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Engineered in situ bioremediation of a petroleum hydrocarbon-contaminated aquifer: assessment of mineralization based on alkalinity, inorganic carbon and stable carbon isotope balances

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Abstract

A concept is proposed to assess in situ petroleum hydrocarbon mineralization by combining data on oxidant consumption, production of reduced species, CH_4, alkalinity and dissolved inorganic carbon (DIC) with measurements of stable isotope ratios. The concept was applied to a diesel fuel contaminated aquifer in Menziken, Switzerland, which was treated by engineered in situ bioremediation. In the contaminated aquifer, added oxidants (O_2 and NO_3^-) were consumed, elevated concentrations of Fe(II), Mn(II), CH_4, alkalinity and DIC were detected and the DIC was generally depleted in ^{13}C compared to the background. The DIC production was larger than expected based on the consumption of dissolved oxidants and the production of reduced species. Stable carbon isotope balances revealed that the DIC production in the aquifer originated mainly from microbial petroleum hydrocarbon mineralization, and that geochemical reactions such as carbonate dissolution produced little DIC. This suggests that petroleum hydrocarbon mineraliza-

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tion can be underestimated if it is determined based on concentrations of dissolved oxidants and reduced species.

Keywords: In situ bioremediation; Petroleum hydrocarbons; Groundwater; Stable carbon isotopes

1. Introduction

In situ bioremediation is considered to be an environmentally sound and cost-effective technology because ideally petroleum hydrocarbons (designated as PHC) are mineralized and the costly excavation, transportation and disposal of contaminated material can be avoided. In PHC contaminated aquifers, microorganisms that can mineralize PHC usually are present. Engineered in situ bioremediation relies on an external supply of oxidants (O_2, H_2O_2 or NO_3^-) and nutrients (N, P) to stimulate microbial metabolism. The performance of aerobic in situ bioremediation is often limited by the insufficient availability of O_2 due to its low solubility and rapid consumption (Lee et al., 1988; Bouwer, 1992). However, within the last years, many studies have shown that the mineralization of PHC does not rely exclusively on O_2 but can also be coupled to the reduction of NO_3^-, Mn(IV), Fe(III), SO_4^{2-} and CO_2 (for a review see Holliger and Zehnder, 1996).

In Menziken, Switzerland, a diesel fuel contaminated aquifer has been remediated by injection of groundwater supplemented with oxidants (O_2, NO_3^-) and nutrients (NH_4^+, PO_4^{3-}, Hunkeler et al., 1995; Höhener et al., 1998). Microcosm studies (Bregnard et al., 1996; Bregnard et al., 1997) and enrichment culture studies (Häner et al., 1995; Häner et al., 1997) have shown that microorganisms from this aquifer were able to mineralize PHC under various conditions and that mineralization was stoichiometrically coupled to oxidant consumption. The processes in this aquifer were simulated in two independent laboratory aquifer column studies under aerobic/denitrifying (Hess et al., 1996) and anaerobic conditions (Hunkeler et al., 1998). For both column studies, a carbon mass balance was established. The decrease of the amount of residual PHC in the columns corresponded to the total elution of carbon compounds (PHC, PHC metabolites, dissolved inorganic carbon (DIC), CH_4) and the increase of microbial biomass in the column. Also, the consumption of oxidants matched stoichiometrically with the production of DIC. In addition, the formation of DIC by microbial mineralization of PHC could be verified using stable carbon isotope balances.

However, in order to demonstrate the efficacy of an in situ bioremediation, mineralization of PHC needs to be proven in situ. In heterogeneous open field systems, it is hardly possible to establish a complete mass balance of PHC and its degradation products (Madsen, 1991). Therefore, a variety of single monitoring parameters to assess PHC degradation have been proposed including concentrations of educts (PHC, oxidants) and products (reduced species, CH_4, DIC, metabolites) of PHC degradation (Heitzer and Sayler, 1993). However, the concentration of these reactants can change due to physical (e.g., dilution, gas exchange) rather than biological processes. In addition, the concentration of some of the reactants (oxidants, reduced species, DIC) may be influenced by interactions with inorganic solid phases such as the oxidation of

FeS_2 by NO_3^-, precipitation of FeS, or dissolution of $CaCO_3$ (Postma et al., 1991; Bennett et al., 1993; Stumm and Morgan, 1996). While some of the monitoring parameters only indicate biodegradation (oxidants, reduced species, metabolites), others (DIC) demonstrate that complete mineralization of the PHC took place (Heitzer and Sayler, 1993). An alternative approach to assess PHC degradation consists of measuring isotope ratios of degradation products (DIC, CH_4) rather than their concentration. The determination of carbon isotope ratios ($\delta^{13}C$, Coplen, 1996) of DIC allows one to differentiate between DIC from non-methanogenic PHC mineralization, DIC from methanogenic PHC mineralization, DIC from CH_4 oxidation and DIC produced by carbonate dissolution (Aggarwal and Hinchee, 1991; Revesz et al., 1995; Landmeyer et al., 1996; Grossman, 1997). Furthermore, microbial CH_4 can be distinguished from thermogenic CH_4 based on $\delta^{13}C$ values (Whiticar et al., 1986; Aravena et al., 1995).

In this study, concentrations of educts (oxidants) and products (reduced species, CH_4, alkalinity, DIC) of PHC mineralization and stable carbon isotope ratios are combined to assess PHC mineralization at the field scale. The approach is applied to the field case Menziken. At first, the spreading of the injected water and the chemical composition of the groundwater in the contaminated aquifer are characterized. Then, the DIC production expected based on the turnover of oxidants and reduced species is compared to the observed DIC production taking into account concentration changes due to dilution and geochemical processes. Finally, the origin of the produced DIC is verified by calculating alkalinity and stable carbon isotope balances.

2. Site description

The field site (Fig. 1) is located in Menziken, Switzerland, at 555 m above sea level and represents a typical hydrogeological situation of the perialpine belt of Switzerland. The shallow valley-fill aquifer consists of glaciofluvial outwash deposits (Fig. 2a and b). Core material showed 3–9 m of interbedded layers of poorly sorted sand and gravel. The unconfined aquifer is underlain by an aquitard, which consists of tightly packed till, and is covered by a 2–3-m thick layer of loamy sediments. The water-saturated zone exhibits a thickness of 2–8 m. The water-table is at 3–4 m below surface, fluctuates seasonally by up to 0.4 m and exhibits a hydraulic gradient of 2–3% from south to north. The hydraulic conductivity of the aquifer determined by single well pumping tests (Logan, 1964) was between 0.25 and 1.0×10^{-3} m s^{-1}.

A diesel fuel contamination caused by the leakage of a storage tank was discovered in January 1988. It has been estimated that 10,000 to 12,000 l of diesel fuel had percolated into the subsurface. After that discovery, remediation measures were enforced by the cantonal authorities. A total of 18 boreholes were drilled and diesel fuel in nonaqueous phase was found in 11 of them. The zone within which these 11 bore holes were situated is designated (ASTM, 1995) as the source area (Fig. 1). The vertical extension of the source area in drill cores was between 0.45 and 1.8 m due to the fluctuations of the water-table and capillary forces. The PHC concentrations varied between 0.9 and 2.5 g kg^{-1} in drilling cores from the source area, between 5.5 and 8.6 g kg^{-1} in samples from

