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Engineered and subsequent intrinsic in situ bioremediation of a diesel fuel contaminated aquifer

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Abstract

A diesel fuel contaminated aquifer in Menziken, Switzerland was treated for 4.5 years by injecting aerated groundwater, supplemented with KNO_3 and $NH_4H_2PO_4$ to stimulate indigenous populations of petroleum hydrocarbon (PHC) degrading microorganisms. After dissolved PHC concentrations had stabilized at a low level, engineered in situ bioremediation was terminated. The main objective of this study was to evaluate the efficacy of intrinsic in situ bioremediation as a follow-up measure to remove PHC remaining in the aquifer after terminating engineered in situ bioremediation. In the first 7 months of intrinsic in situ bioremediation, redox conditions in the source area became more reducing as indicated by lower concentrations of SO_4^{2-} and higher concentrations of Fe(II) and CH_4. In the core of the source area, strongly reducing conditions prevailed during the remaining study period (3 years) and dissolved PHC concentrations were higher than during engineered in situ bioremediation. This suggests that biodegradation in the core zone was limited by the availability of oxidants. In lateral zones of the source area, however, gradually more oxidized conditions were reestablished again, suggesting that PHC availability increasingly limited biodegradation. The total DIC production rate in the aquifer decreased within 2 years to about 25% of that during engineered in situ bioremediation and remained at that level. Stable carbon isotope analysis confirmed that the produced DIC mainly originated from PHC mineralization. The total rate of DIC and CH_4 production in the source area was more than 300 times larger than the rate of PHC elution. This indicates that biodegradation coupled to consumption of naturally occurring oxidants was an important process for removal of PHC which remained in the aquifer after terminating engineered measures.

Keywords: Bioremediation; Petroleum hydrocarbons; Groundwater; Redox processes

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1. Introduction

In aquifers contaminated with petroleum hydrocarbons (PHC), microbial populations usually develop that are able to degrade PHC both under aerobic and anaerobic conditions (Chapelle, 1999). Initially, the rate of PHC dissolution in the source area (=area with PHC in nonaqueous phase) is higher than the rate of biodegradation and thus a plume of dissolved contaminants evolves (Mackay et al., 1985). However, biodegradation coupled to the use of naturally available oxidants may be sufficient to keep contaminant plumes short and prevent migration of contaminants to receptors (Wiedemeier et al., 1999). Natural biodegradation of contaminants in the absence of external intervention has been designated as intrinsic in situ bioremediation (US National Research Council, 1993). If drinking water wells are located close to the source area, engineered in situ bioremediation may be applied to minimize plume sizes and increase the rate of source removal (Norris, 1994). This strategy usually includes an external supply of oxidants and nutrients to increase the biodegradation rate and a hydraulic containment to prevent the spreading of dissolved PHC (Norris, 1994; US National Research Council, 1993).

As in situ bioremediation progresses, the bioavailability of PHC in the source zone of an aquifer usually decreases due to (i) depletion of highly soluble PHC compounds, (ii) a decrease of the PHC phase–water contact area, (iii) sorption of PHC to the matrix and/or (iv) diffusion of PHC into micropores (Beck and Kevin, 1995; Bosma et al., 1997; Brown et al., 1995; Brusseau, 1992; Powers et al., 1994; Schluep et al., 2001). Furthermore, residual PHC may be located in low permeability zones of aquifers and are therefore poorly accessible by supplied oxidants (Beck and Kevin, 1995). The decreasing bioavailability of PHC leads to a declining PHC degradation rate (Bosma et al., 1997). However, if the degradation rate is limited by PHC availability, neither the addition of oxidants and nutrients nor a hydraulic containment may be necessary even if a substantial amount of PHC is still present in the source area. In such a situation, intrinsic in situ bioremediation can serve as a follow-up measure that keeps PHC concentrations low and leads to a further removal of PHC in the source area (Wiedemeier et al., 1999).

The main aim of this study was to investigate the efficacy of intrinsic in situ bioremediation as a follow-up measure to engineered in situ bioremediation. The study site was a diesel fuel contaminated aquifer in Menziken, Switzerland. Concentrations of oxidants, reduced species, biodegradation end products (DIC, CH_4) and dissolved PHC were determined over a period of 4.4 years during transition from engineered to intrinsic in situ bioremediation. In addition, total rates of consumption or production of these species in the source area were estimated, which made it possible to compare total rates of PHC mineralization in the source area during and after engineered in situ bioremediation.

2. Site description and methods

2.1. Site description

The study site is an aquifer in Menziken, Switzerland, in which a diesel fuel contamination was detected in January 1988. After removal of diesel fuel in free phase, excavation of contamination in the unsaturated zone below the former location of the tank and installation of a cutoff wall upgradient of the contaminated zone (Fig. 1), the remaining contamination was treated by engineered in situ bioremediation (Hunkeler et al., 1999). Studies with

