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BIODEGRADATION OF BTEX AND OTHER PETROLEUM HYDROCARBONS BY ENHANCED AND CONTROLLED SULFATE REDUCTION

FINAL REPORT

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ABSTRACT

High concentrations of sulfide in the groundwater at a field site near South Lovedale, OK, were inhibiting sulfate reducing bacteria (SRB) that are known to degrade contaminants including benzene, toluene, ethylbenzene, and m+p-xylenes (BTEX). Microcosms were established in the laboratory using groundwater and sediment collected from the field site and amended with various nutrient, substrate, and inhibitor treatments. All microcosms were initially amended with $FeCl_2$ to induce FeS precipitation and, thereby, reduce sulfide concentrations. Complete removal of BTEX was observed within 39 days in treatments with various combinations of nutrient and substrate amendments. Results indicate that elevated concentration of sulfide is a limiting factor to BTEX biodegradation at this site, and that treating the groundwater with $FeCl_2$ is an effective remedy to facilitate and enhance BTEX degradation by the indigenous SRB population.

On another site in Moore, OK, studies were conducted to investigate barium in the groundwater. BTEX biodegradation by SRB is suspected to mobilize barium from its precipitants in groundwater. Data from microcosms demonstrated instantaneous precipitation of barium when sulfate was added; however, barium was detected redissolving for a short period and precipitating eventually, when active sulfate reduction was occurring and BTEX was degraded through the process. SEM elemental spectra of the evolved show that sulfur was not present, which may exclude $BaSO_4$ and BaS as a possible precipitates. The XRD analysis suggests that barium probably ended in BaS complexing with other amorphous species. Results from this study suggest that SRB may be able to use the sulfate from barite ($BaSO_4$) as an electron acceptor, resulting in the release of free barium ions (Ba^{2+}), and re-precipitate it in BaS, which exposes more toxicity to human and ecological health.

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EXECUTIVE SUMMARY

Groundwater impacted by benzene, toluene, ethylbenzene, and xylenes (BTEX) is a common environmental concern. BTEX is known to be biodegraded anaerobically by sulfate-reducing bacteria (SRB), which produce hydrogen sulfide from sulfate. High concentrations of hydrogen sulfide are toxic and inhibitory to SRB, resulting in incomplete removal of BTEX. This condition is observed at the South Lovedale, OK site where insufficient ions are present to immobilize sulfide produced from sulfate reduction. Microcosms were established with various nutrient, additional substrate, and inhibitor treatments. All microcosms were treated with $FeCl_2$ to precipitate sulfide in the water. Complete removal of BTEX was observed in treatments amended with nutrients, no nutrients or substrate, and nitrite, indicating Fe addition immobilized the sulfide produced from sulfate reduction, eliminating toxicity.

BTEX biodegradation by SRB is suspected to mobilize barium from its precipitants in groundwater on another site in Moore, OK. Data from microcosms demonstrated that precipitated barium may be remobilized during active sulfate reduction that uses BTEX as the substrate. SEM elemental spectra of the evolved show that sulfur was not present, which may exclude $BaSO_4$ and BaS as a possible precipitates. The XRD analysis suggests that barium probably ended in BaS complexing with other amorphous species. Results from this study suggest that SRB may be able to use the sulfate from barite ($BaSO_4$) as an electron acceptor, resulting in the release of free barium ions (Ba^{2+}), and re-precipitate it in BaS, which exposes more toxicity to human and ecological health.

INTRODUCTION

Crude oil impacted groundwater is often the result of petroleum industry operations. Aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (BTEX) are major components of crude oil. BTEX can be degraded under aerobic conditions; however, oxygen depletion due to natural attenuation usually drives the geochemical environment to anaerobic conditions in the groundwater. Numerous studies have demonstrated that BTEX can be degraded anaerobically. Though some studies have reported denitrification as the primary metabolic pathway (Al-Bashir et al., 1990; Hutchins et al., 1991; Rabus and Widdel, 1995), several studies have shown successful BTEX biodegradation under sulfate-reducing conditions (Aeckersberg et al., 1991; Beller et al., 1992; Beller and Spormann, 1997; Coates et al., 1996 and 1997; Lovley et al., 1995; Noh et al., 2003; Rueter et al, 1994; Elshahed and McInerney, 2001; Schmitt et al., 1996), In the environments abundant of sulfate, BTEX can be oxidized by sulfate-reducing bacteria (SRB) and sulfate serves as the terminal electron acceptor. This pathway results in hydrogen sulfide production, which is a potential concern due to its inhibitory effect to microbial metabolism.

Hydrogen sulfide in elevated concentrations was reported to be inhibitory to various anaerobic hydrocarbon-degrading microorganisms including SRB (Koster et al., 1986; McCartney and Oleskiewicz, 1991; O'Flaherty et al., 1998; Okabe et al., 1992; Oleskiewicz et al., 1989; Reis et al., 1992; Roychoudury et al., 2003). The presence of sulfide may result in incomplete biodegration of hydrocarbon contaminants. Sulfide from sulfate reduction has been utilized to precipitate metals from water systems (Canty, 1998; Dvorak et al., 1992; Johnson and Hallberg, 2005; Tabak et al., 2003). By the same mechanism, the addition of divalent transition metal salts, such as ferrous salts, can precipitate out sulfide in waters.

An additional potential concern with anaerobic degradation of BTEX is the mobilization of metals such as barium. Barium occurs in nature mainly as barite ($BaSO_4$) and witherite ($BaCO_3$). Barite has low solubility in water (2.47 mg/l at 25^{o}C); however barium can be mobilized as Ba^{2+} by microbial activity. SRB has been reported to use barite as a sulfate source for respiration (Baldi et al., 1996; Bolze et al., 1974; McCready and Krouse, 1980), resulting in free dissolved Ba^{2+} which is known to cause muscular paralysis (Reeves, 1986).

Site 1: South Lovedale, OK

Site assessments suggested that this site had both been contaminated by BTEX and other petroleum constituents for many years. As a result the groundwater at this site is under anaerobic and reducing conditions, and evidence of anaerobic hydrocarbon degradation was observed. Gypsum beds provide a native source of sulfate to support sulfate reducing processes that can be exploited to facilitate contaminant removal. However, BTEX contamination remained potentially due to nitrogen or phosphorus limitations. Elevated concentrations of sulfide in the groundwater (~63 mg S/L in water and 500 mg H_2S/L dissipating from the well head) at the field site near South

Lovedale, OK, were inhibiting SRB that degrade benzene, toluene, ethylbenzene, and m+p-xylenes in the groundwater. There were also insufficient ions to complex with and immobilize sulfide that had been produced by the sulfate-reducing process.

Site 2: Moore, OK

At this site, the majority of petroleum contaminants have been degraded; however, barium has become a risk driver due to the biogeochemical changes caused by the BTEX degradation. Barium was mobilized under the anaerobic and reducing conditions caused by the natural hydrocarbon degradation, and there were insufficient ions present to complex barium.

In summary, the following study investigated three techniques for each site in the laboratory and partially in the field. These techniques included: 1) amending nitrogen nutrient to enhance sulfate reduction; 2) amending nitrogen nutrient and sulfide-complexing agent (ferrous iron) to enhance sulfate reduction and remove sulfide; and 3) amending sulfate to achieve metal precipitations in a post hydrocarbon degradation environment.

MATERIALS AND METHODS

Chemicals

Potassium nitrate (KNO_3), potassium nitrite (KNO_2), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), sodium molybdate, ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), benzene, and toluene were all acquired from Aldrich (Milwaukee, WI). Methanol was from Sigma (St. Louis, MO). Potassium phosphate monobasic (K_2HPO_4) and ammonium chloride were from Fisher Scientific (Fair Lawn, NJ).

Analytical Methods

Nitrate, nitrite, phosphate, and sulfate were analyzed by DIONEX DX-100 Ion Chromatograph. Sulfide and ammonium were analyzed by standard colorimetric methods (methylene blue) using Shimadzu UV-vis spectrophotometer. BTEX in water were analyzed by GC-MS equipped with a purge-and-trap system. Barium was analyzed by ICP-MS.

South Lovedale Microcosm Studies

Anaerobic microcosm studies were set up using the MW-15 groundwater and sediments. Each microcosm contained 5.0 wt% sediments based on the mass of water in the microcosms. To each microcosm, two times the stoichiometric requirement of $FeCl_2$ were added to each microcosm based on the concentrations of sulfide and sulfate. $FeCl_2$ was added to precipitate sulfide already in the groundwater and potentially precipitate out sulfide produced by sulfate reduction. Based on the concentration of GRO, the stoichiometric requirement of sulfate to degrade BTEX and any other GRO was approximately 100 times less than what was already present in the groundwater/sediment mixture. Table 1 summarizes the treatments that were applied to the microcosms for this site study. Nitrate was added in stoichiometric requirement amount based on GRO and additional substrate. Methanol was added as extra substrate in an equal molar amount as the GRO to corresponding treatments. NH_4Cl and K_2HPO_4 were used as the nitrogen and phosphorus sources, respectively. The nutrients were added in molar C:N:P ratios of 100:10:2. Na_2MoO_4 (20 mM MoO_4^{2-}) was added as a specific inhibitor to sulfate reduction (Oremland and Capone, 1988) and 3 mM nitrite, which is a competitive inhibitor of sulfate reducers (Greene et al., 2003; Haveman et al., 2003, 2004; Jenneman et al., 1986a, 1986b; Londry and Suflita, 1999), were also added to corresponding microcosm treatments. Microcosms were incubated at room temperature and stored in the dark.

Table 1. List of treatments for controlled sulfate-reduction and BTEX biodegradation study

1. Nitrate Amended
2. Nitrate + Methanol Amended
3. No Amendments
4. Nutrients Amended
5. Methanol + Nutrients Amended
6. Methanol + Nutrients + Molybdate Amended
7. Nitrite Amended
8. Nitrite + Nutrients Amended
9. Nitrite + Methanol Amended

Moore Microcosm Studies

Anaerobic microcosm studies were set up using the MW-06 groundwater and sediments. Each microcosm contained 5.0 wt% sediments based on the mass of water in the microcosms. Table 2 summarizes the treatments that were applied to the microcosms. 3 mM nitrite were added to inhibit sulfate reduction, and sulfate was added three times stoichiometric requirement based on barium concentration. Two separate sets of treatments were applied the same way as previously mentioned, except one set was spiked with 1 μl of benzene and the other set with 1 μl of toluene.

Table 2. List of treatments for Moore groundwater microcosm study

1. No Amendments
2. Sulfate Amended
3. Nitrite Amended
4. Nitrite + Sulfate Amended

MW-02 microcosms were treated the same way as MW-06 microcosms, except treatments 2 and 4 were omitted since the concentration of sulfate was more than three times the stoichiometric requirement based on barium.

Mitigation of Barium: BTEX Threshold of Barium Release

Baseline analysis of the MW-06 water yielded 3041.3 g Ba^{2+}/l. Microcosms containing the MW-06 water were established anaerobically. SO_4^{2-} (in form of $FeSO_4 \cdot 7H_2O$) was added in stoichiometric amount to all microcosms in the following set of treatments: 1) Control (no amendments), 2) toluene, 3) toluene plus nutrients, 4) BTEX, and 5) BTEX plus nutrients. Another set of the same treatments were established except the SO_4^{2-} was added in three times the stoichiometric amount required to immobilize barium. Nitrogen and phosphorous nutrients, in the form of NH_4Cl and KH_2PO4, respectively, were added to corresponding treatments in the C:N:P molar ratio of 100:30:3. BTEX (p-xylene used to represent xylenes) were added to individual solubilities (700 mg/l for benzene and toluene; 100 mg/l for ethylbenzene; 200 mg/l for p-xylene).

