. Experimental set-up

The columns (Fig. 1A) were identical to those described elsewhere (Hess et al., 1996). They had an inner diameter of 5 cm and a total length of 47 cm. 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. The aquifer material used to fill the columns was excavated from a diesel fuel contaminated aquifer in Menziken, Switzerland in which Mn-reducing, Fe-reducing, SO_4^{2-} -reducing and methanogenic conditions were observed (Hunkeler et al., 1995). The excavated material was sieved (< 4.5 mm). 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) and contained about 40% w/w carbonates (mainly $CaCO_3$). The concentration of weathered diesel fuel was 950 mg kg^{-1} dry weight. The material was amended with fresh diesel fuel (Esso, Switzerland) to yield a total hydrocarbon concentration of 1410 mg kg^{-1} dry weight. Water-saturated material was homogenized by vigorous stirring and subsequently used to fill two columns. After allowing the material to settle for 2 days, the column filling covered a length of 39 cm and had a total porosity of 0.21, calculated from the volume, weight and density of the material. The hydrocarbon-amended aquifer material, which was used to fill the columns, is denoted as initial aquifer material throughout the text.

The columns were operated at $25 \pm 0.5^\circ C$ in a vertical position under upflow conditions (flow rates of 10.0 ± 0.5 ml h^{-1}). Artificial groundwater consisted of 6.00 mM $NaHCO_3$, 0.20 mM Na_2SO_4 and 0.05 mM $MgSO_4 \cdot 7H_2O$ in distilled water. It was autoclaved at $120^\circ C$ for 20 min. Sterile solutions of $CaCl_2 \cdot 2H_2O$ (0.25 mM final conc.) and KH_2PO_4 (0.01 mM final conc.) were added after autoclaving. The pH was adjusted to 7.5 using 1 M sterile HCl. Concentrations of dissolved oxygen were lowered to < 0.1 mg l^{-1} by passing the artificial groundwater through silicone tubes of 2 m length in a bottle that was continuously flushed with N_2 (Zeyer et al., 1986). Two identical columns were operated over 65 days. Transport parameters were characterized by adding 1 mM NaBr to the artificial groundwater at day 39 of operation. Breakthrough curves were obtained by measuring Br^- -concentrations and analyzed using the computer code CXTFIT2 (Toride et al., 1995). The calculations yielded an effective porosity of 0.18, an

2.2. Sampling and analysis of dissolved species

Depending on the chemical species, 3 to 7 concentration profiles were measured during 65 days of column operation. The stable carbon isotope composition ($\delta^{13}\text{C}$) of the DIC was measured once on day 48. Water samples were taken using 10 ml glass syringes equipped with stainless steel valves (Merck, Dietikon, Switzerland) to avoid loss of volatile compounds. The syringes were filled without headspace at the same rate as the flow rate in the column. Samples were taken in the inverse direction to the water flow starting at the column outlet to minimize the disturbance. Unless stated otherwise, water samples from the columns were filtered using 0.22 μm Millipore PTFE filters (Millipore, Volketswil, Switzerland). The sums of Ca, Mg, Fe and Mn in the form of free ions and complexes with redox state +II are denoted as Ca(II), Mg(II), Fe(II) and Mn(II). Dissolved S(-II) corresponds to the sum of H_2S , HS^- and S^{2-} . For the measurement of dissolved Ca(II), Mg(II), Fe(II), Mn(II), and dissolved organic carbon (DOC), samples were acidified with distilled HNO_3 to yield a final concentration of 0.025 M HNO_3 . Concentrations of SO_4^{2-} , Cl^- , Br^- , Ca(II) and Mg(II) were determined using a Dionex DX-100 ion chromatograph (Dionex, Sunnyvale, USA). Ammonium and dissolved S(-II) were measured colorimetrically (APHA, 1989). Fe and Mn were quantified by atomic absorption spectroscopy (AAS; Varian SpectraAA 400, Varian Techtron, Springvale, Australia). Since the solubility of amorphous FeOOH in the absence of strong ligands is $< 1 \mu\text{M}$ at pH 6 to 8 (Stumm and Morgan, 1996), it was assumed that the measured concentration of dissolved Fe corresponds to the concentration of Fe^{2+} in the form of free ions and complexes in the original sample. DOC concentrations were determined with a Horiba TOC-analyzer (Horiba, Kyoto, Japan). Before analysis, the acidified samples were vigorously bubbled with N_2 to ensure complete stripping of DIC. It has to be taken into account that volatile hydrocarbons (e.g., monoaromatic compounds) and volatile metabolites are stripped as well. Concentrations of dissolved CO_2 and CH_4 were determined using 10 ml glass syringes that were filled with 4 ml sample without contact with the atmosphere and an additional 2 ml N_2 headspace. After shaking the syringes and equilibration for 4 h, the partial pressure of the gases were measured by gas chromatography (Fisons Model 8000; Fisons, Rodano, Italy) on a HayeSep D column (Alltech, Deerfield, IL) at 70°C with N_2 as carrier and a Carlo Erba thermal conductivity detector (Fisons, Rodano, Italy). Concentrations of dissolved gases were calculated from Henry's law using the following Henry constants at 25°C: CO_2 : 0.0339 M atm^{-1} , CH_4 : 0.00129 M atm^{-1} (Stumm and Morgan, 1996). Alkalinity was measured by potentiometric titration using Gran plots for graphi-

Fig. 1. (A) Schematic illustration of column that was operated in upright position. (B) Arithmetic mean of concentrations of dissolved species measured between days 31 and 65 and $\delta^{13}\text{C}$ of DIC measured at day 48. The concentrations given at 0 cm column length corresponds to the concentrations measured in the bottle with the artificial groundwater. For DIC and the $\delta^{13}\text{C}$ of the DIC, no values are given at 0 cm column length because the DIC concentration and $\delta^{13}\text{C}$ changed during the passage of the water through the silicone tube in the gas exchange bottle. The concentrations given at 39 cm column length correspond to the concentrations measured in the effluent of the column.

cal determination of the end point (Stumm and Morgan, 1996). The DIC concentration and pH was calculated based on the alkalinity and the concentration of dissolved CO₂ (Stumm and Morgan, 1996).