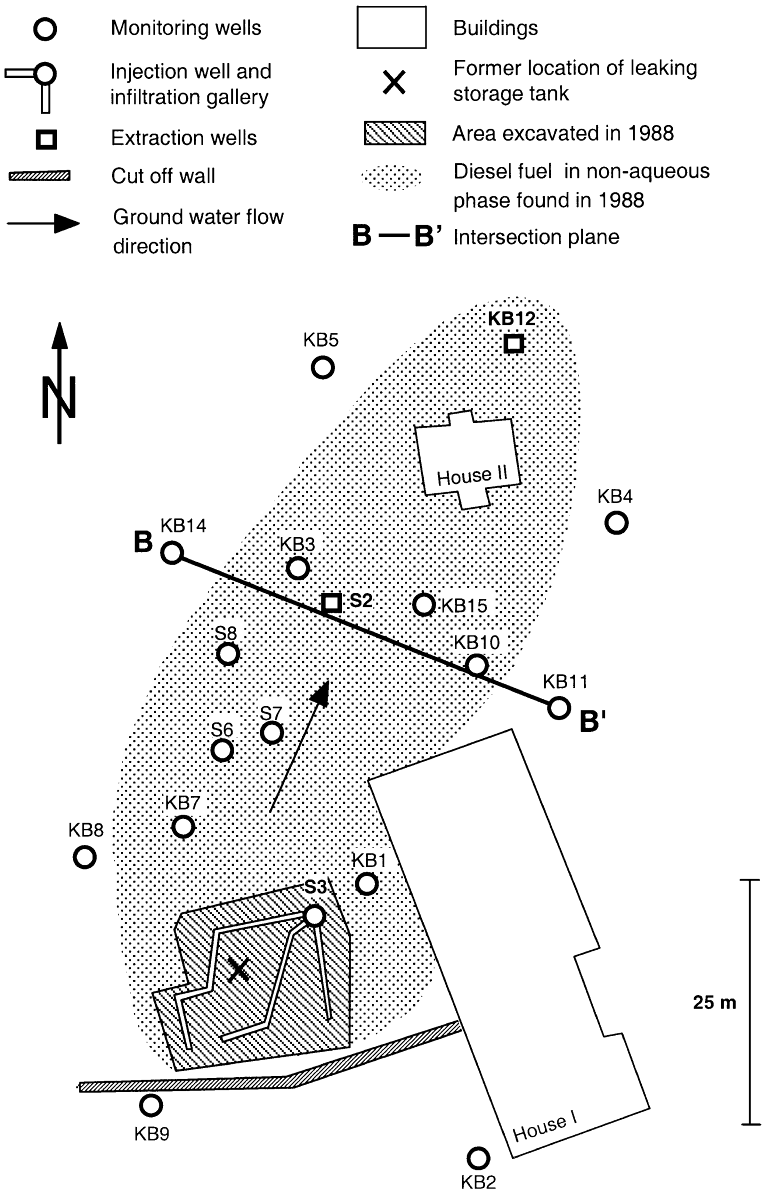

Fig. 1. Map of study site with intersection plane B–B'. During engineered in situ bioremediation, groundwater was extracted at S2 (May 1989–April 1991) or KB12 (May 1991–February 1994), respectively, supplemented with oxidants and nutrients and reinjected in S3.

laboratory aquifer columns (Hess et al., 1996; Hunkeler et al., 1998), microcosms (Bregnard, 1996; Bregnard et al., 1997) and enrichment cultures (Häner et al., 1995, 1997) showed that microorganisms from the contaminated aquifer were able to mineralize PHC under various redox conditions. 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 day^{-1}. This groundwater was aerated and re-injected into well S3, which was connected to an infiltration gallery. When pumping at KB12, about 11% of the injected groundwater was recovered (Hunkeler et al., 1999). During the months May to December, the injected groundwater was supplemented with KNO_3 (average concentration about 1.36 mM based on a decision by the state authorities) and $NH_4H_2PO_4$ (average concentration about 0.08 mM). Between October 1994 and May 1995, no oxidants and nutrients were added, and therefore, this period is not considered as period of engineered in situ bioremediation. A total amount of 5.9 t of KNO_3 and 2 t of O_2 were injected into the aquifer from 1988 to December 1993. In 1993, the concentration of total dissolved PHC had stabilized at a low level (<0.3 mg l^{-1}), and therefore, the state authorities decided to terminate engineered in situ bioremediation and to rely on intrinsic in situ bioremediation. Aquifer material samples taken at the core zone in November 1992 revealed that the remaining contamination mainly consisted of branched alkanes and an unresolved complex mixture of compounds (Bregnard et al., 1996). Investigations using a new partitioning tracer method based on naturally occurring ^{222}Rn (Hunkeler et al., 1997) suggested that in 1995, PHC were still present in the source area at an average concentration of about 1 g kg^{-1}. For this study, groundwater samples were taken three times during the period of oxidant and nutrient injection (July 27, August 24 and September 13, 1993) and six times after the end of injection (April 6, July 19 and September 12, 1994; October 18, 1995; October 29, 1996; November 3, 1997) in the monitoring wells shown in Fig. 1. A complete set of data for the years 1993 to 1996 can be found in Hunkeler (1997).

2.2. Chemical analysis

The methods used for groundwater sampling and analyses corresponded to those described in Hunkeler et al. (1999) except for dissolved PHC which was determined as described in Hunkeler (1997). Dissolved Mn(II) and Fe(II) refers to the sum of all dissolved species in the form of free ions and complexes with the redox state +II, P_T to the sum of inorganic phosphate present as $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}.

