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Degradation of BTX by dissimilatory iron-reducing cultures

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Abstract The ability of indigenous bacteria to anaerobically degrade monoaromatic hydrocarbons has received attention as a potential strategy to remediate polluted aquifers. Despite the fact that iron-reducing conditions are often dominating in contaminated sediment, most of the studies have focussed on degradation of this class of pollutants with other terminal acceptors. In this work, we enriched bacteria from an iron-reducing aquifer in which a plume of pollution has developed over several decades and we show that benzene, toluene, *meta*- and *para*-xylene (BTX) could be degraded by the enriched cultures containing intrinsic iron-reducing microorganisms. To our knowledge, this is the first time that *para*-xylene degradation by dissimilatory iron-reducing bacteria has been reported in sediment free enrichment cultures. BTX degradation rates in enrichment cultures progressively increased in time and were found in good agreement with theoretical values calculated assuming complete BTX oxidation with Fe(II) as final electron acceptor. In addition, using labelled ($^{13}C_1$) benzene and toluene we could unambiguously identify intermediates of their respective degradation pathways. We provide evidence for benzene degradation via phenol formation under iron-reducing conditions, whereas toluene and *meta*-xylene were transformed into the corresponding benzylsuccinates.

Keywords Aquifer · Biodegradation · BTX · Iron reduction · Metabolic pathway

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Introduction

Benzene, toluene, ethylbenzene and the three xylene isomers (BTEX) are a group of monoaromatic petroleum derived pollutants often found in contaminated aquifers. The ability of intrinsic microorganisms to actively degrade these compounds has gained attention as an efficient and inexpensive tool for remediation of polluted areas. However, slow biodegradation rates in anaerobic environments, particularly under iron-reducing conditions, have made it difficult to obtain degrading cultures or isolates with which to unravel the mechanisms and pathways that this class of pollutants follow. Degradation of toluene by diverse bacteria has been extensively reported (Coates et al. 2001; Zheng et al. 2002) and is well understood but benzene (Kazumi et al. 1997; Lovley et al. 1996; Rooney-Varga et al. 1999) and

xylenes (Jahn et al. 2005) degrading cultures that utilise iron as final electron acceptor are more difficult to obtain and cultivate due to the stability of these molecules towards anaerobic oxidation and the low conversion rates that they undergo. Most of the studies in which metabolites have been detected were carried out under sulphate and nitrate conditions (Beller et al. 1996, 1997a, b; Chakraborty et al. 2004; Phelps et al. 2001) and, to date, no benzene or xylene degrading pure culture of dissimilatory iron-reducing bacteria could be isolated. However, there are indications of the involvement of bacteria belonging to the *Geobacteraceae* family in the oxidation of monoaromatic compounds (Anderson et al. 1998; Lovley et al. 2004; Snoeyenbos-West et al. 2000) as well as evidence for their dominance downstream of the aquifer under investigation in this research (Röling et al. 2001).

In the present study, we have investigated the BTX biodegradation process in enrichment cultures obtained from microcosms previously inoculated with polluted sediment or groundwater of an aquifer in which a plume of pollution has originated from the nearby landfill (Banisveld, Boxtel, The Netherlands) (Breukelen et al. 2003).

In the original microcosms (Botton et al. 2006), degradation of benzene, toluene and each of the xylene isomers was observed after incubation under iron-reducing conditions for periods comprised between 100–600 days. Microcosms were incubated with one of the BTX compounds as only carbon source and iron as terminal electron acceptor. The gradual increase in degradation rates measured in time suggested the adaptation of microbial communities towards this class of pollutants (Botton et al. 2006). A step further from the above mentioned work is represented by the research presented here, with which we aimed at gaining further insight into the BTX anaerobic degradation process with respect to pollutant removal in relation with iron as electron acceptor and metabolite formation. The aqueous fraction of active microcosms was then transferred into fresh medium under strict iron-reducing conditions and the ability to oxidise BTX pollutants was maintained in all sediment free inoculations with the only exception of *ortho*-xylene. In order to detect BTX degradation metabolites, enrichment cultures were amended with labelled substrates and putative byproducts of benzene, toluene and *meta*-xylene were detected.