For stable carbon isotope analysis of the DIC, 10 ml of water were collected in glass syringes without headspace. The DIC was precipitated as BaCO₃ by the addition of 0.1 ml of a CO₂-free NaOH solution (1.5 M) and 0.4 ml of a CO₂-free BaCl solution (1.2 mM). After 10 h of equilibration, the sample was discharged from the syringe through a 0.22 μm Millipore PTFE filter. The filtrate was dried at 105°C for 12 h. The BaCO₃ was converted to CO₂ under a vacuum by the addition of H₃PO₄. The ¹³C:¹²C ratios were measured with a Fisons Prism isotope-ratio mass spectrometer (Fisons, Middlewich, Cheshire, UK) and are reported in the usual delta notation (δ¹³C) referenced to the PDB (Peedee belemnite) standard (Coplen, 1996).

To quantify the elution of hydrocarbons from the column, 80 to 420 ml sample were taken at the outlet of the column using 500 ml separatory funnels. The separatory funnels contained 5 to 10 ml pentane with 1-chlorooctane as an internal standard and were chilled on ice to avoid evaporation of the pentane. The hydrocarbons were extracted into the pentane by vigorous shaking. The organic phase was analyzed by gas chromatography (GC) using a capillary (Bregnard et al., 1996) as well as a packed separatory column (Häner et al., 1995). The packed column allowed separation of *m*- and *p*-xylene, which could not be separated on the capillary column. Hydrocarbons were identified and quantified using external standards. The peaks that could not be identified were quantified using the average response factor of the identified peaks. Some of the unidentified peaks may have consisted of volatile metabolites. Benzene could not be quantified due to poor separation from the solvent.

2.3. Sampling and analysis of column matrix

At day 65, the column operation was stopped, and one column was frozen at -18°C. The frozen material was extruded and sliced as shown in Fig. 1A. The frozen slices were immediately transferred to an anaerobic chamber containing a N₂ atmosphere. All data of compounds in the solid and water phase shown in the results section were obtained from this column.

Hydrocarbons were extracted by sonication using CH₂Cl₂ as the solvent according to the EPA method 3550 (US Environmental Protection Agency, 1989). The total hydrocarbon concentration and the concentration of single compounds were determined by capillary GC analysis of the extracts as described in detail by Bregnard et al. (1996).

For stable carbon isotope analysis of the weathered diesel fuel, contaminated aquifer material that had not been amended with fresh diesel fuel was extracted with CH₂Cl₂. The extract was evaporated to a constant weight, and 10 μl of the residue was combusted in an evacuated quartz tube at 950°C for 3 h using 1 g CuO as an oxidant. The CO₂ that was produced was analyzed with a Fisons Optima isotope-ratio mass spectrometer (Fisons, Middlewich, Cheshire, UK). The δ¹³C of the fresh diesel fuel was determined identically as that of the extracted hydrocarbons. For stable carbon isotope analysis of the carbonates in the aquifer material, a sample of aquifer material was ground, and the δ¹³C was determined identically as that of the precipitated BaCO₃.

FeS was extracted with 0.5 M HCl according to Heron et al. (1994). The extraction was performed at 25°C to avoid oxidation of H₂S by Fe³⁺ (Von Gunten and Zobrist, 1993). In the extracts, Fe(II) was analyzed using the phenanthroline method and S(-II) using the methylene blue method (APHA, 1989).

Microbial cells were removed from aquifer solids by vortexing 1 g of aquifer material in 10-ml filtered artificial groundwater containing 0.1% Na₄P₂O₇ (Balkwill et al., 1988). The aquifer solids were allowed to settle down for 2 min; 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).

2.4. Calculations

Saturation calculations were performed with MICROQL (Westall, 1986) using stability constants from Thorstenson and Plummer (1977), Matsunaga et al. (1993) and Stumm and Morgan (1996). The stability constants were corrected for temperature and ionic strength using the van't Hoff equation and the Güntelberg approximation, respectively (Stumm and Morgan, 1996). The consumption or production of dissolved species during 65 days of column operation was quantified by multiplying the difference between the average concentration in the effluent, and the inflow with the flow rate and the length of the operation time. The average concentration of hydrocarbons, S(-II), and Fe(II) in the solid phase after termination of column operation was obtained by multiplying the concentration in each slice with the weight of the slice, adding the amounts of all eight slices and dividing the sum by the total weight of the aquifer material. The total elution of DOC and dissolved hydrocarbons from the column was obtained by integrating the concentration vs. time curves of the effluent. The reported uncertainties of measured values are standard uncertainties determined according to International Organization for Standardization (1993). Uncertainties on calculated values were estimated using the law of propagation of uncertainty (International Organization for Standardization, 1993).

3. Results

3.1. Concentrations of dissolved species

The average of the concentrations of dissolved species measured between days 31 and 65 are shown in Fig. 1B. Between days 31 and 65, the general shape of the concentration profiles remained constant. In the first 26 cm of the column, SO₄²⁻ was almost completely consumed and the average S(II) concentrations were higher than 1 μM (Fig. 1B). The Fe(II) concentration was constant between 2 and 20 cm column length, but increased at column length > 20 cm. The Mn(II), CH₄, alkalinity and DIC concentrations steadily increased with increasing column length. The δ¹³C of the DIC increased by 3.1‰ (Fig. 1 B). The pH increased until 14 cm column length and then decreased again.

For CH_4 and CO_2 , the partial pressures were calculated from the concentrations of dissolved gases and the Henry coefficients. The partial pressure of CH_4 increased to 0.2 atm at the column end. The partial pressure of CO_2 was 0.006 ± 0.001 atm, and the partial pressure of N_2 was 0.98 atm, assuming that the water was in equilibrium with respect to N_2 at the column inlet, and N_2 was neither consumed nor produced in the column. Thus, the total partial pressure of the dissolved gases increased to greater than 1 atm due to the production of CH_4 . This increase explains the observed formation of gas bubbles in the aquifer material. The gas bubbles were mainly found at column lengths > 20 cm.

