

Intrinsic Aromatic Hydrocarbon Biodegradation for Groundwater Remediation

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Résumé — Biodégradation intrinsèque des hydrocarbures aromatiques pour la réhabilitation de nappes aquifères — La biodégradation intrinsèque, qui représente le processus clé de l'atténuation naturelle, est de plus en plus couramment envisagée comme une alternative aux procédés plus actifs pour la réhabilitation de sites contaminés. Dans cet article, la biodégradation intrinsèque des BTEX et des HAP est discutée. La première partie passe en revue la compréhension actuelle de la dégradation microbienne des hydrocarbures aromatiques ainsi que les méthodes disponibles pour l'évaluation de la bioréhabilitation intrinsèque. Le concept et les résultats obtenus lors d'une étude de cas sont présentés dans une seconde partie.

La biodégradation anaérobie et la biodégradation aérobie contribuent toutes deux à l'élimination des polluants de sites contaminés, comme les anciennes usines à gaz ou les décharges polluées par des huiles de pyrolyse. Les processus de biodégradation intrinsèque engendrent habituellement une suite de zones de redox (méthanogène, sulfatoréductrice, Fe(III)-réductrice, dénitrifiante, aérobie) dans le panache des polluants dans l'aquifère en aval de la source de contamination. Les méthodes d'évaluation dans ces différentes zones de redox comprennent l'analyse hydro- et géochimique, la mesure des potentiels redox et la détermination de l'hydrogène. La biodégradation des polluants visés peut être démontrée par la modification des profils de polluant, par le fractionnement isotopique, par des produits spécifiques du métabolisme et par des études en microcosmes sur des échantillons du terrain. Les études en microcosmes constituent un outil particulièrement utile pour identifier les mécanismes de dégradation et pour étudier le rôle d'accepteurs d'électrons spécifiques et des conditions redox.

Lors d'une étude de cas, nous avons examiné la biodégradation intrinsèque d'un site pollué par des huiles de pyrolyse. Du fait de la faible capacité de sorption de l'aquifère, la diminution de la concentration en polluant le long du panache d'eau polluée a été majoritairement attribuée à de la biodégradation. La réduction des sulfates et la réduction de Fe(III) étaient les principaux processus redox au cœur du panache d'eau souterraine. Les changements de profil des polluants le long du panache indiquaient l'existence de processus de biodégradation actifs, par exemple, la biodégradation du toluène et du naphthalène dans des zones anaérobies. Dans des microcosmes pollués avec des polluants modèles, la biodégradation du toluène et de l'éthylbenzène a été observée en conditions sulfatoréductrices. La dégradation du toluène, de l'éthylbenzène, du benzène et du naphthalène avait lieu en présence de Fe(III). En conditions aérobies, tous les BTEX et HAP étaient rapidement dégradés.

Abstract — Intrinsic Aromatic Hydrocarbon Biodegradation for Groundwater Remediation — *Intrinsic biodegradation, representing the key process in natural attenuation, is increasingly considered for the remediation of contaminated sites as an alternative to more active measures. In this paper, intrinsic biodegradation is discussed with respect to BTEX and PAH. In the first part, an overview is*

given summarizing the current understanding of microbial aromatic hydrocarbon degradation and the methods available for the assessment of intrinsic bioremediation. In the second part, the concept and selected results of a case study are presented.

Both aerobic and anaerobic biodegradation of aromatic hydrocarbons contribute to pollutant elimination at contaminated sites such as former manufactured gas plants and tar-oil polluted disposal sites. Intrinsic biodegradation processes usually result in a sequence of redox zones (methanogenic, sulfate-reducing, Fe(III)-reducing, denitrifying, aerobic) in the groundwater plume downgradient the source of contamination. Methods to assess redox zonation include hydro- and geochemical analysis, measurement of the redox potential, and determination of hydrogen. Biodegradation of target pollutants can be demonstrated by alterations in the pollutant profiles, isotopic fractionation, specific metabolic products, and by microcosm studies with authentic field samples. Microcosm studies in particular are a useful tool to identify degradation mechanisms and to understand the role of specific electron acceptors and redox conditions.

In a case study, intrinsic biodegradation was examined at a tar-oil polluted disposal site. Due to the low sorption capacity of the aquifer, decreasing pollutant concentrations with increasing plume length were attributed predominantly to biodegradation. Sulfate reduction and Fe(III) reduction were the most important redox processes in the anaerobic core of the groundwater plume. Changing pollutant profiles with increasing plume length indicated active biodegradation processes, e.g. biodegradation of toluene and naphthalene in the anaerobic zones. In microcosms amended with model pollutants, biodegradation of toluene and ethylbenzene was observed under sulfate-reducing conditions. Degradation of toluene, ethylbenzene, benzene and naphthalene occurred in the presence of Fe(III). Under aerobic conditions, all BTEX and PAH were rapidly degraded.

ABBREVIATIONS

ACE	acenaphthene
B	benzene
BTEX	sum of benzene, toluene, ethylbenzene, and xylenes
E	ethylbenzene
FLU	fluorene
NAP	naphthalene
PAH	poly(cyclic) aromatic hydrocarbons
PHE	phenanthrene
PYR	pyrene
T	toluene
TEA	terminal electron acceptor
US-EPA	United States Environmental Protection Agency
X	xylenes (sum of <i>o</i> -, <i>m</i> - and <i>p</i> -xylene)

INTRODUCTION

Intrinsic biodegradation, sometimes used synonymously with natural attenuation, represents an increasingly important component of contaminated site treatment strategies. At first maligned as a “do-nothing alternative”, intrinsic biodegradation is now being recognized as a legitimate remediation option that can supplement and sometimes even supplant more costly active measures such as pump-and-treat. However, as compared to the classical site remediation techniques, intrinsic bioremediation requires a better understanding of the pollutant elimination processes taking

place in aquifers and of their assimilative capacity. Pollutant elimination depends on the metabolic activity of the native microorganisms, that is triggered by the hydrogeology and geochemistry of aquifers. A careful and site-specific evaluation and monitoring is a prerequisite for the responsible application of the intrinsic bioremediation concept.

It is the objective of this paper to discuss the intrinsic bioremediation concept with respect to an application for aromatic hydrocarbon polluted sites.