Materials and methods

Enrichment cultures

Enrichment cultures were obtained by transferring 5–10 ml of liquid from the parent microcosms into 40 ml of fresh anaerobic medium prepared as described in (Botton et al. 2006). Once inoculated, enrichment cultures were spiked with one of the BTX contaminant from anaerobic aqueous stock solutions in concentrations ranging from 10–30 μM for benzene to 300–400 μM for toluene and xylenes. The amorphous iron provided as terminal electron acceptor was prepared by neutralizing a 0.4 M solution of $FeCl_3$ with NaOH (1.0 M) until pH = 7 (Lovley et al. 1990).

The established enrichment cultures were monitored in time for pollutant and iron concentrations and further amended with either carbon source or electron acceptor when they became depleted.

Analytical techniques

Contaminant concentrations were determined by analysing 250 μl of headspace with a GC (HP 5890 Series II) connected to a flame ionisation detector (FID), as reported in (Botton et al. 2006). Gaseous samples were injected in split mode and separated through a DB-5 (J&W Scientific) column (30 m $\times$ 0.32 mm, 0.25 μm film thickness) kept isothermally at 50°C with helium as carrier gas. BTX headspace concentrations were quantified by comparison with standards prepared and stored under the same conditions as the inoculations.

Iron concentration in the liquid phase of the cultures was quantified by spectrophotometric analysis (A_{562}) after treatment of the samples with the ferrozine colorant. The ferrozine method allows to measure both the total amount of iron and the reduced Fe(II) in the liquid suspension and the difference between these values represents the microbially reducible Fe(III) (Lovley et al. 1986, Viollier et al. 2000). Because ferrozine

binds with the reduced form of iron, the total iron measurement is carried out after reduction of the samples with hydroxylamine. Each measurement was done in duplicate.

Metabolite detection

In order to detect metabolite formation and trends in time, enrichment cultures in which removal of benzene, toluene and *meta*-xylene was observed were further transferred into fresh medium, spiked with labelled or unlabelled compounds and sacrificed at different stages of the degradation process (approximately after degradation of 10–30–60–80% of the initial concentration) according to the following procedure: cultures (approximately 50 ml) were acidified to pH ≤ 2, spiked with the internal standard (fluorobenzoic acid) and extracted three times with diethyl ether in a separatory funnel. The solvent was collected, dried with anhydrous sodium sulphate and concentrated to 1 ml. Samples were then derivatised with diazomethane and finally dissolved into trimethylpentane (Beller et al. 1996). Analyses were carried out by GC-MS (Trace MS Finnigan) equipped with RTX-5Sil MS column (30 m, 0.25 mm, film thickness 1 μm) preceded by a retention gap (2 m, 0.53 mm internal diameter). Samples were injected cold on-column with helium as carrier gas (constant linear gas velocity 42 cm/s) and the following oven program was applied: 70°C for 1 min, 40°C/min until 120°C and 10°C/min up to 300°C. The ionisation mode was electron impact (70 eV).

Full scan analysis of derivatised sample extracts was used for a preliminary screening and for retention time determination, which enabled the successive higher resolution analysis by selected ion monitoring (SIM). Identification of unlabelled BTX metabolites (as derivatised esters) was carried out by comparison with original standards with the exception of (3-methylbenzyl)succinic acid for the detection of which previously published chromatograms were utilised (Beller et al. 1995, 1997a, b). For the identification of labelled metabolites, the same reference chromatograms were applied with the only difference that an increase of 1 in the m/z ratio was taken into account. Metabolites analysis in SIM was obtained by screening the following m/z values: for phenol 65–66, 78–79, 108–109; for benzylsuccinic acid (BSA) 91–92, 117–118, 131–132, 145–146, 236–237 and for methyl-BSA 91, 105, 115, 131, 145, 159, 177, 190, 250.

Labelled benzene and toluene ($^{13}C_1$, 99% purity) and all the other chemicals were purchased from Aldrich.

Results

BTX degradation

Benzene

The ability to utilise benzene as growth substrate was maintained in the enrichment cultures, as shown in Table 1 and Fig. 1a, and the removal of the contaminant could be observed after a lag phase of 50 days, significantly shorter than in the parent microcosms (230 days) (Botton et al. 2006).

