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Contribution of anaerobic microbial activity to natural attenuation of benzene in groundwater

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Abstract

Anaerobic biodegradation of hydrocarbons, using a variety of terminal electron acceptors (TEAs), is increasingly being reported both in laboratory studies and in the field. Of all the petroleum hydrocarbons, benzene is considered the most problematical due to its high toxicity and relatively high aqueous solubility. These, combined with its peculiarly stable structure, mean that it has long been considered recalcitrant in all but aerobic conditions. There is now a small, but growing, literature to suggest that this may not in fact be the case. We present an assessment of the field, encompassing reviews up to 1997 and original papers published since then. It appears that benzene is indeed degraded anaerobically, but that organisms capable of doing so are not ubiquitous. In addition, benzene degradation may be competitively inhibited by the presence of more readily degraded compounds such as toluene. Certainly, the occurrence and rate of benzene attenuation under anaerobic conditions is far more site-specific than for other benzene, toluene, ethylbenzene and xylenes (BTEX) compounds. We discuss a mathematical method for modelling redox-dependent, differential degradation rates.

Keywords: Benzene; BTEX; Anaerobic degradation; Nitrate-reduction; Iron-reduction; Sulphate reduction; Methanogenesis; Modelling

1. Introduction

Petroleum contains, in addition to many other hydrocarbon constituents, benzene, toluene, ethylbenzene and xylenes (BTEX). These are the most significant components in terms of pollution potential as they are the most soluble. Leaks of petroleum, leading to contamination of soil and groundwater by BTEX compounds, are widespread. Thus, dissolved BTEX compounds in the subsurface environment are candidates for removal via naturally occurring processes, whereby redox reactions mediated by autochthonous microorganisms result in the production of less harmful, even benign, products (Wiedemeier et al., 1996).

Benzene typically makes up less than 2% of petroleum (Irwin, 1997), but is important since it is considered the most toxic and persistent of all petroleum components. Its solubility in water is only 1.78 g l^{-1} (Stephen and Stephen, 1963), yet it is the most soluble of petroleum hydrocarbons (Alexander, 1999). In addition, its structure and shape make it difficult to oxidise and degrade. Reasonable evidence exists showing that the TEX compounds all degrade naturally in groundwater systems (Aronson and Howard, 1997), whereas for benzene the picture is mixed.

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Indeed, it is thought that benzene degradation may be inhibited in the presence of other hydrocarbons, such as toluene (Krumholz et al., 1996), though the mechanism for this is unclear.

BTEX degradation occurs most rapidly under aerobic conditions. However, aquifers are often anoxic. In the absence of dissolved oxygen in groundwater, benzene degradation rates decrease or can stop altogether. The aerobic degradation of benzene, via catechol, is well established (Aronson and Howard, 1997) and will not be further considered here. The application of oxygen to anoxic soil, sediments and groundwater is possible by a variety of means (biopiles, injection of O_2/air/aerated water/hydrogen peroxide or the injection of chlorite, which is degraded by perchlorate-reducing bacteria to yield oxygen in situ (Coates et al., 1998)). These are intrusive and, therefore, relatively expensive measures. Hence, where feasible, monitored natural attenuation (intrinsic bioremediation) is likely to remain the most widespread remediation technique for BTEX-contaminated aquifers.

Natural attenuation encompasses a host of physical processes (e.g. dispersion, dilution, sorption and volatilisation) as well as chemical and biological degradation. Biodegradation depends on microbial activity that varies with hydrogeological site characteristics and aquifer geochemistry (Allard and Neilson, 1997). Here, anaerobic benzene biodegradation is examined, considering evidence from both laboratory and the field. Literature surveys reveal conflicting evidence on conditions required for its degradation (Harwood and Gibson, 1997; Aronson and Howard, 1997; Heider et al., 1999). Thus, it is difficult to predict a priori the occurrence/rate of benzene removal without a detailed understanding of aquifer conditions.