2.3. Quantification of total rates of consumption or production of reactive species

Total rates of consumption of oxidants and production of reduced species, CH_4 and DIC were quantified for a section of the aquifer situated between the infiltration gallery and an intersection plane B–B′ perpendicular to the flow of groundwater (Fig. 1). This plane B–B′ was chosen because (i) the number of monitoring wells along cross-section B–B′ was sufficient to obtain concentration profiles of dissolved species across the source area, (ii) wells KB14, S8, KB15, KB10 and KB11 reach approximately to a similar depth of the aquifer, covering the entire vertical extension of contamination (Hunkeler et al., 1999), and (iii) a large part of the contamination was expected to be located upgradient of

B–B′. The aim of the calculations was to compare total rates determined for different times rather than to obtain accurate absolute values.

2.3.1. Flux of groundwater through intersection plane

The specific discharge of groundwater across the intersection plane B–B′ during engineered in situ bioremediation was determined using nonreactive tracers as described in Hunkeler et al. (1999). Based on the flow velocities and the measured hydraulic gradients during the tracer test, the average hydraulic conductivity was estimated using Darcy's law and assuming a porosity of 0.15. The calculation yielded an average hydraulic conductivity of 6×10^{-4} m s^{-1}, which is in the range of the hydraulic conductivity determined by pumping tests ($4.5\pm2.5\times10^{-4}$ m s^{-1}, n=3). The specific discharge at natural gradient flow was estimated based on the average measured hydraulic conductivity and hydraulic gradients derived from water level measurements.

2.3.2. Consumption or production of reactive species upgradient of intersection plane

Total rates of consumption or production of reactive species i upgradient of intersection plane B–B′ (ΔF_i) were obtained based on the specific discharge across the intersection plane, measured concentrations of the species and hypothetical concentrations of the species if no reactive processes would take place upgradient of the intersection plane:

$$\Delta F_i = \int_0^w (c_i(y) - c_i^*(y))q(y)\mathrm{d}(y)\mathrm{d}y \tag{1}$$

where y is the horizontal coordinate in direction of the intersection plane, $c_i(y)$ is the measured concentration of species i, $c_i^*(y)$ is the hypothetical concentration of species i if no reactive processes would take place in the source area, $q(y)$ is the specific discharge, $\mathrm{d}(y)$ is the screen length of the monitoring wells and w is the width of the intersection plane. The rate of oxidant supply, F, to the contaminated zone upgradient of the intersection plane was calculated using the following equation:

$$F_i = \int_0^w c_i^*(y)q(y)\mathrm{d}(y)\mathrm{d}y \tag{2}$$

Values for $c_i(y)$, $c_i^*(y)$, $q(y)$ and $\mathrm{d}(y)$ between monitoring wells were calculated using linear interpolation. Hypothetical concentrations, c_i^*, at each monitoring well were calculated as follows:

$$c_i^* = Dc_i^{\mathrm{inj}} + (1 - D)c_i^{\mathrm{bg}} \tag{3}$$

where c_i^{inj} is the concentration of species i in the injected water, c_i^{bg} is the concentration of species i in groundwater upgradient of the source area and D is the fraction of injected water. For engineered in situ bioremediation, D was determined based on results of a tracer test (Hunkeler et al., 1999). For intrinsic in situ bioremediation, D is zero, i.e., the hypothetical concentrations correspond to c_i^{bg}. The calculated consumption and production of reactive species are only approximative since dispersive fluxes across the boundaries and exchange of compounds between saturated and unsaturated zone were neglected. The DIC production by PHC mineralization $\Delta F_{\mathrm{DIC}}^{\mathrm{obs}}$ was determined by subtracting DIC production due to carbonate dissolution from the total rate of DIC production calculated according to Eq. (1). DIC prodution due to carbonate dissolution was calculated based on production of dissolved Ca^{2+} and Mg^{2+}, which were quantified using Eq. (1). The origin of the DIC was verified by calculating the $\delta^{13}C$ of $\Delta F_{\mathrm{DIC}}^{\mathrm{obs}}$ as described in Hunkeler et al. (1999).

2.3.3. Verification of mass flux concept

The mass flux calculation relies on the assumption that specific discharge and concentrations of reactive species determined at the five monitoring wells of the intersection plane B–B′ are representative for the plume intersecting B–B′, and values between wells can be approximated by linear interpolation. Regarding specific discharge, the validity of these assumptions can be tested by calculating the Cl^- mass flux across intersection plane B–B′ during engineered in situ bioremediation and comparing it to the amount of Cl^- added at S3. The mass flux of chloride (Cl^-) across the intersection plane B–B′ was calculated using Eq. (1) by inserting the background Cl^- concentration for $c_i^*(y)$ and the measured concentration at each well for $c_i(y)$. A mass flux of Cl^- across the intersection of 69 mol day^{-1} was obtained, which corresponded well to the amount of Cl^- added at S3 (72 mol day^{-1}). This indicates that the specific discharge determined for the five wells is representative for the plume. Furthermore, it indicates that the intersection plane covered the entire lateral and vertical extension of the plume of injected water. Regarding the concentrations of reactive species, the relatively smooth concentration profiles for many parameters and the occurrence of more reduced conditions in the centre of the intersection plane (see below) suggest that the wells reasonably represent the plume of reactive species. An evaluation of the uncertainty of the mass flux calculations due to the limited number of monitoring wells would require additional sampling points to get more detailed concentration profiles.