During an incubation period of almost 300 days, the degradation of 44.5 μM of benzene was coupled to the formation of 0.7 mM of Fe(II), whereas, according to the stoichiometry of the complete oxidation of benzene with iron as terminal electron acceptor, 1.1 mM of iron should be reduced. The difference between the measured and theoretical values suggests that benzene was not completely mineralised, as discussed later.

An additional series of enrichment cultures was inoculated with a microcosm in which both *meta*-xylene and benzene were degraded. However, in the successive transfer depicted in Fig. 1b, benzene was provided as the only carbon source. Also in this case, the removal of the pollutant was carried out under iron-reducing conditions and the increase of Fe(II) was equal to 0.6 mM, in agreement with the theoretical value of 0.5 mM.

In either series of enrichment cultures the average degradation rate increased in time with similar values and trends as reported in Table 1.

Toluene

Two series of enrichment cultures were obtained from groundwater and sediment microcosms

Table 1 BTX degradation rates and comparison between observed and theoretical electron acceptor versus donor ratios*

Pollutant removed	Concentration removed (μM)	Degradation rate (μM/d)		Ratio e acceptor/e donor		*n*
		1st spike	2nd spike	Observed	Theoretical	
Benzene (Fig 1a)	45.5	0.11 ± 0.05	0.22 ± 0.07	15 ± 0.7	30	2
Benzene (Fig 1b)	15.1	0.14 ± 0.02	0.22 ± 0.01	36.8 ± 12.8	30	2
Toluene (Fig. 1c)	349.7 (Q)	3.97 ± 1.7	6.3 ± 0.1	29.7 ± 3.6	36	2
	354.8	4.0 ± 0.1	6.4 ± 0.0	21.4 ± 7.5	36	2
Toluene (Fig. 1e)	707.1	2.09 ± 0.08	5.06 ± 0.86	26.14 ± 0.43	36	3
m-xylene (Fig. 1g, h)	134.5	0.3 ± 0.01	0.57	20.8	42	2
	634.9	1.39 ± 0.26	7.69±2.87	17.0 ± 1.84	42	2
p-xylene (Fig. 1f)	66.2	0.21 ± 0.08		20.2 ± 5.59	42	2

* Theoretical ratios were calculated assuming complete oxidation of the carbon source (one of the BTX) with Fe(II) as terminal electron acceptor, see Botton et al. (2006) for reactions. "*n*" is the number of replicate incubations considered for these calculations

previously exposed to toluene and in which degradation of this pollutant was already taking place. After groundwater microcosms were transferred into fresh medium, some of the enrichment cultures were additionally amended with 0.1 mM of quinone (anthrahydroquinone-2,6-disulfonate, AQDS), a humic acid analogue, in order to test whether the presence of such compound would enhance the biodegradation rate of toluene, as already observed in other studies (Jahn et al. 2005; Lovley et al. 1998, 1999). Toluene was immediately utilised with a faster biodegradation rate than in the parent microcosm (from 3μM/d to 4.0 μM/d) (Fig. 1c).

During the course of the experiment a black precipitate accumulated at the bottom of the incubation bottles, most likely due to the formation of Fe(II)/Fe(III) complexes, as already observed and described in previous studies (Lovley et al. 1987), and, at the same time, the total iron concentration—the hydroxylamine-extractable iron—was found to decrease in time (Tot Fe and Tot Fe + Q in Fig. 1d). As the method used for the measurement of the total iron is designed to extract only the microbially reducible fraction (Lovley et al. 1987), if the iron precipitates in forms that are not longer accessible to bacteria, it becomes no longer detectable.

On the basis of this consideration, which implies that the iron precipitates are not hydroxylamine extractable (not bioavailable), the Fe(II) concentration was recalculated (Fe(II) corrected and Fe(II) + Q corrected in Fig. 1d) assuming that the total amount of iron remained constant during the experiment, which is indeed the case, as the systems are closed. By applying this correction, the stoichiometry of toluene degradation under iron-reducing condition was found to be closer to the theoretical ratios, as reported Table 1.