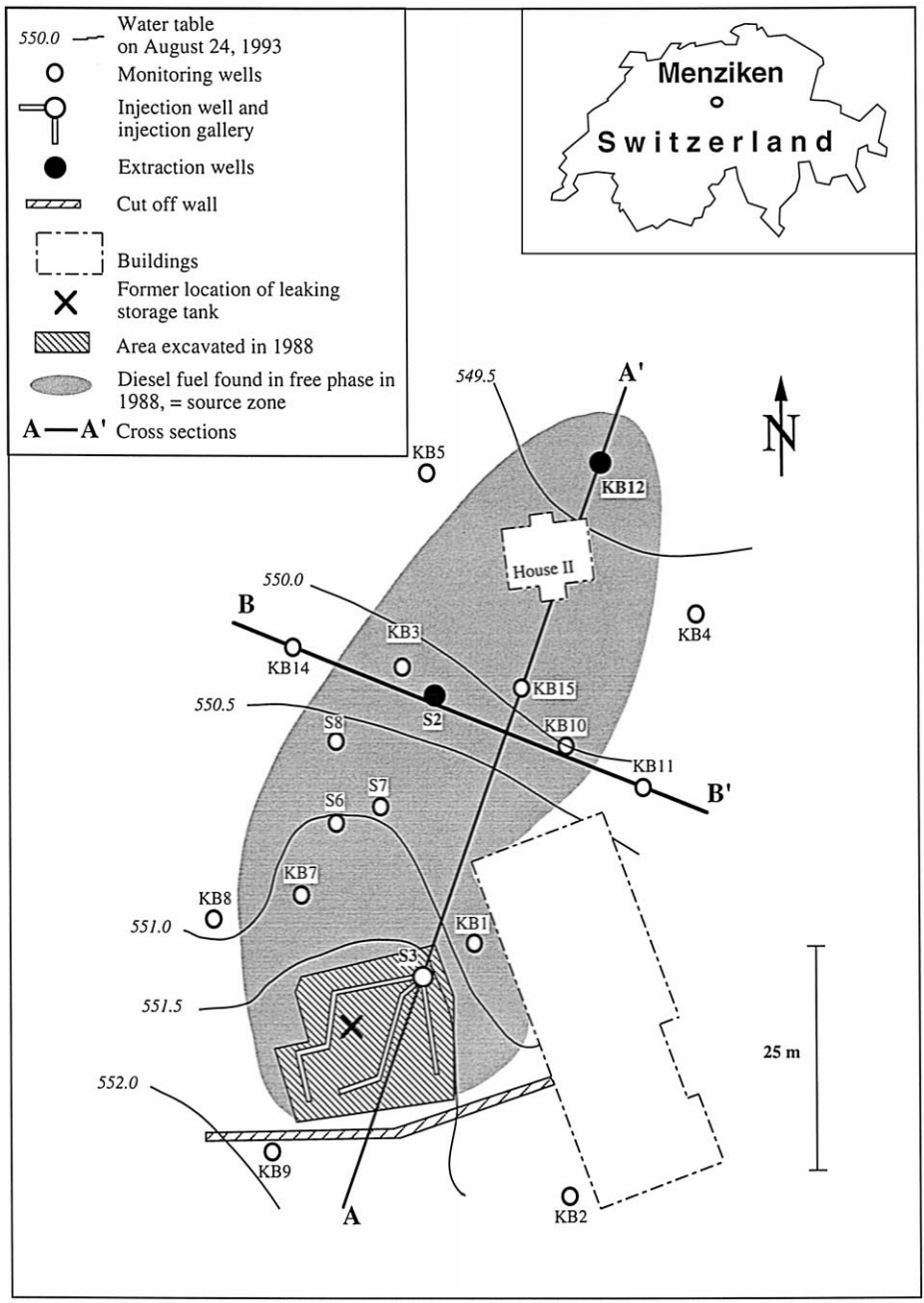

Fig. 1. Map of the study site with water-table on August 24, 1993 influenced by extraction of groundwater at a rate of 80 $m^3\ d^{-1}$ at KB12 and injection at S3 at the same rate.

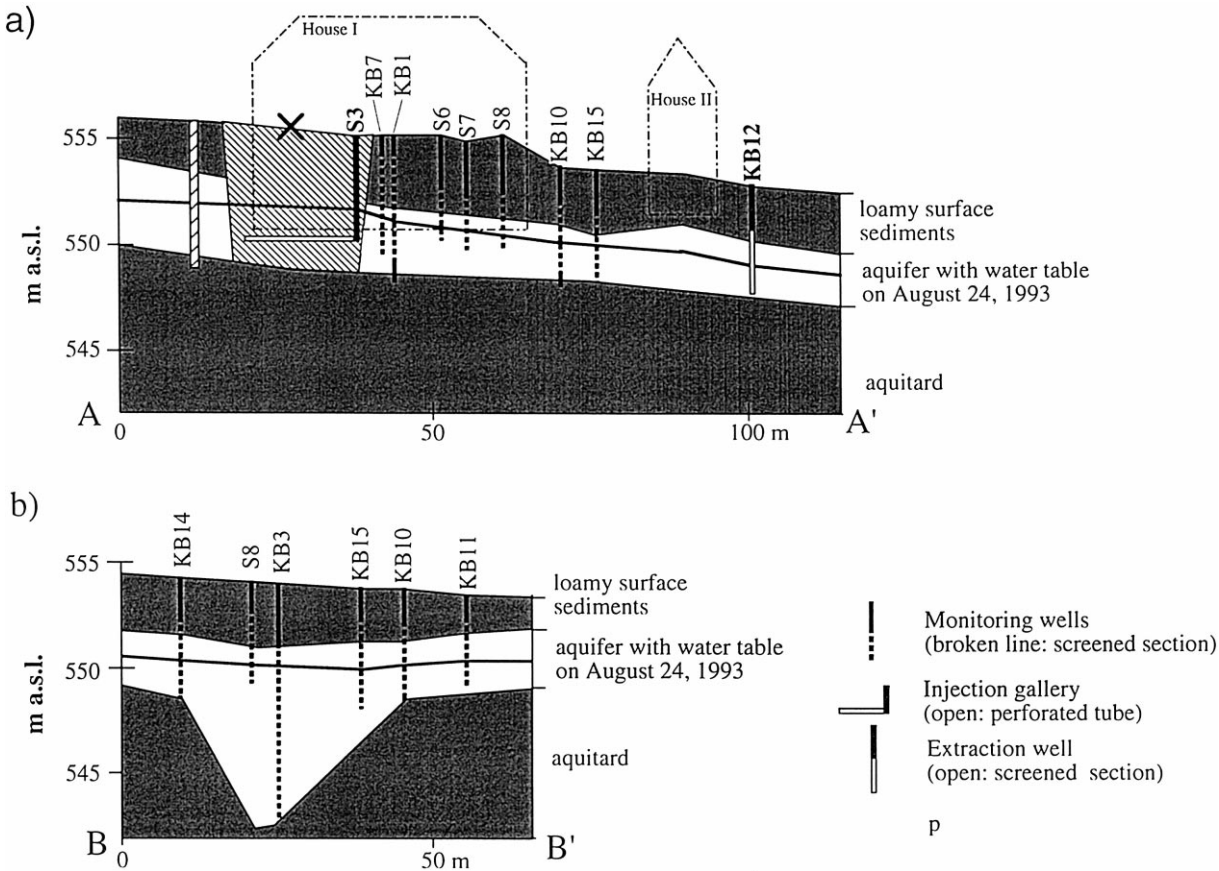

Fig. 2. Cross-sections along A–A′ and B–B′ (Fig. 1) with projected buildings and monitoring wells.

the northern boundary of the excavated zone, and was up to 72 g kg^{-1} in the vadose zone just underneath the leaking tank. The bore holes were equipped with PVC piezometer tubes (diameter 11.4 cm) that were screened in the saturated part of the aquifer (Table 1). Five of the wells (KB1, KB3, KB4, KB10 and KB14) reached to the aquitard, all others only partially penetrated the aquifer. The position and depth of the wells as well as the screen lengths had been chosen by the local authorities prior to and independently from this study.

Between January and May 1988, about 1300 l of diesel fuel were recovered by pumping in S2 and KB12. In February 1988, 250 m^3 soil and subsoil in the vicinity of the tank (Fig. 1) were excavated to a depth of 4.5 m, removing an estimated 4500 l

Table 1
Wells at Menziken[a] and results of the tracer test

	Water-table Aug. 24, 1993 (m a.s.l.)	Screen length below water-table (m)	Mean residence time of injected groundwater (d)	Fraction of injected groundwater (F)[b] (−)
Pristine groundwater				
KB2	551.21	1.03	n.d.	0.00
Source area and ≥ 70% of injected water				
S3	551.80	0.8[c]	< 0.1	1.00
S6	551.06	0.78	0.9	0.95 ± 0.03
S7	550.88	0.89	0.9	0.88 ± 0.04
S8	550.71	0.55	2.2	0.80 ± 0.13
KB1	550.94	2.21	4.4	0.75 ± 0.17
KB3	550.12	5.89	1.9	0.70 ± 0.12
KB7	551.09	1.44	4.2	0.79 ± 0.21
KB15	549.72	1.55	2.5	0.72 ± 0.18
Border zones				
KB4	549.92	1.99	3.0	0.02 ± 0.01
KB5	549.83	2.09	5.0	> 0.30
KB8	550.92	1.08	n.d.	0.00
KB9	551.87	0.94	1.7	0.80 ± 0.08
KB10	550.00	1.15	2.3	0.42 ± 0.10
KB11	550.19	1.13	2.8	0.19 ± 0.06
KB12	549.40	–	–	0.11 ± 0.01
KB14	550.29	1.43	8.3	0.33 ± 0.16

[a] Well S2 was not accessible after 1992.