A 1997 review of likely mechanisms (Harwood and Gibson, 1997) suggested that benzene could be degraded via benzoate under a range of conditions. No single microbial species had been shown to degrade completely the compound under anaerobic conditions, although stable benzene-degrading enrichment cultures were known. Toluene-degrading organisms, however, had been identified, and included members of the nitrate-reducing genera *Azoarcus* and *Thauera*, and the iron-reducing *Geobacter metallireducens* as well as a variety of unnamed sulphate-reducers. The only organisms known to degrade BTEX compounds anaerobically are bacteria, but it has been suggested that the currently poorly studied anaerobic fungi might prove to be involved.

Aronson and Howard (1997) reviewed and tabulated a large number of laboratory and field investigations. The majority of published studies failed to demonstrate anaerobic benzene degradation. Those that did indicated that benzene was degraded under nitrate-, Fe(III)- and manganese-reducing, and sometimes under methanogenic conditions. Reinhard et al. (1997) examined BTEX removal from groundwater in the presence of nitrate and sulphate and showed that benzene and *o*-xylene were the most recalcitrant. Under nitrate-reducing conditions, ethylbenzene, toluene and *m*-xylene were removed within 6 days. In the presence of sulphate, toluene and xylenes were removed after 60 days. Many authors attributed the lack of degradation to insufficient residence time. Others suggested that since benzene degradation appears to be inhibited in the presence of other carbon sources, it might be that the degradation seen in the field was due to aerobic degradation at the plume periphery.

Since these reviews, a number of pertinent papers have been published. Space does not allow for the comprehensive review of papers published prior to 1997. We therefore suggest this paper should be read in conjunction with the earlier reviews (Aronson and Howard, 1997; Harwood and Gibson, 1997; Heider et al., 1999).

2. Benzene degradation under different redox regimes

Cellular respiration, the process by which living cells obtain energy to support metabolic processes, comprises a chain of oxidation–reduction couples, whereby energy is extracted via a stepwise oxidation (i.e., removal of electrons) of organic and inorganic molecules. In order to proceed, there needs to be a relatively more oxidised chemical species available at each step to prevent the accumulation of electrons that would hinder the reaction kinetics. The compounds that provide a ‘‘sink’’ for the electrons expelled at the end of the chain of reactions are known as terminal electron acceptors (TEAs). In

aerobic respiration, the TEA is molecular oxygen, but in the absence of oxygen, a number of less highly oxidised compounds may serve, assuming organisms capable of making use of them are present. Available TEAs are generally used in the environment in decreasing order of oxidation–reduction potential. Possible TEAs include NO_3^-, Fe(III), Mn(IV), SO_4^{2-} and CO_2. Lovley (2000) noted that benzene degradation had been reported with all these common electron acceptors.

2.1. Nitrate-reducing conditions

Benzene has long been considered recalcitrant in the field under nitrate-reducing conditions. Where it has been seen, it has been much slower than under aerobic conditions and it appears that O_2 is still required as a substrate for the oxygenases that mediate the oxidative cleavage of the aromatic ring, even if it is not used as a TEA (Anid et al., 1993; Durant et al., 1999). Benzoate is often considered to be a central metabolite in the degradation of monoaromatic hydrocarbons, and it has been shown to be degraded under denitrifying conditions (Harwood and Gibson, 1997) though some workers still point to the apparent inability of nitrate-reducers to degrade benzene (Kao and Borden, 1997). Nales et al. (1998) demonstrated benzene degradation under nitrate-reducing conditions, but found that TEX substrates competitively inhibited its degradation. They also demonstrated benzene degradation under sulphate- and Fe(III)-reducing conditions, but not with methanogenesis. Burland and Edwards (1999) link benzene degradation to reduction of nitrate to nitrite (but not to conversion to gaseous nitrogen).

The most noteworthy paper in this field in recent years described benzene oxidation by two strains of the genus *Dechloromonas* with nitrate as the sole electron acceptor (Coates et al., 2001), the first time this has been shown in a single organism, rather than in enrichment cultures or sediment studies. Other than the ability to degrade benzene, the two strains were isolated on the basis of very different metabolic capabilities—one was isolated by its ability to reduce (per)chlorate, the other on its ability to oxidise humic matter. This, along with the demonstrated ubiquity of members of the genus, is pointed to by the authors as an indication that they hold potential for treatment of benzene-contaminated environments.