3. Results

3.1. Spatial variation of NO_3^-

Nitrate concentrations during and after oxidant and nutrient injection are displayed in Fig. 2. On August 24, 1993, the NO_3^- concentration in the injected water was 1.04 mM while background NO_3^- concentrations upgradient of the contaminated zone were 0.3 mM. The NO_3^- concentration is <0.1 mM in all of the monitoring wells within the source area. An elevated NO_3^- concentration was found upgradient of the cutoff wall indicating that the cutoff wall was leaking. On September 12, 1994, 7 months after terminating engineered in situ bioremediation, NO_3^- concentrations were below <0.1 mM in all monitoring wells in the source area except KB1. Between September 12, 1994 and November 3, 1997, the number of monitoring wells with <0.1 mg l^{-1} NO_3^- decreased from seven to four (Fig. 2).

3.2. Composition of groundwater samples taken at S6 and KB15

To illustrate changes in groundwater composition after terminating engineered in situ bioremediation in more detail, the values of selected chemical parameters are shown for

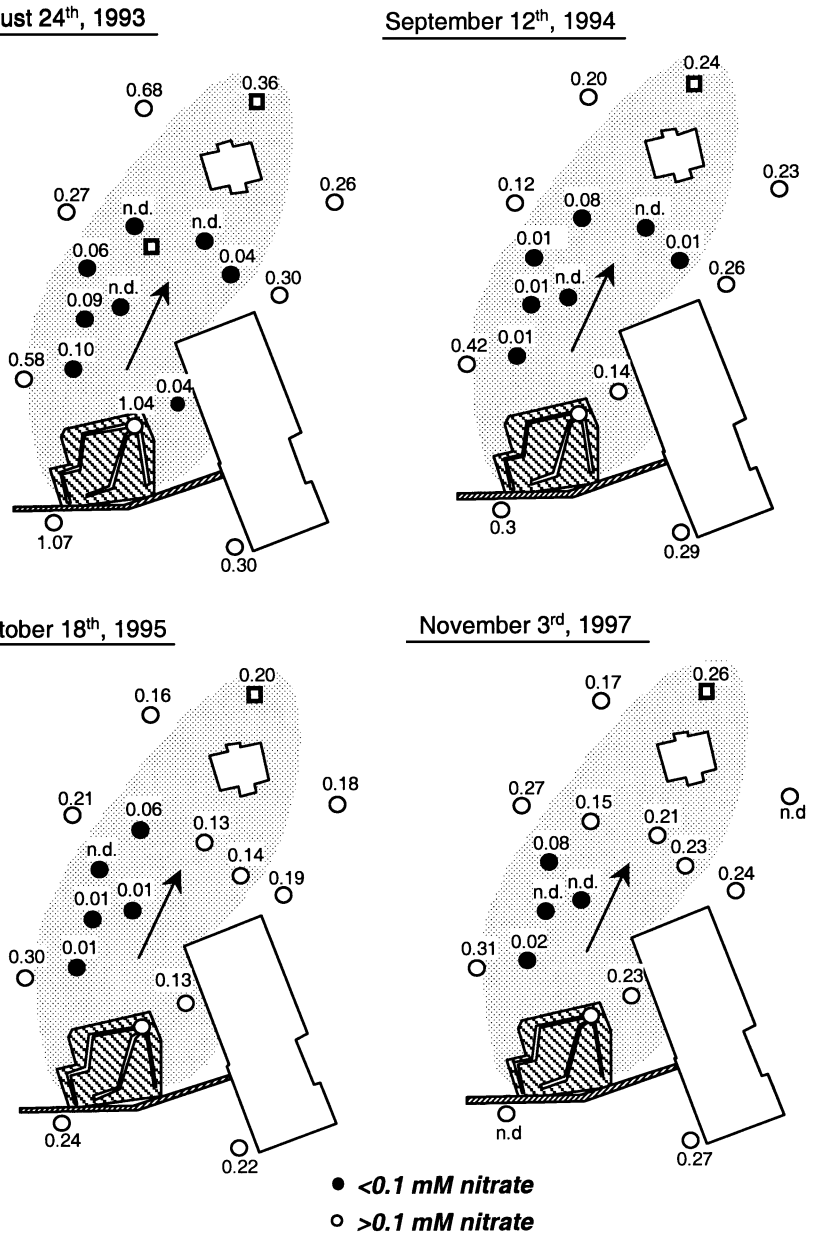

Fig. 2. Nitrate concentrations during (August 24, 1993) and after (September 12, 1994; October 18, 1995; November 3, 1997) engineered in situ bioremediation. Monitoring wells with nitrate concentrations <0.1 mM (●) and >0.1 mM (O); n.d.: nitrate concentration below detection limit (0.003 mM).

two monitoring wells, S6 and KB15 (Fig. 3). S6 is located 20 m downgradient of the infiltration gallery in a zone that was initially heavily contaminated (Fig. 1). KB15 lies 35 m downgradient where the aquifer was less contaminated.