[b] Determined by measuring the concentration of the constantly added Cl^- after a constant concentration had been reached in the monitoring well using:

$$F = \frac{c_{\text{tracer}} - c^{\text{pr}}_{\text{tracer}}}{c^{\text{inj}}_{\text{tracer}} - c^{\text{pr}}_{\text{tracer}}} \quad (1)$$

c_{tracer} = measured tracer concentration in monitoring well (mol l^{-1}); $c^{\text{pr}}_{\text{tracer}}$ = measured tracer concentration in pristine groundwater (KB2, mol l^{-1}); $c^{\text{inj}}_{\text{tracer}}$ = measured tracer concentration in injected groundwater (mol l^{-1}).

[c] Location of seeping pipes of injection gallery (m below water-table).

n.d. = Tracer not detected.

– = not measured.

diesel fuel. Prior to filling the excavated zone with clean gravel, an injection gallery was installed by connecting horizontal seeping pipes of a total length of 50 m to the vertical injection well S3 (Fig. 1). A cut-off wall reaching the aquitard at a depth of 6.5 m was built upgradient of the contaminated zone to reduce further spreading of the diesel fuel plume (Fig. 2a). Groundwater was pumped out of well S2 (May 1989–April 1991) or well KB12 (May 1991–February 1994 and October 1994–May 1995) at a rate of 72–115 $m^3\ d^{-1}$. This groundwater was aerated and re-injected into well S3. During the months of May to December, it was supplemented with KNO_3 (average concentration about 1.36 mM) and $NH_4H_2PO_4$ (average concentration about 0.08 mM). The upper allowed concentration limit of KNO_3 was imposed by the cantonal authorities.

3. Field and laboratory methods

3.1. Application of artificial tracers

Between January 3 and March 16 of 1995, a tracer test with Cl^- (NaCl), uranine (Na-fluorescein) and Br^- (NaBr, all from Siegfried, Zofingen, Switzerland) was carried out to determine the spreading and dilution of the groundwater that was injected at S3. The hydraulic conditions during the tracer test in 1995 were similar to those during the measurement of the chemical composition of the groundwater. The rate of groundwater injection was 86 $m^{-3}\ d^{-1}$. Uranine (50 g) and NaBr (9 kg) were dissolved in 20 l of tap water and injected within a quarter of an hour on January 3, 1995. From January 3 to March 16, NaCl was added at a constant concentration of 1.2 mM to the injected groundwater. Samples were taken at all monitoring wells every 2 to 5 h for 1 week after the beginning of the tracer test and every 2 to 7 days during the following month until tracer concentrations reached background levels again. The mean residence time of the injected groundwater between the injection gallery and the monitoring wells was obtained by calculating the time after which 50% of the mass of Br^- observed locally at each monitoring well had passed (Matthess and Ubell, 1983). The fraction of injected groundwater sampled in each monitoring well was calculated (Table 1) based on the measured Cl^- concentrations.

3.2. Groundwater sampling techniques

Groundwater samples were collected using Whale Superline 991 downhole submersible electrical pumps (Munster Simms Engineering, Bangor, Northern Ireland) with FEP tubing (Maagtechnic, Dübendorf, Switzerland). At least 1.5 well volumes were pumped before sampling. The pre-pumping volume was determined by analyzing chemical species as a function of pre-pumping time and/or by continuously monitoring temperature, pH, O_2 and electric conductivity in a flow cell connected to the pump. The flow cell was equipped with electrodes which allowed simultaneous measurement of temperature, pH (WTW pH 95), O_2 (Clark type electrode, WTW Oxi 96) and conductivity (WTW LF 92, all from WTW, Weilheim, Germany). The groundwater samples showed no visible turbidity. Samples for analysis of dissolved species were filtered in the field immediately after sampling, using 0.22 μm polyvinylidenefluoride filters

(Millipore, Bedford, MA). Samples for the analysis of cations were acidified with 0.1% distilled concentrated HNO_3, samples for S(-II) analysis were fixed using zinc acetate solution (APHA, 1989). For alkalinity determinations and gas analysis, 117 ml serum bottles were filled with unfiltered samples and closed without head space using butyl rubber stoppers. Within 6 h after sampling, a 20-ml head space was introduced along with removal of 20 ml of liquid by N_2. The removed liquid was used for alkalinity determination. In the resulting head space, gases were measured after shaking followed by 12 h of equilibration at 7°C (Bossard et al., 1981). Alkalinity was also determined in some filtered samples. No significant differences in the alkalinity between filtered and unfiltered samples was observed. For stable carbon isotope analysis of the DIC, 1-l glass bottles were filled with unfiltered groundwater and closed without head space using rubber stoppers. Within 6 h after sampling, the DIC was precipitated as $BaCO_3$ by adding 10 ml of a CO_2-free NaOH (2 M) and 40 ml of a CO_2-free $BaCl_2$ solution (1.2 M). After more than 12 h of equilibration, the precipitate was filtered under a stream of N_2 to avoid changes of the sample due to contamination with atmospheric CO_2 and dried at 105°C during 12 h. For stable carbon isotope analysis of the CH_4, 1-l glass bottles were filled with unfiltered groundwater and closed without head space using rubber stoppers. Within 6 h after sampling, 20 ml of He was added, displacing water. CO_2 was removed from the head space by raising the pH of the water to 12 using 10 M NaOH. CH_4 analyses are described below.

3.3. Chemical analysis

Concentrations of the anions NO_3^-, SO_4^{2-}, Cl^- and Br^- and the cations Na^+, K^+, Mg^{2+} and Ca^{2+} were determined with a Dionex DX-100 ion chromatograph (Dionex, Sunnyvale, USA). Dissolved Fe and Mn were quantified by atomic absorption spectroscopy in an air-acetylene flame (Varian SpectrAA 400, Varian Techtron, Springvale, Australia). It was assumed that the dissolved Fe and Mn consisted mainly of Fe(II) and Mn(II) because the solubility of Fe(III) is low at $pH > 6$ (Stumm and Morgan, 1996). The terms Fe(II) and Mn(II), respectively, correspond to the sum of all dissolved species with the redox state II. S(-II) represents the sum of H_2S, HS^- and S^{2-} and was determined colorimetrically (APHA, 1989). Within 12 h after sampling, NH_4^+ and NO_2^- were determined colorimetrically according to standard methods (APHA, 1989), and alkalinity was measured by potentiometric titration using Gran plots for graphical determination of the end point (Stumm and Morgan, 1996). CO_2, N_2O, CH_4 and C_2H_6 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, Micromass, Rodano, I) on a HayeSep D column using N_2 as carrier and a Carlo Erba thermal conductivity detector. Concentrations of dissolved gases were calculated according to Bossard et al. (1981), based on Henry's law using the following Henry constants which are corrected for 7°C: CO_2: 0.0594 mol l^{-1} atm^{-1} (Stumm and Morgan, 1996); N_2O: 0.0449 mol l^{-1} atm^{-1} (Weiss and Price, 1980); CH_4: 0.00206 mol l^{-1} atm^{-1} (Yamamoto et al., 1976); C_2H_6: 0.0032 mol l^{-1} atm^{-1} (Schwarzenbach et al., 1993). DIC concentrations were calculated from alkalinity and pH (Stumm and Morgan, 1996). Calculation of DIC using the partial pressure of CO_2 instead of pH and

quantifications using the yield of $BaCO_3$ in some precipitated samples gave values with less than 7% deviation. Charge balances were calculated to assure the accuracy of the analyses. The deviation between the sums of negative and positive charges was always less than 4%. Detection limits of the analyses are given in Table 2.

