

Anaerobic Bio-Oxidation of MTBE and 1,2-DCA

David B. Vance (ARCADIS G&M Midland, Texas), Mark Lupo (ARCADIS G&M Houston, Texas), Neal McHugh (Kinder Morgan Liquid Terminals LLC., Carteret, New Jersey)

ABSTRACT

Many hydrocarbons are biodegradable by oxidation. This includes petroleum hydrocarbons, di- and mono-substituted halogenated hydrocarbons, and other xenobiotic hydrocarbons such as MTBE. In addition to aerobic, anaerobic bio-oxidation processes can be stimulated using alternate electron acceptors such as nitrate, ferric iron, or sulfate.

A field scale anaerobic bio-oxidation system has been designed and installed at a large storage terminal along the Houston Ship Channel. A discrete plume of MTBE and a mixed plume of benzene and 1,2 DCA are the focus of a remediation program. Each of the constituent systems were evaluated in two bench scale design studies in which the bio-oxidation systems tested included: oxygen; nitrate; ferric chloride; ferric sulfate; sodium sulfate; and humate-catalyzed ferric chloride and ferric sulfate. Data describing the heterogeneous response to these stimulation regimes will be presented from the bench scale design study and the on-going field pilot applications.

INTRODUCTION

A broad range of hydrocarbons are biodegradable by bio-oxidation. This includes petroleum hydrocarbons, di- and mono-substituted halogenated hydrocarbons, and other xenobiotic hydrocarbons such as Methyl *tert*-Butyl Ether (MTBE). The dominant bio-oxidation system under near-surface conditions is driven by oxygen. However, other bio-oxidation processes also take place under anaerobic conditions using alternate electron acceptors such as nitrate, ferric iron, or sulfate. These microbiological degradation pathways date to the initial development of microbial life early in the Precambrian. Subsurface organisms that can exploit those pathways are ubiquitous, but exhibit a high degree of spatial variation.

One primary advantage to the use of stimulated anaerobic bio-oxidation for remediation is that the stimulating reagents are water soluble. Perhaps more importantly, in many instances these reagents are only reactive with the impacting hydrocarbons. The properties allow for ready conservative transport without consumption by abiotic or biological side reactions. Aerobic bio-oxidation requires oxygen. Which is particularly labile in the subsurface, readily reacting with minerals in the geologic matrix.

Kinder Morgan and ARCADIS have designed and are implementing a field scale anaerobic bio-oxidation system at a large liquid storage terminal along the Houston Ship Channel for a discrete plume of MTBE. Extensive galleries of buried screened pipes have been installed to deliver treatment fluids in-situ for treatment of the MTBE plume. A mixed plume of benzene and 1,2-dichloroethane (DCA) is also present at another location.

This paper reviews anaerobic bio-oxidation processes and presents the results from two bench scale design studies: one evaluating the degradation of MTBE and its daughter product *tert*-butyl alcohol (TBA); and the second the degradation of a mixture of DCA and benzene. The bio-oxidation systems evaluated in a benchscale study included oxygen, nitrate, ferric chloride (for ferric iron alone), ferric sulfate (with both constituents potential electron acceptors), potassium sulfate (for sulfate alone), and humate-catalyzed ferric chloride and ferric sulfate. The MTBE and benzene were rapidly degraded by oxygen. MTBE is degraded by humate-catalyzed ferric iron almost as rapidly as with oxygen, however TBA the MTBE daughter product was not degraded. Ferric chloride alone degraded MTBE and TBA completely at a rate about 20% that of oxygen. Ferric sulfate, and sulfate also slowly degraded MTBE. The nitrate was completely ineffective. In the case of benzene and DCA, the humate-catalyzed ferric chloride is as effective as oxygen for benzene, and also degraded the DCA. The oxygen did not stimulate DCA degradation. All of the anaerobic systems did degrade the 1,2-DCA within 190 days, with the humate-catalyzed ferric chloride the most rapid of the set.