Oxygen was depleted to concentrations <0.05 mM in S6 and KB15, until September 12, 1994. Afterwards, oxygen concentrations gradually increased in KB15 while remain-

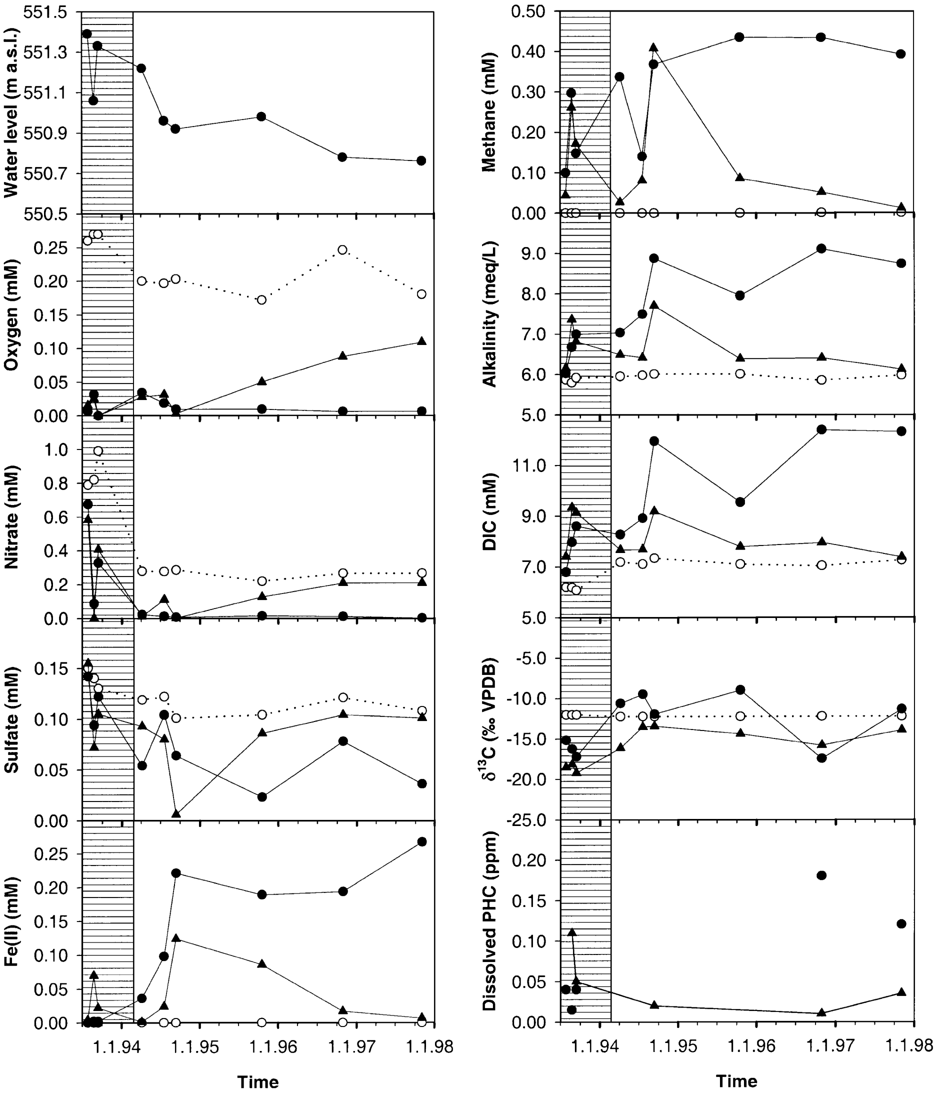

Fig. 3. Values of selected parameters for monitoring wells S6 (●) and KB15 (▲) during and after engineered in situ bioremediation. Expected values at S6 if the species entering the contaminated zone had behaved like a non-reactive tracer (○). The expected values were calculated according to Eq. (3). Shaded area: engineered in situ bioremediation in operation.

ing low in S6. The concentration of NO_3^- was highly variable throughout 1993 in both wells, probably due to fluctuations of the water level. When the water table was >551.1 m a.s.l. at S6, NO_3^- concentrations were high since part of the NO_3^- rich water was flowing above the contaminated zone. In S6, NO_3^- concentration remained low throughout the period of intrinsic in situ bioremediation, whereas in KB15, the concentration gradually increased towards background values. Concentrations of NO_2^- and N_2O were always below 0.04 mM (data not shown). The SO_4^{2-} concentration decreased after terminating engineered in situ bioremediation and subsequently reached background values again in KB15 while it remained low in S6. Fe(II), CH_4, alkalinity, DIC (Fig. 3) and Mn(II) (data not shown) increased after terminating engineered in situ bioremediation, and decreased again in KB15 only. During engineered in situ bioremediation, the $\delta^{13}C$ of the DIC was in both wells more negative than that of the background water. After terminating engineered in situ bioremediation, the $\delta^{13}C$ of DIC in S6 shifted to more positive values except for October 29, 1996, while it remained more negative than background values in KB15. The concentration of dissolved PHC at S6 was higher after terminating engineered bioremediation. At KB15, the concentration of dissolved PHC was below 0.05 mg l^{-1} during the entire period of intrinsic in situ bioremediation.

3.3. Composition of groundwater samples taken at monitoring wells along intersection plane B–B′

The data at selected sampling days are shown for the monitoring wells located in the vicinity of the intersection plane B–B′ (KB14, S8, KB15, KB10, KB11) in Fig. 4. Monitoring well KB3 was not included since it has a much longer screened interval than the other monitoring wells (Hunkeler et al., 1999). The composition of groundwater samples taken at monitoring wells along intersection plane B–B′ is illustrated for one sampling day during engineered in situ bioremediation (August 24, 1993) and three sampling days after terminating engineered in situ bioremediation (September 12, 1994; October 18, 1995; November 3, 1997). In addition, for dissolved PHC, concentrations measured in December 1991 are shown.