In the first part of the paper, an overview is given, addressing:

- microbial aromatic hydrocarbon degradation processes under varying redox conditions;
- methods to assess redox zonation in the field;
- methods to evaluate intrinsic bioremediation.

In the second part:

- a case study is presented focussing on intrinsic biodegradation in the contaminated groundwater plume of a tar-oil polluted landfill.

1 DEFINITION OF INTRINSIC BIODEGRADATION AND NATURAL ATTENUATION

“Intrinsic biodegradation” is defined as the decomposition of organic compounds by indigenous microorganisms without artificial enhancement. The basic concept behind intrinsic bioremediation is to use the capacity of naturally occurring microorganisms to degrade contaminants that have been released into the environment.

The more general concept of natural attenuation includes all processes contributing to a reduction in pollutant concentration and mass (Wiedemeier *et al.*, 1999). According to the *Environmental Protection Agency (US-EPA)*, Natural attenuation is defined as “the reliance on natural attenuation processes to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by other more active methods. The natural attenuation processes that are at work in such a remediation approach include a variety of physical, chemical, or biological processes that, ... , act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. These *in-situ* processes include biodegradation; dispersion; dilution; sorption; volatilization; ...” (US-EPA, 1999; US-NRC, 2000).

Obviously, most of the natural attenuation processes, *e.g.* physical dilution, physicochemical sorption and ion exchange, chemical dissolution/precipitation or complexation, result in a redistribution of the pollutants in the environment. A real pollutant elimination can be achieved by intrinsic biodegradation, representing the key process in natural attenuation.

2 BIODEGRADATION OF BTEX AND PAH

2.1 Interrelation of Redox Conditions and Degradation of Aromatic Hydrocarbons

Microorganisms obtain energy for cell production and maintenance by catalyzing the transfer of electrons from electron donors to electron acceptors. Important electron acceptors in aquifers include dissolved oxygen, nitrate, sulfate, and Fe(III) minerals in the aquifer material. After these electron acceptors are depleted, microorganisms may ferment organic compounds through reactions that produce methane and carbon dioxide (Chapelle, 2001).

The microbial energy yield obtained by pollutant degradation depends on the redox regime. Due to

thermodynamic considerations, aerobic biodegradation is the most efficient process for the breakdown of aromatic hydrocarbons. Under anaerobic conditions, denitrification and metal-reduction are favoured to sulfate reduction, and methanogenesis (Table 1). Therefore, in general a sequential utilization of the TEA is observed: aerobic degradation is followed by denitrification, iron and manganese reduction, sulfate reduction and methanogenesis (Bouwer, 1992). At contaminated sites with high levels of biodegradable material, most of the TEA present in the upgradient groundwater, are rapidly consumed in the source. Therefore, downgradient and adjacent to the source zone often very low redox potentials, resulting in methanogenic or sulfidogenic conditions, are observed that increase with increasing plume length (Christensen *et al.*, 2001). Microbial activity governs the redox regime by consumption of TEA (*e.g.* sulfate) and formation of reduced respiration products (*e.g.* sulfide). On the other hand, the redox regime significantly affects the microbial utilization of specific pollutants.

In the presence of oxygen, the BTEX (Fritsche and Hofrichter, 2000; Kästner, 2000) and most PAH with up to 5 rings (Cerniglia, 1992; Kästner, 2000) are biodegradable. Biodegradation rates of PAH decrease with increasing molecular weight (Tiehm and Fritsche, 1995; Tiehm, *et al.* 1997; Tiehm and Stieber, 2001) and additional growth substrates might be necessary as for cometabolic degradation of benzo[a]pyrene (Juhász and Naidu, 2000). Under aerobic conditions, oxygen is used not only as terminal electron acceptor, but also as chemical reactant for the enzymatic oxygenase reactions that result in the cleavage of the aromatic ring structure (Fritsche and Hofrichter, 2000; Kästner, 2000).

Since a cleavage of the aromatic ring by oxygenase reactions is not possible in the absence of oxygen, other enzymatic mechanisms must be undergone such as coenzyme-A-ligase reactions, carboxylations, reductive dehydroxylations, or methyl oxidations (Heider and Fuchs, 1997). The anaerobic

TABLE 1

Stoichiometry and standard free energy changes under various redox conditions (McFarland and Sims, 1991, modified)

Degradation process	Reaction: mineralization of naphthalene at pH 7 and 25°C	ΔG° (kcal/eeq)
Aerobic Respiration	$C_{10}H_8 + 12 O_2 \rightarrow 10 CO_2 + 4 H_2O$	-25.43
Denitrification	$C_{10}H_8 + 9.6 NO_3^- + 9.6 H^+ \rightarrow CO_2 + 4.8 N_2 + 8.8 H_2O$	-23.88
Fe(III) reduction	$C_{10}H_8 + 20 H_2O + 48 Fe^{3+} \rightarrow 10 CO_2 + 48 Fe^{2+} + 48 H^+$ $C_{10}H_8 + 48 FeOOH(s) + 24 HCO_3^- + 48 H^+ \rightarrow 10 CO_2 + 48 FeCO_3 + 76 H_2O$	-24.53* -5.66**
Sulfate Reduction	$C_{10}H_8 + 6 SO_4^{2-} + 9 H^+ \rightarrow 10 CO_2 + 3 H_2S + 4 H_2O$	-1.67
Methanogenesis	$C_{10}H_8 + 12 H_2O \rightarrow 4 CO_2 + 6 CH_4$	-0.99

eeq: electron equivalent.

* assuming a soluble species is available at neutral pH, *e.g.* by microbial chelation.

** assuming a bicarbonate concentration of 10^{-3} M.

degradation of the alkylbenzenes, *e.g.* toluene, ethylbenzene, and the xylenes, has been studied for many years, and the enzymatic degradation pathways are well known (Fuchs *et al.*, 1994; Harwood *et al.*, 1999; Heider *et al.*, 1999; Schink, 2000). In contrast, anaerobic biodegradation of aromatic hydrocarbons without additional functional groups, *i.e.* benzene and PAH, was considered unlikely due to the lack of destabilizing ring substituents. Only in recent years, oxygen independent hydroxylation and degradation of benzene was repeatedly observed, even though the degrading bacteria could not yet be isolated in most cases (Grbic-Galic, 1990; Weiner *et al.*, 1998; Burland and Edwards, 1999; Schink, 2000). Degradation of PAH in the absence of oxygen has been documented only in a few reports. Carboxy derivatives have been observed as the first intermediates (Zhang and Young, 1997; Meckenstock *et al.*, 2000), and recently a reductive 2-naphthoic acid pathway was proposed for naphthalene degradation under sulfate reducing conditions (Annweiler *et al.*, 2002).