Review of Bio-Oxidation Processes

The Case for the Stimulation of Anaerobic Bio-Oxidation Made by Natural Attenuation

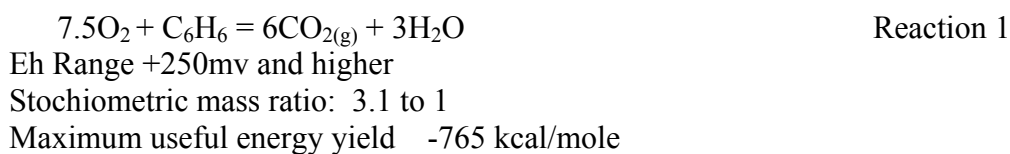
Numerous studies have been performed for almost a decade to evaluate and quantify the effects of both aerobic and anaerobic biodegradation of organic compounds at a variety of sites under natural groundwater flow conditions (1,2,3 and 4).

Aerobic, intrinsic remediation requires that adequate oxygen be available for the metabolic processes to occur. However, since the kinetics of oxygen utilization is essentially instantaneous compared to the relatively slow movement of groundwater, oxygen usually cannot be continuously supplied under natural flow conditions and most often becomes depleted in an area of hydrocarbon-impacted groundwater. A hydrocarbon-impacted area typically then reverts to anaerobic processes. Anaerobic metabolism is evidenced by use of alternative redox couples such as nitrate reduction, ferric iron reduction, sulfate reduction, and methanogenesis if corresponding reducing conditions (i.e., redox potential) are suitable. Since anaerobic processes occur at kinetic rates slower than the kinetic rate of oxygen utilization, these alternate electron acceptors can sometimes be continuously supplied by migrating groundwater.

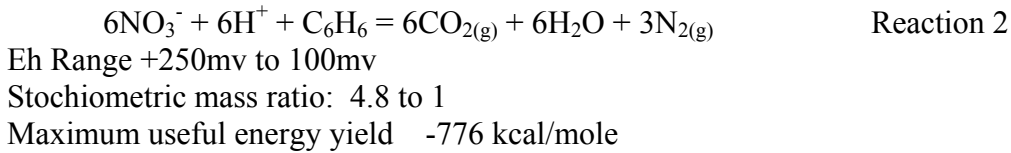
Oxidation is the dominant intrinsic degradation processes for petroleum hydrocarbons, whether under aerobic or anaerobic conditions. Oxidation and reduction reactions fundamentally involve the transfer of electrons. A substance that is oxidized loses electrons, and one that is reduced gains them. An oxidizing agent is a substance that readily accepts electrons causing oxidation of the substance that is the electron donor and is oxidized. The most familiar oxidizing agent (electron acceptor) is oxygen, however, any process that removes electrons from other substances is causing oxidation.

Using benzene as an example compound the following reaction pathways illustrate the: bio-oxidation reactions; products created; the Oxidization Reduction Potential (ORP) conditions under which they occur; the stoichiometry of the reactions; and the potential energy yield. Note that each of these bio-oxidation reactions takes place at increasingly lower redox conditions and in each case carbon dioxide is produced as one of the primary end products.