The comparison between the two cultures—with or without the addition of AQDS (Q)—after 100 days of incubation revealed that residual toluene concentration in the quinone amendemends was 50 μM lower than the culture without humic analogue. The increase of Fe(II) was also found to reflect the presence of the humic acid analogue: a higher concentration of iron was reduced in the cultures in which quinone was supplemented (10.4 mM) than in the cultures without (7.6 mM).

Enrichment cultures obtained from sediment microcosms (Fig. 1e) showed an immediate removal of toluene as well as an increase of the average rate of toluene degradation: from 0.3 μM/d in the parent sediment microcosm up to 2.1 and 5.1 μM/d after 50 and 120 days of inoculation respectively in the enrichment cultures. Toluene degradation occurred under iron-reducing condition, as demonstrated by the increase of Fe(II) in all the inoculations plotted in Fig. 1e.

Xylenes

When *meta*-xylene degrading microcosms were transferred in sediment free enrichments, the degradation of the pollutant resumed after a lag phase of approximately 100 days (Fig. 1g).

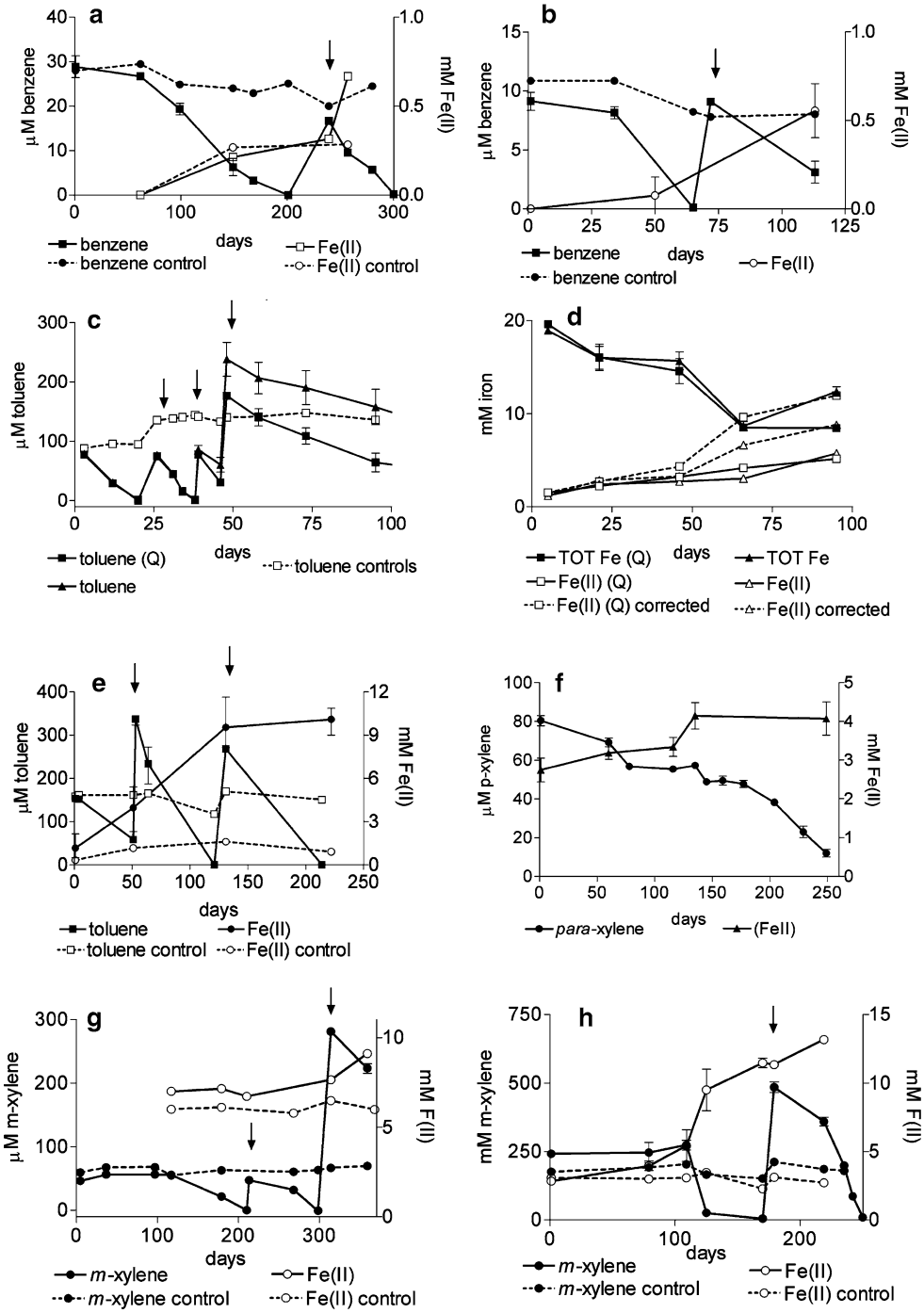

Fig. 1 Degradation of BTEX in enrichment cultures coupled to Fe(II) formation. Benzene removal in (**a**) and (**b**); toluene degradation in ennrichment cultures (**c**)—some of which amended with AQDS (Q)—and iron trend (**d**); *para*-xylene (**e**) and *meta*-xylene in successive enrichment cultures (first and second transfers from the same parent microcosms in (**g**) and (**h**) respectively). Values represent the average of two or three inoculations (see Table 1) and arrows indicate when samples were spiked again

Cultures were further transferred and the average degradation rate in the successive enrichments was higher than in the parent cultures (Table 1) and reached 7.7 μM/d after the cultures were spiked again on day 180 (Fig. 1h). In each incubation, the removal of *m*-xylene occurred concurrently to Fe(III) reduction, however the precipitation of insoluble iron, similarly to toluene degrading cultures, may account for the low electron donor-acceptor ratios observed (Table 1).

para-Xylene degrading sediment microcosms were transferred and used as inocula for new enrichment cultures in which the removal of the pollutant started without lag phase, Fig. 1f. *p*-Xylene degradation occurred under iron-reducing conditions: 1.3 ± 0.4 mM of Fe(II) were formed during the 250 days of incubation. The mineralisation of this compound would imply the reduction of 2.1 mM of electron acceptor, however the lower Fe(II) concentration measured could be due to the incomplete oxidation of xylene to CO_2.