The general shape of the concentration profiles along the intersection plane B–B′ is similar during and after engineered in situ bioremediation (Fig. 4). Oxidant concentrations (NO_3^-, SO_4^{2-}) were generally higher in wells outside the source area (KB14, KB11) than in wells within the source area (S8, KB15, KB10). In contrast, concentrations of Fe(II), CH_4 and DIC were lower outside than inside the source area. NO_2^- concentrations were always lower than 0.025 mM and N_2O concentrations lower than 0.002 mM (data not shown). P_T concentrations (S8, KB15 and KB10; data not shown) were 0.001 to 0.025 mM during engineered in situ bioremediation (August 24, 1993) and 0.001 to 0.006 mM after terminating engineered in situ bioremediation (October 18, 1995).

On September 12, 1994, 7 months after terminating engineered in situ bioremediation, concentrations of SO_4^{2-} in monitoring wells S8 and KB15 were lower and concentrations of Fe(II), CH_4 and DIC higher compared to the values measured on August 24, 1993. At S8, concentrations of oxidants remained low and concentrations of reduced species, CH_4 and DIC elevated compared to values at KB11, while at KB10 and KB15, all parameters approached background values again.

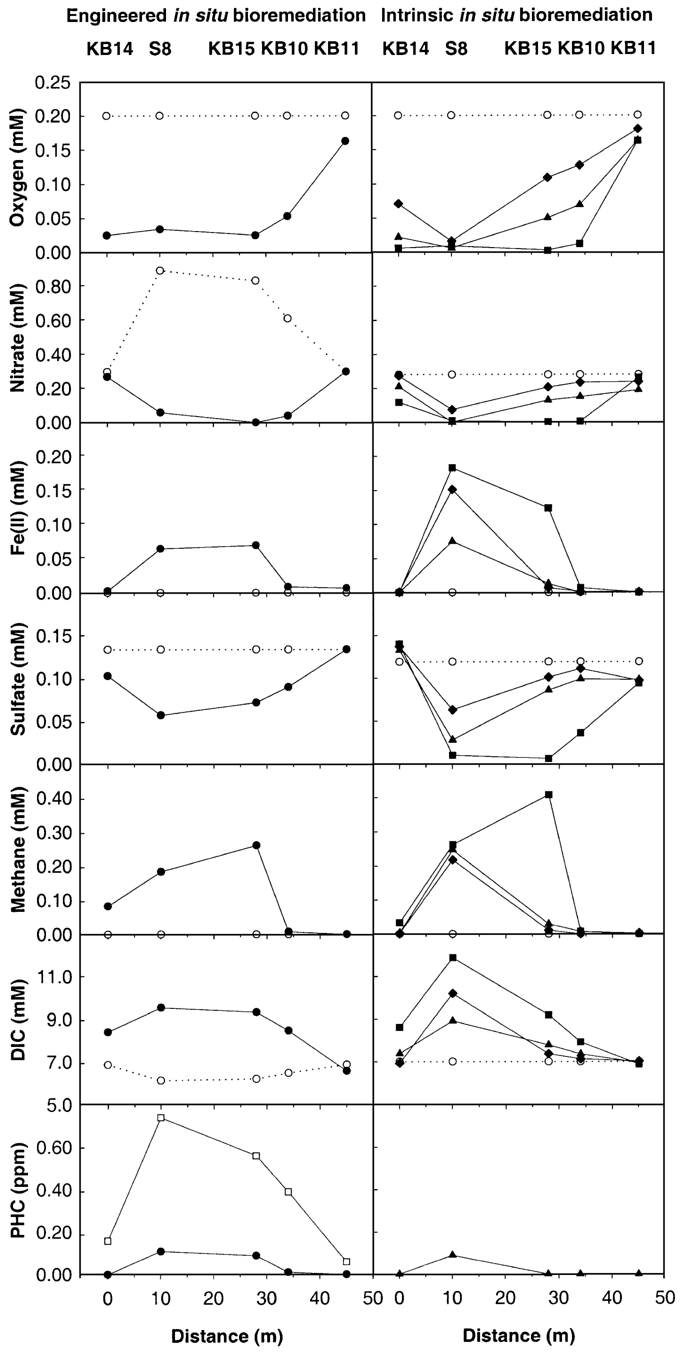
Engineered *in situ* bioremediation
Intrinsic *in situ* bioremediation
KB14 S8 KB15 KB10 KB11
KB14 S8 KB15 KB10 KB11
Oxygen (mM)
0.25
0.20
0.15
0.10
0.05
0.00
Nitrate (mM)
0.80
0.60
0.40
0.20
0.00
Fe(II) (mM)
0.20
0.15
0.10
0.05
0.00
Sulfate (mM)
0.15
0.10
0.05
0.00
Methane (mM)
0.40
0.30
0.20
0.10
0.00
DIC (mM)
11.0
9.0
7.0
5.0
PHC (ppm)
0.60
0.40
0.20
0.00
0
10
20
30
40
50
0
10
20
30
40
50
Distance (m)
Distance (m)

Table 1
Rate of supply (*F*) and rates of consumption or production (Δ*F*) of species involved in microbial processes in the subsurface