Sediments/precipitates were analyzed to identify and semi-quantify forms of mineral barium. Analysis was conducted by scanning electron microscope (SEM) for physical appearance, wavelength dispersive spectroscopy (WDS) for qualitative chemical composition, and by X-ray diffraction (XRD) for identification of mineral phases.

Table 3. Treatments for BTEX threshold study

Treatment	Nutrients	Toluene	BTEX	3 x Stoichiometric Sulfate Requirement
1				
2		■		
3	■	■		
4			■	
5	■		■	
6				■
7		■		■
8	■	■		■
9			■	■
10	■		■	■

Microbiological Mobilization of Barium from Barite: Microcosm Study

Microcosms were established in 125-ml serum bottles. Table shows the treatments that were used in this study. Water (1.0 ml) from treatment 6A from the BTEX threshold study was used as the SRB inoculation. Barite ($BaSO_4$; Sigma-Aldrich) was used as a source of sulfate and

3.0 g were added to corresponding microcosms. Benzene (Sigma-Aldrich) was added to concentration of 874 mg/l, and sodium lactate (Sigma-Aldrich) was added to concentration of 20 mM. Na_2SO_4 (Sigma-Aldrich) was used as a source of free dissolved sulfate and 0.93 g was added to corresponding microcosms. All microcosms were filled to top of serum bottle with minimal salts solution (preparation method described in the next paragraph) and were capped in anaerobic glove box (N_2 atmosphere). The microcosms were stored in the dark at room temperature.

A salts solution was prepared by adding the following amounts of salts per 100 ml: 0.2 g NaCl and 0.2 g $CaCl_2$. The minimal salts solution was prepared by adding the following per 1 L: 3.8 g KH_2PO_4, 12.5 g K_2HPO_4, 1.5 g $(NH_4)_2HPO4$, and 1.0 ml of the salts solution. The solution was boiled and sparged with N_2 to eliminate oxygen.

Table 4. Treatments for barium-release from barite study

Treatment ID	SRB Inoculation	Lactate	Benzene	Barite	$NaSO_4$
Ba 1			▨	▨	
Ba 2	▨			▨	
Ba 3	▨		▨		
Ba 4	▨		▨	▨	
Ba 5	▨	▨		▨	
Ba-S 1			▨	▨	▨
Ba-S 2	▨			▨	▨
Ba-S 3	▨		▨		
Ba-S 4	▨		▨	▨	▨
Ba-S 5	▨	▨		▨	▨
S 1			▨		▨
S 2	▨				▨
S 3	▨		▨		
S 4	▨		▨		▨
S 5	▨	▨			▨

RESULTS AND DISCUSSION

Groundwater Characterization

Groundwater and sediments were collected from monitoring wells (MW) at their corresponding sites (MW-15 for South Lovedale; MW-06 and MW-02 for Moore). Baseline characterization analyses were conducted upon arrival at our laboratory, which are summarized in Tables 5 and 6.

Table 5. Groundwater baseline organics concentrations

MW	Benzene, μg/l	Ethylbenzene, μg/l	m+p-Xylenes, μg/l	Other BTEX, μg/l	GRO, mg/l
15	700	220	310	<5	4.3
06	48	<5	<5	<5	
02	<5	<5	<5	<5	

Table 6. Groundwater baseline inorganics concentrations

MW	SO_4^{2-}, mg/l	NO_3^--N, mg/l	NH_3-N, mg/l	S^{2-}, mg/l	Ba, μg/l	Fe, mg/l
15	1730	4.3	2.0	8.928		
06	28.490			0.129	2300	0.3
02	1242.575			0.072	228	0.1

South Lovedale Microcosm Studies

Change in the benzene concentrations over 210-day period is shown in Figure 1. Benzene concentrations decreased slowly in non-amended, nutrients only and nutrients-methanol treated microcosms by 100% after 127 days. However sulfate concentration decreased by only 50% in the non-amended, decreased down by 76.7% in nutrients-only and completely removed in the nutrients-methanol amended microcosms (Figure 2). The lower sulfate reduction observed in the non-amended microcosm experiments and the incomplete sulfate reduction in the nutrients amended microcosms suggest that the media was nutrients limited and may be needing a simple organic carbon source to enhance the benzene degradation.

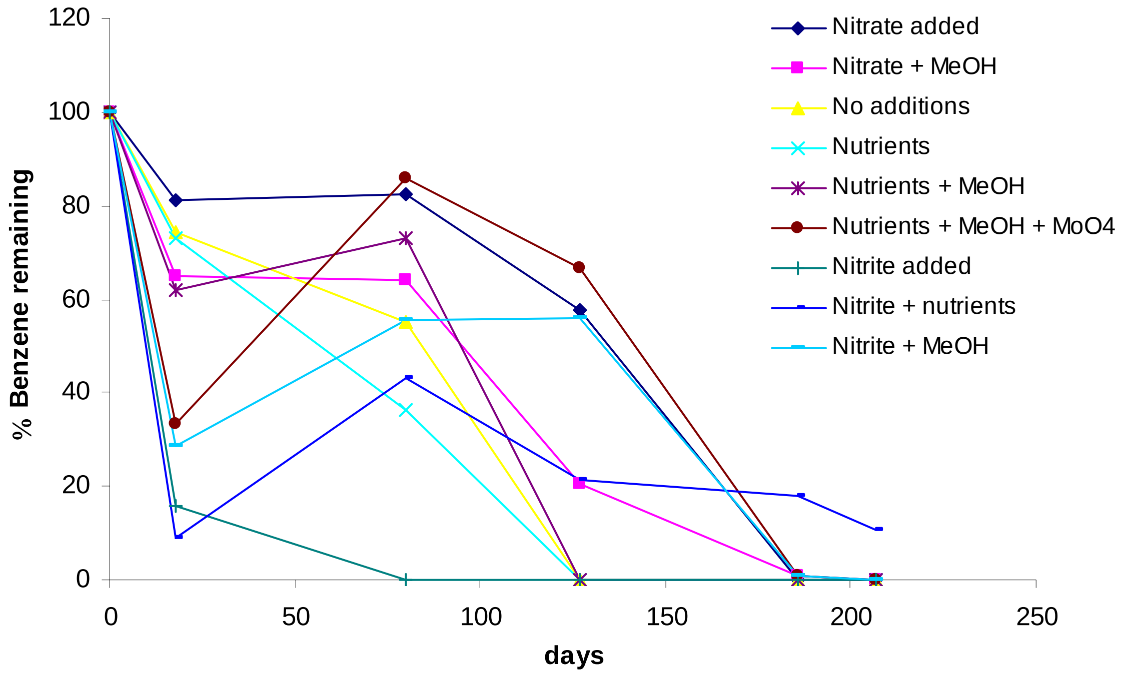


Figure 1. Biodegradation of benzene from various groundwater microcosm treatments

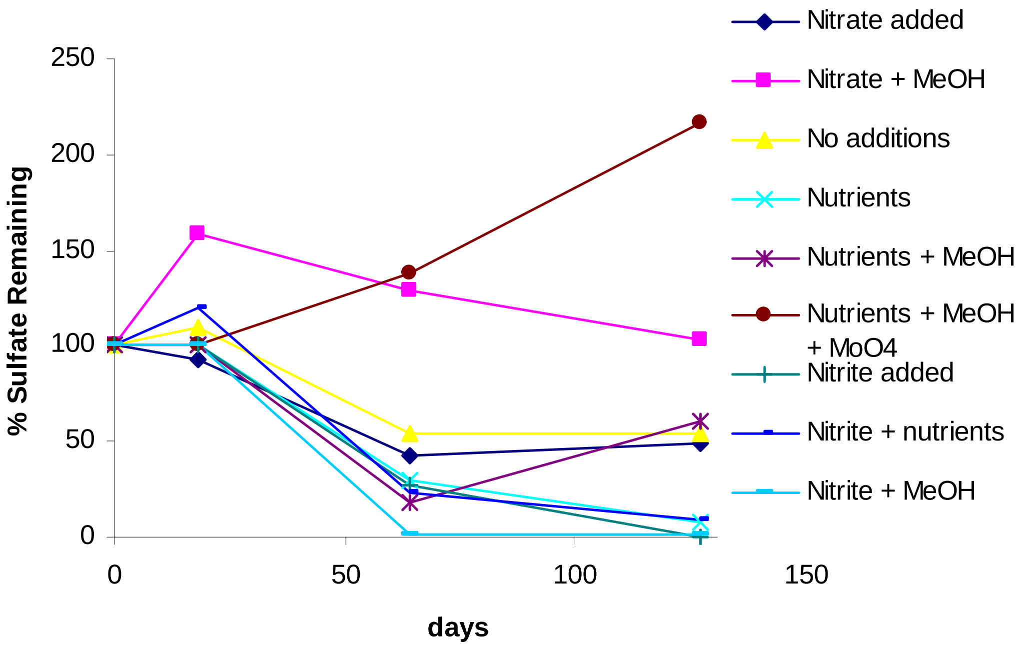


Figure 2. Sulfate reduction in various treatments

In the molybdate added microcosms to investigate the inhibition of sulfate reduction, a 66.5% decrease in benzene concentrations were observed after 18 days, however it was increased by 34.2% to 52.4% in the next 109 days of the experiment. Approximately 60% increase was observed in the nutrients-methanol-molybdate amended microcosms at the end of 70 days. However no sulfate reduction was observed. The increase in the sulfate concentration was thought to be due to possible desorption of sulfate from the sediments.

An 18.9% decrease in benzene concentrations were observed in the nitrate-amended microcosms in the first 80 days, followed by a sharper degradation rate of 24% in the next 47days. 50% sulfate reduction was observed in this microcosm indicating bacteria were capable of switching to denitrification when electron source is available.

Benzene concentrations were decreased by 35% after 18 days in the nitrate-methanol amended microcosms, then no change in benzene concentrations were observed over the next 62 days and a decrease by 44% was observed after an additional 47 days. Sulfate concentrations in these microcosms were increased by 60% during the first 20 days of the experiment, which may be due to desorption of sulfate from the sediments and started to decrease afterwards achieving a 30% decrease in the next 50 days. The decrease observed may be indicating that nitrate in the media may become limited and bacteria started to use sulfate as their electron acceptor again.

The concentration of the ethylbenzene decreased by 84.7 to 100% over the 127-day period for all the experiments as shown in Figure 3, except for the nitrite-nutrients amended treatment, and the concentrations of m+p-xylenes decreased by 93.9 to 100.0% over the 127-day period for all of the experiments (Figure 4).