Metabolite detection

Benzene

Benzene degrading enrichment cultures described above were further transferred into fresh medium, spiked again with benzene or ^{13}C-labelled-benzene and sacrificed at decreasing residual concentrations. Phenol was detected in each of the culture extracts and ^{13}C-phenol was recovered in those cultures that were previously provided with labelled benzene, indicating that this compound was a product of the metabolic activity of dissimilatory iron-reducing microorganisms. The concentration of phenol was too low for quantification, even after analysing in SIM and the formation of the subsequent putative metabolite, 4-hydroxybenzoate, could not be observed. However, we could measure progressively increasing concentrations of benzoate in those cultures spiked with unlabelled benzene (Fig. 2a). The increase in benzoate concentration did not stop even when cultures were benzene depleted, similarly to the trend recently observed in methanogenic consortium (Ulrich et al. 2005), as if this compound could not be readily degraded. Labelled benzoate could not be identified in ^{13}C-benzene amended cultures probably because when the cultures were sacrificed the residual concentration of benzene was still above 50% of the initial value—whereas a higher percentage of pollutant had been degraded when cultures spiked with unlabelled benzene were extracted: a slow conversion rate of phenol into the following product could therefore account the lack of labelled benzoate.

Low transformation rates would also explain the fact that the phenol detected in ^{13}C-benzene amended cultures was just partially labelled, as shown in Fig. 2b, from which it appears that the phenol mass spectrum consists approximately of equal amounts of labelled and unlabelled fragments.

We could not detect other putative metabolites such as toluene or benzylsuccinate in any of the benzene degrading culture extracts. However, when benzylsuccinate was provided as carbon source it was immediately degraded (data not shown), indicating that the ability of the enriched cultures to utilise this compound, but we could not establish whether the same benzene degraders were involved or other bacteria were present that could degrade it.