3.2. Concentrations of inorganic species in solid phase and saturation calculations

At the end of the column experiment, the highest S(-II) concentrations in the solid phase were found between 8 and 26 cm column length (Fig. 2B) while the Fe(II) concentrations were elevated by about 1.3 ± 0.3 mmol kg^{-1} dry weight compared to the initial aquifer material over the entire length of the column. Saturation calculations were performed based on the measurements of dissolved species made between day 38 and 49 depending on the species. Samples were saturated with respect to FeS at sampling ports located at 8, 14 and 20 cm column length and saturated or oversaturated with respect to FeCO_3 (Siderite) at 26, 35 and 39 cm column length (Fig. 2C). Samples of all sampling ports were undersaturated with respect to MnCO_3 (Rhodocrosite) and MnS (data not shown). Furthermore, all samples were saturated with respect to CaCO_3 (Calcite) and low-magnesium calcites, and undersaturated with respect to $\text{CaMg}(\text{CO}_3)_2$ (Dolomite) and magnesium calcites containing more than 7% mole magnesium carbonate. Direct cell counts resulted in $6.7 \pm 1.0 \cdot 10^7$ cells g^{-1} dry aquifer material. No significant change between the cell numbers at the beginning and the end of the experiment was observed.

3.3. Concentrations of hydrocarbons

At the end of the experiment, total hydrocarbon concentrations in the solid phase were found to be lower than in the initial aquifer material in all slices between 0 and 35 cm column length (Fig. 2C). However, in the last slice, the total hydrocarbon concentration was elevated. The largest decrease of the total hydrocarbon concentration was observed in the first slice. In the initial aquifer material, *n*-alkanes as well as *i*-alkanes (isoprenoid alkanes, e.g., farnesane, norpristane, pristane, phytane) were present (Fig. 3A). After 65 days of column operation, *n*-alkane concentrations were below the detection limit in all slices, while the *i*-alkanes were still present (Fig. 3B). In total, 200 mg kg^{-1} dry weight hydrocarbons were removed (Table 1). One third of the decrease of the hydrocarbon concentration in the column could be attributed to the removal of *n*-alkanes, while the remaining two-thirds was due to the removal of unidentified compounds. The *i*-alkane concentration remained constant (Table 1). The $\delta^{13}\text{C}$ of the weathered diesel fuel in the aquifer material was -29.7% , the $\delta^{13}\text{C}$ of the added diesel fuel -30.1% .

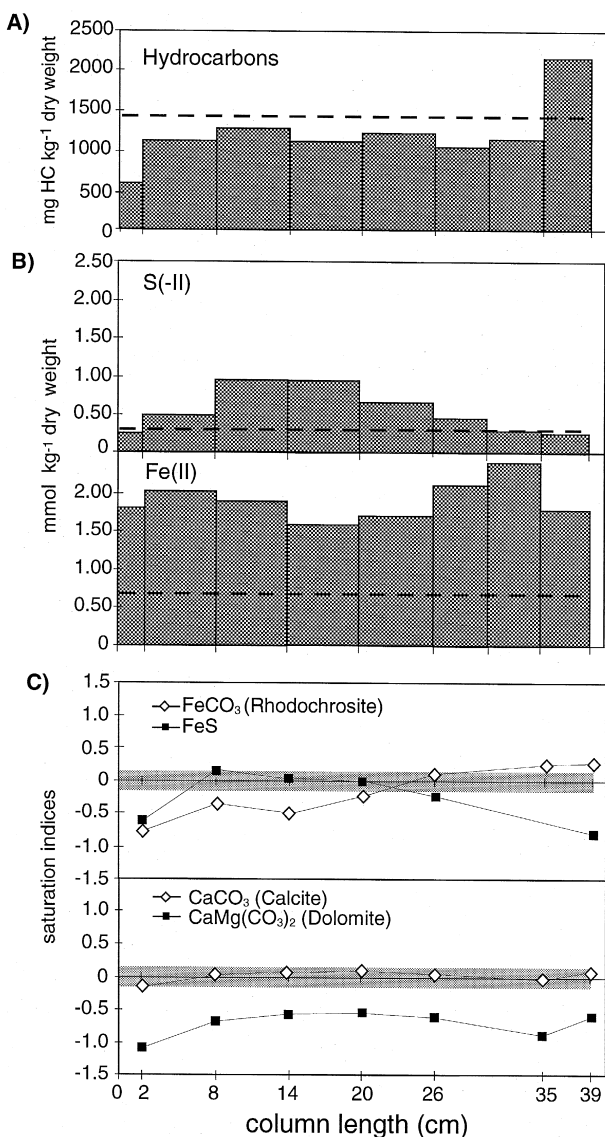


Fig. 2. (A) Hydrocarbon concentration in the aquifer material before (broken line) and after 65 days of column operation. (B) $S(-II)$ and $Fe(II)$ concentration in the aquifer material before (broken line) and after 65 days of column operation. (C) Saturation indices of $FeCO_3$, FeS , $CaCO_3$ and $CaMg(CO_3)_2$ calculated from data measured between day 38 and 49. Positive values indicate oversaturation; negative values indicate undersaturation. The hatched areas correspond to the range of uncertainty of the calculations.

The concentration of the hydrocarbons detected in the effluent of the column was 1.5 mg l^{-1} at day 1 (Fig. 4A). It decreased to 0.7 mg l^{-1} at day 20, and then remained at a constant level of $0.64 \pm 0.03 \text{ mg l}^{-1}$ until the last measurement at day 55. Approxi-