Pollutant degradability is most significantly affected by the availability of the different TEA. However, in the field also the catabolic potential of the indigenous microflora, the availability of nutrients, and other environmental factors determine which pollutants are eventually degraded.

2.2 Anoxic Biodegradability of BTEX and PAH

With nitrate as TEA, biodegradation has been reported for ethylbenzene, toluene, and xylenes (Altenschmitt and Fuchs, 1991; Hutchins, 1991; Schocher *et al.*, 1991; Evans *et al.*, 1992; Fries *et al.*, 1994; Seyfried *et al.*, 1994; Biegert and Fuchs, 1995; Rabus and Widdel, 1995; Ball *et al.*, 1996; Beller and Spormann, 1997). Benzene degradation under denitrifying conditions is still controversially discussed (Burland and Edwards, 1999; Lovley, 2000). Nitrate dependent anoxic naphthalene mineralization has been demonstrated recently for pure cultures (Rockne *et al.*, 2000). Biodegradation of PAH by mixed cultures was reported in several cases, notably for naphthalene (Milhelcic and Luthy, 1988a; Milhelcic and Luthy, 1988b; Al-Bashir *et al.* 1990), but also for some other 2- to 4-ring PAH—*e.g.* phenanthrene (Milhelcic and Luthy, 1988a, 1988b); acenaphthene, acenaphthylene, fluorene, anthracene (Leduc *et al.* 1992); anthracene, phenanthrene, pyrene (McNally *et al.*, 1998).

Field studies indicate that degradation of aromatic hydrocarbons is possible also with Fe(III)—*e.g.* Vejen, Denmark (Lynkilde and Christensen, 1992); Bimidji, Minnesota (Anderson and Lovley, 1999); Düsseldorf, Germany (Schmitt *et al.*, 1996)—, which is generally the most abundant potential TEA in soils and sediments (Lovley 1991). In laboratory studies, biodegradation of BTEX and PAH under Fe(III) reducing conditions has been observed for toluene (Lovley *et al.*, 1989), benzene and toluene (Lovley *et al.* 1994b), naphthalene and benzene (Anderson and

Lovley, 1999), and recently also for naphthalene and phenanthrene (Ramsey *et al.*, 2001). Although increasing regard is given to Fe(III)-reducing processes, only a few studies deal with the degradation mechanisms (Lovley and Loney, 1990). Utilization of Mn(IV) as electron acceptor was observed during biodegradation of naphthalene (Langenhoff *et al.*, 1996).

In the presence of sulfate, the degradation of alkylbenzenes has been demonstrated with pure cultures—*e.g.* toluene (Rabus *et al.*, 1993; Beller *et al.*, 1996; Harms *et al.*, 1999); *o*-xylene and *m*-xylene (Harms *et al.*, 1999). The biodegradation of benzene and some PAH has been shown with sediment samples or enrichment cultures—all BTEX (Phelps and Young, 1999); benzene (Lovley *et al.*, 1995; Weiner *et al.*, 1998); naphthalene (Bedessem *et al.* 1997; Coates *et al.*, 1997; Zhang and Young, 1997; Galushko *et al.*, 1999; Meckenstock *et al.*, 2000), phenanthrene (Coates *et al.*, 1997; Zhang and Young, 1997); fluorene, fluoranthene (Coates *et al.*, 1997).

Under methanogenic conditions, biotransformation was observed for BTEX—*e.g.* toluene (Wilson *et al.*, 1986; Grbic-Galic and Vogel, 1987; Phelps and Young, 1999), ethylbenzene (Phelps and Young, 1999), and benzene (Wilson *et al.*, 1986; Grbic-Galic and Vogel, 1987). Grbic-Galic (1990) also reported the degradation of naphthalene and acenaphthene by methanogenic consortia. In other publications, a decrease of PAH indicated limited PAH transformation under methanogenic conditions (*e.g.* naphthalene (Govind *et al.*, 1991; Parker and Monteith 1995; Sharak-Genthner *et al.*, 1997); acenaphthylene, anthracene, phenanthrene (Parker and Monteith, 1995), but neither of these studies was based on enrichment cultures or isolated strains.

3 ASSESSMENT OF REDOX ZONES

Since the biodegradability of groundwater pollutants differs significantly in different redox environments, the redox characterisation of polluted aquifers is essential to understand and to predict intrinsic biodegradation. At present, redox conditions are routinely analysed by hydrogeochemical parameters and redox potential. Analysis of hydrogen concentrations in groundwater has been developed recently, and might further improve the interpretation of field data in the future.

3.1 Hydrochemical and Geochemical Analysis

The depletion of oxygen, nitrate, and sulfate within a plume compared to concentrations outside the hydrocarbon plume, and the elevated concentration of ferrous iron and methane produced within the plume can be used to identify redox zones and to roughly estimate the total amount of electron acceptors available in groundwater (Borden *et al.*, 1995).

However, calculation of pollutant mass degradation with respect to electron acceptor consumption and by-product production is hampered by precipitation and abiotic reactions of particular compounds in the subsurface. For instance, elevated concentrations of ferrous iron only reflect iron(III) reduction if there is minimal sulfate reduction, because generation of sulfide results in the formation of iron sulfide precipitates. Fe(II) also may precipitate as ferrous carbonate (Heron and Christensen, 1995). The actual distribution of Fe(II) will be controlled by the groundwater chemistry and the sediment type. The precipitates increase the reduction capacity of the sediment. This will dramatically increase the demand for oxygen, if a stimulated bioremediation of the plume by the addition of oxygen is considered (Heron and Christensen, 1995; Hartog *et al.*, 2002).