Toluene

The identification of the first metabolites in the toluene degradation pathway under iron-reducing conditions was carried out with a similar approach as for benzene: labelled and unlabelled toluene were separately added to second generation of enrichment cultures that were sacrificed and extracted at decreasing toluene residual concentrations. The analysis of sample extracts revealed the formation of benzylsuccinate (BSA) and ^{13}C-benzylsuccinate in toluene and ^{13}C-toluene degrading cultures respectively, but the successive transformation of BSA into benzoate could only be observed in unlabelled incubations.

Benzoate and BSA concentrations were below 1 μM with the latter following a steadily decreasing trend while benzoate initially accumulated in the cultures and later started decreasing: such trends seem to confirm that

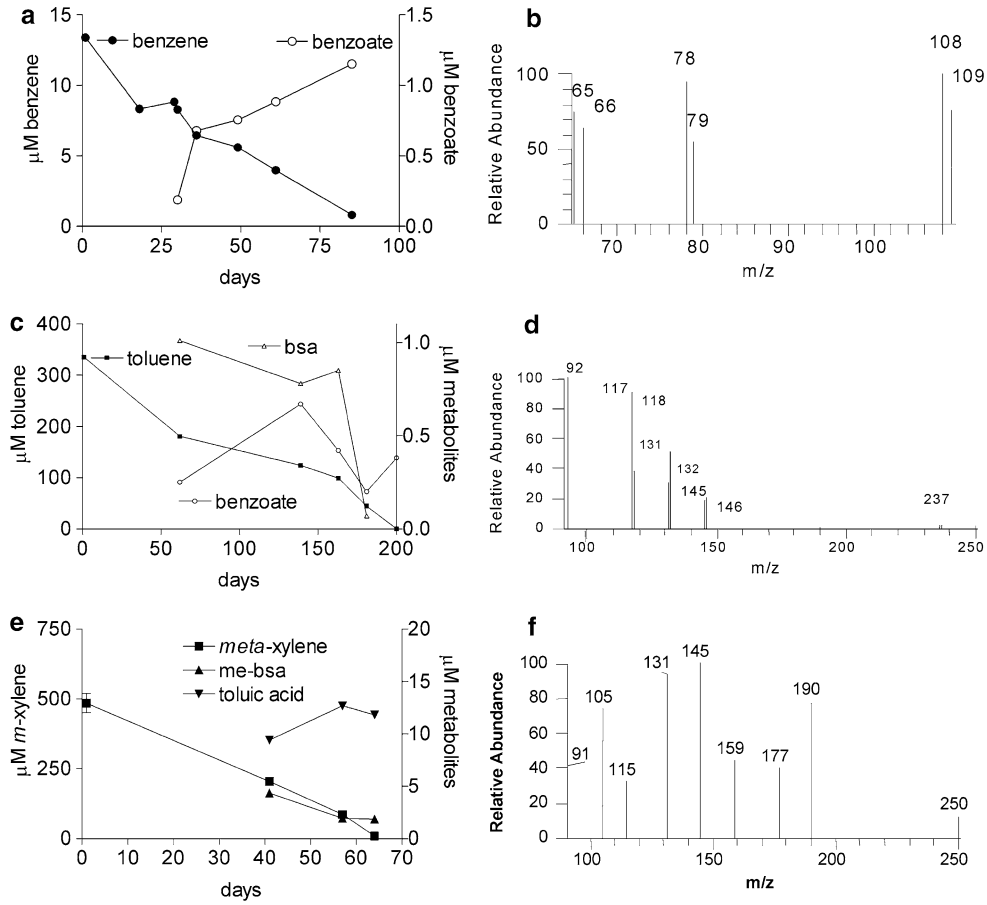

Fig. 2 Metabolites formation in BTX degrading iron-reducing cultures. Degradation of benzene and concomitant benzoate accumulation (**a**). Mass spectrum of phenol in ^{13}C-benzene degrading cultures (**b**). Degradation of toluene and parallel BSA and benzoate formation (**c**). Mass spectrum of BSA in ^{13}C toluene degrading cultures (**d**). Degradation of *meta*-xylene and concomitant meBSA and toluic acid formation (**e**). Mass spectrum of meBSA (**f**). The spectra were obtained after screening in selected ion monitoring as described in Material and methods section

concentrations of these two intermediates are linked (Fig. 2c)