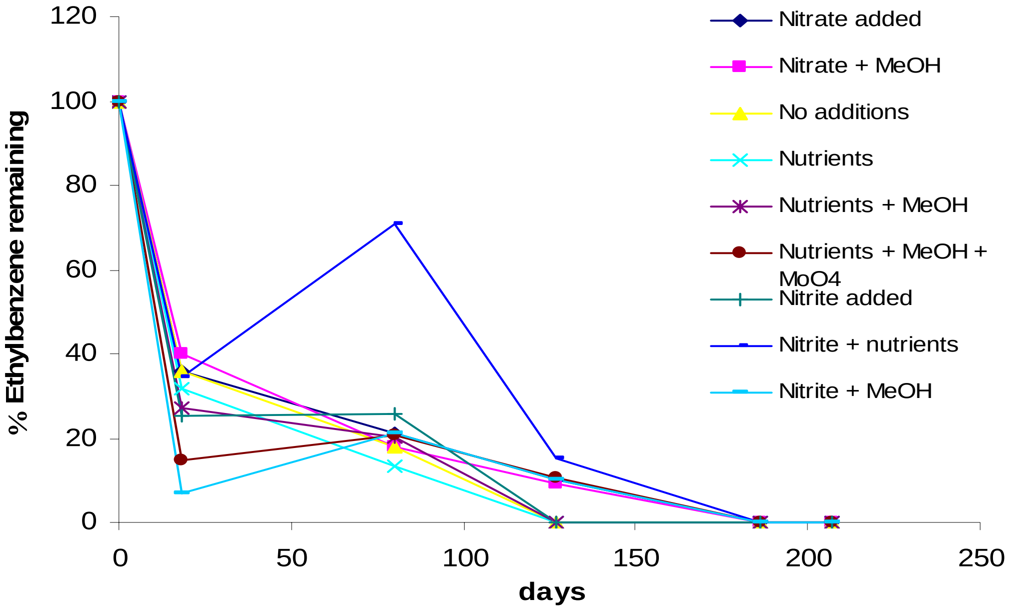


Figure 3. Biodegradation of ethylbenzene from various groundwater microcosm treatments

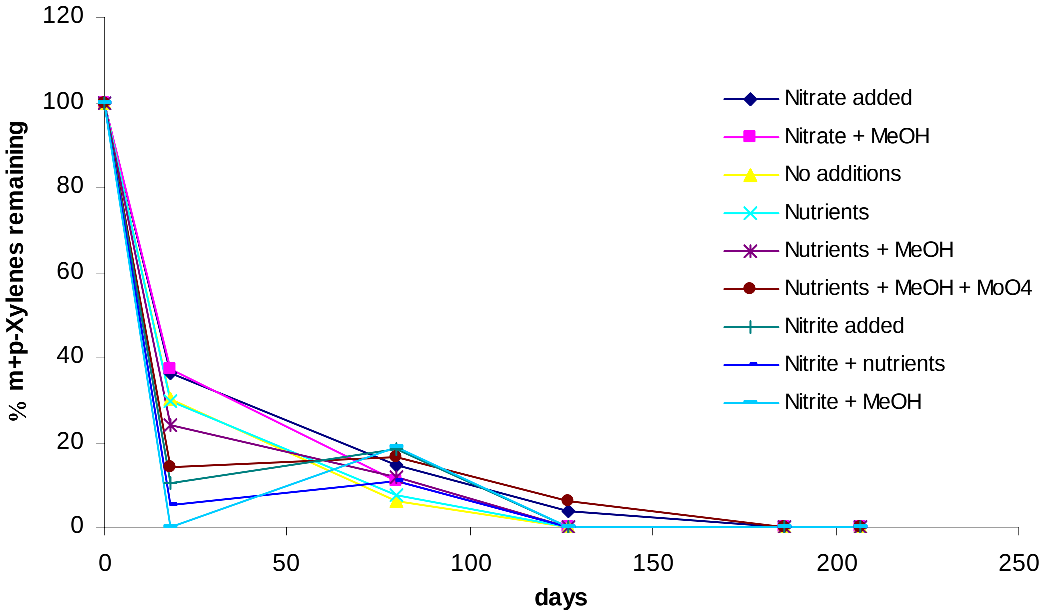


Figure 4. Biodegradation of m+p-xylenes from various groundwater microcosm treatments

Benzene, ethylbenzene, and m+p-xylenes degradation was observed in the nitrate plus nutrients amended microcosms; however, sulfate concentration did not decrease in those microcosms. The addition of nitrate and methanol as substrate may have stimulated denitrifying bacteria activity, resulting in the denitrifying bacteria out-competing the SRB.

Nitrite was used as an inhibitor of sulfate-reduction; however microcosms containing nitrite exhibited sulfate reduction, evidenced by removal of sulfate, benzene, ethylbenzene and m+p-xylenes. Recent SRB studies have reported that some SRB can produce periplasmic nitrite reductase, resulting in SRB resistance to nitrite (Greene et al., 2003; Haveman et al., 2004). The SRB present in the South Lovedale site groundwater may produce nitrite reductase which may explain the SRB resistance and continued hydrocarbon degrading activity.

In summary, complete removal of benzene, ethylbenzene and m+p-xylenes were observed in treatments containing nutrients, no nutrient or substrate additions, and nitrite. This indicates that with iron addition, sulfide no longer was inhibiting hydrocarbon degradation due to sulfide precipitation. The 4.3 mg/L background concentration of ammonium provided sufficient amount of nitrogen to be utilized by the SRB. This is indicated by the similar rate of hydrocarbon depletion with the nutrient amended treatment. Therefore suggesting with iron addition benzene, ethylbenzene and m+p-xylenes can be removed by simple natural attenuation.

Field-scale studies started in February 2005, and water samples from monitoring wells (MW) have been collected and analyzed at three points of time post-treatment. Tables 7 and 8 summarize the analysis results for MW-15 and MW-32.

Table 7. Field results from Monitoring Well 15 (MW-15).

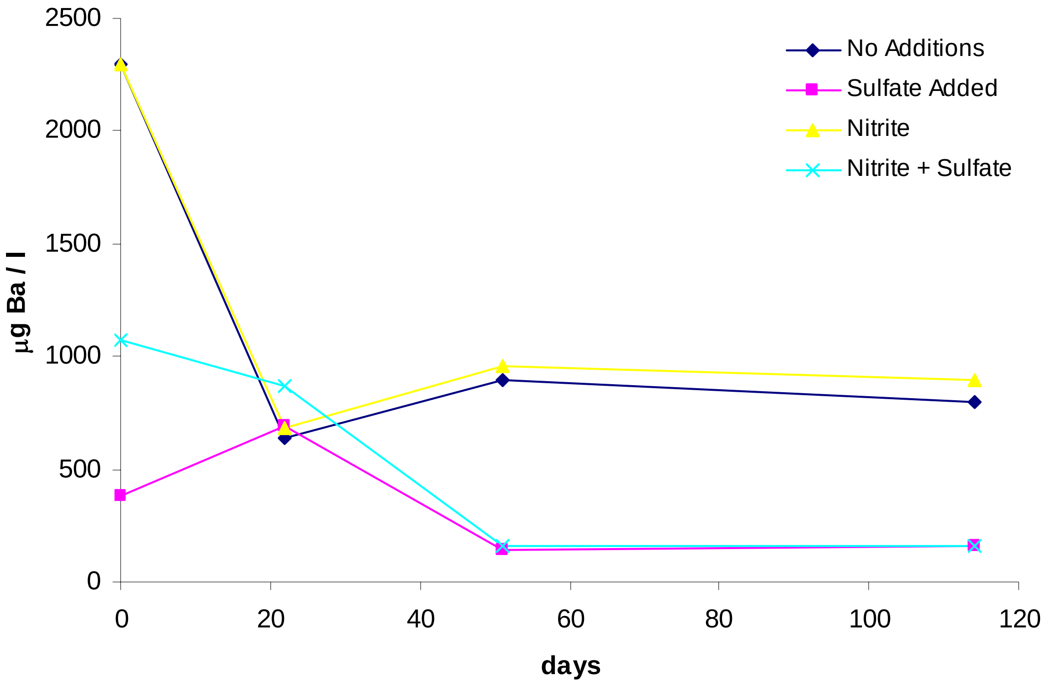
Parameter	2/4/2005	2/25/2005	3/9/2005	5/4/2005
Alkalinity, mg/l CaCO3	90	60	260	220
NO2/NO3, mg/l	9.7	7.844	8.009	<0.100
SO4, mg/l	1730	1203.14	1539.72	1280.028
NH3, mg/l	2	0.108	2.04	0.86
CH4, mg/l	0.189	0.35	0.426	
TPH, mg/l	4.2	7	5.4	
Benzene, ug/l	700	600	620	
Toluene, ug/l	<5	2.6	<10	
Ethylbenzene, ug/l	220	290	330	
m+p-Xylenes, ug/l	310	320	530	

Table 8. Field results from Monitoring Well 32 (MW-32).

Parameter	2/4/2005	2/25/2005	3/9/2005	5/4/2005
Alkalinity, mg/l CaCO3	103	70	280	280
NO2/NO3, mg/l	18.7	12.817	21.396	<0.100
SO4, mg/l	1500	1582.31	1446.45	1485.15
NH3, mg/l	1	0.507	1.426	0.43
CH4, mg/l	0.389	0.007	0.706	
TPH, mg/l	<1.1	<1.1	<1.1	
Benzene, ug/l	<2.5	6.2	<1.0	
Toluene, ug/l	<2.5	<1.0	<1.0	
Ethylbenzene, ug/l	<2.5	4.5	<1.0	
m+p-Xylenes, ug/l	<2.5	<1.0	<1.0	

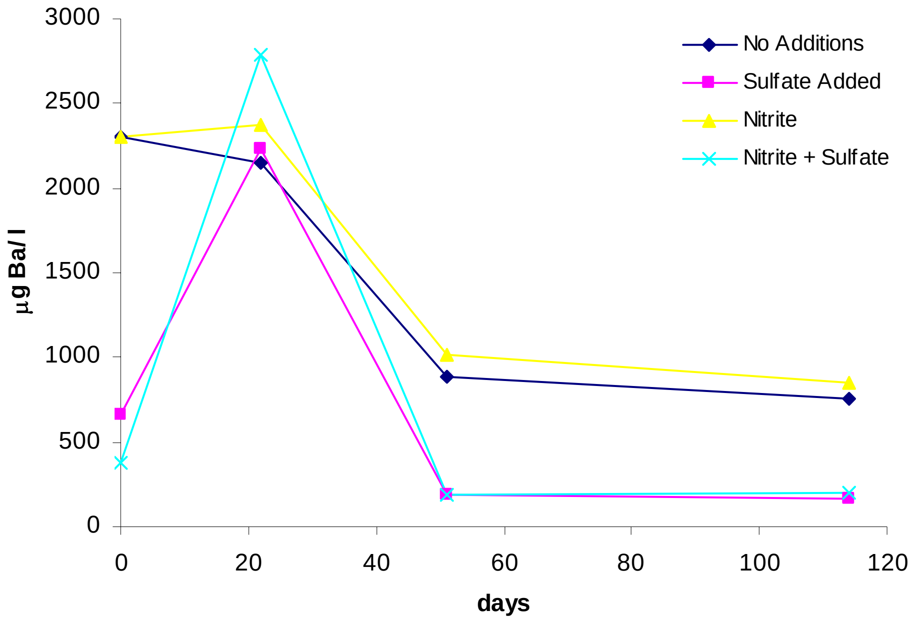
Moore Microcosm Studies

Initial decrease in barium is evident after the addition of iron sulfate in the treatments with benzene, toluene, and no benzene/toluene amendments (Figure 5); however, barium eventually decreases by roughly 1000 µg/l in the treatments with no iron sulfate addition. This may be the result of adsorption of barium to the sediments. These treatments still had higher barium concentrations (800-900 µg/l) than the treatments amended with sulfate (160-200 µg/l). Degradation of benzene and toluene was evident in the benzene and toluene amended treatments. Complete degradation occurred in treatments with no amendments and treatments amended with nitrite only (Figure 6). Incomplete degradation (36-38 µg/l remaining after 114 days) occurred in treatments with sulfate amendments. The presence of small amount of BTEX has not shown negative influence on barium sulfate. Nitrite was degraded in all corresponding treatments, suggesting the SRB may be resistant to nitrite and may produce nitrite reductase to consume nitrite.