3.2 Redox Potential

The electrochemical measurement of the redox potential is often considered for redox characterization of natural environments. The advantages of this method are the quick measurement and the limited costs to obtain spatially and temporally resolved field data. In principle, the oxidation-reduction potential of groundwater is a measure of electron activity and is an indicator of the relative tendency to accept or transfer electrons. The upper range is attributed to oxygen reduction with potential values about 800 mV and the lower range to carbonate—and H^+ —reduction with values about -300 to -400 mV (Zehnder and Stumm, 1988).

Interpretation of the redox potential of groundwater samples is difficult, since the measured value is a mixed potential of many reactions and cannot be used for quantitative purposes (Stumm and Morgan, 1996). Furthermore, reliable and reproducible values require sufficient exchange currents at the electrode and redox equilibrium at the electrode and in the groundwater. Unfortunately, electrodes often do not respond to the relevant redox couples or equilibration kinetics are too slow (Sigg, 2000). However, electrochemical redox measurements seem to be useful in Fe(II)/(III) dominated waters, because this redox couple underlies fast equilibration kinetics (DVWK, 1989). In other cases, additional analysis of hydro- and geochemical parameters has been recommended for a reliable description of the redox conditions (Christensen *et al.*, 2000).

3.3 Hydrogen

An alternative—but at present poorly available and expensive—indicator for the different redox processes occurring in the field is the measurement of hydrogen concentration (Lovley and Goodwin, 1988; Lovley *et al.*, 1994a; Chapelle *et al.*, 1995; Chapelle *et al.*, 1997). The hydrogen method is based on the ecological concept of interspecies hydrogen transfer by microorganisms. Because

each microbial TEA process in groundwater systems is associated with a characteristic hydrogen concentration, concentrations of dissolved hydrogen can be used to evaluate redox processes.

Meer *et al.* (2001) compared hydrogeochemical and hydrogen analysis at seventeen different sites. In conclusion, there was no single best method to identify redox conditions. Due to the particular limitations of each individual redox indicating parameter, in practice it is preferable to analyse redox conditions in the field on the basis of several lines of evidence in order to obtain a comprehensive figure of the field conditions.

4 METHODS TO ASSESS INTRINSIC BIODEGRADATION

Regulatory acceptance of monitored natural attenuation as a groundwater remediation strategy is often dependent on credible evidence that decreases in the concentrations of contaminants truly represent intrinsic biodegradation of these compounds rather than abiotic processes. Different methods have been developed to assess intrinsic bioremediation under field conditions. In many cases, several techniques are applied simultaneously in order to demonstrate biological pollutant degradation by multiple lines of evidence (Chapelle *et al.*, 1995; Cho *et al.*, 1997; Levine *et al.*, 1997; Christensen *et al.*, 2001).

4.1 Pollutant Profiles

The assessment of pollutant biodegradation by the monitoring of a single compound, *e.g.* benzene, is not possible since abiotic processes such as sorption, dilution, and volatilization have also to be considered. However, conclusions can be drawn from changing ratios of particular BTEX and PAH in a plume if their physicochemical properties (*Table 2*) are taken into account. A more rapid decline of a less hydrophobic and therefore less retarded compound indicates that intrinsic biodegradation occurs.

The spatial distribution of contaminants, but also of specific metabolic products, electron acceptors, and respiratory products, can be used to evaluate the occurrence of a given biodegradation reaction. For example, at a hydrocarbon polluted site, the plume lengths of ethylbenzene and benzene were compared. The considerably shorter plume length of ethylbenzene as compared to benzene pointed to a faster intrinsic biodegradation of ethylbenzene in the anaerobic redox zones (Volkering *et al.*, 2001). At another site, in particular toluene and *o*-xylene decreased rapidly in the anaerobic zone, and slower reduction of ethylbenzene and benzene was observed (Borden *et al.*, 1995). In a gasoline plume, toluene and ethylbenzene declined most rapidly with distance from the source followed

by *m-p*-xylene and then *o*-xylene and benzene (Borden *et al.*, 1997). These examples illustrate that the horizontal and vertical concentration of pollutants provides evidence for biodegradation processes. Since pollutant concentration data are available at most contaminated sites, the evaluation of changing pollutant profiles represents a readily available line of evidence demonstrating intrinsic biodegradation.

TABLE 2
Physicochemical properties of BTEX* and PAH**

Compound	Molecular Weight	Water solubility	Octanol-water partition coefficient
		[mg l ⁻¹]	log K _{OW}
Benzene (B)	78	1789	2.13
Toluene (T)	92	579	2.65
Ethylbenzene (E)	106	187	3.13
<i>o</i> -xylene (<i>o</i> -X)	106	221	3.13
<i>m</i> -xylene (<i>m</i> -X)	106	160	3.20
<i>p</i> -xylene (<i>p</i> -X)	106	215	3.18
Naphthalene (NAP)	128	30.0	3.37
Acenaphthylene (ACY)	152	3.93; 16.1***	4.07
Acenaphthene (ACE)	154	3.47	4.33
Fluorene (FLU)	166	1.98; 1.8***	4.18
Phenanthrene (PHE)	178	1.29	4.46
Anthracene (ANT)	178	0.07	4.45
Fluoranthene (FLA)	202	0.26	5.33
Pyrene (PYR)	202	0.14	5.32
Benzo[a]anthracene (BaA)	228	0.014	5.61
Chrysene (CHR)	228	0.002; 0.0006***	5.61
Benzo[b]fluoranthene (BbF)	252	0.0012	6.57
Benzo[k]fluoranthene (BkF)	252	0.0006	6.84
Benzo[a]pyrene (BaP)	228	0.0038	6.04
Dibenzo[a,h]anthracene (DBA)	278	0.0005	6.75
Benzo[g,h,i]perylene (PER)	276	0.00026	7.23
Indeno[1,2,3-cd]pyrene (IND)	276	0.062	7.66

* data obtained from Miller *et al.* (1985).