As was the case for phenol, BSA recovered in ^{13}C-toluene degrading cultures was not completely labelled but was instead a mixture of ^{12}C and ^{13}C-BSA, as if a longer period was necessary before the intermediate of labelled toluene transformation could completely replace BSA previously formed from unlabelled substrate (Fig. 2d).

meta-Xylene

meta-Xylene degrading cultures described above were further spiked with pollutant and sacrificed when the residual concentration was less then 50% of the initial value. Methylbenzylsuccinate (meBSA) (Fig. 2f) and toluic acid were detected in samples extracts and, as already observed for toluene, decreasing concentrations of meBSA corresponded to an initially rising trend for toluic acid that later started to decrease, as expected for successive metabolic intermediates (Fig. 2e).

Discussion

BTX degradation

The data presented above indicate that indigenous BTX degrading bacteria could be enriched from the polluted aquifer developed downstream

of the Banisveld landfill under conditions—strictly anaerobic, with iron as terminal electron acceptor and without iron chelating molecules—that possibly resemble the original conditions in the aquifer (Breukelen et al. 2003). Despite the low degradation rates associated with the enriched dissimilatory iron reducers, we could, by successive transfer of liquid fraction of microcosms (Botton et al. 2006), establish BTX degrading cultures characterised by a progressively enhanced ability to oxidise monoaromatic hydrocarbons with iron as terminal electron acceptor (Table 1).

Degradation of *para*-xylene in sediment free iron-reducing cultures has not been reported previously and the ability to utilise this compound in absence of oxygen was only observed under nitrate (Haner et al. 1995) or sulphate (Edwards et al. 1992) reducing conditions. In another study, *p*-xylene loss was observed in enrichment cultures established after inoculating iron-reducing medium with polluted sediment (Phelps et al. 1999) but the removal of the pollutant could not be linked to iron reduction, as iron concentration was not monitored.

Anaerobic removal of the three xylene isomers was previously observed to occur under various conditions that included sulphate-reducing cultures (Edwards et al. 1992; Morasch et al. 2001) and pure isolates (Harms et al. 1999; Meckenstock et al. 2004), denitrifying bacteria (Fries et al.1994; Hess et al. 1997; Morasch et al. 2001; Rabus et al. 1995) and methanogenic consortia (Edwards et al. 1994). Under iron-reducing conditions, degradation of xylenes appears to be more difficult and the ability to oxidize all BTEX including *o*-xylene and ethylbenzene by iron-reducing bacteria was only recently reported in enrichment cultures obtained from a contaminated aquifer (Jahn et al. 2005).

The observed slow degradation rates and incomplete transformation of the carbon source to CO_2 within the monitored time frame may account for the lower concentration of Fe(II) measured in benzene and *p*-xylene degrading cultures. In addition, the formation of iron complexes, probably originating from the reaction between microbially reduced Fe(II) and Fe(II) oxides provided in the growth medium, in some cases could hinder a proper evaluation in terms of stoichiometric rates, as observed in toluene and *m*-xylene degrading cultures. Since the rate of Fe(III) reduction was found to correlate positively with the solubility of different Fe(III) oxyhydroxides, including the amorphous Fe(III) oxide, solubility appears to be a rate-controlling parameter in the microbial reduction of Fe(III) oxyhydroxides (Bonneville et al. 2004). Consequently, the precipitation of iron complexes needs to be taken into account especially when calculating stoichiometric ratios and degradation rates. It is thus conceivable to relate the decrease in toluene average degradation rate observed in the last period of the experiment (after day 50) with the precipitation of iron and hence with the reduced availability of the electron acceptor towards microbial reduction.

Metabolites formation

Enriched benzene, toluene and xylene degrading cultures were further monitored in order to detect the first metabolites of the respective degradation pathways. As expected, oxidation of toluene and *meta*-xylene proceeded with the addition of a fumarate molecule to give the correspondent benzylsuccinate and methylbenzylsuccinate. However, benzene degradation followed a different route that involved the hydroxylation of the aromatic ring to form phenol. These results were validated by ^{13}C labelled substrates that allowed to unequivocally link benzene and toluene oxidation to the formation of labelled phenol and BSA respectively.