2.2. Iron-reducing conditions

Iron is considered to be especially significant in hydrocarbon degradation in marine sediments, with Fe(III), chelated to a variety of compounds, shown to stimulate benzene oxidation in anaerobic sediment (Lovley et al., 1996; Caldwell et al., 1999). Kazumi et al. (1997) showed that benzene was degraded in methanogenic, sulphate-reducing and iron-reducing conditions. Benzene loss also occurred in the presence of Fe(III) in sediments from freshwater environments. Heider et al. (1999) noted that benzene was degraded under iron-reducing conditions but that no single benzene-degrading organism had been isolated. A community including members of the genus *Geobacter* was implicated. Many primitive benzene-degrading bacteria (hyperthermophiles), previously thought to require SO_4^{2-}, have been shown to grow using Fe(III) as an electron acceptor (Anderson et al., 1998; Rooney-Varga et al., 1999). Caldwell and Suflita (2000) found evolution of phenol and benzoate under a range of conditions (Fe(III)- and sulphate-reduction, and with methanogenesis), supporting the theory that benzoate is a central metabolite in anaerobic degradation of aromatic compounds.

3. Sulphate-reducing conditions

Several workers have demonstrated benzene degradation under sulphate-reducing conditions in soil collected from contaminated sites (e.g. Phelps et al., 1996). Chaudhuri and Wiesmann (1995) showed that degradation was via benzoate. Benzene degradation was comprehensively demonstrated in sulphate-reducing sediments from San Diego Bay (Lovley et al., 1995). Reinhard et al. (1997) investigated BTEX degradation under a range of redox conditions, but benzene degradation was only associated with sulphate reduction. Enrichment of aquatic sediments with known benzene-degraders leads to degradation of benzene and growth of benzene-degrading organisms, suggesting that the lack of benzene degradation in some aquifers is due to failure of appropriate

organisms to colonise the aquifer, rather than adverse environmental conditions (Weiner and Lovley, 1998a). A sulphate-reducing consortium was found to remain relatively complex despite the culture's long exposure to benzene as the only carbon and energy source (over 3 years) and repeated dilutions of the original enrichment (Phelps et al., 1998). Conversely, complete mineralisation of benzene to CO_2 has been demonstrated, apparently within single cells, in microcosms (Lovley et al., 1995), and more recently in a contaminated aquifer (Anderson and Lovley, 2000).

3.1. Methanogenesis

Where no electron acceptors other than CO_2 are present, it is suggested that benzene might be degraded to CO_2 and methane. In such a situation, it would not be possible to mineralise all the benzene. However, in the absence of any other TEA, this might play a role in limiting the extent of the contaminant plume. Benzene has been shown to be converted to CH_4 and CO_2 with no lag phase in the absence of other electron acceptors (Grbic-Galic and Vogel, 1987; Kazumi et al., 1997; Weiner and Lovley, 1998b).

4. Modelling differential degradation rates

Modelling of the complex biogeochemical interactions is a valuable means of quantifying the varied and complex interactions between contaminants, the local hydrogeology (groundwater flow) and the local hydrogeochemistry and, ultimately, making predictions of (i) the viability of natural attenuation as a remediation technique for BTEX compounds in aquifers and (ii) the feasibility/efficiency of enhanced remediation schemes. Here, we provide a modelling approach for microbial degradation of organic contaminants. The approach could be used in conjunction with flow, transport and geochemical modelling to describe the fate of such contaminants in groundwater systems (Barry et al., 2002). For the quantification of hydrocarbon compounds, models of different levels of complexity exist. Probably the most common mathematical formulation is based on linking the removal of organic compounds to the microbial growth rate. For a single organic compound, single microbial species (or a consortium that is not differentiated into different species) and single TEA, this growth rate can be expressed by