** data obtained from Sims and Overcash (1983), unless otherwise noted.

*** data obtained from Kästner (2000).

4.2 Isotopic Fractionation

Another approach to assess intrinsic biodegradation is to monitor different isotopes of the same compound instead of analysing the changes in pollutant profiles. Isotopes are atoms from the same element with a different number of neutrons in the nucleus, resulting in different atomic weight. Stable isotopes, such as ¹³C and the common isotope ¹²C, are not subject to radioactive decay, and behave slightly differently in various physical, chemical, and biological processes. The change in isotope composition of molecules due to differences in reaction rates is called isotope fractionation. The fractionation causes the residual pollution to become enriched in the heavy isotope. In the context of intrinsic bioremediation, isotopic fractionation of ¹³C/¹²C has been demonstrated repeatedly for toluene (Morasch *et al.*, 2001; Pelz *et al.*, 2001; Richnow *et al.*, 2001) and first indications concerning an isotopic fractionation of benzene have been presented (Volkering *et al.*, 2001). Also isotopic fractionation of deuterium/hydrogen has been reported (Morasch *et al.*, 2001; Pond *et al.*, 2002). The highest isotope fractionation factors are associated with reducing conditions and the lowest ratios are associated with aerobic and nitrate-reducing environments. Therefore, isotopic fractionation has also been suggested to delineate redox zones (Levine *et al.*, 1997). On the other hand, to select an appropriate isotope fractionation factor for the calculation of biodegradation rates, information of the biochemical conditions such as temperature and redox conditions in the contaminated aquifer must be available (Richnow *et al.*, 2001).

4.3 Specific Metabolic Products

Intermediate products of hydrocarbon biodegradation provide biochemical evidence of microbial transformations. Therefore, monitoring of distinctive metabolites has been proposed for documenting *in situ* biodegradation in hydrocarbon-contaminated aquifers. Such metabolites should have the following favorable characteristics:

- an unequivocal and unique biochemical relationship to their parent hydrocarbons;
- no commercial or industrial uses;
- sufficient biological and chemical stability (Beller *et al.*, 1995). Presently, limited field data exist on the concentration, stability, fate and transport of metabolites in hydrocarbon polluted plumes.

Under anaerobic conditions, metabolic by-products include benzoic acid, one to three methylbenzoic acids and other aromatic acids that are structurally related to alkylbenzene precursors, alicyclic acids and low molecular weight aliphatic acids (Cozzarelli *et al.*, 1994; Beller *et al.*, 1995; Cozzarelli *et al.*, 1995). It has been shown recently for a fuel contaminated aquifer, that *e.g.* benzylsuccinates and methylbenzylsuccinates are metabolites that can indicate

anaerobic degradation of toluene and xylene, respectively (Beller, 2002). At another site, neither benzyl succinic nor benzyl fumaric acid were detected in the anaerobic petroleum hydrocarbon degrading zones, but benzene acetic acid and methyl benzoic acids were found (Levine *et al.*, 1997).

Obviously, specific metabolites might be used as molecular markers of biological contaminant degradation. However, since the intermediates are subject to further degradation, their absence does not indicate that intrinsic biodegradation does not occur. Another important obstacle in applying this technique is the labor-intensive and time-consuming analytical procedure (Levine *et al.*, 1997; Beller, 2002).

4.4 Microcosm Studies

Aquifer materials placed into laboratory vessels such as test tubes or septated serum vials for measurement of microbial activity are referred to as microcosms. A microcosm is defined as a community or other small unity that is representative of a larger unity. The reasoning for microcosms is that by understanding the activity in a small portion of an aquifer, much can be learned about the aquifer as a whole. Microcosms have been used extensively in studies of subsurface microbial ecology and are especially useful in assaying indigenous microbial communities for specific activities, *e.g.* degradation of xenobiotic pollutants (Chapelle, 2001). Whereas the field techniques such as measuring pollutant profiles or isotopic fractionation represent integral parameters to assess intrinsic biodegradation, microcosm studies are an appropriate tool to identify the degradation mechanisms.

In direct field measurements, the identification of the redox conditions suitable for the degradation of a particular pollutant is hampered by several obstacles:

- in the subsurface microcompartments are likely to exist with varying redox conditions occurring within short distances and integrated by the sampling of groundwater;
- usually even on a larger scale an overlap of redox zones is observed in the plume;
- the formation of different redox zones by microbial degradation does not necessarily mean that degradation of the target pollutants occurs in all zones. Often, the target pollutants constitute only a small part of the overall biodegradable organic contamination in the plume, *e.g.* in the plumes of landfills (Christensen *et al.*, 1994) and disposal sites rich in organic material (Schulze *et al.*, 2001). Therefore, additional studies in closed systems and under known redox conditions are required to identify the degradation mechanisms.

Laboratory (Hunt *et al.*, 1997; Bjerg *et al.*, 1999; Althoff *et al.*, 2001) and *in situ* microcosms (Gillham *et al.*, 1990a; Gillham *et al.*, 1990b; Acton and Barker, 1992; Bjerg *et al.*,

1996; Bjerg *et al.*, 1999) have frequently been used to demonstrate biodegradation of contaminants by indigenous microorganisms under conditions closely resembling ambient. For example, in a laboratory biodegradation study comparing alkylbenzene biodegradation at two sites, the evaluation of degradation patterns in microcosms constructed with sediment and groundwater from several areas of the plume allowed for a reasonable comparison with field data throughout the plume length. At the first site studied, toluene was rapidly degraded in the microcosms, and was also found to decay most rapidly in the field. At the second site, toluene, *o*-xylene, *m*-xylene, and benzene were degraded under anaerobic conditions, and the microcosms indicated that iron-reduction was the dominant degradation mechanism (Hunt *et al.*, 1997). Bjerg *et al.*, (1999) studied the biodegradation of aromatic and chlorinated aliphatic compounds in *in situ* microcosms and laboratory batch experiments. Both approaches gave results comparable to those obtained in a field injection experiment performed in the same plume. It was confirmed that laboratory microcosms, that are easier to run than *in situ* microcosms, are a useful tool in determining the degradability of pollutants under specific redox conditions.

5 CASE STUDY

The case study was performed in order to investigate intrinsic bioremediation in a PAH and BTEX contaminated plume. In the field, the pollutant concentrations in groundwater and sediment, and the redox zonation were determined. Laboratory microcosms were performed to identify the most important degradation mechanisms. In the following paragraphs, selected results (see also Tiehm *et al.*, 2001) are presented that illustrate the concept of the microbiological site examination.