	O_2		NO_3^-		NH_4^+		Mn(II)	Fe(II)	SO_4^{2-}		CH_4	DIC		DIC+CH_4	PHC
	F	ΔF	F	ΔF	F	ΔF	ΔF	ΔF	F	ΔF	ΔF	ΔF_{DIC}^{obs}	$\delta^{13}C^a$	ΔF	ΔF
Aug. 24, 1993	32	−26	89	−80	4.5	−1.9	1.0	5.6	17	−5.6	19	286	−34.8	305	0.5
Sept. 12, 1994	17	−15	24	−21	0	3.4	0.8	8.1	12	−9.2	19	184	−21.6	203	–
Oct. 18, 1995	16	−11	21	−10	0	2.9	0.1	2.4	10	−2.6	7.8	57	−33.5	65	0.2
Oct. 29, 1996	21	−14	22	−7	0	1.7	0.4	3.5	10	−1.5	7.8	94	−32.9	102	0.2
Nov. 3, 1997	20	−13	22	−7	0	0.5	0.3	3.9	10	−2.2	5.8	73	–	79	–

All rates are in mol/day. –: not determined.

[a] Carbon isotope composition ($\delta^{13}C$) of produced DIC (ΔF_{DIC}^{obs}). The $\delta^{13}C$ of DIC was calculated as described in Hunkeler et al. (1999).

In December 1991, dissolved PHC were detected in all five monitoring wells along intersection plane B–B′, whereas on August 24, 1993, dissolved PHC were only detected in the three wells in the middle of the intersection plane at concentrations at least seven times smaller than in 1991. On October 18, 1995, dissolved PHC were only detected in S8 (Fig. 4).

3.4. Total rates of consumption and production of selected species

To be able to compare consumption or production of species involved in PHC mineralization during and after engineered in situ bioremediation, total rates of these processes upgradient of B–B′ were estimated using Eq. (1) (Table 1). The calculations are based on differences between measured and hypothetical (no reactive processes) concentrations of these species in monitoring wells along intersection plane B–B′, which are illustrated in Fig. 4. During engineered in situ bioremediation, most of the available O_2 and NO_3^- and about one-third of the sulfate was consumed (Table 1). After terminating engineered in situ bioremediation, the supply of O_2, NO_3^- and SO_4^{2-} decreased due to the lower specific discharge of groundwater and lower concentrations of O_2 and NO_3^-. The rates of O_2 and NO_3^- consumption were lower and the rates of SO_4^{2-} consumption and Fe(II) production higher on September 12, 1994 than on August 24, 1993. The Fe(II) production may even have been higher than calculated since precipitation of Fe(II) as FeS or $FeCO_3$ (Matsunaga et al., 1993; Von Gunten and Zobrist, 1993) was not included. On October 18, 1995, October 29, 1996 and November 3, 1997, the consumption of NO_3^-, SO_4^{2-} and the production of Fe(II) and Mn(II) was again lower than on September 12,

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Fig. 4. Values of selected parameters for monitoring wells along intersection plane B–B′on August 24, 1993 (●), September 12, 1994 (■), October 18, 1995 (▲) and November 3, 1997 (◆). Expected values if species entering the contaminated zone had behaved like a conservative tracer (○). In addition, PHC concentrations measured in December 1991 during engineered in situ bioremediation are shown (□).

1994. The CH_4 production was similar on August 24, 1993 and September 12, 1994, and then decreased (Table 1). On September 12, 1994, the DIC production was 64% of that during engineered in situ bioremediation, and then decreased to about 25% of the DIC production during engineered in situ bioremediation (Table 1). On August 24, 1993 during engineered in situ bioremediation, a net consumption of NH_4^+ was observed. After terminating engineered in situ bioremediation, NH_4^+ was produced. Except for September 12, 1994, the $\delta^{13}C$ of the produced DIC ($\Delta F_{\mathrm{DIC}}^{\mathrm{obs}}$) was slightly more negative than the $\delta^{13}C$ of the PHC in the aquifer (−29.7 ‰). On September 12, 1994, the $\delta^{13}C$ of the DIC was more positive than the $\delta^{13}C$ of the PHC.

4. Discussion

4.1. Engineered in situ bioremediation

The added oxidants were almost completely consumed on August 24, 1993, during the last year of engineered in situ bioremediation, and dissolved Fe(II), Mn(II) and CH_4 were detected. Therefore, it can be concluded that engineered in situ bioremediation was not successful in shifting the redox state of groundwater in the core of the contaminated zone towards oxidized conditions. The availability of oxidants probably still limited the total rate of biodegradation in the source area during the final period of engineered in situ bioremediation. However, the total flux of dissolved PHC across the intersection plane decreased from 5 mol C day^{-1} in 1991 to 0.5 mol C day^{-1} on August 24, 1993. The rate of DIC and CH_4 production (Table 1) was much larger than the rate of PHC elution, which indicates that PHC were mainly removed by mineralization from the source area during engineered in situ bioremediation. The $\delta^{13}C$ of produced DIC ($\Delta F_{\mathrm{DIC}}^{\mathrm{obs}}$) was in a similar range (−34.8 ‰, Table 1) as the $\delta^{13}C$ of PHC (−29.7 ‰) confirming that the DIC originated from PHC mineralization. The depletion of the $\delta^{13}C$ in DIC by 5.1 ‰ compared to PHC is consistent with recent laboratory studies, which showed that the $\delta^{13}C$ of DIC from aerobic benzene mineralization is 3.6 ‰ to 6.2 ‰ depleted in ^{13}C compared to the initial $\delta^{13}C$ of benzene (Hunkeler et al., 2002).