$$\frac{\mathrm{d}X_{\mathrm{growth}}}{\mathrm{d}t} = v_{\max}\frac{C_{\mathrm{org}}}{K_{\mathrm{org}}+C_{\mathrm{org}}}\frac{C_{\mathrm{ea}}}{K_{\mathrm{ea}}+C_{\mathrm{ea}}}X, \tag{1}$$

where t is time, X is the local microbial concentration (subject to both growth and decay), $v_{\max}$ is an asymptotic maximum specific uptake rate, C_{org} and C_{ea} are the aqueous concentrations of the organic compound (substrate) and TEA, respectively. K_{org} and K_{ea} are the half-saturation constants for the organic compound and the TEA, respectively. For the complete mass-balance of the bacterial group, microbial decay needs to be considered, leading to

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{\mathrm{d}X_{\mathrm{growth}}}{\mathrm{d}t} + \frac{\mathrm{d}X_{\mathrm{decay}}}{\mathrm{d}t}, \tag{2}$$

with

$$\frac{\mathrm{d}X_{\mathrm{decay}}}{\mathrm{d}t} = -v_{\mathrm{dec}}X, \tag{3}$$

where v_{dec} is a decay rate constant (which could be replaced with an experimentally derived function). During microbial growth, both organic substrate and TEA are consumed at rates that are proportional to $v_{\max}$. Thus, for a known reaction stoichiometry, the degradation rate can be easily determined from

$$\frac{\mathrm{d}C_{\mathrm{org}}}{\mathrm{d}t} = Y_{\mathrm{org}}\frac{\mathrm{d}X_{\mathrm{growth}}}{\mathrm{d}t}, \tag{4}$$

for the substrate and, similarly, for the TEA,

$$\frac{\mathrm{d}C_{\mathrm{ea}}}{\mathrm{d}t} = Y_{\mathrm{ea}}\frac{\mathrm{d}X_{\mathrm{growth}}}{\mathrm{d}t}, \tag{5}$$

where Y is an appropriate stoichiometric factor. As written, the above formulation treats multiple hydrocarbon compounds as one single compound with similar physico-chemical properties and, consequently cannot mimic the above-discussed differential degradation (or recalcitrance) of compounds

under varying redox conditions. To simulate this, Eq. (1) needs to be modified to

$$\frac{dX}{dt} = \left[\left(\sum_{n=1,\, n_{org}} \frac{dX_n}{dt} \right) - v_{dec} \right] X, \quad (6)$$

where each of the growth terms dX_n/dt is derived from a compound-specific term similar to Eq. (1) where the uptake rates v_{max} can then differ between different substrates. The decay rate, however, is not expected to vary, and so appears only once in Eq. (6). If more than one TEA is involved in the degradation process, this will typically require simulation of concentrations of multiple microbial groups, each associated with a particular TEA. To inhibit growth of bacteria in the presence of a thermodynamically more favourable TEA, one or more inhibition terms of the form

$$I_{inh,ea} = \frac{K_{inh,ea}}{K_{inh,ea} + C_{inh,ea}} \quad (7)$$

can be included as a factor in Eq. (1) and/or Eq. (6), where $C_{inh,ea}$ is the concentration of a more favourable TEA and $K_{inh,ea}$ is an inhibition constant that needs to be much smaller than typical concentrations of the more favourable TEA. The Monod-type inhibition term $I_{inh,ea}$ will then remain near 0 as long as the more favourable TEA is present in significant amounts but reaches its maximum value (of unity) as soon as the more favourable TEA is depleted (no growth-inhibition).

Finally, it is possible to take into account the release of nutrients and metabolic products from decaying cells using:

$$\frac{dC_{prod}}{dt} = Y_{prod} \frac{dX_{dec}}{dt}. \quad (8)$$

If initial X, C_{org}, C_{ea} and C_{prod} are known, the system of ordinary differential equations given in this section may be solved by standard numerical methods, e.g. the Runge–Kutta method (Abramowitz and Stegun, 1972). With the above equations incorporated into a numerical flow and transport model, e.g. Prommer et al. (1999, 2000a,b), it is then possible to simulate redox-dependent, differential degradation rates, e.g. benzene might degrade at the same or faster rate as toluene under aerobic conditions whereas under selected anaerobic conditions it might degrade more slowly or not at all.