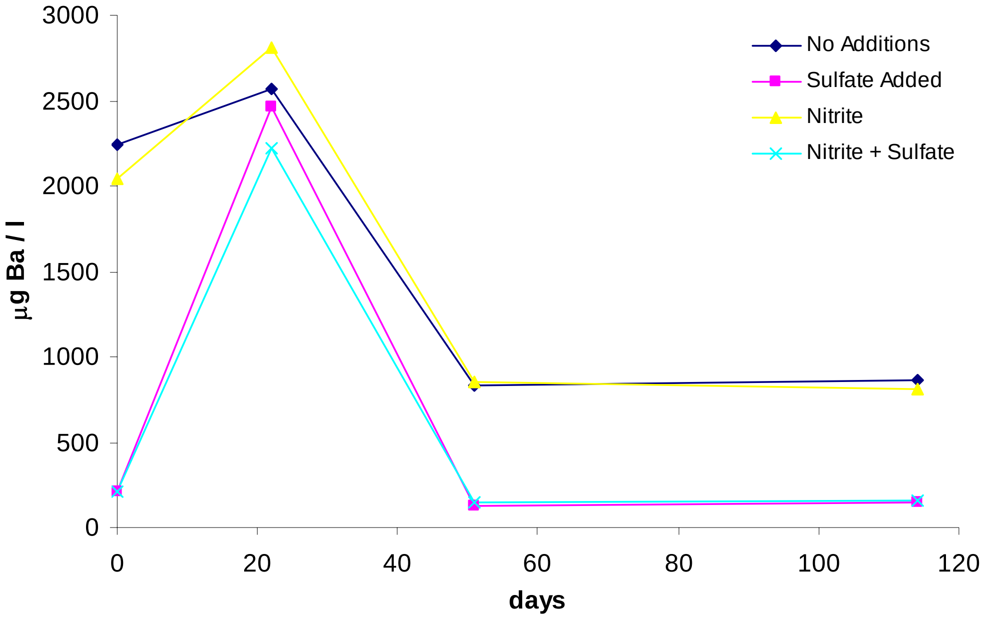
(a)

Figure 5 (a). Impact treatments on dissolved barium for groundwater collected from MW-06 which were not amended with aromatic hydrocarbons.



(b)

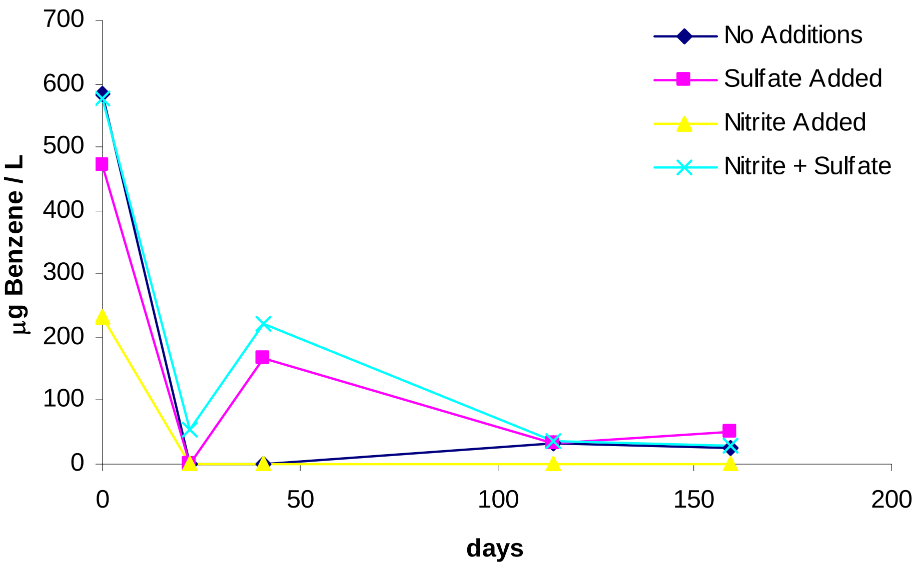
Figure 5 (b) Impact of treatments on dissolved barium for groundwater collected from MW-06 which were amended with benzene.



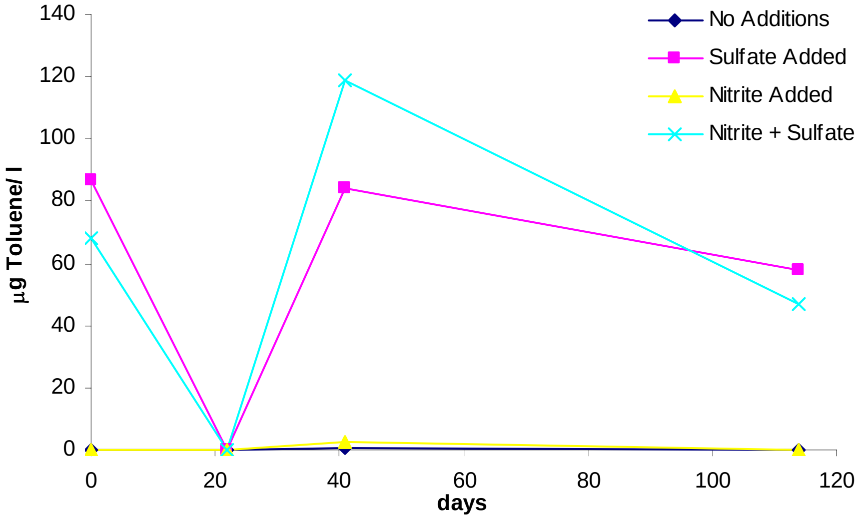
(c)

Figure 5 (c). Impact of treatments on dissolved barium for groundwater collected from MW-06 which were amended with toluene.

Complete degradation of benzene and toluene was observed within 22 days in all microcosms (Figure 6). Benzene and toluene were added to microcosms treated with sulfate to determine if free barium levels would be affected by biodegradation. Biodegradation of benzene and toluene was observed, but it did not affect free barium levels (Figure 5). The amount of excess sulfate in solution was adequate for removing benzene and toluene, without the SRB using sulfate from barite. The biodegradation of the re-amended toluene occurred at a slower rate, possibly due to insufficient amount of nutrients available to the microbes.



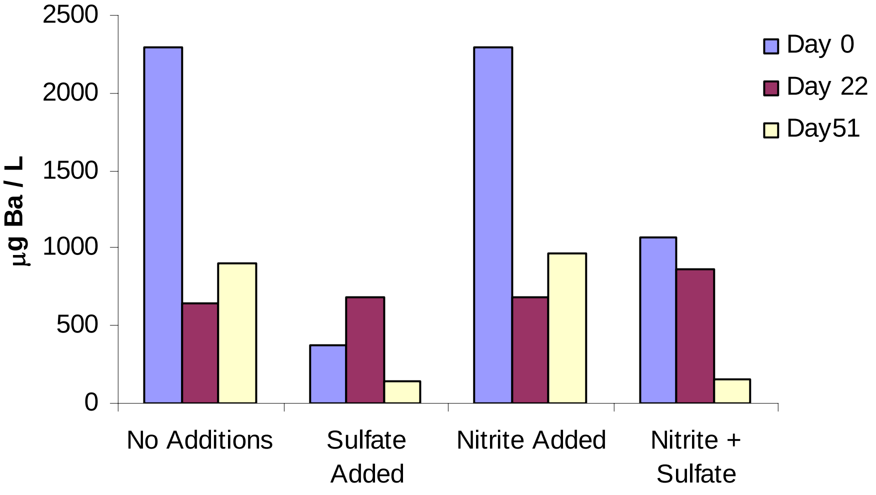
(a)



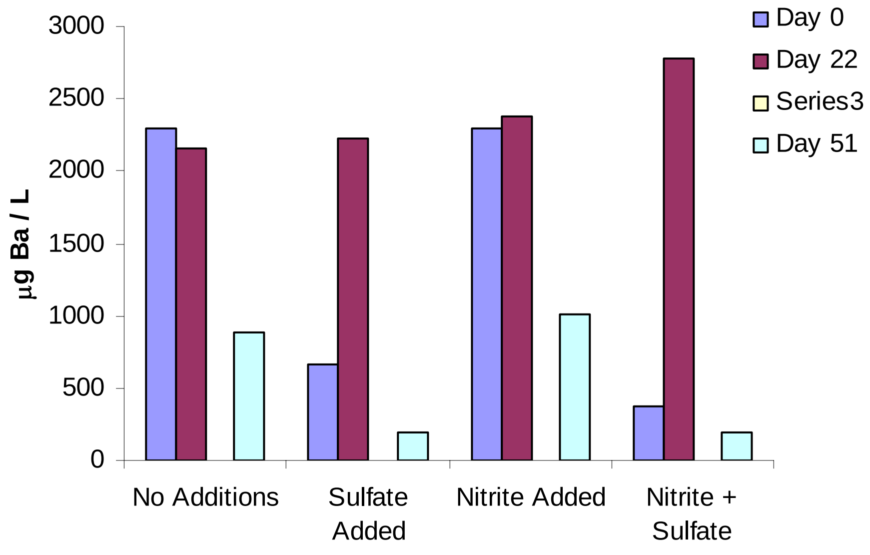
(b)

Figure 6. Biodegradation of (a) benzene and (b) toluene in corresponding amended microcosms

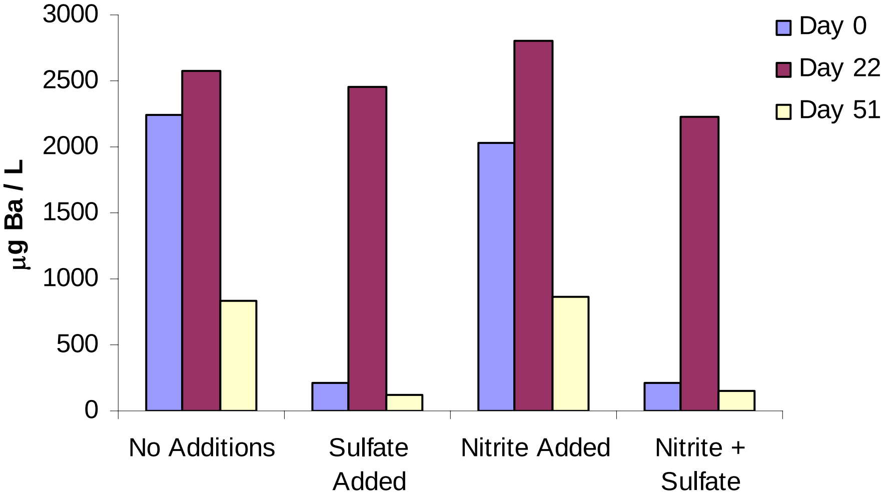
Sulfate concentration in the MW-02 water was high enough to complex barium. Only 66 to 76 µg Ba / l was found in the water samples, which is significantly lower than the >2000 µg Ba / l found in the MW-06 samples. The amount of sulfate was higher than the three times the stoichiometric requirement to complex barium. Results for the MW-02 samples show little change in Ba concentration after 91 days (Figure 6).