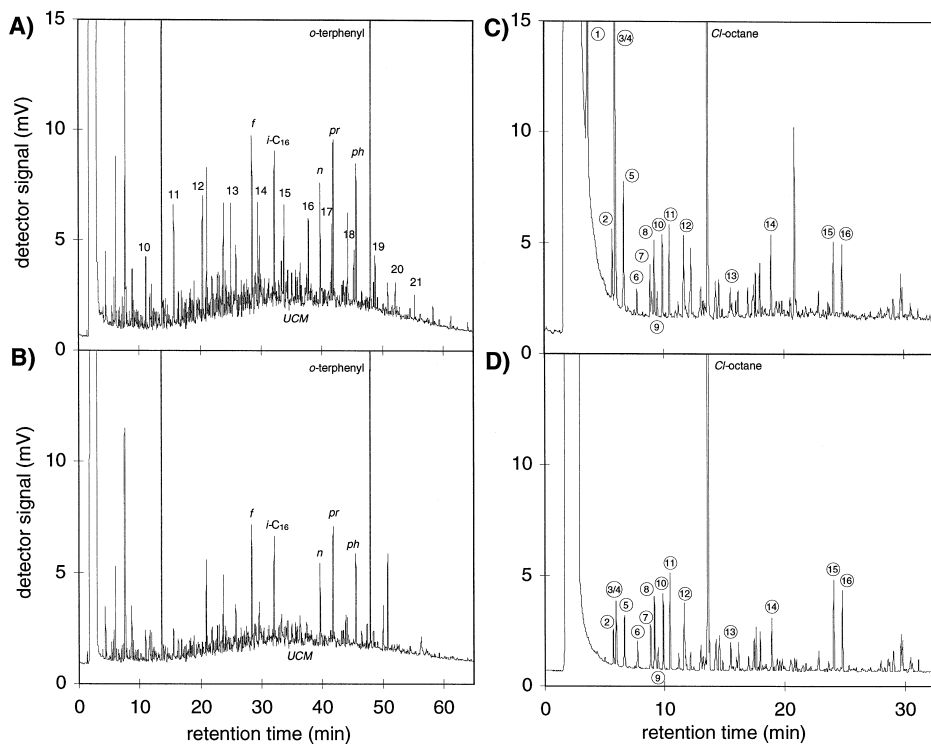


Fig. 3. GC analysis of hydrocarbons in the aquifer material at the start of the experiment (A) and in the aquifer material of slice 2–8 cm after 65 days of column operation (B). UCM: unresolved complex mixture, 10–21: carbon numbers of *n*-alkanes, f = farnesane (2,6,10-trimethyltridecane), *i*-C₁₆ = C₁₆-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). *o*-terphenyl: internal standard. GC analysis of dissolved hydrocarbons in effluent samples taken at day 1 (C) and day 55 (D) of column operation. 1–16: compound number according to Table 2. Cl-octane: internal standard.

mately 50% of the mass of the hydrocarbons detected at day 1 consisted of toluene, ethylbenzene, and xylenes. Further 20% of the mass of detected hydrocarbons were other monoaromatic and di-aromatic compounds (Table 2). The remaining 30% could

Table 1
Quantification of diesel fuel hydrocarbons (HC) by gas chromatography

Diesel fuel components	Initial concentration (mg HC kg ⁻¹ dry weight)	Average concentration after 65 days (mg HC kg ⁻¹ dry weight)
Total hydrocarbons	1410	1210
Total <i>n</i> -alkanes (<i>n</i> -C ₁₀ to <i>n</i> -C ₂₁)	70	< 6
Total <i>i</i> -alkanes (f, <i>i</i> -C ₁₆ , n, pr, ph)	65	62
Unresolved complex mixture UCM	1270	1140

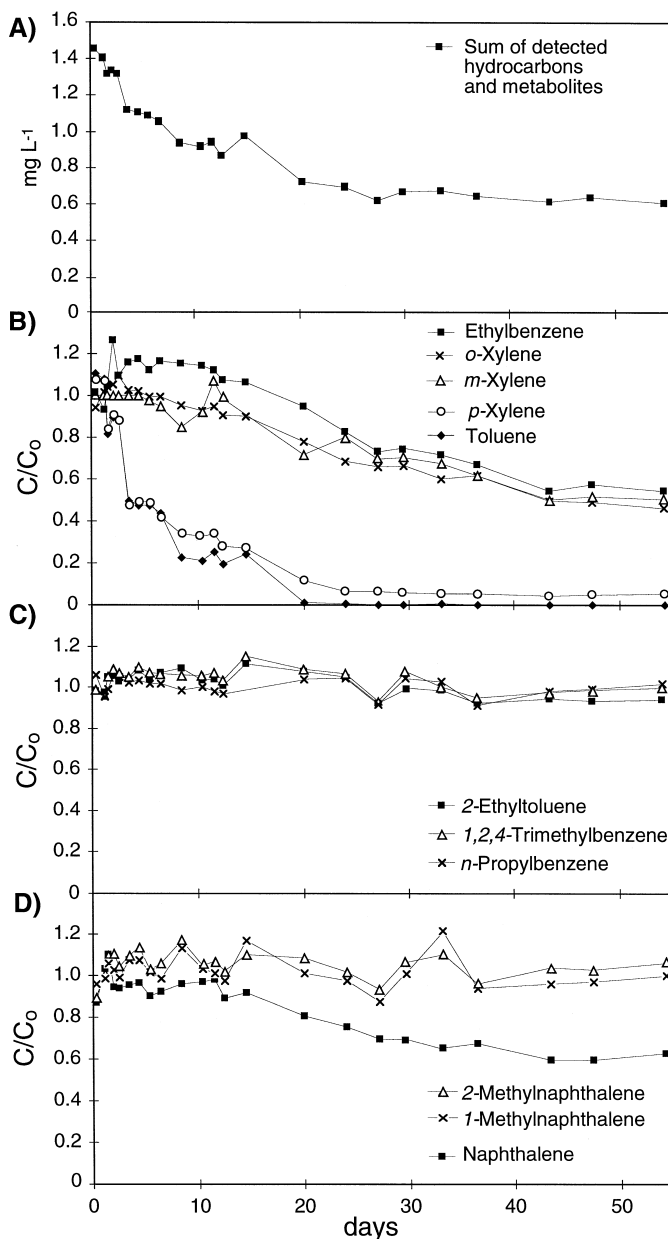


Fig. 4. Sum of detected hydrocarbons in the effluent of the column (A). Ratio of the actual concentration to the initial concentration of various mono- (B and C) and di-aromatic (D) hydrocarbons. The temporal variations of the concentrations of isopropylbenzene, 3- and 4-ethyltoluene, 1,2,3- and 1,2,5-trimethylbenzene and 1,2,4,5-tetramethylbenzene were similar to those shown in C.