3.4. Stable carbon isotope analysis

All measured $^{13}C/^{12}C$ ratios are reported in the delta notation ($\delta^{13}C$) referenced to the VPDB standard (Coplen, 1996). The $\delta^{13}C$ value is defined as:

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R_{sample} and $R_{standard}$ are the carbon isotope ratios in the sample and the standard, respectively. For stable carbon isotope analysis of the DIC, the dried $BaCO_3$ was converted to CO_2 at 90°C in an automated acid bath preparation system using H_3PO_4 and was measured on a Micromass-Prism isotope-ratio mass spectrometer (Micromass, Middlewich, Cheshire, UK). The analytical reproducibility was $< 0.2‰$. For stable carbon isotope analysis of the carbonates in the aquifer material, a sample of aquifer material was ground and the $\delta^{13}C$ was determined identically as that of the precipitated $BaCO_3$. The $\delta^{13}C$ of the PHC was measured in a sample extracted with CCl_2H_2 from contaminated aquifer material. The solvent was evaporated to constant weight. About 10 μl of the extract were combusted in an evacuated quartz tube at 950°C for 3 h using 1 g CuO as an oxidant. The produced CO_2 was cryogenically purified on a vacuum line and analyzed with a Micromass Optima isotope-ratio mass spectrometer. For determination of the $\delta^{13}C$ of the CH_4, the head space gas of the 1-l glass bottles was removed with a syringe and injected into a vacuum line containing CuO heated to 850°C to oxidize the CH_4 to CO_2. After 30 min reaction time, the produced CO_2 was cryogenically purified and the $\delta^{13}C$ was measured with a Micromass Optima isotope-ratio mass spectrometer (Micromass). To determine the reproducibility of the measurement, CH_4 was added at different concentrations to 1-l glass bottles filled with tap water except for a 20-ml head space of He. The standard deviation of the $\delta^{13}C$ measurements performed as described above was 1.5‰ ($n = 6$).

3.5. Saturation calculations

Saturation calculations were performed with MICROQL (Westall, 1986) using stability constants from Matsunaga et al. (1993) and Stumm and Morgan (1996), taking into account the formation of complexes. The stability constants were corrected for temperature and ionic strength using the Güntelberg approximation (Stumm and Morgan, 1996).

3.6. Uncertainties

The reported uncertainties of measured values are standard uncertainties. Uncertainties on calculated values were estimated using the law of propagation of uncertainty (Taylor, 1997).

Table 2
Water temperature and groundwater chemistry of the Menziken site during in situ bioremediation on August 24, 1993

	Temp. (°C)	pH	Alk (meq l^{-1})	DIC (mM)	O_2 (mM)	NO_3^- (mM)	NO_2^- (mM)	N_2O (mM)	NH_4^+ (mM)	SO_4^{2-} (mM)	Fe(II) (mM)	Mn(II) (mM)	CH_4 (mM)	Cl^- (mM)	PO_4^{3-} (mM)	Na^+ (mM)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	$\delta^{13}C_{DIC}$ (‰)	$\delta^{13}C_{CH_4}$ (‰)[a]
Pristine groundwater																					
KB2	11.5	7.15	5.83	6.88	0.191	0.30	< d.l.	< d.l.	0.004	0.140	< d.l.	< d.l.	< d.l.	0.259	0.001	0.40	0.04	0.58	2.45	− 12.1	–
Source area and ≥ 70% injected groundwater[b]																					
S3	12.3	7.87	5.78	5.95	0.306	1.04	0.001	< d.l.	0.095	0.137	0.002	< d.l.	< d.l.	0.254	0.064	0.58	0.85	0.61	2.23	− 11.7	–
S6	13.6	7.10	6.68	7.97	0.031	0.09	< d.l.	< d.l.	0.026	0.094	0.020	0.004	0.297	0.221	0.018	0.50	0.58	0.59	2.35	− 16.2	–
S7	13.7	6.98	7.18	9.01	0.025	< d.l.	< d.l.	< d.l.	0.018	0.110	0.020	0.005	0.196	0.233	0.028	0.50	0.61	0.62	2.51	− 21.2	–
S8	13.6	6.99	7.65	9.55	0.034	0.06	0.001	< d.l.	0.035	0.057	0.065	0.007	0.184	0.205	0.025	0.54	0.54	0.66	2.59	− 16.2	− 50.0
KB1	13.9	7.20	6.81	7.85	0.031	0.04	< d.l.	< d.l.	0.038	0.059	0.052	0.004	0.607	0.258	0.013	0.45	0.64	0.59	2.28	− 9.4	− 68.0
KB7	14.2	7.19	6.80	7.85	0.025	0.10	0.002	< d.l.	0.021	0.103	0.018	0.004	0.363	0.254	0.007	0.50	0.68	0.62	2.44	− 15.0	− 58.0
KB15	13.3	6.96	7.37	9.35	0.025	< d.l.	< d.l.	< d.l.	0.031	0.072	0.070	0.015	0.261	0.255	0.014	0.52	0.57	0.58	2.55	− 18.1	–
Border zones																					
KB4	12.3	7.18	5.83	6.79	0.194	0.26	< d.l.	< d.l.	0.013	0.125	< d.l.	< d.l.	< d.l.	0.228	0.001	0.55	0.08	0.72	2.15	− 12.3	–
KB5	12.8	7.29	5.89	6.63	0.038	0.68	< d.l.	< d.l.	< d.l.	0.133	< d.l.	< d.l.	< d.l.	0.258	0.017	0.47	0.45	0.58	2.43	− 12.5	–
KB8	11.3	7.13	5.83	6.93	0.122	0.58	< d.l.	< d.l.	< d.l.	0.160	< d.l.	< d.l.	0.007	0.303	0.012	0.48	0.27	0.49	2.65	− 13.5	–
KB9	13.6	7.54	5.73	6.12	0.141	1.07	< d.l.	< d.l.	< d.l.	0.135	< d.l.	< d.l.	< d.l.	0.255	0.049	0.48	0.83	0.59	2.35	− 12.4	–
KB10	13.4	6.99	6.78	8.47	0.053	0.04	< d.l.	< d.l.	0.002	0.090	0.009	0.002	0.007	0.185	0.002	0.42	0.38	0.50	2.61	− 22.1	–
KB11	12.1	7.17	5.63	6.58	0.163	0.30	< d.l.	< d.l.	0.001	0.134	0.007	0.002	< d.l.	0.241	0.002	0.48	0.07	0.62	2.34	− 12.3	–
KB12	11.9	7.17	5.83	6.82	0.097	0.36	< d.l.	0.005	< d.l.	0.140	0.002	< d.l.	< d.l.	0.267	0.001	0.49	0.18	0.59	2.43	− 12.9	–
KB14	12.8	7.01	6.78	8.42	0.025	0.27	< d.l.	< d.l.	0.001	0.103	0.003	0.010	0.083	0.259	0.005	0.53	0.41	0.60	2.64	− 14.9	–
Detection limit			0.010	0.010	0.004	0.002	0.001	0.001	0.001	0.002	0.002	0.002	0.001	0.002	0.001	0.003	0.003	0.003	0.003		

[a]Measured on December 12, 1993.
[b]KB3 was not taken into account since the screen length is much longer than that of the other monitoring wells.
< d.l. = Below detection limit.
– = not determined.

4. Results and discussion

4.1. Spreading and dilution of the injected groundwater

The mean residence time of the injected groundwater in the aquifer before reaching the monitoring wells and the fraction of injected groundwater that was found at each monitoring well are given in Table 1. Monitoring wells S6 and S7 were reached first by the injected groundwater after an average residence time in the aquifer of 0.9 days. This corresponds to mean flow velocities of 22 m d^{-1}. The mean residence time of the injected groundwater between the injection gallery and the monitoring wells of the cross-section B–B′ (Figs. 1 and 2b) was between 1.9 and 2.8 days with the exception of KB14. Although monitoring wells KB1 and KB7 are close to the injection gallery, the injected groundwater passed these wells only after 4.2 and 4.4 days, respectively. At KB1, the flow was influenced by the foundation of house I and the cut-off wall (Fig. 1). KB7 may have been influenced by a local zone of low permeability. Surprisingly, the tracers were also detected at KB9, indicating that the cut-off wall was permeable somewhere near this well.