(a)



(b)



(c)

Figure 7. Barium concentrations in MW-02 groundwater microcosms which were (a) not amended with aromatic hydrocarbons, (b) amended with benzene, and (c) amended with toluene.

The addition of sulfate was successful in removing free barium ion, bringing the Ba^{2+} concentration below drinking water standard of 2 mg/l. The amount of barium ion decreased for microsms not amended with sulfate, which may be due to other precipitations with divalent anions such as S^{2-} and CO_3^{2-} . The formation of transient BaS from the S^{2-} produced during sulfate reduction has been observed in other studies (Baldi et al., 1996). BaS is stable in anaerobic conditions and is stabilized by colloidal or organic surfaces.

In summary, the formation of BaS is possible. Though the decrease of Ba^{2+} (down to ~800-900 µg/l) was observed in the microcosms not amended with sulfate, those with sulfate added were lower in Ba^{2+} concentration (160-200 µg/l). Also, degradation of benzene and toluene by SRB did not effect barium concentration in the microcosms.

Mitigation of Barium: BTEX Threshold of Barium Release

Baseline analysis of the MW-06 water yielded 3041.3 µg Ba^{2+}/l. Microcosms containing the MW-06 water were established anaerobically in 125-ml serum bottles. The treatments were set up as described in Table 7. SO_4^{2-} was added in the form of $FeSO_4{\cdot}7H_2O$ to immobilize free barium. Nitrogen and phosphorous nutrients, in the form of NH_4Cl and KH_2PO4, respectively, were added to corresponding treatments in the C:N:P molar ratio of 100:30:3. BTEX (p-xylene used to represent xylenes) were added to individual solubilities (700 mg/l for benzene and toluene; 100 mg/l for ethylbenzene; 200 mg/l for p-xylene).

The addition of sulfate did not have an initial effect on free barium concentration (Figure 8). Free barium concentration remained relatively stable for Treatments 4 and 9 despite the apparent sulfate reduction (Figure 9) and degradation of BTEX (Figures 10, 11, 12, and 13). Free barium concentration decreased from 3041 μg/l to 2655, 1447, 1340, and 700 μg/l in Treatments 2, 3, 7, and 8 after one month of incubation. However, the barium concentration increased in conjunction with the significant decrease of sulfate and toluene concentrations, suggesting the SRB are utilizing sulfate from the barite precipitates. Barium concentration continued to decrease in Treatments 5 and 10.

Solid samples from each microcosm were collected and analyzed for mineral barium identification. SEM/WDS (Figure 14) indicated that less than 1% of the weight in the solid phases of treatments 5 and 10 was that of Ba minerals. SEM elemental spectra showed that sulfur was not present, which may exclude BaS as a possible precipitate; however, the analysis did show barium was present in the solid form. Inconclusive identification of the barium species suggests that the precipitated form of barium should not assumed to be stable. The XRD analysis (Figure 15) could not find a match for $BaSO_4$, $BaCO_3$, and BaO. Studies have assumed that the transient species BaS is formed during sulfate reduction since it is stable in anaerobic conditions and in presence of colloidal surfaces but also had inconclusive XRD data (Baldi et al., 1996).

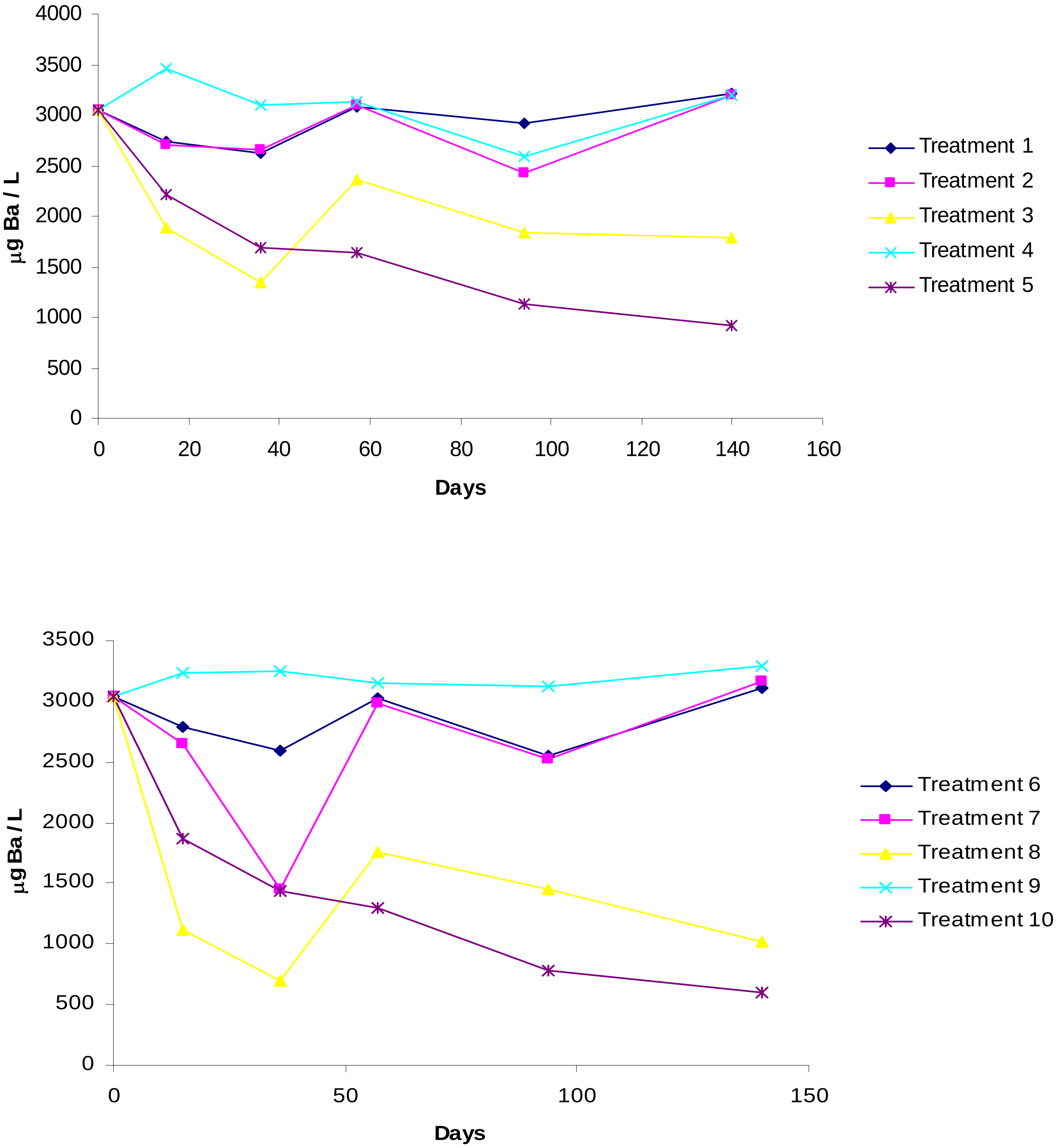
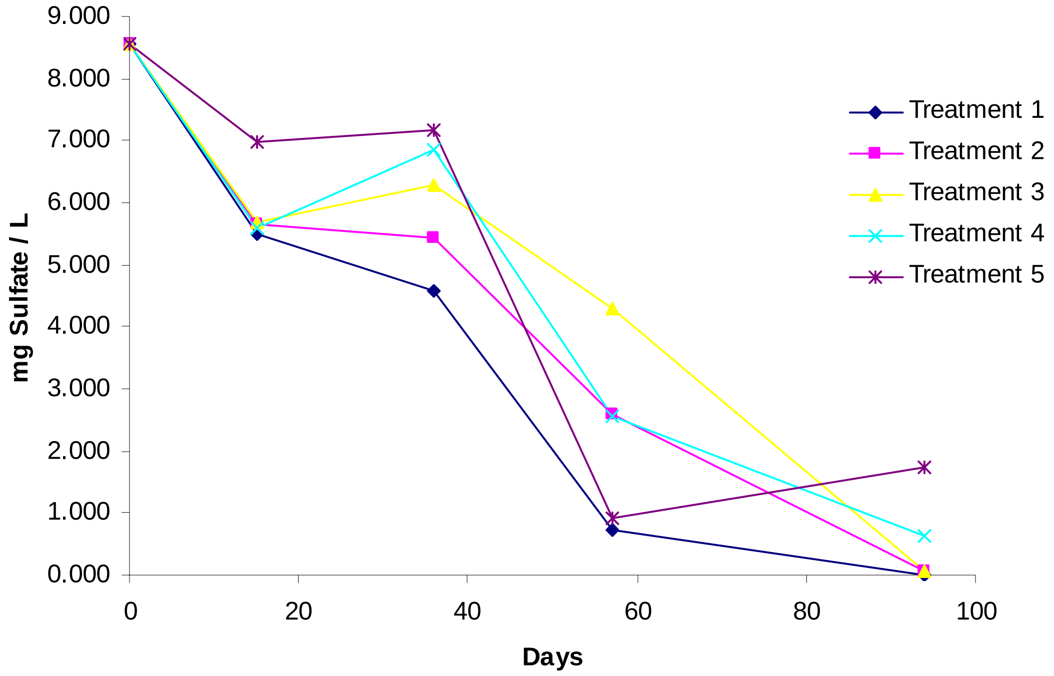