Table 2
Concentration of mono- and di-aromatic hydrocarbons in the effluent of the column at day 1 and 55

Compound		Effluent concentration		
No.	Name	Day 1 ($\mu\text{g l}^{-1}$)	Day 55 ($\mu\text{g l}^{-1}$)	$\log K_{\text{OW}}^{\text{a}}$ (l^{-1})
1	Toluene	314	< 2	2.65
2	Ethylbenzene	37	19	3.13
3	<i>p</i> -Xylene	223	6	3.18
4	<i>m</i> -Xylene	64	29	3.20
5	<i>o</i> -Xylene	70	31	3.13
6	Isopropylbenzene	11	9	3.66
7	<i>n</i> -Propylbenzene	25	23	3.69
8	3- and 4-Ethyltoluene	48	43	—
9	1,3,5-Trimethylbenzene	12	8	3.55
10	2-Ethyltoluene	37	34	3.53
11	1,2,4-Trimethylbenzene	48	47	3.58
12	1,2,3-Trimethylbenzene	37	33	3.58
13	1,2,4,5-Tetramethylbenzene	13	11	4.00
14	Naphthalene	39	26	3.36
15	2-Methylnaphthalene	39	46	3.86
16	1-Methylnaphthalene	36	36	3.87
	Not identified	403	198	
	Total	1456	599	

^aFrom Eastcott et al., 1988.

Octanol–water partitioning coefficient (K_{OW}) at 25°C of the hydrocarbons.

not be identified. The decrease of the hydrocarbon concentration in the effluent was mainly due to decreasing concentrations of toluene, ethylbenzene, and xylenes (Fig. 3C,D; Table 2). The DOC concentration in the effluent decreased from about 2 mg l⁻¹ (day 14) to 0.2 mg l⁻¹ (day 56).

4. Discussion

4.1. Microbial processes

In this study, hydrocarbon mineralization was assessed by calculating the expected DIC and alkalinity production based on the observed consumption of oxidants and production of reduced species, and by comparing it to the observed DIC and alkalinity production (see below). The chosen approach corresponded to that applied in a related field study (Hunkeler et al., 1997). The calculation of the expected DIC and alkalinity production requires the postulation of microbial hydrocarbon mineralization processes (Table 3). In aquifers, the microbial reduction of Fe- and Mn-oxides is generally more important than the abiotic dissolution of these minerals (Stone and Morgan, 1984; Lovley et al., 1991). Therefore, the increase of the concentration of dissolved Fe(II) and Mn(II) in the column was attributed to the action of Fe- and Mn-reducing microorganisms (Processes 1 and 2, Table 3). Under the conditions created in this study, the consumption of SO_4^{2-} is most likely due to microbial reduction of SO_4^{2-} to S(-II)

Table 3

Stoichiometric equations of selected processes involved in anaerobic mineralization of hydrocarbon. Contributions to DIC production, contribution to alkalinity production and $\delta^{13}\text{C}$ of the DIC.

No. <i>n</i>	process ^a	A contribution to DIC ^b v_{DIC}^n	B contribution to alkalinity ^b v_{Alk}^n	C $\delta^{13}\text{C}$ of DIC $\delta^{13}\text{C}_{\text{DIC}}^n$ (‰)
<i>Microbial hydrocarbon mineralization</i>				
1	$0.17 \langle \text{CH}_{1.85} \rangle + \text{FeOOH}(s) + 2 \text{H}^+ \rightarrow 0.17 \text{CO}_2 + \mathbf{Fe}^{2+} + 1.66 \text{H}_2\text{O}$	+0.17	+2	-29.9 ^c
2	$0.34 \langle \text{CH}_{1.85} \rangle + \text{MnO}_2(s) + 2 \text{H}^+ \rightarrow 0.34 \text{CO}_2 + \mathbf{Mn}^{2+} + 1.31 \text{H}_2\text{O}$	+0.34	+2	-29.9 ^c
3	$1.37 \langle \text{CH}_{1.85} \rangle + \mathbf{SO}_4^{2-} + 2 \text{H}^+ \rightarrow 1.37 \text{CO}_2 + \text{H}_2\text{S} + 1.27 \text{H}_2\text{O}$	+1.37	+2	-29.9 ^c
4	$1.37 \langle \text{CH}_{1.85} \rangle + 0.74 \text{H}_2\text{O} \rightarrow 0.37 \text{CO}_2 + \mathbf{CH}_4$	+0.37	0	+50 ^d
<i>Geochemical processes</i>				
5a	$0.67 \text{FeOOH}(s) + \text{H}_2\text{S} \rightarrow 0.67 \text{FeS}(s) + 0.33 \text{S}(0) + 1.33 \text{H}_2\text{O}$	0	0	-
5b	$\text{Fe}^{2+} + \text{H}_2\text{S} \rightarrow \text{FeS}(s) + 2 \text{H}^+$	0	-2	-
6	$\text{CaCO}_3(s) + 2 \text{H}^+ \leftrightarrow \text{CO}_2 + \mathbf{Ca}^{2+} + \text{H}_2\text{O}$	+1	+2	+0.7 ^e
7	$\text{MgCO}_3(s)^f + 2 \text{H}^+ \leftrightarrow \text{CO}_2 + \mathbf{Mg}^{2+} + \text{H}_2\text{O}$	+1	+2	+0.7 ^e
8	$\text{CO}_2 + \text{Fe}^{2+} + \text{H}_2\text{O} \leftrightarrow \text{FeCO}_3(s) + 2 \text{H}^+$	-1	-2	^g

^aAll species are given in the form in which they exist at the reference point of the alkalinity titration (pH = 4.3). Thus, the number of protons that are produced or consumed corresponds to alkalinity consumption or production. The species printed bold were used to quantify the processes.

^bMoles per mole stoichiometric turnover.

^cThe $\delta^{13}\text{C}$ of the DIC produced coupled to Fe(III)-, Mn(IV)- and SO_4^{2-} -reduction was assumed to correspond to the $\delta^{13}\text{C}$ of the hydrocarbons present in the column although there is a debate about possible isotope fractionation during SO_4^{2-} -reducing hydrocarbon mineralization (Landmeyer et al., 1996).

^dThe $\delta^{13}\text{C}$ of the DIC produced by fermentative-methanogenic hydrocarbon mineralization was calculated assuming that the hydrocarbons were completely degraded to CO_2 and CH_4 in the ratio given by the stoichiometric equation (Edwards and Grbic-Galic, 1994). The $\delta^{13}\text{C}$ of the CH_4 was assumed to be -60‰, which is typical for microbially produced CH_4 (Whiticar et al., 1986).

^eMeasured stable carbon isotope composition of carbonate in a sample of the aquifer material.

^fMay have been present as magnesium calcite or dolomite.

^gFor the stable carbon isotope balances, the $\delta^{13}\text{C}$ of the precipitated FeCO_3 was calculated for each segment of the column based on the $\delta^{13}\text{C}$ of the DIC and the isotope fractionation factor between DIC and FeCO_3 (Carothers et al., 1988). Fractionation equilibrium was assumed.