The average fraction of injected groundwater recovered in the monitoring wells generally decreased with increasing residence time in the source area (Table 1). This decrease was probably due to dilution of injected groundwater with surrounding groundwater during the flow of the water through the aquifer. Part of the dilution may also have been caused by mixing of groundwater from different depths during sampling. Except for KB10 and KB12, all monitoring wells within the source area received at least 70% of injected groundwater. Thus, the plume of injected groundwater corresponded well to the source area. However, only about 11% of the injected groundwater was recovered in the extraction well KB12.

Based on the location of the source area and the results of the tracer test, the monitoring wells at Menziken were attributed to one of the following three groups (Table 1):

1. pristine groundwater upgradient: neither tracer nor PHC detected: KB2
2. source area and $\geq 70\%$ of injected groundwater: S3 (injection gallery) and wells S6, S7, S8, KB1, KB3, KB7 and KB15
3. border zones: all other wells.

4.2. Groundwater chemistry

The results of the chemical and isotopical analyses of dissolved groundwater components are given in Table 2. Pristine groundwater from upstream of the source area (KB2) was aerobic (O_2 saturation = 59%) and contained NO_3^- and SO_4^{2-}, indicating that oxidant consumption due to natural organic matter or reduced solid phases is low in this aquifer. In the monitoring wells within the source area, concentrations of O_2 and NO_3^- were low, and dissolved Fe(II), dissolved Mn(II) and CH_4 were detected indicating the activity of aerobic and anaerobic microorganisms. The alkalinity and the DIC concentrations were elevated in all monitoring wells within the source area. Except for KB1, the $\delta^{13}C$ of the DIC decreased to values below those of the DIC in the injected groundwater

(−11.7‰) and in the groundwater upgradient of the source area (−12.1‰). At KB1, the $\delta^{13}C$ of the DIC was more positive than in the injected groundwater. The $\delta^{13}C$ of the CH_4 determined in samples taken on December 12, 1993 was −68‰ at KB1, −58‰ at KB7 and −50‰ at S8. These values are typical for microbially produced CH_4 (Whiticar et al., 1986).

In monitoring wells in the border zones, generally higher concentration of O_2 and NO_3^- were found than within the source area. The SO_4^{2-} concentration, the DIC concentration and the $\delta^{13}C$ of the DIC corresponded to that found upgradient of the source area (KB2) except for KB10 and KB14.

Since the main goal of this study was to assess the biodegradation of the residual diesel fuel in this aquifer, the following paragraphs focus on the microbial and geochemical processes in the source area. Processes in the border zones are not discussed in detail.

4.3. Consumption of oxidants and production of reduced species in source area

The concentrations of oxidants and other parameters measured on August 24, 1993 and the average Cl^- concentration determined during the tracer test are plotted vs. the mean residence time of the injected groundwater in Fig. 3. In addition, the expected concentration assuming only dilution of the injected groundwater with pristine groundwater having a chemical composition observed at KB2 are shown. This allows a distinction between concentration changes due to dilution and concentration changes caused by reactive processes. The concentrations of the added oxidants (O_2, NO_3^-) decreased by more than 95% within less than 0.9 days residence time (Fig. 3). The decrease was much quicker than expected taking into account only dilution which indicates consumption of the added oxidants. Degassing of O_2 is unlikely since the injected groundwater was not oversaturated with respect to O_2. Since the decrease of NO_3^- concentrations was far larger than increases in NH_4^+, NO_2^- and N_2O concentrations, it can be concluded that most of the NO_3^- was reduced to other N-compounds, probably N_2. Sulfate concentrations decreased to a minimal concentration of 0.057 mM, which is 40% of the concentration in pristine groundwater. Although SO_4^{2-} concentrations decreased, concentrations of S(-II), the product of microbial SO_4^{2-}-reduction (Zehnder and Zinder, 1980), were below detection limit. The S(-II) may have precipitated with Fe(II) or reacted with Fe oxides (Von Gunten and Zobrist, 1993; Furrer et al., 1996). Dissolved Fe(II) and Mn(II) probably originated from microbial processes since the rate of microbial Fe- and Mn-reduction usually exceeds that of the corresponding abiotic processes (Stone and Morgan, 1984; Lovley et al., 1991). However, abiotic processes that consume Fe(II) and Mn(II) may have influenced the concentrations of dissolved Fe(II) and Mn(II) as discussed below. The highest methane concentrations were observed at KB1 where the longest residence time of the injected groundwater was observed.

4.4. Geochemical equilibria in the source area

Saturation of groundwater samples with respect to various minerals were calculated for groundwater samples from the source area in order to identify geochemical reactions

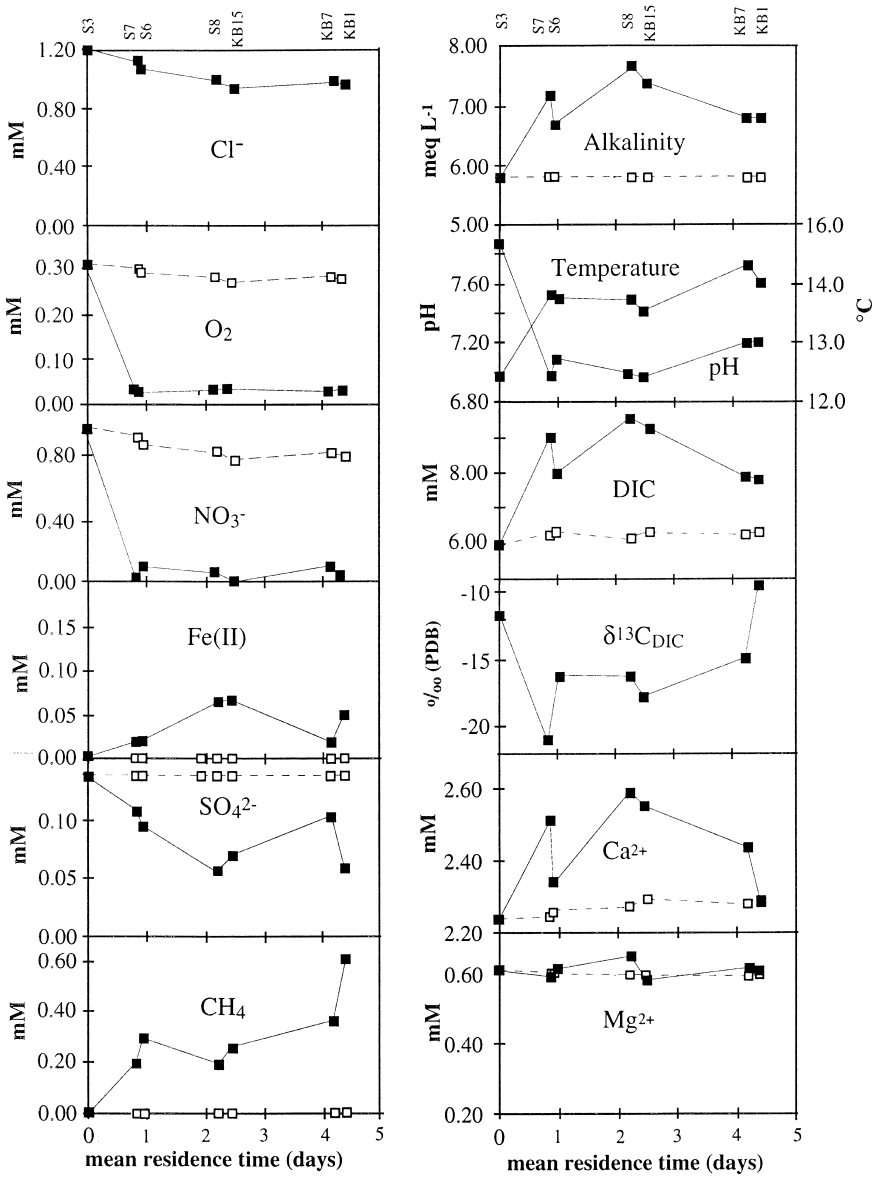

Fig. 3. Chemistry and temperature of groundwater samples in monitoring wells in the source zone on August 24, 1993 and average Cl^- concentration during the tracer test vs. the mean residence time of the injected water. The solid line connects measured values (■), the broken line hypothetical concentrations (□) considering only dilution, calculated using:

$$\hat{c}_i = F \times c_i^{\text{inj}} + (1 - F) \times c_i^{\text{pr}} \quad (2)$$