Figure 8. Barium profiles in microcosms



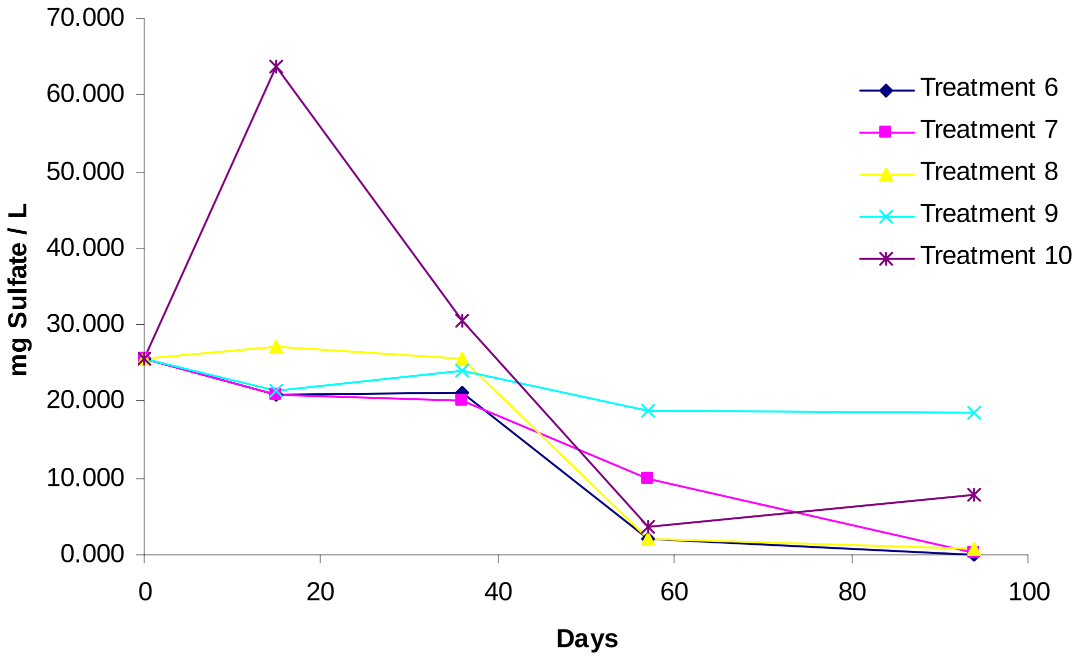
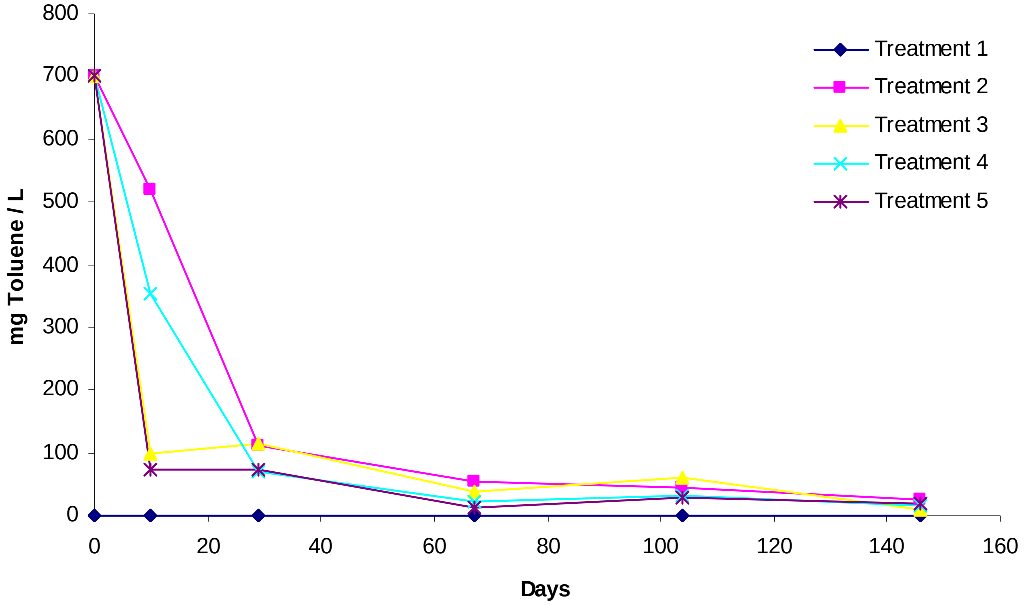


Figure 9. Sulfate profiles in microcosms



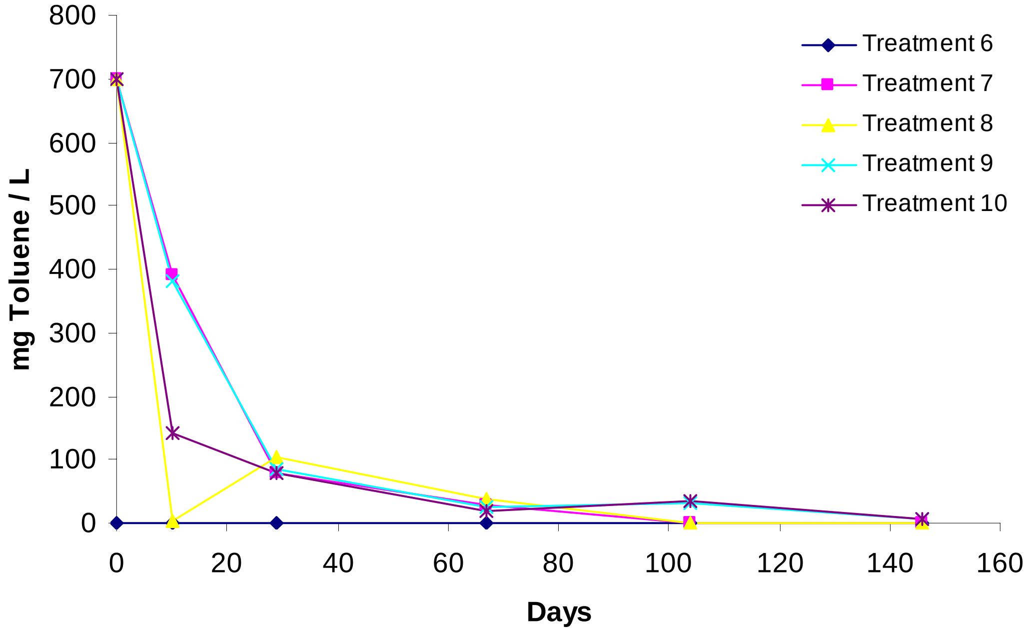
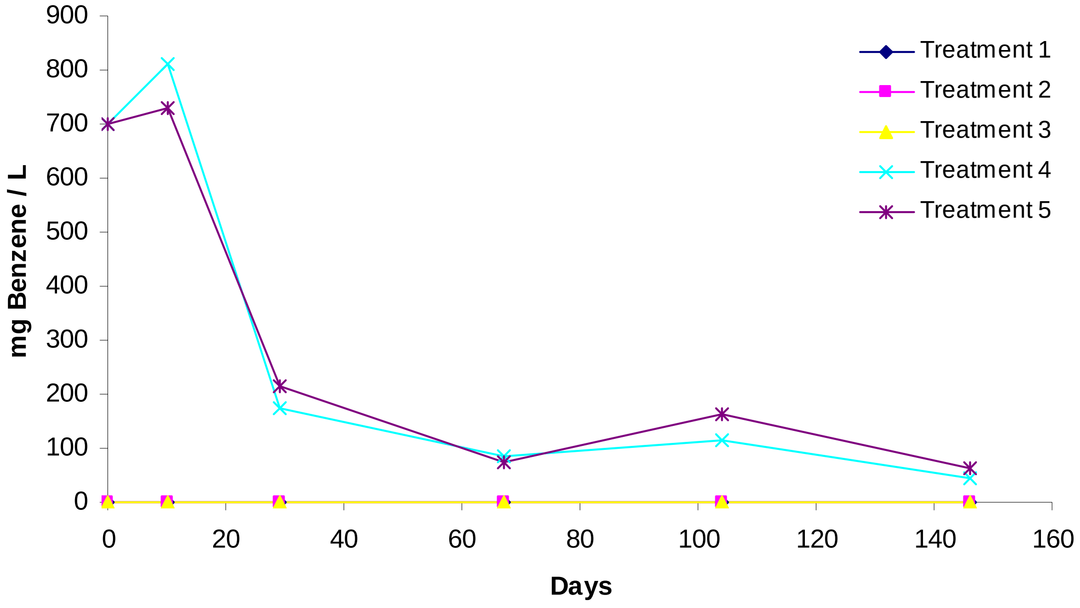


Figure 10. Toluene profiles in microcosms



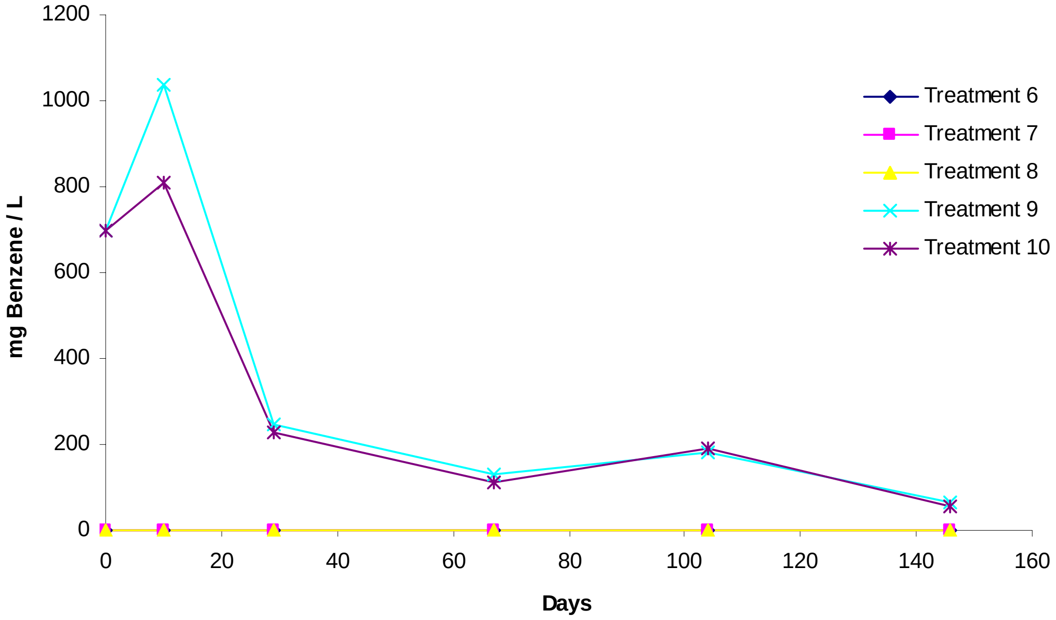
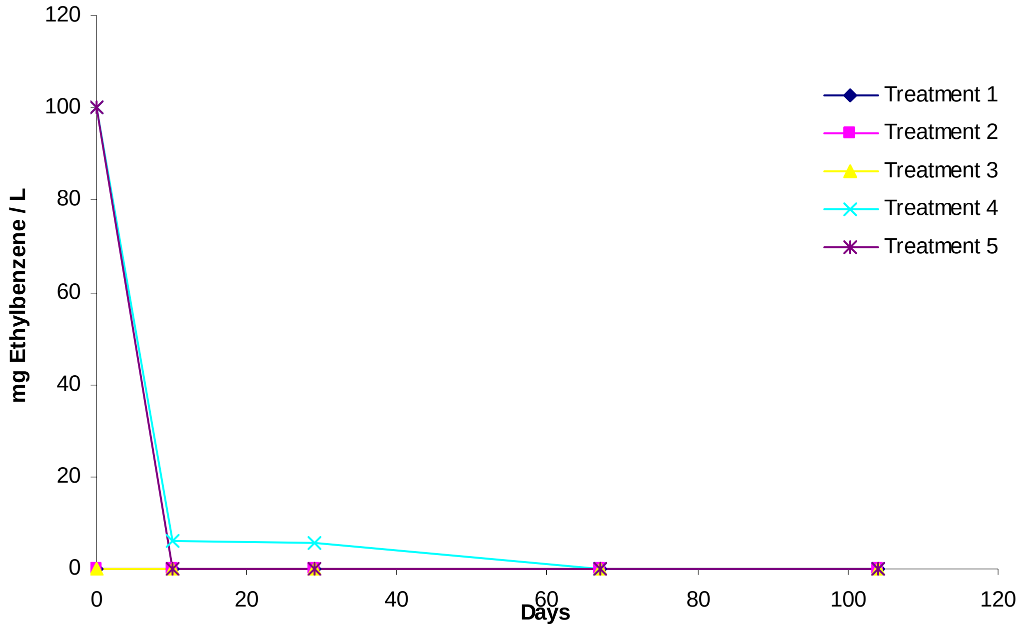