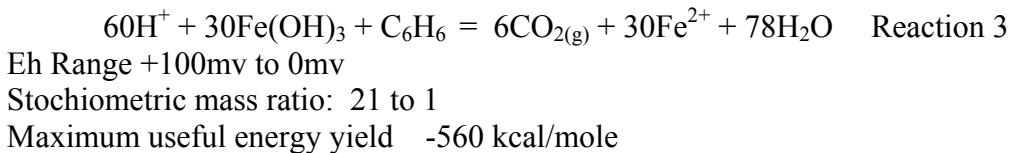
Aerobic Respiration



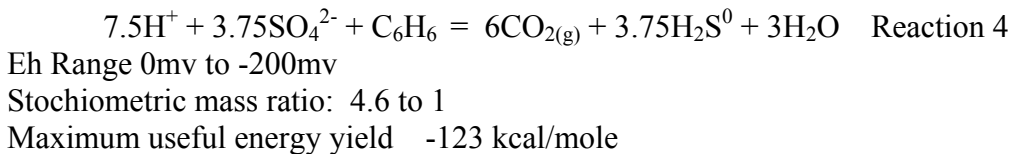
Denitrification



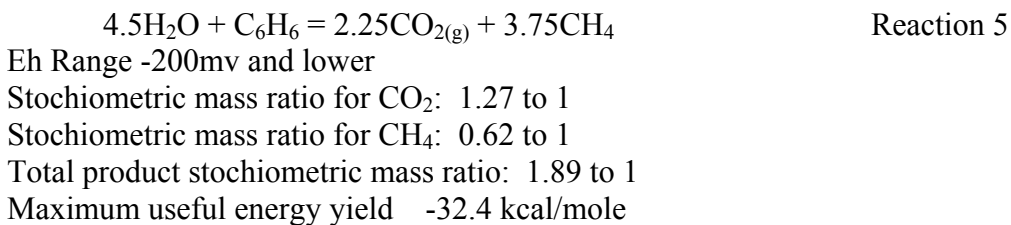
Iron Reduction



Sulfate Reduction



Methanogenesis



It is important to consider the energy yield of the reactions. The greater the energy yield the more productive is the process to the utilizing bacteria. Aerobic degradation and denitrification are almost equally energetic. The ferric iron reduction system is slightly less energetic and there is a significant reduction in energy yield associated with sulfate reduction. Lowest of all is the energy yielded from methanogenesis (over 17 times less than the ferric iron system). Energy yield is important in that it ultimately impacts the kinetics of the overall biogeochemical system. More available energy generally means more rapid growth and higher levels of biological activity.

However even though the anaerobic bio-oxidation systems yield slightly to significantly less energy, they still offer broad potential for biodegradation to take place under a wide range of conditions and also offer specific degradation pathways exploiting bacterial enzymatic systems that are particularly suited for the degradation of the types of compounds that prove to be resistant to aerobic degradation (such as polynuclear aromatic hydrocarbons).

In addition, ferric iron and to a more limited extent sulfate may be part of the mineral matrix and are available for direct reaction in an impacted zone without reliance on advective groundwater transport. In many such cases anaerobic processes that are directly responsible for the major portion of biodegradation of petroleum compounds in groundwater, also contribute to the degradation of chlorinated and other xenobiotic hydrocarbons through what are generally more complex processes. Benzene has been reported to degrade under ferric iron reducing conditions (5, 6, and 7). After nitrate and bio-available iron have been reduced, sulfate reduction may occur, resulting in the production of sulfide. Benzene also degrades under sulfate reducing conditions (8 and 9).

After DO has been depleted, available nitrate may be used as an alternate electron acceptor for anaerobic biodegradation. This process is known as denitrification. At the Kinder Morgan facility, denitrification is taking place, but it appears to take the step to nitrite and then stops, nitrite accumulates. In some instances reported in the literature, when nitrate reduction has been documented to be associated with the degradation of benzene, the nitrogen reduction chemistry slows at the production of nitrite, with nitrite typically accumulating before ultimate conversion to nitrogen gas (10). With the presence of benzene this could be a reason for trace nitrite accumulation observed in groundwater at Kinder Morgan. From the perspective of the energy yield iron and sulfate reduction are viable biochemical processes. However, nitrate reduction of benzene does not directly supply sufficient energy to support growth (11). There are many more sites in which the degradation of benzene by nitrate is reported to not occur (12, 13 and 14) than sites where it does take place. This is one example of the spatial variability of anaerobic bio-oxidation bacteria.