$\hat{c}_i$ = hypothetical concentration (mol l^{-1}) of i considering only dilution; c_i^{inj} = measured concentration (mol l^{-1}) of i in injected groundwater; c_i^{pr} = measured concentration (mol l^{-1}) of i in pristine groundwater upgradient; F = fraction of injected groundwater withdrawn (Table 1).

which may change the concentrations of DIC, Fe(II), Mn(II) and S(-II). According to Matsunaga et al. (1993), $FeCO_3$ (siderite), amorphous FeS, and $MnCO_3$ (rhodochrosite) preferentially precipitate in anaerobic aquifers. The formation of MnS is less likely since the solubility of MnS is much higher than the solubility of amorphous FeS (Matsunaga et al., 1993). In a laboratory column study, Von Gunten and Zobrist (1993) found that the reaction of S(-II) with FeOOH is the dominant process removing S(-II) from the aqueous phase even if the water is oversaturated with respect to amorphous FeS. In addition to these minerals, $CaCO_3$ (calcite) and $MgCa(CO_3)_2$ were taken into consideration since water samples contained Ca^{2+} and Mg^{2+} and the aquifer material was rich in carbonate (Bregnard et al., 1996). The results of the saturation calculations are given in Fig. 4. Because S(-II) concentrations were below the detection limit, it was not possible to calculate the saturation index of amorphous FeS.

Samples of the two monitoring wells reached first by the injected groundwater were undersaturated with respect to $FeCO_3$, and samples of some of the monitoring wells reached later were slightly oversaturated (KB1). Samples of all monitoring wells were

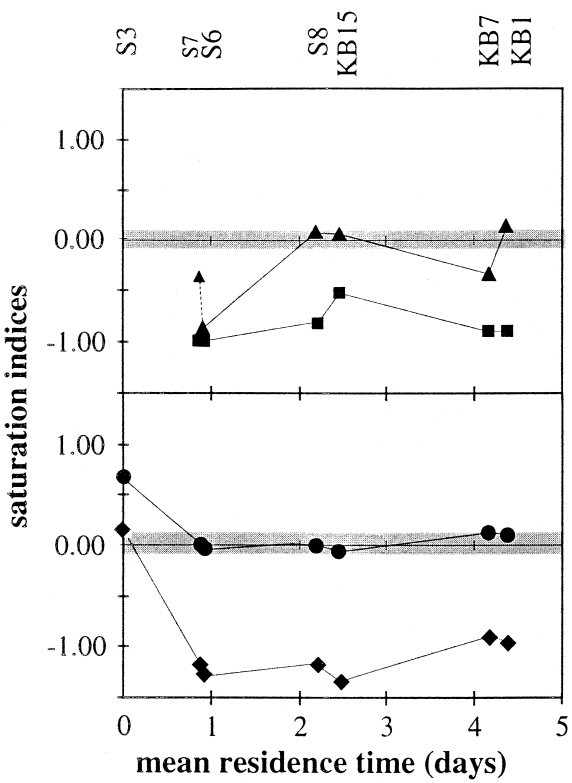

Fig. 4. Saturation indices (= log[ion product/solubility product]) of $FeCO_3$ (siderite, ▲), $MnCO_3$ (rhodochrosite, ■), $CaCO_3$ (calcite, ●) and $MgCa(CO_3)_2$ (dolomite, ◆). Positive values indicate oversaturation, negative value undersaturation. The hatched area corresponds to the range of uncertainty.

undersaturated with respect to $MnCO_3$ and $CaMg(CO_3)_2$ (dolomite) and saturated with respect to $CaCO_3$ (calcite). The injected groundwater was supersaturated with respect to $CaCO_3$ (calcite) and $CaMg(CO_3)_2$. The reason for the supersaturation probably was degassing of CO_2 during aeration of the injected groundwater. Aeration resulted in an increase of the pH value and a concomitant increase in the concentration of CO_3^{2-}.

4.5. Predominant microbial and geochemical processes in the source area

In order to be able to calculate the expected DIC production based on the consumption of oxidants and production of reduced species, microbial and geochemical processes were postulated (Table 3). The scheme of processes had been successfully applied for mass balances in laboratory aquifer columns and microcosms (Bregnard et al., 1996; Hess et al., 1996; Hunkeler et al., 1998). The processes were selected based on the observed turnover of oxidants and reduced species and the saturation calculations. The following assumptions were made concerning the microbial and geochemical processes: (i) The H/C ratio of mineralized PHC corresponded to the average H/C ratio in diesel fuel which is 1.85 (Millner et al., 1992); (ii) Fe(II) and Mn(II) were generated by microbial reduction of Fe and Mn oxides; (iii) both major pathways of methanogenesis, via acetate fermentation and reduction of CO_2 by H_2, can be represented by the same net stoichiometric equation since the net productions of CO_2 are the same in both cases if the PHC are completely mineralized (Grossman et al., 1989; Herczeg et al., 1991; Grossman, 1997); (iv) the production of microbial biomass and the consumption of NO_3^- to incorporate N in microbial biomass can be neglected; and (v) S(-II) was mainly removed by reaction with FeOOH as observed in column studies by Von Gunten and Zobrist (1993).

4.6. Stoichiometric coupling of DIC production to microbial oxidations of PHC

In order to verify that the oxidants were used to mineralize PHC, the DIC production expected based on the consumption of oxidants and production of reduced species was calculated for the flow paths between S3 and the monitoring wells in the source area. The expected DIC production was compared to the observed DIC production. To simplify the calculations, the following effects were not included: (i) variation of processes with time, since temporal variation at the site occur at a seasonal scale whereas the mean residence times of the groundwater between the injection gallery and the monitoring wells taken into consideration is < 4.4 days; (ii) oxidation of other compounds than PHC, since oxidized conditions upstream of the contaminated zone indicate that other electron donors are lacking in this aquifer, and (iii) gas exchange between the saturated and the unsaturated zone, since the residence time of the groundwater between the injection gallery and the monitoring wells is short. The consequences of simplifications (i) and (ii) on the balances can be considered as minor for the reasons given above, while the effect of simplification (iii) must be taken into account and is discussed further down in this text.

The concentration difference Δc_i, which reflects either consumption of oxidants, the production of reduced species or production of DIC, was calculated by subtracting the

Table 3
Stoichiometries of selected processes involved in hydrocarbon mineralization, contributions to DIC, contributions to alkalinity and stable carbon isotope ratio ($\delta^{13}C$) of produced DIC