(Process 3, Table 3; Zehnder and Zinder, 1980). Methanogenesis mainly occur through two pathways, acetate fermentation and CO_2 reduction (Whiticar et al., 1986). If the hydrocarbons are completely mineralized, similar amounts of CO_2 are produced independent of the pathway (Process 4, Table 3; Grossman et al., 1989; Herczeg et al., 1991; Grossman, 1997).

4.2. Geochemical processes

In addition to microbial processes, geochemical processes that influence the concentrations of reduced species [$\text{S}(-\text{II})$, $\text{Fe}(\text{II})$, $\text{Mn}(\text{II})$] and produce or consume alkalinity

and DIC have to be included when calculating the expected DIC and alkalinity production. The geochemical processes (Processes 5 to 8, Table 3) were postulated based on saturation calculations and analysis of the solid phase as discussed in this paragraph.

Since the dissolved S(-II) concentrations were lower than expected based on the consumption of SO_4^{2-} and only about 1% of the SO_4^{2-} was recovered as S(-II) in the effluent of the column, it can be concluded that the dissolved S(-II) was removed from the water phase close to location of its generation. The removal of S(-II) from the water phase was confirmed by analyzing the distribution of the S(-II) in the solid phase (Fig. 2 B): Elevated S(-II) concentrations were mainly found in the zone of high SO_4^{2-} reduction (0–26 cm). Dissolved S(-II) can be removed from the water phase by precipitation with Fe^{2+} and Mn^{2+} (Matsunaga et al., 1993) or reaction with Fe- and Mn-oxides (Dos Santos Afonso and Stumm, 1992). Precipitation of MnS is unlikely because all samples were undersaturated with respect to MnS. Precipitation of FeS (Process 5b, Table 3) may have occurred because some samples were saturated with respect to FeS (Fig. 2C). However, the reaction of S(-II) with FeOOH (Process 5a, Table 3) can be the predominant S(-II) removing process even when the water phase is oversaturated with respect to FeS (Von Gunten and Zobrist, 1993; Furrer et al., 1996). In this study, we assumed that S(-II) was also mainly removed by reaction with FeOOH to form FeS and S(0). The column filling appeared increasingly black with time, confirming that FeS was formed. However, the recovery of S(-II) in the solid phase was only 25% of the S(-II) expected by process 5a. The remaining S(-II) may have been transformed to other S species (e.g. $\text{S}_2\text{O}_3^{2-}$, polysulfides).

At column length > 20 cm, the dissolved Fe(II) concentration may have been influenced by the precipitation of FeCO_3 (Process 8, Table 3), since samples were oversaturated with respect to FeCO_3 . The amount of FeCO_3 that had precipitated can be roughly estimated by subtracting the observed amount of S(-II) in the solid phase from the total amount of Fe(II) extracted by 0.5 M HCl (Heron et al., 1994). This results in 0.8 mmol for the zone with oversaturation with respect to FeCO_3 . Thus, in the column up to 0.8 mmol, FeCO_3 may have precipitated. In addition to precipitation of FeCO_3 , dissolution of Ca and Mg carbonate (Processes 6 and 7, Table 3) was taken into account based on the changes of concentrations of Ca(II) and Mg(II) in water samples (Fig. 1B).

4.3. DIC and alkalinity balances

The contributions of the postulated processes to DIC and alkalinity production were quantified based on average changes in concentrations of dissolved species, and the stoichiometric coefficients given in columns A and B of Table 3. For quantification of process 1, the Fe^{2+} precipitated as FeCO_3 was considered in addition to the increase of the dissolved Fe(II) concentration. It was assumed that 0.8 mmol FeCO_3 had precipitated at a constant rate in the zone of the column oversaturated with respect to FeCO_3 . The calculations rely on the assumption that the oxidants were exclusively used to mineralize hydrocarbons. Furthermore, an average H:C ratio of 1.85 was assumed, which is typical for hydrocarbons in diesel fuel (Millner et al., 1992).

The expected and observed DIC production agreed well in the first half of the column (Fig. 5A). In the second half, the observed DIC production was higher than expected. However, the deviation was within the range of uncertainty. The expected alkalinity production corresponded well to the measured alkalinity production throughout the whole column (Fig. 5B). The agreement between the expected and observed DIC and alkalinity production indicates that the postulated processes and the assumption that the oxidants were exclusively used for hydrocarbon mineralization are plausible. However, it has to be considered that the relative standard uncertainty of the observed DIC and alkalinity production is relatively large (29% and 24%, respectively). Furthermore, other

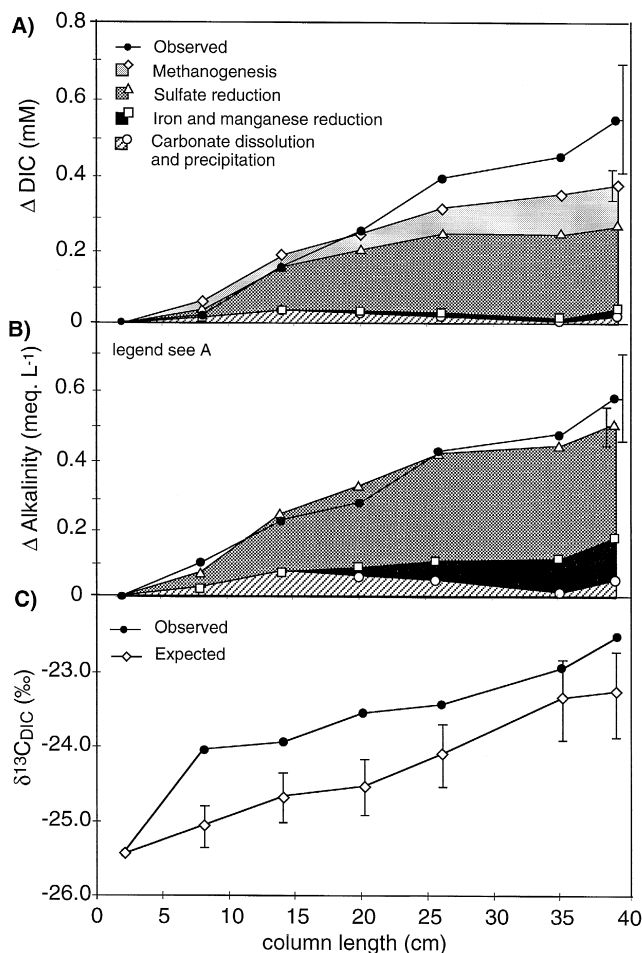


Fig. 5. (A) Expected inorganic carbon production (standard uncertainty ± 0.04 mM) and observed inorganic carbon production (standard uncertainty ± 0.16 mM). (B) Expected alkalinity production (standard uncertainty ± 0.06 meq l^{-1}) and observed alkalinity production (standard uncertainty ± 0.14 meq l^{-1}). (C) Expected and observed $\delta^{13}C$ of the dissolved inorganic carbon (DIC). The standard uncertainty of the observed $\delta^{13}C$ corresponds to the size of the marker.