5.1 Site Description

The study site “Stürmlinger Sandgrube” is located in Karlsruhe, in the south-west of Germany. The aquifer consists of porous sand and gravel representing a typical composition for the Upper Rhine Valley. The groundwater velocity averages 0.8 m/day, the coefficient of permeability (K_f value) is about 2×10^{-3} m/s, and the groundwater temperature, 11-13°C. Between 1925 and 1956, the former sand pit was filled with municipal and industrial waste, including slags, combustion residues, and gas works residues. The disposal site covers a surface area about 1500 m². The bottom of the dump reaches the groundwater table in about 8 m depth. In the northern part of the dump, tar oil was found as nonaqueous phase liquid in the fluctuation zone of the groundwater table.

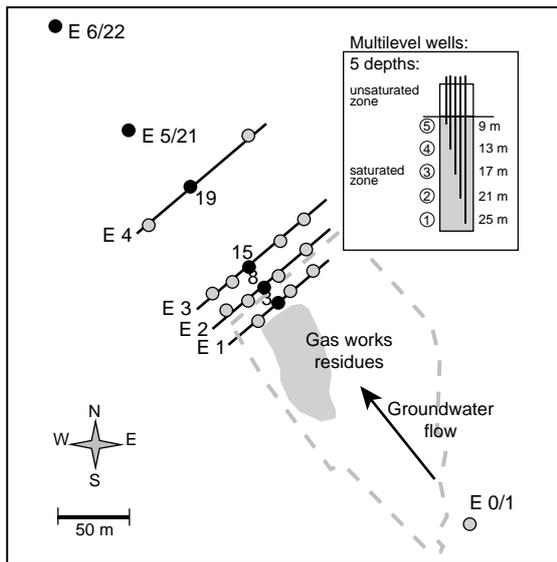


Figure 1

Location of the multilevel sampling wells at the disposal site "Stürmlinger Sandgrube".

In the plume, a net of multilevel wells was installed. The wells consist of 5 separate pipes ending in about 9, 13, 17, 21, and 25 m depth. Twenty wells are arranged in lines perpendicular to the flow direction with increasing distance to the disposal site (E1 to E4: 5 m, 20 m, 40 m, and 110 m, respectively). Two additional wells are located further downgradient (E5/21: 170 m, and E6/22: 260 m). Well E0/1 was installed upgradient the dump in order to measure background concentrations (Fig. 1).

5.2 Pollutants

After passage of the dump, the groundwater is heavily contaminated with BTEX and PAH. Adjacent to the source, the maximum concentration of the dissolved BTEX and PAH reaches values of 50 000 $\mu\text{g/l}$ and 17 000 $\mu\text{g/l}$, respectively. The maximum contamination is found in the sampling depths of 9 m nearby the source and 13 m at most of the multilevel sampling wells further downgradient.

The decline of BTEX and selected PAHs along the plume is shown in Fig. 2. In this figure, pollutant concentrations are charted as average concentrations over all 5 sampling depths

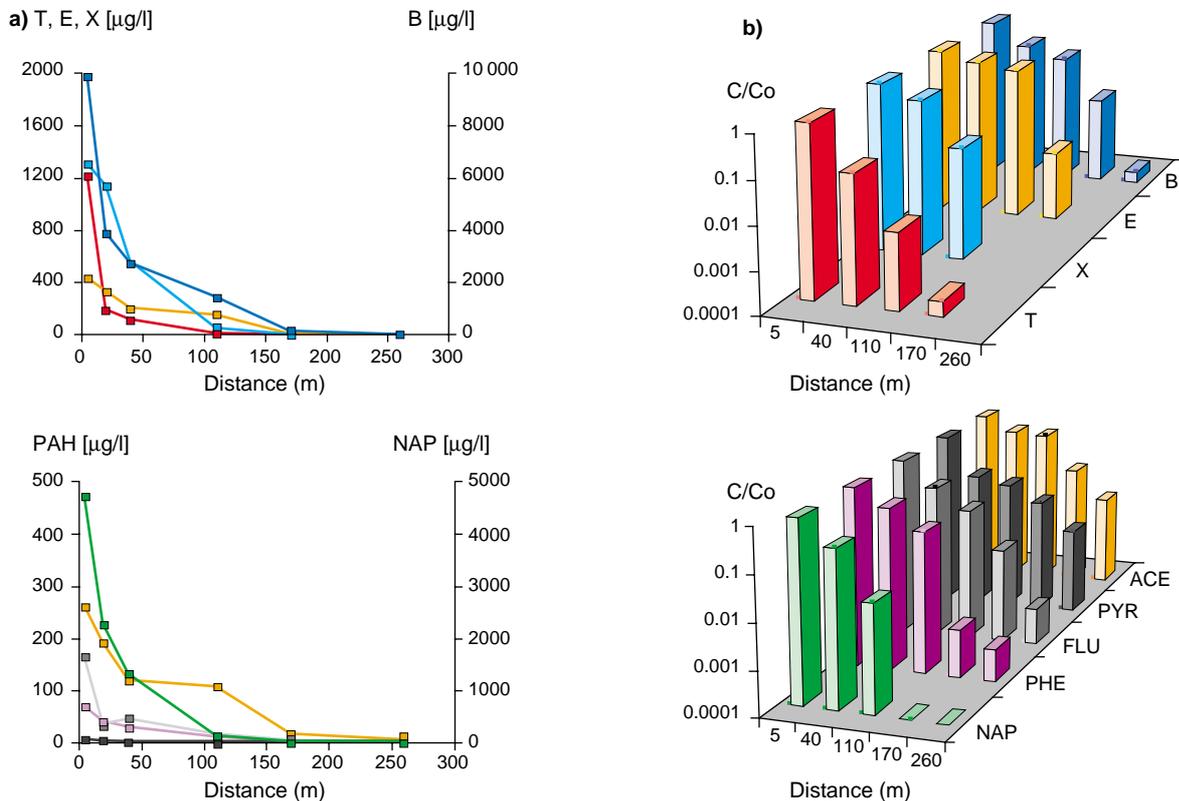


Figure 2

BTEX (top) and PAH (bottom) in the contaminated groundwater plume. Absolute (a) and relative C/C_0 (b) (semilogarithmic plot) average vertical concentrations are shown.