Process (n)		Contribution to DIC[a] (ν^n_{DIC})	Contribution to alkalinity[a] (ν^n_{Alk})	$\delta^{13}C$ of DIC ($\delta^{13}C^n_{DIC}$ (‰))
Microbial hydrocarbon mineralization[b]				
$0.68\ \langle CH_{1.85}\rangle + \mathbf{O_2}$	$\rightarrow\ 0.68\ CO_2 + 0.63\ H_2O$	+0.68	0	−29.7[c]
$0.85\ \langle CH_{1.85}\rangle + \mathbf{NO_3^-} + H^+$	$\rightarrow\ 0.85\ CO_2 + 0.5\ N_2 + 1.29\ H_2O$	+0.85	+1	−29.7[c]
$0.34\ \langle CH_{1.85}\rangle + MnO_{2(s)} + 2H^+$	$\rightarrow\ 0.34\ CO_2 + \mathbf{Mn^{2+}} + 1.31\ H_2O$	+0.34	+2	−29.7[c]
$0.17\ \langle CH_{1.85}\rangle + FeOOH_{(s)} + 2H^+$	$\rightarrow\ 0.17\ CO_2 + \mathbf{Fe^{2+}} + 1.66\ H_2O$	+0.17	+2	−29.7[c]
$1.37\ \langle CH_{1.85}\rangle + \mathbf{SO_4^{2-}} + 2H^+$	$\rightarrow\ 1.37\ CO_2 + H_2S + 1.26\ H_2O$	+1.37	+2	−29.7[c]
$1.37\ \langle CH_{1.85}\rangle + 0.74\ H_2O$	$\rightarrow\ 0.37\ CO_2 + \mathbf{CH_4}$	+0.37	0	+38[d]
Geochemical processes				
$CaCO_3(s) + 2H^+$	$\leftrightarrow\ CO_2 + \mathbf{Ca^{2+}} + H_2O$	+1	+2	+0.7[e]
$2/3\ FeOOH_{(s)} + H_2S$	$\rightarrow\ 2/3\ FeS_{(s)} + 1/3\ S(0) + 4/3\ H_2O$	0	0	–[f]
$CO_2 + Fe^{2+} + H_2O$	$\leftrightarrow\ FeCO_{3(s)} + 2H^+$	−1	−2	

[a] Moles per mole stoichiometric turnover.
[b] From data of Millner et al. (1992), it was calculated that hydrocarbons in diesel fuels have an average H/C ratio of 1.85 (denoted as $\langle CH_{1.85}\rangle$). All 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.
[c] Measured $\delta^{13}C$ of residual hydrocarbons from Menziken.
[d] Methanogenic PHC mineralization is reported to enrich $\delta^{13}C$ of DIC to up to +38‰ (Grossman, 1997).
[e] Measured $\delta^{13}C$ of carbonate minerals in a sample from the contaminated aquifer. Value is only valid if $CaCO_3$ is dissolving.
[f] Depends on $\delta^{13}C$ of the precipitating DIC and fractionation factor between the DIC and the $FeCO_3$ (Deines, 1980).

expected concentration if only dilution would take place ($\hat{c}_i$, see Fig. 3) from the measured concentration c_i:

$$\Delta c_i = c_i - \hat{c}_i. \tag{3}$$

The expected production of DIC ($\Delta c_{\mathrm{DIC}}^{\mathrm{exp}}$) due to microbial PHC mineralization was then calculated by multiplying the consumption of oxidants or production of reduced species with the stoichiometric factor ν_{DIC}^{n} in Table 3. The contributions of all processes were added:

$$\Delta c_{\mathrm{DIC}}^{\mathrm{exp}} = \sum_n \Delta c_{\mathrm{DIC}}^{n} = \sum_n \nu_{\mathrm{DIC}}^{n} |\Delta c_i| \tag{4}$$

$\Delta c_{\mathrm{DIC}}^{n}$ (mol l^{-1}): DIC production by process n (Table 3); ν_{DIC}^{n} (−): contribution of process n per stoichiometric turnover (Table 3).

The observed DIC production ($\Delta c_{\mathrm{DIC}}^{\mathrm{obs}}$) was calculated by subtracting the DIC production by $CaCO_3$ dissolution from the total DIC production. The DIC production due to carbonate dissolution was quantified based on the Ca^{2+} and Mg^{2+} concentrations.

$$\Delta c_{\mathrm{DIC}}^{\mathrm{obs}} = c_{\mathrm{DIC}} - \hat{c}_{\mathrm{DIC}} - \Delta c_{\mathrm{DIC}}^{\mathrm{carb}} = c_{\mathrm{DIC}} - \left[F \times c_{\mathrm{DIC}}^{\mathrm{inj}} + (1 - F) \times c_{\mathrm{DIC}}^{\mathrm{pr}} \right] - \Delta c_{\mathrm{DIC}}^{\mathrm{carb}} \tag{5}$$

$\Delta c_{\mathrm{DIC}}^{\mathrm{obs}}$ (mol l^{-1}): observed DIC production; $\Delta c_{\mathrm{DIC}}^{\mathrm{carb}}$ (mol l^{-1}): DIC production due to carbonate dissolution (Table 3).

Expected and observed DIC productions are illustrated for two selected flow paths, S3–S6 and S3–KB1, representing mean groundwater residence times of 0.9 and 4.4 days, respectively (Fig. 5). The expected production of DIC is significantly smaller (by 45% for S3–S6 and by 30% for S3–KB1, respectively) than the observed production of DIC for both flow paths (Fig. 5). A similar discrepancy was also found for all other monitoring wells. There are several explanations for the discrepancy between observed and expected DIC production, among them: (1) PHC mineralization was underestimated based on concentrations of dissolved oxidants and reduced species because reduced species (Fe(II), Mn(II)) adsorbed to surfaces or precipitated, (2) dissolution of carbonates was underestimated, or (3) gas exchange with the unsaturated zone cannot be neglected since CH_4 was lost by degassing. The third reason may have especially contributed to the discrepancy at the flow path S3–KB1 because the highest CH_4 concentration and longest mean residence time of the injected groundwater was found for that flow path. Degassing of significant quantities of CH_4 is more likely than degassing of CO_2 because the partial pressure of CH_4 in some wells was up to 20 times larger than the partial pressure of CO_2. If CH_4 is lost by degassing, PHC mineralization coupled to methanogenesis is underestimated if it is quantified based on CH_4 concentrations in the aqueous phase.

A larger production of DIC than expected based on the turnover of measured oxidants and reduced species was also observed in other studies of aquifers polluted with organic compounds (Herczeg et al., 1991; Bennett et al., 1993). In the study by Herczeg et al.

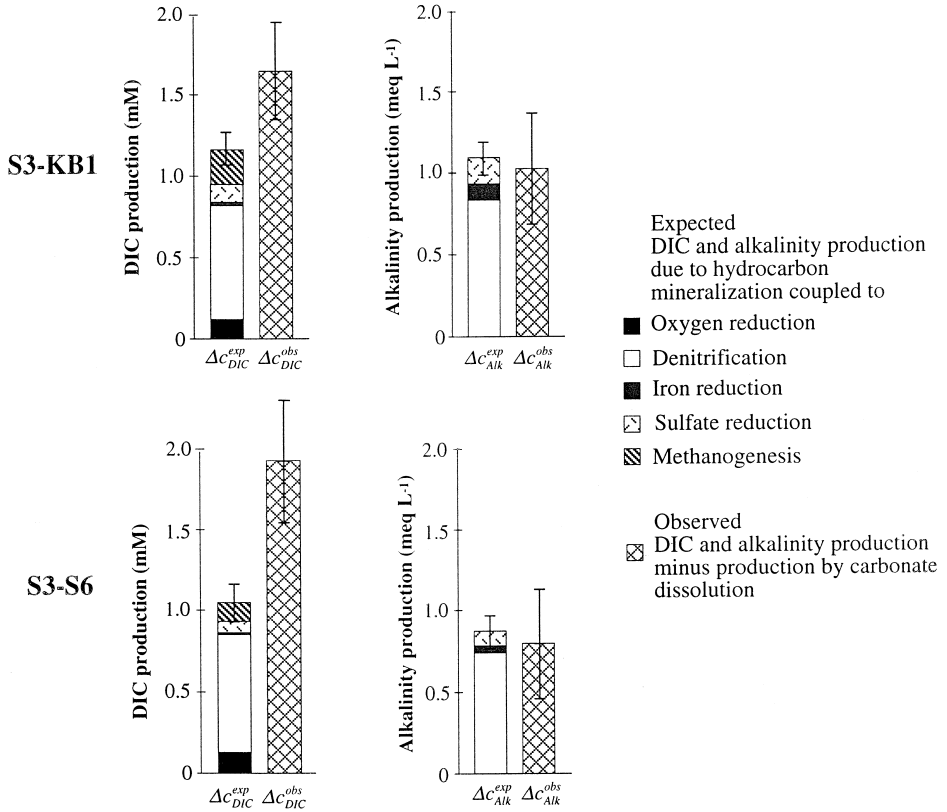

Fig. 5. Expected and observed DIC and alkalinity production between S3 and the monitoring wells KB1 and S6 calculated according to Eqs. (3)–(5). The contribution of hydrocarbon mineralization coupled to Mn reduction is too small to be visible.