processes than postulated in Table 3 may have occurred as well. For example, reduction of FeOOH by S(-II) could also have lead to the formation of $S_2O_3^{2-}$ or polysulfides in addition to S(0) and FeS (Dos Santos Afonso and Stumm, 1992; Peiffer et al., 1992).

The DIC balance (Fig. 5A) suggests that 67% of the DIC was produced by hydrocarbon mineralization coupled to SO_4^{2-} reduction, 30% coupled to methanogenesis, and 3% coupled to Fe(III) and Mn(IV) reduction. If CH_4 is considered as end product of PHC mineralization in addition to DIC, hydrocarbon mineralization coupled to SO_4^{2-} reduction and methanogenesis equally contributed to hydrocarbon mineralization. DIC production by Ca- and Mg-carbonate dissolution corresponded approximately with DIC removal by the precipitation of $FeCO_3$. The main process producing alkalinity was SO_4^{2-} reduction.

If S(-II) had been removed by precipitation with Fe^{2+} (Process 5b, Table 3) instead of reaction with FeOOH (Process 5a, Table 3), the expected alkalinity production would have been the same, since both processes are not tied to a net alkalinity production or consumption. The expected DIC production, however, would have been larger if one assumes that the reduction of Fe(III) to produce the Fe^{2+} necessary for the precipitation of the FeS was coupled to hydrocarbon mineralization. Hydrocarbon mineralization coupled to Fe(III) reduction would account for 11% instead of 3% of the expected DIC production. If S(-II) had reacted with FeOOH to form Fe(II) and $S_2O_3^{2-}$ or polysulfides (S_4^{2-} , S_5^{2-}) instead of FeS and S(0), the contribution of Fe(III) reduction to hydrocarbon mineralization would have been smaller, since the produced Fe(II) would have partly originated from an abiotic process that is not coupled to hydrocarbon mineralization.

4.4. Stable carbon isotope balance

Since the $\delta^{13}C$ of DIC is dependent on the process responsible for its production, stable carbon isotope balances of the DIC can be used to verify the postulated processes. The $\delta^{13}C$ of the expected DIC was calculated based on two mass balance equations for ^{12}C and ^{13}C (Hunkeler et al., 1997) and compared to the measured $\delta^{13}C$.

$$C_{DIC}^{k+1} = C_{DIC}^k + \sum_n \Delta C_{DIC}^n \quad (1)$$

$$C_{DIC}^{k+1} \cdot \delta^{13}C_{DIC}^{k+1} = C_{DIC}^k \cdot \delta^{13}C_{DIC}^k + \sum_n \Delta C_{DIC}^n \cdot \delta^{13}C_{DIC}^n \quad (2)$$

where C_{DIC}^{k+1} , C_{DIC}^k (mM) is the expected DIC concentration at sampling ports $k+1$ and k , respectively; $\delta^{13}C_{DIC}^{k+1}$, $\delta^{13}C_{DIC}^k$ (‰) is the expected $\delta^{13}C$ at sampling ports $k+1$ and k , respectively; ΔC_{DIC}^n (mM) is the expected DIC production by process n (Table 3) between sampling ports k and $k+1$, which was quantified as discussed above; and $\delta^{13}C_{DIC}^n$ (‰) is the $\delta^{13}C$ of DIC produced by process n (Table 3).

The calculation resulted in an increase of the $\delta^{13}C$ of the DIC by 2.2‰ between the sampling port at 2 cm and the column outlet (Fig. 5C). Except for the shift between the sampling port at 2 and 8 cm, the calculated and measured curves correspond well (Fig. 5C). The increase of the $\delta^{13}C$ was mainly caused by DIC production coupled to methanogenesis. The agreement between calculated and measured $\delta^{13}C$ in this study suggests that the sources of DIC that were both postulated and quantified based on the

turnover of oxidants and reduced species are plausible. A shift to a more positive $\delta^{13}\text{C}$ was also observed in field studies where methanogenic conditions were found (Baedecker et al., 1993; Landmeyer et al., 1996).

4.5. Carbon balance

A carbon balance was performed over the whole period of 65 days of column operation (Fig. 6), considering both mineralization of hydrocarbons to DIC and CH_4 , and elution of hydrocarbons and metabolites from the column. The total DIC production was calculated based on the observed DIC production, and was corrected for carbonate dissolution and precipitation. The elution of non-volatile metabolites from the column was assumed to be reflected in the measured DOC concentrations in the effluent. Since DOC concentrations were measured after vigorously bubbling the samples, it was assumed that DOC measurements did not reflect the elution of volatile aromatic hydrocarbons and volatile metabolites. Therefore, elution of these compounds was considered separately based on concentrations of dissolved hydrocarbons and metabolites measured in the pentane extract of the effluent.

The sum of carbon compounds in the effluent over the whole period of operation corresponded to the observed decrease of the hydrocarbon content in the aquifer material (Fig. 6). The carbon balance indicates that about 65% of the removed hydrocarbons were mineralized to DIC, 20% to CH_4 , and 15% were eluted from the column as unaltered hydrocarbons or metabolites. Thus, microbial mineralization was the predominant hydrocarbon removing process in the column, accounting for 85% of the decrease of the hydrocarbon content. In total, about 120 mg C kg^{-1} dry weight were mineralized within 65 days.