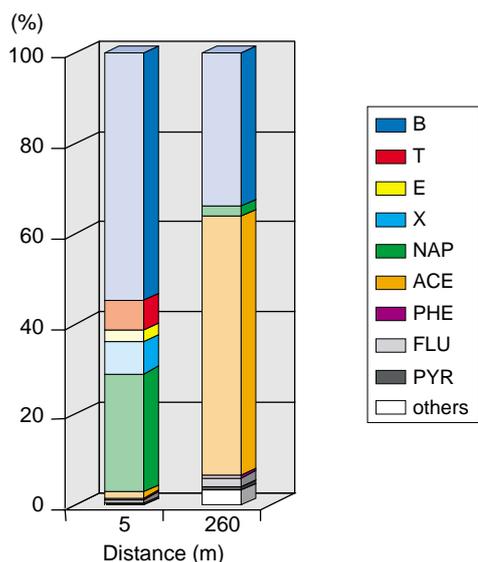


Figure 3

Composition of the BTEX and PAH in the groundwater plume at 5 and 260 m distance to the source.

of the multilevel wells, to eliminate flow path deviations and to increase statistical certainty. All pollutants decrease significantly with increasing distance to the disposal site. The xylenes are no longer detectable (corresponding to a concentration $< 1 \mu\text{g/l}$) at 170 m distance. Toluene and ethylbenzene are below the detection limit 260 m downgradient (Fig. 2b). At this distance, the concentration of naphthalene is below $1 \mu\text{g/l}$, despite the high initial aqueous phase concentration of about $5000 \mu\text{g/l}$.

The concentration of naphthalene in the aqueous phase decreased more rapidly (4 orders of magnitude within 170 m) than the other, more hydrophobic and more sorptive PAH (2 to 3 orders of magnitude), and toluene decay occurred faster as compared to the other alkylbenzenes. This finding is one line of evidence for active biodegradation processes in the anaerobic zones of the plume.

Adjacent to the source zone, 55% of the total mass of the BTEX and PAH were attributed to benzene, 26% to naphthalene, and 16% to toluene, ethylbenzene, and the xylenes (Fig. 3). 260 m downgradient, still 34% of the contamination was attributed to benzene, but naphthalene and the alkylbenzenes represented only 2% of the remaining pollutants. The relative contribution of acenaphthene increased from 1% at a distance of 5 m to 57% after 260 m. Obviously, both the retardation and the biodegradation of acenaphthene in the groundwater plume are poor resulting in an increasing dominance of this compound. Therefore, acenaphthene might be a suitable analytical standard to monitor the maximum plume length.

5.3 Redox Zones

Dissolution of organic contaminants from the dump into groundwater and subsequent microbiological degradation influence the redox sensitive groundwater constituents. In the plume, different redox zones were detected in terms of reduced concentrations of electron acceptors and the occurrence of the corresponding respiration products (Fig. 4).

Oxygen and nitrate were detected in the upper groundwater layer, adjacent to the water table (depth 9 m). 260 m downgradient, these electron acceptors were also available in the deeper horizons (Fig. 4a). In an extended area of the plume, elevated Fe(II)-concentrations indicated Fe(III)-reduction (Fig. 4b). Sulfate reduction was the predominating redox process in the highly contaminated areas of the plume near the disposal site (Fig. 4c). Due to the spatial proximity of iron- and sulfate reducing processes resulting in FeS precipitation, only low concentrations of Fe(II) and sulfide were found. Elevated CH_4 concentrations were restricted to a small area near the source (Fig. 4d). The redox zones were not sharply separated and transition zones were observed indicating that groundwater samples might have represented composite samples of different microenvironments.

5.4 Identification of the Degradation Mechanisms

Microcosms were designed to study degradation of BTEX and PAH in the presence of different electron acceptors. Groundwater was taken from the plume about 20 m downgradient of the sand pit, in a depth of 17 m. Sulfate reduction was the predominating redox process in this zone of the plume. Benzene, toluene, ethylbenzene, naphthalene, phenanthrene, and pyrene were added as model pollutants. Each microcosm study was performed with 2 l of groundwater. In order to inoculate also surface-attached indigenous microorganisms, 100 g sediment from the same sampling point were added to each microcosm. Incubation was done in 2.25 l bottles at groundwater temperature ($12 \pm 2^\circ\text{C}$) in the dark. The bottles were shaken by hand every 2-3 days. Abiotic losses were analysed in control bottles inactivated by the addition of sodium azide. Anaerobic microbial degradation was monitored in the original groundwater containing about 130 mg/l sulfate, and after dosage of alternative TEA. Fe(III)-reducing conditions were adjusted by the addition of amorphous Fe(III)-hydroxide (Phillips and Lovley, 1986), and aerobic conditions were obtained by the addition of air.

Results show that the degradation pattern of BTEX and PAH differed significantly under the various redox conditions. Under sulfate reducing conditions, toluene and ethylbenzene were degraded (Fig. 5), whereas benzene and the PAH were not metabolized during 100 days of incubation.