Some Specific Features of the Iron Bio-Oxidation System

Some background information concerning the biogeochemistry of iron (and to a lesser extent sulfur) in soil and groundwater is of value to aid in the understanding of ferric iron driven anaerobic bio-oxidation. Iron is the one of the most biologically labile (reactive and available) substance in the environment of biological systems (iron is a key factor in blood and photosynthesis for example). Depending on pH, oxidation state, or the presence of complexing agents, iron can be present in groundwater as a soluble species or a colloid. Iron in near surface environments is almost always in the ferric oxidation state, and as such relatively immobile in groundwater systems. Impacting hydrocarbons stimulate iron-reducing bacteria, which can convert some portion of the bio-available iron in the geologic matrix to soluble ferrous iron. The ferrous iron can in turn be rapidly re-oxidized back insoluble ferric iron. However, iron that goes through the solubilization cycle from ferric, to ferrous, then back to ferric; is often colloidal in form and still mobile in the groundwater system. Elevated concentrations of total iron are therefore indicative of biological activity, with the ratios of ferrous, ferric, and total iron diagnostic of immediate geochemical processes and conditions in a monitor well or the pore space of the adjacent formation.

The re-oxidation of the ferrous iron daughter product of ferric iron bio-oxidation can be accomplished using oxygen or nitrate (15). Nitrate is particularly promising since it is a soluble reagent resistant to many side reactions in a geologic matrix, unlike oxygen. The nitrate iron oxidation process has been recognized only in the last decade by geomicrobiologist as being responsible for the formation of the massive banded iron formations in the Precambrian. Regeneration may be a key feature of a remediation system using ferric iron anaerobic bio-oxidation; the iron can be reasonably regenerated into a bioactive form in situ. In contrast oxygen and nitrate are irreversibly consumed when used as electron acceptors during bio-oxidation processes.

If iron reducing anaerobic bio-oxidation is to be stimulated by the addition of ferric iron salts to soil or groundwater, it may be of value to use chelating agents as an aid for soluble transport. Conventional chelators such as citrate or gluconate can be used. However, soluble humic or fulvic materials also offer powerful chelating capacity in a form that is more indigenous to natural biogeochemical systems in nature. Two advantages present themselves: first, these soluble humates are highly mineralized and are refractory to further biodegradation; and secondly, the iron chelating capacity is extremely strong, yet the iron in these complexes is easily available to biological processes.

Another even more important property is imparted to a ferric iron anaerobic bio-oxidation system by the presence of soluble humates. The humates actually help to catalyze the anaerobic bio-oxidation reactions. This catalytic effect not only speeds up the degradation reactions, but also aids in the completion of the degradation process to a more complete end point. This catalytic phenomenon is well demonstrated in the bench scale design work that ARCADIS has done with these systems to date. The effect is equally applicable to MTBE, Benzene, and DCA and is extensively reported in the literature (16, 5, and 17).

Sulfur is another chemical that is extremely bioactive. The dominant form in nature is sulfate, followed by hydrogen sulfide. Sulfate may be present as a dissolved anion in groundwater, it can also be present in the mineralogy of the geologic matrix of the water-bearing zone. When sulfate alone is used to support anaerobic bio-oxidation, the end product is hydrogen sulfide, a potentially toxic gas. In a native groundwater system there is usually a source of bio-available iron so that the sulfur reactions will terminate in the formation of iron pyrite, a crystalline solid with relatively innocuous characteristics.

The Evaluation of Stimulated Anaerobic Bio-Oxidation Along the Houston Ship Channel

Field work has been performed nationwide by the EPA and ARCADIS G&M using nitrate salts to stimulate the biodegradation of BTEX. Ferric iron and sulfate driven anaerobic oxidation of petroleum hydrocarbons has also been well demonstrated

in the peer reviewed literature. However, the focus has been on iron and sulfate in naturally occurring forms and concentrations.