4.2. Transition from engineered to intrinsic in situ bioremediation

The investigated period of intrinsic in situ bioremediation can be divided into two phases based on temporal variations in concentrations of reactive species (Fig. 3): A period of rapid concentration changes indicating more reducing conditions (1994) and a period of steady concentrations (S6) or gradual changes (e.g., KB15) towards more oxidizing conditions (1995–1997). In this paragraph, the first phase is discussed which characterizes the initial reaction of the system to the transition from engineered to intrinsic in situ bioremediation.

After terminating engineered in situ bioremediation, SO_4^{2-} concentrations decreased and Fe(II), Mn(II) and CH_4 concentrations increased (Figs. 3 and 4). These shifts can be explained by the lower supply of O_2 and NO_3^- and the longer residence time of the groundwater in the contaminated zone. The total rate of sulfate reduction and Fe(II) production increased. In addition, a net production of NH_4^+ was observed indicating that NO_3^- was not only reduced to N_2 but also to NH_4^+, which is expected under NO_3^- limited conditions (Tiedje, 1988). In contrast to Fe(II) production and SO_4^{2-} reduction, the total rate of CH_4 production was similar on September 12, 1994 as during engineered in situ bioremediation (data from August 24, 1993). However, CH_4 may have escaped to the unsaturated zone due to higher CH_4 concentrations and longer residence times of the groundwater in the source area, which leads to an underestimation of methanogensis. Losses of CH_4 to the unsaturated zone were also observed in other studies of methanogenic aquifers (Barber et al., 1990; Herczeg et al., 1991). The shift of the $\delta^{13}C$ of the DIC to more positive values (Fig. 3; Table 1) from August 24, 1993 to September 12, 1994 indicates that the importance of fermentative-methanogenic relative to non-methanogenic PHC mineralization increased after terminating engineered in situ bioremediation. Fermentative-methanogenic PHC degradation leads to production of DIC with $\delta^{13}C > 0‰$ (Grossmann, 1997) while the $\delta^{13}C$ of DIC produced during non-methanogenic PHC mineralization is slightly more negative than the $\delta^{13}C$ of PHC (Hunkeler et al., 2002). A progressive shift of the $\delta^{13}C$ of the DIC to more positive values and a simultaneous increase of the CH_4 concentration was also observed at another field site (Baedecker et al., 1993).

Although the lower supply of O_2 and NO_3^- was partly compensated by a higher consumption of other oxidants, the total rate of PHC degradation upgradient of B–B′ was probably limited by the availability of oxidants as indicated by the almost complete consumption of O_2, NO_3^- and SO_4^{2-} upgradient of S8 and KB15 and the high concentrations of Fe(II) and CH_4.

4.3. Intrinsic in situ bioremediation

Between September 12, 1994 and November 3, 1997, the consumption of dissolved oxidants and the production of dissolved reduced species upgradient of intersection plane B–B′ decreased again (Table 1). The zone of highly reducing conditions began to shrink (Fig. 2) and concentrations of redox sensitive species in KB10 and KB15 approached background values (Fig. 4). Furthermore, the average $\delta^{13}C$ of the DIC became more negative indicating that fermentative-methanogenic degradation became less important again (Table 1). The decreasing consumption of oxidants could have been due to a decreasing availability of (i) nutrients (N, P), (ii) oxidants (Fe- and Mn-oxides), or (iii) PHC. The most likely explanation for the decrease in oxidant consumption is that PHC availability became limiting in parts of the aquifer. The supply of nitrogen and phosphorus probably did not limit biodegradation since in October 18, 1995, NH_4^+ and P_T were detected in most of the monitoring wells even though they were not added any more. P_T may have desorbed from surfaces (Stumm and Morgan, 1996). NH_4^+ probably mainly originated from microbial reduction of NO_3^- (Tiedje, 1988). Oxidant availability probably was not limiting either since not only the rate of Mn- and Fe-reduction decreased but also the consumption of dissolved oxidants which entered the source area at a constant rate during intrinsic in situ bioremediation. The hypothesis that oxidant consumption decreased due to limited availability of PHC in parts of the aquifer is also supported by the pattern of PHC concentrations. In monitoring wells in which a decrease of the oxidant consumption was observed, no dissolved PHC were found any more on October 18, 1995 and October 29, 1996 (KB10 and KB15). On the other hand, in monitoring wells in which oxidant concentrations remained low and concentrations of reduced species elevated (S6 and S8), dissolved hydrocarbons were detected (Figs. 3 and 4). Upgradient of these monitoring wells, biodegradation probably was still limited by the availability of oxidants.

The rate of DIC and CH_4 production decreased between September 12, 1994 and November 3, 1997. However, on November 3, 1997, the rate of DIC and CH_4 production was still about 25% of that during engineered in situ bioremediation. Furthermore, the rate of DIC and CH_4 production remained much larger than the rate of PHC elution (Table 1). This demonstrates that biodegradation was clearly the most important process removing weathered PHC, also in the absence of an external supply of oxidants. Thus, this study suggests that intrinsic in situ bioremediation can serve as an effective follow-up measure to mineralize PHC that remain in aquifers after terminating engineered in situ bioremediation.

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