Figure 11. Benzene profiles in microcosms



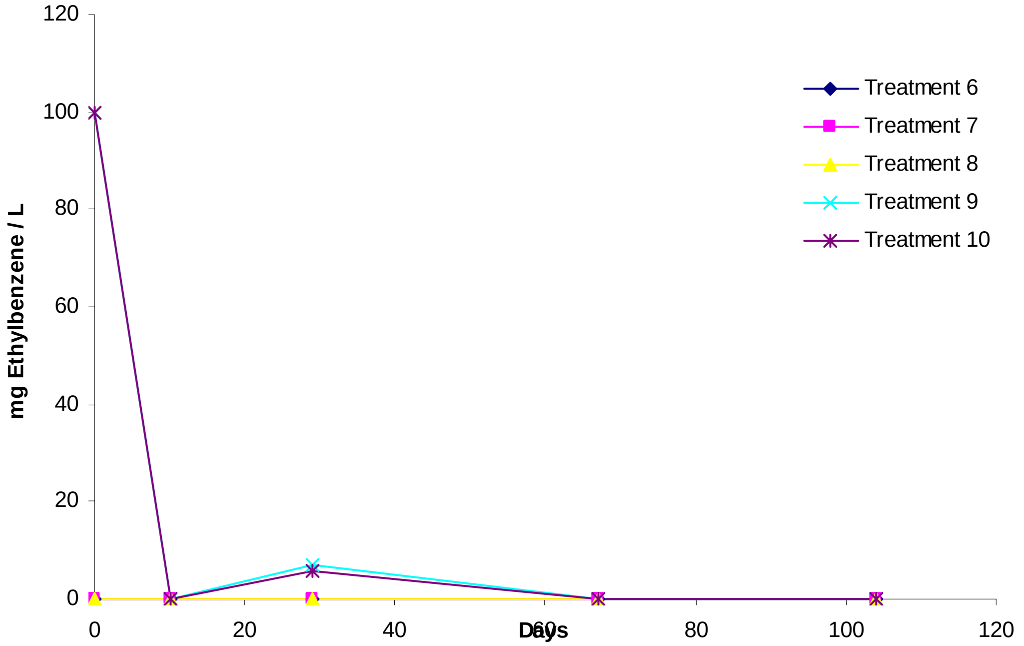
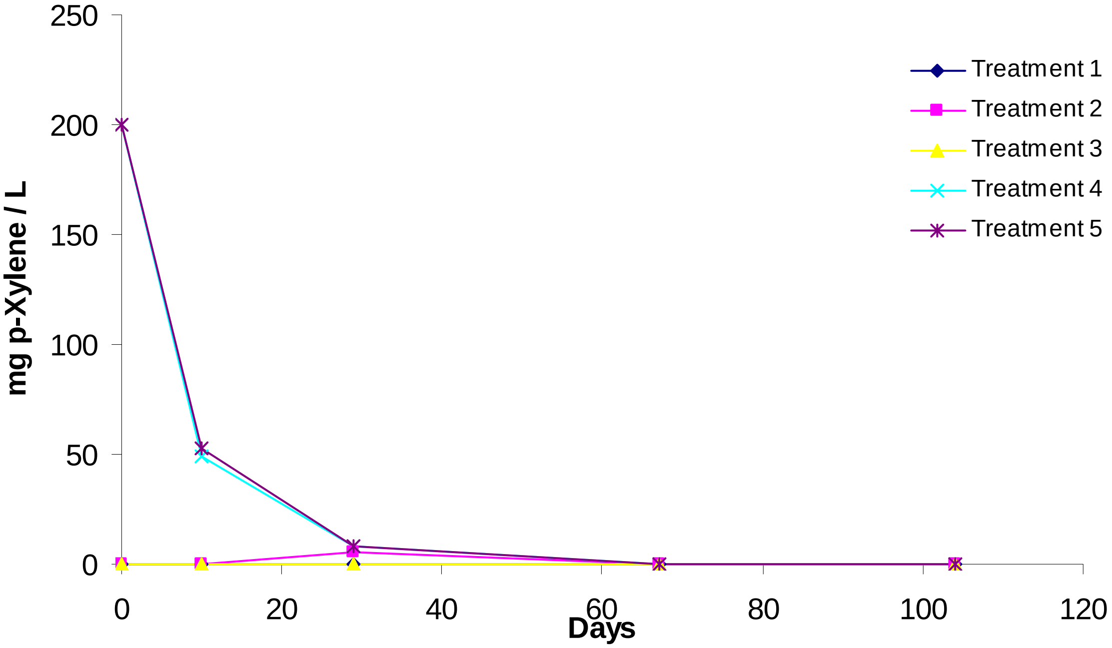


Figure 12. Ethylbenzene profiles in microcosms



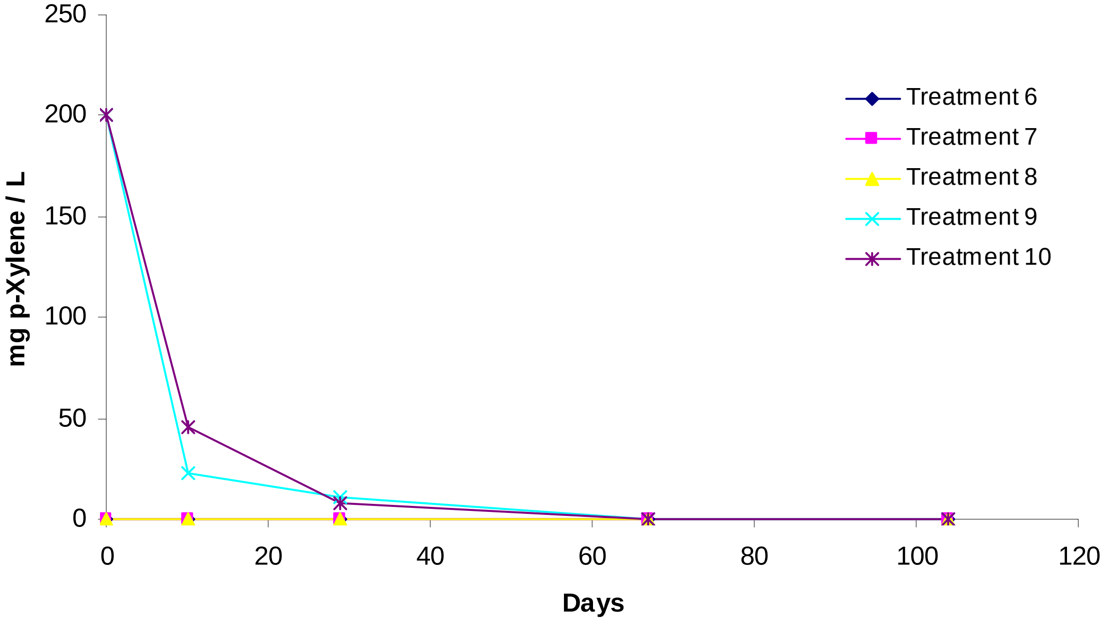


Figure 13. p-Xylene profiles in microcosms

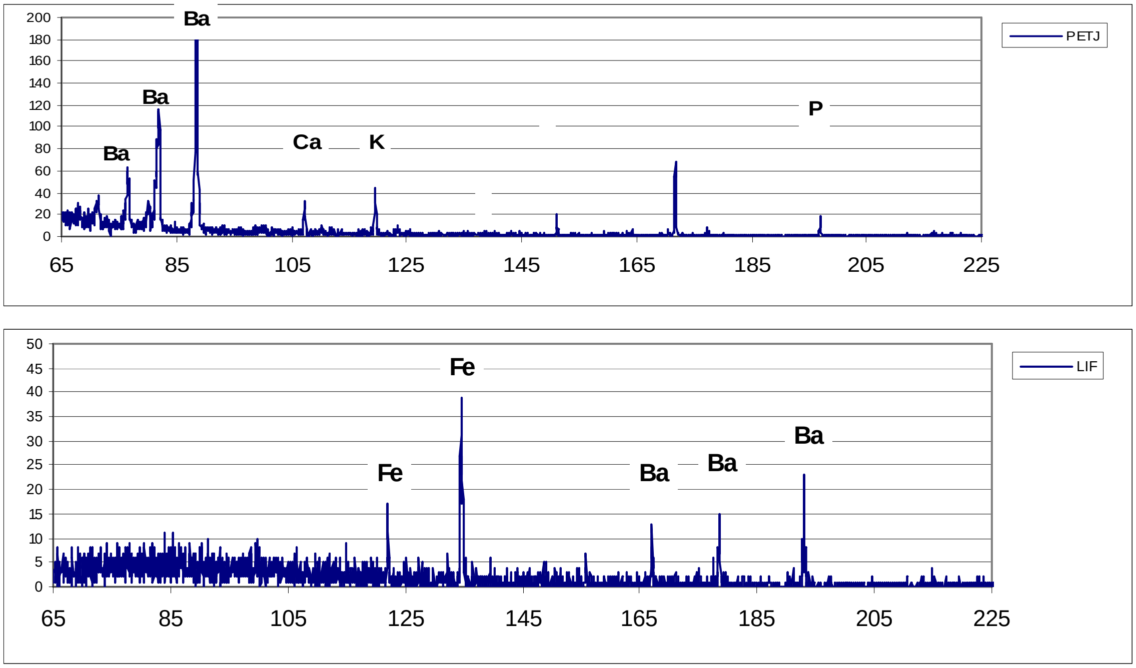


Figure 14. Elemental spectra of precipitate evolved in microcosms

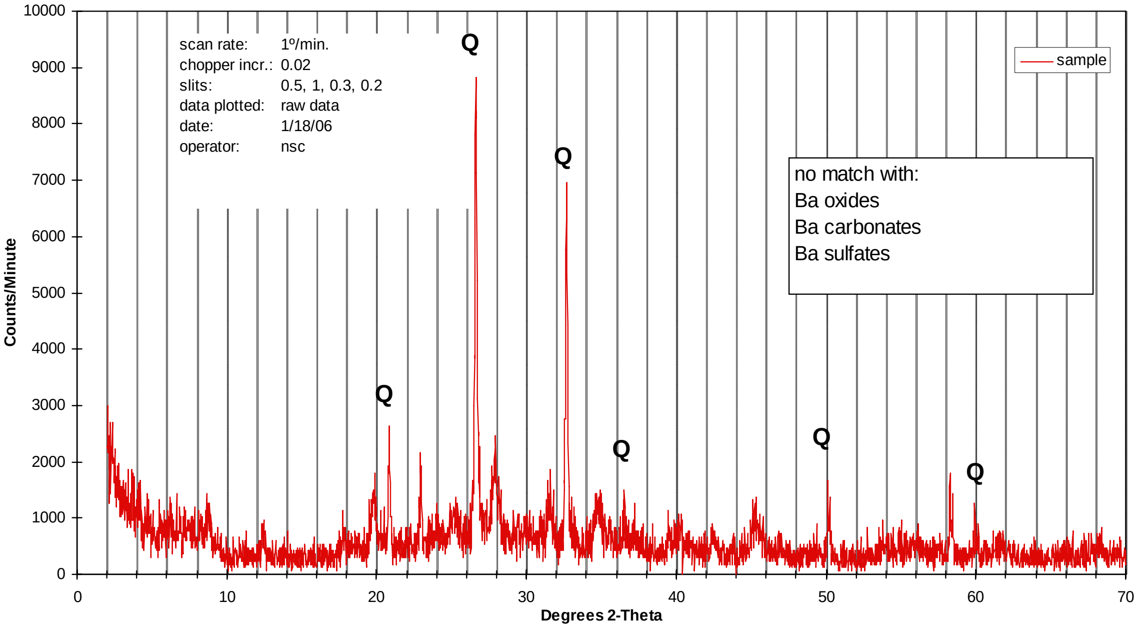


Figure 15. X-ray diffraction of precipitate evolved in microcosms

Microbiological Mobilization of Barium from Barite: Microcosm Study

Microbial mobilization of barium from barite was not apparent from the microcosm study results (Figure 16). The amount of barium that dissolved was less than the maximum solubility of barite (2.47 mg/l) and not significant difference was observed between the inoculated and non-inoculated microcosms, suggesting abiotic dissolution. Additionally, no sulfate-reduction was observed in the microcosms amended with free sulfate. Since there is no data available for benzene degradation, it is difficult to conclude whether or not the inoculation provided viable cells for corresponding treatments. Overall, data from this experiment are inconclusive.