According to recent findings there are at least two pathways for anaerobic benzene degradation depending on the redox conditions under which the process is carried out and consequently upon the different bacteria involved. In fact, phenol and benzoate have been identified as intermediates of benzene degradation in nitrate-reducing isolates (Chakraborty et al. 2005) but in enrichment cultures of nitrate reducers amended with ^{13}C-benzene methylation to form toluene followed by BSA and benzoate was also observed (Ulrich et al. 2005). In addition, both toluene and phenol were detected as first intermediates in methanogenic cultures, suggesting the existence

of two parallel pathways operating within the methanogenic consortium (Ulrich et al. 2005). According to these studies, benzoate appears to be a key intermediate in benzene anaerobic degradation, as already proved by means of deuterated benzene amendments of a sulphidogenic consortium (Phelp et al. 2001).

The results provided in this work are in accordance with the pathway already proposed by Caldwell and Suflita (Caldwell et al. 2000) which, to our knowledge, is the only study in which metabolites of benzene degradation under iron-reducing conditions were detected and identified as phenol and benzoate. When cultures were spiked with labelled benzene, only labelled phenol was recovered and similarly to our data, the phenol spectrum initially corresponded to a mixed ^{12}C–^{13}C-phenol signal and only later could a clear ^{13}C-phenol spectrum be obtained. Furthermore, in the same study, although benzoate was detected in each of the incubations, the labelled form could be recovered only in sulphate-reducing cultures but not in cultures grown under methanogenic and iron-reducing conditions (Caldwell et al. 2000), suggesting that under these redox environments a slower conversion of benzene into the following intermediates takes place.

In fact, both labelled phenol and BSA were detected but the respective unlabelled compounds were still present, and benzoate but no ^{13}C-benzoate was recovered, indicating that a longer incubation might be needed before benzene and toluene are fully converted and finally oxidised to CO_2. This observation is further supported by the fact that in the same benzene and toluene degrading cultures utilised for metabolite detection, the concentration of Fe(II) formed was found to be lower than the expected stoichiometric values calculated assuming a complete mineralization.

The observed metabolic pathway of toluene via BSA and benzoate is consistent with the mechanism already known in literature. The successive steps that bacteria utilise to gain energy from toluene seem not to be affected by different redox conditions: BSA is formed and then undergoes further transformation to benzoate as reported in many studies (Beller et al. 1997a, b, 2000; Beller 2000; Biegert et al. 1996) that also include iron-reducing bacteria (Kane et al. 2002). In fact, due to the unambiguous origin of BSA as a product of toluene degradation and its occurrence under each redox condition, BSA represents a feasible biomarker for the detection of toluene degradation in situ (Beller et al. 1995, 2000; Beller 2000; Young et al. 2005).

Degradation of xylenes has been mostly studied under nitrate and sulphate-reducing conditions and just recently an iron-reducing enrichment culture was obtained able to degrade monoaromatic hydrocarbons including *ortho*-xylene (Jahn et al. 2005). Previous research showed how a denitrifying pure isolate could transform both toluene and *ortho*-xylene (Beller et al. 1997a, b; Biegert et al. 1996) into their corresponding benzylsuccinates and similarly a sulphate reducer would follow the same pathway when growing on toluene and *ortho* or *para*-xylenes (Beller et al. 1996). Although there is no evidence of discrepancies in the metabolic route that xylenes isomers follow under different environments, data presented in this work prove that the same mechanisms of addition of fumarate to the *m*-xylene molecule to give meBSA applies to iron-reducing microorganisms.

Conclusions

With this research we provide new insight of degradation processes occurring under iron-reducing conditions, which are often occurring in polluted aquifers but also less investigated. Data presented here indicate that dissimilatory iron-reducing microbial communities enriched from a contaminated aquifer could utilise a range of monoaromatic compounds as carbon source, including hazardous chemicals such as benzene or contaminants previously considered recalcitrant to microbial attack as *para*-xylene. In addition, we proved that the anaerobic oxidation of benzene proceeds via phenol formation whereas toluene and *meta*-xylene are transformed in BSA and meBSA respectively. The identification of metabolic intermediates will help to properly address BTX biological removal in the field and to establish suitable bioindicators with which occurrence of in situ degradation processes can be assessed.

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