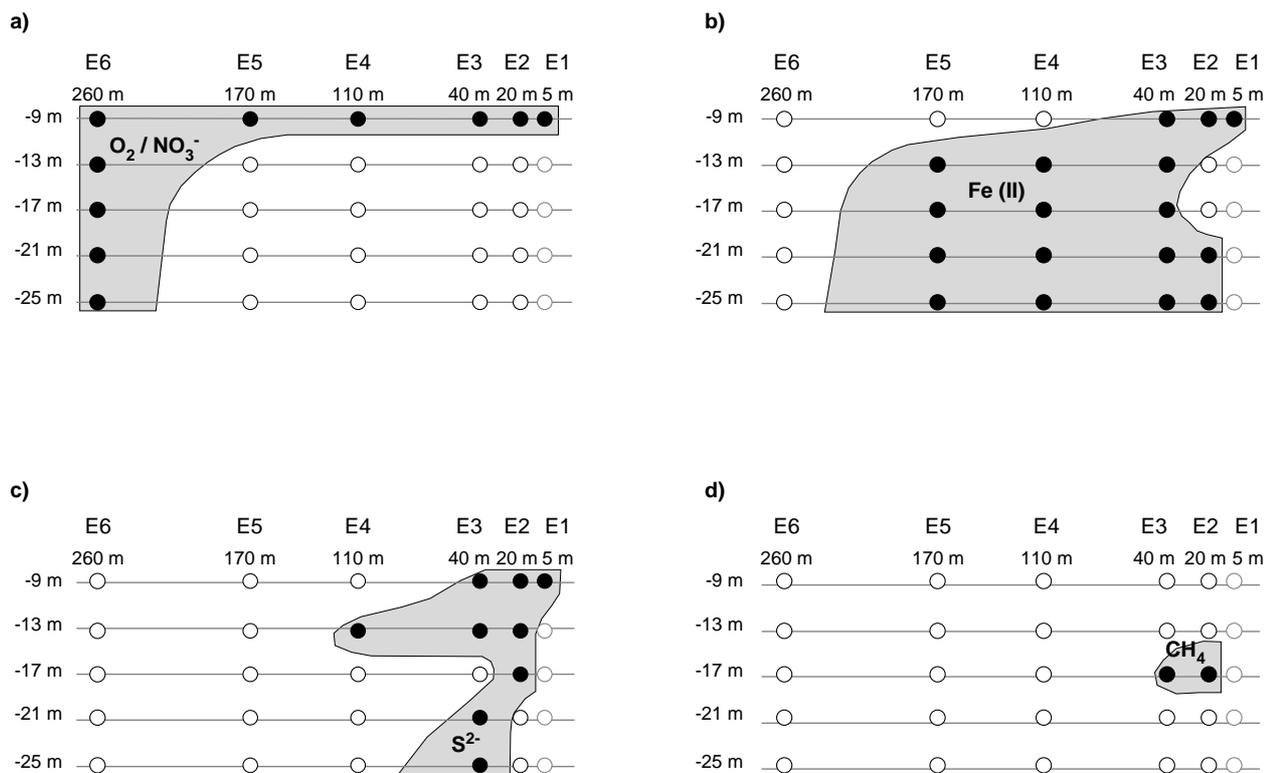


Figure 4

Redox zones in the plume of the disposal site, "Stürmlinger Sandgrube".

- (a) aerobic/denitrifying zone ($O_2 \geq 0.5$ mg/l and $NO_3^- \geq 1$ mg/l); (b) Fe(III)-reducing zone ($Fe(II) \geq 1$ mg/l); (c) sulfate-reducing zone ($S^{2-} \geq 100$ μ g/l); (d) methanogenic zone ($CH_4 \geq 100$ μ g-l).

In the presence of Fe(III), a stimulated biodegradation of the model compounds was observed (Fig. 6). Toluene and ethylbenzene were transformed with faster kinetics than under sulfate-reducing conditions. Additionally, benzene and naphthalene were degraded. The results of the microcosms indicated that, at this site, sulfate reducing and in particular Fe(III)-reducing microbial communities contribute significantly to the elimination of aromatic hydrocarbons in the anaerobic zones of the plume.

The highest degradation potential was observed in the presence of oxygen. After the addition of air, all BTEX and PAH were degraded to concentrations below the detection limit within 14 days (data not shown).

CONCLUSION

The studies published in recent years have clearly demonstrated that, in many cases, monitored intrinsic

biodegradation represents a viable strategy in groundwater remediation. A prerequisite for predicting the future expansion of the contaminated groundwater plumes is the site-specific assessment and understanding of the natural biodegradation processes. From a microbiological point of view, the biodegradation potential in the aquifer and the spatial distribution and availability of electron acceptors are essential.

In a case study, intrinsic biodegradation in the plume of a municipal waste and gas works residues disposal site resulted in the formation of different redox zones. Three-dimensional hydrogeochemical analysis and pollutant profiles indicated that both anaerobic and aerobic processes contributed to BTEX and PAH degradation, but the reduction of aromatic hydrocarbons could not be related to specific redox processes on the basis of field data alone. Corresponding microcosm experiments showed that toluene and ethylbenzene were degradable under sulfate-reducing conditions. In the presence

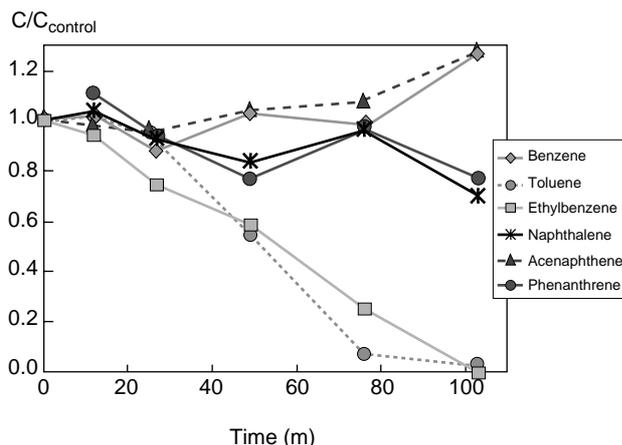


Figure 5
Degradation of BTEX and PAH under sulfate reducing conditions.

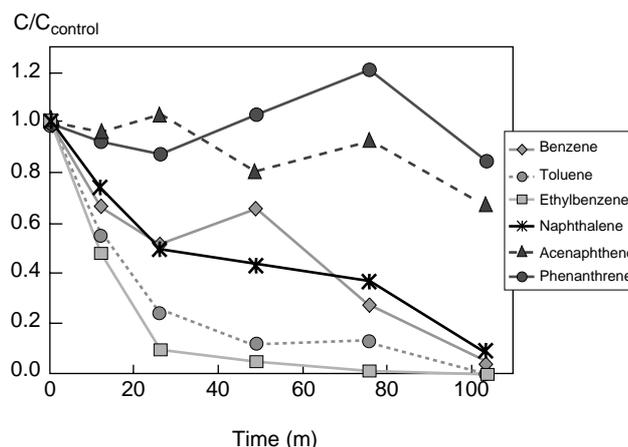


Figure 6
Degradation of BTEX and PAH after the addition of Fe(III).

of Fe(III), additionally benzene and naphthalene were degraded. In the presence of oxygen, the anaerobically recalcitrant pollutants were utilized. Due to its low retardation and slow biodegradation, acenaphthene was a suitable compound to monitor the maximum plume length at this site.

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