Although the consumption of oxidants, the production of DIC and the removal of hydrocarbons indicated microbial hydrocarbon mineralization, no increase of the biomass

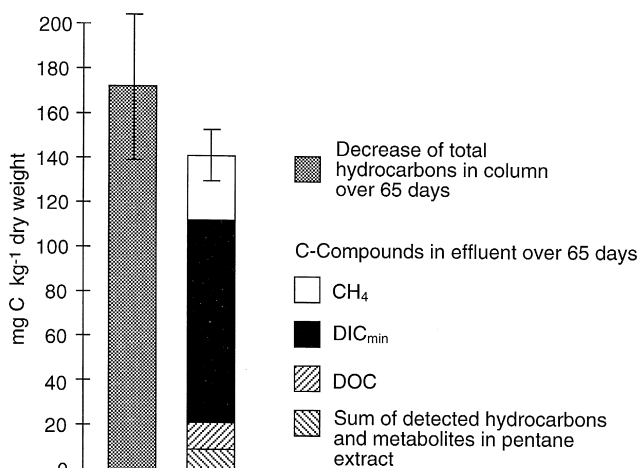


Fig. 6. Carbon balance over 65 days of column operation. DIC_{min} corresponds to the total DIC production minus the DIC produced by carbonate dissolution.

in the column was observed. The increase of the biomass may have been smaller than the range of uncertainty of the detection method, since biomass yields of anaerobic microbial cultures are usually low (Edwards et al., 1992; Edwards and Grbic-Galic, 1994).

4.6. Degradation of selected compounds

Since *n*-alkanes were removed from the aquifer material in contrast to *i*-alkanes (Fig. 3A,B, Table 1), it can be concluded that *n*-alkanes were degraded and possibly mineralized. Their transformation to dissolved metabolites is unlikely because the elution of DOC was 6 times smaller than the decrease of the *n*-alkane content in the aquifer material. Assuming that the alkanes were completely mineralized, half of the consumption of oxidants and production of reduced species can be attributed to the mineralization of *n*-alkanes.

In Fig. 4B,C,D, the ratios of the actual to the initial concentration of various mono- and di-aromatic compounds (denoted as relative concentrations) are shown. The relative concentration of ethylbenzene, *o*-xylene and *m*-xylene decreased to about 0.7 within 65 days of column operation (Fig. 4B). The relative concentration of toluene and *p*-xylene decreased to less than 0.1 within 24 days. Since toluene and *p*-xylene have similar physical-chemical properties (Table 2) as ethylbenzene, *o*-xylene, and *m*-xylene, a similar decrease of the relative concentration would be expected if all compounds were only removed by elution from the column. The much faster decrease of the relative concentration of toluene and *p*-xylene compared to the other compounds suggests that toluene and *p*-xylene were removed by microbial degradation in addition to elution from the column. The relative concentrations of the propylbenzenes, ethylbenzenes, trimethyl- and tetramethylbenzenes, given in Table 2 (Nos. 6–13), remained constant during the entire operation period. In Fig. 4C, the relative concentrations are illustrated for *n*-propylbenzene, 2-ethyltoluene, and 1,2,4-trimethylbenzene. The constant relative concentrations indicate that no significant loss of these compounds during the period of the experiment either by elution or by biodegradation. The relative concentrations of naphthalene decreased to about 0.6 while the ratio of 1- and 2-methylnaphthalene remained constant (Fig. 4D), suggesting that biodegradation of naphthalene probably did occur.

Thus, the analysis of hydrocarbon concentrations in the aquifer material and the effluent of the column indicates that, of the identified compounds, *n*-alkanes, toluene, *p*-xylene and naphthalene were degraded with lag phases of less than 65 days at observable rates. Biodegradation of these compounds in the absence of O_2 and NO_3^- was also observed in various microbial cultures, in microcosms and field studies (Lovley and Lonergan, 1990; Aeckerberg et al., 1991; Edwards et al., 1992; Edwards and Grbic-Galic, 1994; Thierrin et al., 1995; Ball and Reinhard, 1996; Beller et al., 1996; Coates et al., 1996). The elution of other compounds at constant concentrations (e.g., trimethylbenzene) or at constant ratios (e.g., ethylbenzene to *o*-xylene and *m*-xylene) in our study does not mean that these compounds are not biodegradable in the absence of O_2 and NO_3^- . To degrade these compounds, a longer incubation time may be required. In addition, the presence of easily degradable compounds may inhibit the degradation of other compounds. A sequential degradation of monoaromatic compounds (toluene,

p-xylene and *o*-xylene) under SO_4^{2-} reducing conditions was observed in a microcosm study by Edwards et al. (1992). In their study, toluene was degraded first followed by *p*-xylene and finally *o*-xylene.

4.7. Implications for bioremediation

This study demonstrates that in the absence of O_2 and NO_3^- , hydrocarbon mineralization coupled to SO_4^{2-} reduction and methanogenesis can substantially contribute to the removal of hydrocarbons from contaminated aquifer material. Hydrocarbon mineralization coupled to Fe(III) and Mn(IV) reduction was of minor importance in our study. In field cases, a larger percentage of the hydrocarbon mineralization may be coupled to Fe(III) and Mn(IV) reduction due to the longer residence time of the water in the contaminated zone. DIC, alkalinity, and stable carbon isotope balances were shown to be a useful tool to verify hydrocarbon mineralization.

In the columns, selected aromatic compounds of toxicological concern (e.g., toluene, *p*-xylene and naphthalene) and *n*-alkanes were degraded. However, some other compounds such as ethylbenzene, *m*-xylene, *o*-xylene and trimethylbenzenes were not degraded at significant rates, but eluted from the column at a constant concentration over the entire period of the experiment. If a similar degradation pattern is observed in field cases, these compounds may pollute the groundwater over a longer period of time. However, the more recalcitrant compounds may be degraded as well, once the more degradable compounds are mineralized, or they may be degraded in zones of the aquifers where aerobic or denitrifying conditions are re-established due to mixing of polluted with unpolluted groundwater (Eganhouse et al., 1996).

Acknowledgements

We thank J. McKenzie, S. Bernasconi and J. Teranes for providing the facilities for isotope analysis and for helpful discussions; A. Häner and T. Bregnard for the assistance in hydrocarbon analysis; and G.T. Townsend and two referees for reviewing the manuscript. The work was supported by the Swiss National Science Foundation (Priority Programme Environment).

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