5. Discussion and conclusions

It is evident from a number of studies that the prevailing redox, physicochemical and biological conditions all play a part in determining the rate and extent of benzene degradation. Where degradation of hydrocarbons has been seen under anaerobic conditions, it appears that often the reaction pathway is unique to the organisms, compound and TEA, as summarized in Fig. 1.

Heider et al. (1999) noted in their review paper that the enzymes required for the anaerobic metabolism of hydrocarbons are substrate-induced, i.e., they are produced by the organism in response to the presence of the compound. Cells grown on toluene, for instance, will produce toluene-degrading enzymes, cells grown on another carbon/energy source will not. Compared to aerobic metabolism, it appears that enzymes involved in anaerobic metabolism are more substrate-specific.

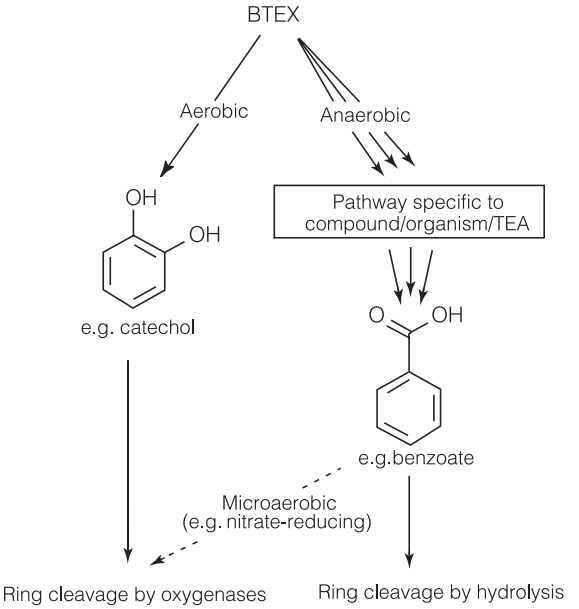

Fig. 1. Generalised BTEX biodegradation pathways.

The overall picture of anaerobic benzene degradation is that it can and does occur in a variety of conditions, but that organisms capable of utilising benzene anaerobically are by no means ubiquitous. There are also contrasting data concerning the use of TEAs in degradation, with studies by Nales et al. (1998) showing benzene degradation under NO_3^-, SO_4^{2-} and Fe(III)-reducing conditions and not under methanogenic conditions, while Kazumi et al. (1997) report degradation under methanogenic, SO_4^{2-} and Fe(III)-reducing conditions only. Where rapid benzene degradation is seen in the field, it is often associated with shallow aquifers and it is suggested that most of this degradation is aerobic, along the margins of contaminant plumes, with a limited amount occurring anaerobically within the plume body (Aronson and Howard, 1997). Anaerobic degradation of benzene is clearly far more site-specific than for the remaining TEX compounds, its extremely stable structure making it less susceptible to microbial attack and thus highly dependant on both biotic and abiotic factors. A high organic fraction has been shown to inhibit anaerobic benzene degradation (Nales et al., 1998), as has the presence of alternative energy sources (Corseuil et al., 1998). In the presence of high concentrations of BTEX compounds, it may be that removal of the more easily degraded TEX component and associated TEA may be responsible for the persistence of benzene in the environment. The use of enrichment cultures may favour faster-growing species at the expense of slower-growing microorganisms which are capable of degrading benzene over longer incubation periods/residence times (Rabus et al., 1999). It is likely that a combination of laboratory experiments and modelling of hydrogeology and hydrogeochemistry will aid in determining the potential for natural attenuation at a given site. While still poorly understood, anaerobic biodegradation is acknowledged as a factor in the natural attenuation of a variety of compounds, including some, such as benzene, once considered to be recalcitrant in all but aerobic conditions.

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