(1991), methanogenesis followed by loss of the CH_4 to the unsaturated zone was postulated to be responsible for the excess DIC. In the study by Bennett et al. (1993), an additional, unknown source of CO_2 had to be assumed to explain the concentration changes along a flow path with a geochemical model. However, at those sites, the residence time of the groundwater in the contaminated zone was much longer than in Menziken.

To differentiate better between the reasons for the difference between observed and expected DIC production (= unaccounted DIC), alkalinity and stable carbon isotope balances were calculated.

4.7. Alkalinity balances

Different sources of DIC can be distinguished based on the ratios of DIC to alkalinity production which is dependent on the DIC source (Herczeg et al., 1991; Stumm and

Morgan, 1996). For example, the generation of 1 mol of DIC by dissolution of carbonates generates two equivalents of alkalinity, whereas the generation of 1 mol of DIC by aerobic PHC mineralization does not generate alkalinity (Table 3). The expected and observed alkalinity production accompanying microbial PHC mineralization were determined analogously (Eqs. (4) and (5)) to the expected and observed DIC production for two selected wells (Fig. 5). The difference between expected and observed alkalinity production was within the range of uncertainty for both monitoring wells. This indicates that the processes producing the unaccounted DIC are not producing alkalinity. Thus, carbonate dissolution is an unlikely source of the unaccounted DIC, since carbonate dissolution is accompanied by alkalinity production.

4.8. Stable carbon isotope balances of DIC

The determination of the $\delta^{13}C$ of DIC allows one to differentiate between different sources of DIC. The $\delta^{13}C$ of the produced DIC depends on the $\delta^{13}C$ of the source of DIC and possible process specific changes of $\delta^{13}C$. The following sources of DIC can be distinguished based on $\delta^{13}C$ values of the DIC: (i) CO_2 from non-methanogenic PHC mineralization which has a $\delta^{13}C$ similar to the degraded PHC (-29.7‰ in this study, Table 3), (ii) CO_2 produced by methanogenic PHC mineralization which is enriched in ^{13}C (up to $+38$‰; Grossman, 1997), (iii) CO_2 from CH_4 oxidation which is depleted in ^{13}C by up to 100 permille compared to DIC in groundwater (Whiticar and Faber, 1986; Whiticar et al., 1986) and (iv) CO_2 from carbonate dissolution which has a $\delta^{13}C$ similar to the $\delta^{13}C$ of the dissolved carbonate ($+0.7$‰ in this study, Table 3).

In this study, the $\delta^{13}C$ of the observed DIC production was calculated using Eqs. (5) and (6) and compared to the $\delta^{13}C$ of the DIC sources given above. Eq. (6) was obtained by multiplying Eq. (5) with the corresponding $\delta^{13}C$ values:

$$\begin{aligned}\Delta c_{\mathrm{DIC}}^{\mathrm{obs}} \times \delta^{13}\mathrm{C}_{\mathrm{DIC}}^{\mathrm{obs}} = {} & c_{\mathrm{DIC}} \times \delta^{13}\mathrm{C}_{\mathrm{DIC}} \\ & - \left[F \times c_{\mathrm{DIC}}^{\mathrm{inj}} \times \delta^{13}\mathrm{C}_{\mathrm{DIC}}^{\mathrm{inj}} + (1-F) \times c_{\mathrm{DIC}}^{\mathrm{pr}} \times \delta^{13}\mathrm{C}_{\mathrm{DIC}}^{\mathrm{pr}} \right] \\ & - \Delta c_{\mathrm{DIC}}^{\mathrm{carb}} \times \delta^{13}\mathrm{C}_{\mathrm{DIC}}^{\mathrm{carb}} \end{aligned} \quad (6)$$

$\delta^{13}C_{\mathrm{DIC}}^{\mathrm{obs}}$ (‰): $\delta^{13}C$ of the $\Delta c_{\mathrm{DIC}}^{\mathrm{obs}}$; $\delta^{13}C_{\mathrm{DIC}}$ (‰): measured $\delta^{13}C$ of DIC in groundwater of monitoring well; $\delta^{13}C_{\mathrm{DIC}}^{\mathrm{inj}}$ (‰): measured $\delta^{13}C$ of DIC in injected groundwater (S3); $\delta^{13}C_{\mathrm{DIC}}^{\mathrm{pr}}$ (‰): measured $\delta^{13}C$ of DIC in pristine groundwater (KB2); $\delta^{13}C_{\mathrm{DIC}}^{\mathrm{carb}}$ (‰): $\delta^{13}C$ of DIC produced by $CaCO_3$ dissolution (Table 3). The $\delta^{13}C_{\mathrm{DIC}}^{\mathrm{obs}}$ was calculated by dividing Eq. (6) by Eq. (5).

The calculated $\delta^{13}C_{\mathrm{DIC}}^{\mathrm{obs}}$ was between -26.6 and -32.8‰ for the flow paths leading from S3 to S6, S8, KB15 and KB7 (Table 4). These values are close to the $\delta^{13}C$ of the PHC present in the aquifer (-29.7‰) indicating that non-methanogenic PHC mineralization was mainly responsible for the production of $\Delta c_{\mathrm{DIC}}^{\mathrm{obs}}$. For the flow path between S3 and KB1, a value of -0.5‰ was obtained which suggests a mixture of methanogenic and non-methanogenic PHC mineralization or carbonate dissolution as the source of the

Table 4
Stable carbon isotope balance of DIC production for flowpaths between injection gallery and monitoring wells in the source area

	Observed DIC production	
	$\Delta c_{DIC}^{obs\ a}$ (mM)	$\delta^{13}C_{DIC}^{obs\ b}$ (‰)
S3–S6	1.88 ± 0.29	-29.8 ± 3.3
S3–S7	2.70 ± 0.31	-44.3 ± 4.1
S3–S8	3.11 ± 0.32	-26.6 ± 1.8
S3–KB1	1.67 ± 0.28	-0.5 ± 2.5
S3–KB7	1.55 ± 0.28	-29.3 ± 3.9
S3–KB15	2.92 ± 0.31	-32.8 ± 2.5

[a]Calculated using Eqs. (3) and (4).
[b]Calculated using Eqs. (5) and (6).

observed DIC production. The second possibility is unlikely since carbonate dissolution is accompanied by alkalinity production and thus would cause a discrepancy in the alkalinity balance (Fig. 5). In addition, the high CH_4 concentration at KB1 indicates that methanogenesis is an important process between S3 and KB1. For the flow path between S3 and S7, a value of -44.3‰ was obtained which suggests the occurrence of CH_4 oxidation. However, it is unlikely that CH_4 oxidation was an important source of DIC since the O_2 availability in the source area was limited. In conclusion, except for the flow path between S3 and S7, stable carbon isotope balances indicate that the entire observed DIC production, including the unaccounted DIC not linked to electron acceptor consumption, originated from PHC mineralization. Since the observed DIC productions, Δc_{DIC}^{obs}, in Table 4, corresponds in the average to $93 \pm 6\%$ of the total DIC production, Δc_{DIC}, it can be concluded that PHC mineralization was the dominant source of DIC in the source area. In other words, the DIC balance (Fig. 5) remains unbalanced due to an underestimation of the electron accepting reactions and not due to an overestimation of the observed DIC production. Possible causes for this discrepancy are the underestimation of solid phase formation (FeS, MnS) as well as the underestimation of CH_4 degassing from the aquifer.

5. Conclusions

This study shows that the assessment of PHC mineralization is more reliable when the microbial DIC production is (i) determined based both on the turnover of oxidants and reduced species and on DIC and (ii) verified by calculating alkalinity and stable carbon isotope balances. This allows one to determine whether the added and/or endogenous oxidants were used to mineralize PHC. The quantification of the PHC mineralization based on the turnover of oxidants and reduced species alone can lead to an underestimation of PHC mineralization. Future studies should address the exchange of gaseous compounds between the unsaturated and the saturated zone as well as the

contribution of geochemical reactions of the solid phases in the aquifer on the mass balance of hydrocarbon mineralization under anaerobic conditions in the field.

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