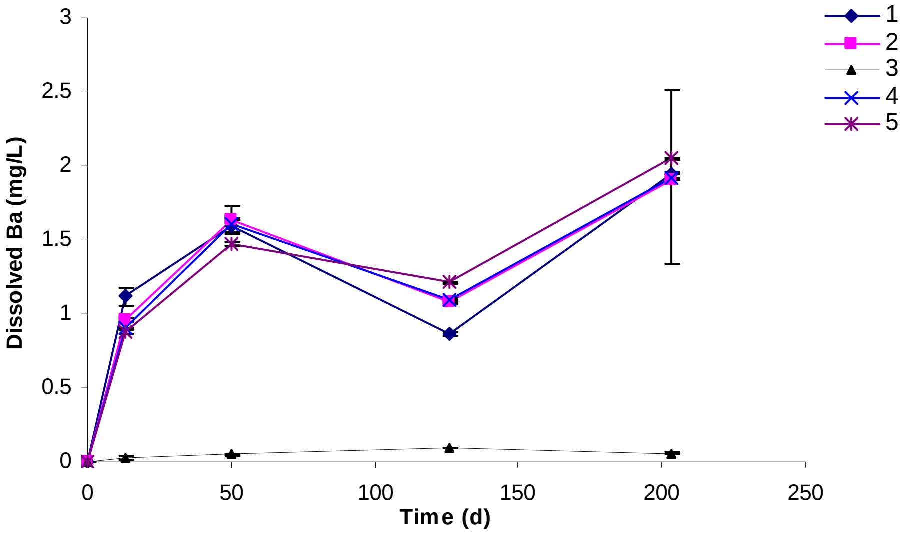


Figure 16. Dissolved barium in microcosms

CONCLUSIONS

All microcosms for the South Lovedale study were initially amended with $FeCl_2$ to induce FeS precipitation and, thereby, reduce sulfide concentrations. Complete removal of BTEX was observed within 39 days in treatments with various combinations of nutrient and substrate amendments including no amendments (other than $FeCl_2$). This indicated that the elevated concentration of sulfide is the only limiting factor to BTEX biodegradation at this site, and that treating the groundwater with $FeCl_2$ can facilitate and enhance BTEX degradation by the indigenous SRB population.

Microcosm studies for the Moore study suggested that dissolved barium may be released during active degradation of organics; however, SEM elemental spectra of the evolved showed that sulfur was not present, which may exclude $BaSO_4$ and BaS as a possible precipitates; however, the analysis, did show barium was present in the solid form. The XRD analysis could not find a match for $BaSO_4$, $BaCO_3$, and BaO. Inconclusive identification of the barium species suggests that the precipitated form of barium should not assumed to be stable during BTEX biodegradation, and that Ba may be precipitating in a different form during this biodegradation.

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REFERENCES

Aeckersberg, F., F. Bak, and F. Widdel. 1991. Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. Arch. Microbiol. 156:5-14.

Al-Bashir, B., T. Cseh, R. Leduc, and R. Samson. 1990. Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soil under denitrifying conditions. Appl. Microbiol. Biotechnol. 34:414-419.

Baldi, F., M. Pepi, D. Burrini, G. Kniewald, D. Scali, and E. Lanciotti. 1996. Dissolution of barium from barite in sewage sludges and cultures of *Desulfovibrio desulfuricans*. Appl. Environ. Microbiol. 62:2398-2404.

Beller, H.R., D. Grbic-Galic, and M. Reinhard. 1992. Microbial degradation of toluene under sulfate-reducing conditions and the influence of iron on the process. Appl. Environ. Microbiol. 50:81-90.

Beller, H.R., and A.M. Spormann. 1997. Benzylsuccinate formation as a means of anaerobic toluene activation by sulfate-reducing strain PRTOL1. Appl. Environ. Microbiol. 63:3729-3731.

Bolze, C.E., P.G. Malone, and M.J. Smith. 1974. Microbial mobilization of barite. Chem. Geol. 13:141-143.

Canty, M. 1998. Overview of the sulfate-reducing bacteria demonstration project under the Mine Waste Technology Program. Min. Pro. Ext. Met. Rev. 19:61-80.

Coates, J.D., R.T. Anderson, and D.R. Lovley. 1996. Anaerobic hydrocarbon degradation in petroleum-contaminated harbor sediments under sulfate-reducing conditions and artificially iron-reducing conditions. Environ. Sci. Technol. 30:2784-2789.

Coates, J.D., J. Woodward, J. Allen, P. Philp, and D.R. Lovley. 1997. Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. Appl. Environ. Microbiol. 63:3589-3593.

Dvorak, D.H., R.S. Hedin, H.M. Edenborn, and P.E. McIntire. 1992. Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot scale reactors. Biotechnol. Bioeng. 40:609-616.

Elshahed, M.S., and M.J. McInerney. 2001. Is interspecies hydrogen transfer needed for toluene degradation under sulfate-reducing conditions? FEMS Microbiol. Ecol. 35:163-169.

Greene, E.A., C. Hubert, M. Nemati, G.E. Jenneman, and G. Voordouw. 2003. Nitrite reductase acitivity of sulfate-reducing bacteria prevents their inhibition by nitrate-reducing, sulfide-oxidizing bacteria. Environ. Microbiol. 5:607-617.

Haveman, S.A., V. Brunelle, J.K. Voordouw, G. Voordouw, J.F. Heidelberg, and R. Rabus. 2003. Gene expression analysis of energy metabolism mutants of *Desulfovibrio vulgaris* Hildenburough indicates an important role for alcohol dehydrogenase. J. Bacteriol. 185:4345-4353.

Haveman, S.A., E.A. Greene, C.P. Stilwell, J.K. Voordouw, and G. Voordouw. 2004. Physiological and gene expression analysis of inhibition of *Desulfovibrio vulgaris* Hildenborough by nitrite. J. Bacteriol. 186:7944-7950.

Hutchins, S.R., G.W. Sewell, D.A. Kovacs, and G.A. Smith. 1991. Biodegradation of aromatic hydrocarbons by aquifer microorganisms under denitrifying conditions. Environ. Sci. Technol. 25:68-76.

Jenneman, G.E., M.J. McInerney, and R.M. Knapp. 1986a. Appl. Environ. Microbiol. 51:1205-1211.

Jenneman,G.E., A.D. Montgomery, and M.J. McInerney. 1986b. Appl. Environ. Microbiol. 51:776-780.

Johnson, D.B. and K.B. Hallberg. 2005. Acid mine drainage remediation options: a review. Sci. Total Environ. 338:3-14.

Koster, I.W., A. Rinzema, A.L. de Vegt, and G. Lettinga. 1986. Sulphide inhibition of the methanogenic activity of granular sludge at different pH levels. Water Research. 12:1561-1567.

Londry, K.L., and J.M. Suflita. 1999. Use of nitrate to control sulfide generation by sulfate-reducing bacteria associated with oily waste. Journal of Industrial Microbiology and Biotechnology. 22:582-589.

Lovley, D.R., J.D. Coates, J.C. Woodward, and E.J.P. Phillips. 1995. Benzene oxidation coupled with sulfate reduction. Appl. Environ. Microbiol. 61:953-958.

McCartney, D.M., and J.A. Oleskiewicz. 1991. Sulphide inhibition of anaerobic degradation of lactate and acetate. Water Research. 25:203-209.

McCready, R.G.L. and H.R. Krouse. 1980. Sulfur isotope fractionation by *Desulfovibrio vulgaris* during metabolism of $BaSO_4$. Geomicrobiol. J. 2:55-62.

Noh, S.L., J.M. Choi, Y.J. An, S.S. Park, and K.S. Cho. 2003. Anaerobic biodegradation of toluene coupled to sulfate reduction in oil-contaminated soils: optimum environmental conditions for field applications. Journal of Environmental Science and Health: Part A—Toxic/Hazardous Substances and Environmental Engineering. A38:1087-1097.

O'Flaherty, V., T. Mahoney, R. O'Kennedy, and E. Colleran. 1998. Effect of pH on growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic, and sulphate-reducing bacteria. Process Biochemistry. 33(5):555-569.

Okabe, S., P.H. Nielson, and W.G. Characklis. 1992. Factors affecting microbial sulphate reduction by *Desulfovibrio desulfuricans* in continuous culture: limiting nutrients and sulfide concentrations. Biotechnology and Bioengineering. 40:725-734.

Oleskiewicz, J.A., T. Marstaller, and D.M. McCartney. 1989. Effects of pH on sulphide toxicity to anaerobic processes. Environmental Technology Letters. 10:815-822.

Oremland, R.S., and D.G. Capone. 1988. Use of "specific" inhibitors in biogeochemistry and microbial ecology. Adv. Microb. Ecol. 10:285-383.

Rabus, R., and F. Widdel. 1995. Anaerobic degradation of ethylbenzene and other aromatic hydrocarbons by new denitrifying bacteria. Arch. Microbiol. 163:96-103.

Reeves, A.L. 1986. Barium, p. 84-94. *In* L. Frieberg, G.F. Nordberg, and V. Vouk (ed.), Handbook on the toxicology of metals, 2nd ed. Elsevier Science Publishers Biomedical Press, New York.

Reis, M.A.M, J.S. Almeida, P.C. Lemos, and M.J.T. Carrondo. 1992. Effect of hydrogen sulphide on the growth of sulphate reducing bacteria. Biotechnology and Bioengineering. 40:593-600.

Rueter, P., R. Rabus, H. Wilkes, F. Aeckersberg, F.A. Rainey, H.W. Jannasch, and F. Widdel. 1994. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. Nature. 372:455-458.

Roychoudury, A.N., P. van Cappellen, J.E. Kostka, and E. Viollier. 2003. Kinetics of microbially mediated reactions: dissimilatory sulfate reduction in saltmarsh sediments (Sapelo Island, Georgia, USA). Estuarine Coastal and Shelf Science. 56:1001-1010.

Schmitt, R., H.R. Langguth, W. Puttmann, H.P. Rohns, P. Eckert, and J. Schubert. 1996. Biodegradation of aromatic hydrocarbons under anoxic conditions in a shallow sand and gravel aquifer of the Lower Rhine Valley, Germany. Org. Geochem. 25:41-50.

Tabak, H.H., R. Scharp, J. Burckle, F.K. Kawahara, and R. Govind. 2003. Advances in biotreatment of acid mine drainage and biorecovery of metals: 1. Metal precipitation for recovery and recycle. Biodegradation. 14:423-436.