Initial Evaluation of Soils

During the initial evaluation of the site conditions associated with two large above ground tanks vadose zone soil samples were collected and evaluated for evidence of natural attenuation processes that may be active towards Methyl *tert*-Butyl Ether (MTBE). The results of that evaluation found Eh conditions indicative of anaerobic activity (as low as -213 mV) and ferrous iron concentrations as high as 26 mg/L in leachate test solutions. The MTBE concentration in the soil sample was 13.7 mg/kg and the concentration of TBA, the first degradation daughter product of MTBE was 61.5 mg/kg. In aggregate, this data was strongly suggestive that natural anaerobic bio-oxidation of MTBE by indigenous ferric iron reducing bacteria was taking place in the MTBE impacted soils beneath the tanks. ARCADIS further recommended that a more detail bench scale evaluation of those processes be performed.

Bench Scale Anaerobic Bio-Oxidation Design Studies

Two bench scale studies have been performed to evaluate the efficacy of the application of anaerobic bio-oxidation at the site. Soils from two different locations at the site that were representative of the impact with MTBE, or benzene and DCA were collected for each evaluation. One study evaluated the degradation of MTBE and its degradation daughter product TBA. The second was focused on the degradation of Benzene and DCA. The rationale of the design work was to determine which anaerobic bio-oxidation pathway presented the optimum choice for stimulation at the sites and the degradation kinetics of the process.

Aside from the different soils and the different contaminants, each study had nine microcosms that were set up and operated under the following conditions:

1. Nitrate Reducing
 - Using potassium nitrate as an electron acceptor, at an initial nitrate concentration of 400 mg/L
2. Iron Reducing
 - Using ferric chloride to provide the electron acceptor, at an initial ferric iron concentration of 200 mg/L.
3. Iron and Sulfate Reducing
 - Using ferric sulfate to simultaneously provide both electron acceptors, at initial concentrations of 200 mg/L ferric iron and approximately 170 mg/L sulfate.
4. Iron Reducing with a Soluble Humate Catalyst
 - Using ferric chloride with soluble humate, at an initial ferric iron concentration of 200 mg/L and 12 mg/L of soluble humate
5. Iron and Sulfate Reducing with a Soluble Humate Catalyst

- Using ferric sulfate to provide both electron acceptors with the presence of soluble humate to catalyze iron reduction reactions, at initial concentrations of 200 mg/L ferric iron, 170 mg/L sulfate, and 12 mg/L soluble humate.
- 6. Sulfate Reducing
 - Using potassium sulfate to provide the electron acceptor, at an initial concentration of 100 mg/L sulfate.
- 7. Aerobic
 - Using air to provide oxygen as the primary electron acceptor.
- 8. Live Control
 - No amendments, but no sterilization procedures either, activity in this system may be due to methanogenesis.

The respective microcosms were spiked with MTBE and with benzene, and DCA at the following concentrations:

- MTBE 12 ppm
- Benzene 8 ppm
- DCA 2 ppm

Results for MTBE

With regards to the degradation of MTBE complete degradation was observed in all of the systems except No. 1 Nitrate Reduction. The degradation of TBA exhibited more complex behavior. Complete degradation of TBA took place in the following microcosms:

- No. 2 Ferric Chloride
- No. 3 Ferric Sulfate
- No. 8 Live Control

Incomplete (over the life of the study at least) TBA degradation was observed in the following microcosms:

- No. 1 Potassium Nitrate
- No. 4 Ferric Chloride with Humate
- No. 5 Ferric Sulfate with Humate
- No. 6 Potassium Sulfate

The degradation rates of MTBE and TBA in each of the systems were variable. For MTBE the time (within the resolution of the respective sampling events) of degradation to a non-detect concentration was as follows:

- No. 2 Ferric Chloride - 130 Days
- No. 3 Ferric Sulfate - 96 Days
- No. 4 Ferric Chloride with Humate – 96 Days

- No. 5 Ferric Sulfate with Humate – 130 Days
- No. 6 Potassium Sulfate – 130 Days
- No. 7 Aerobic – 96 Days

For the TBA the time for degradation to a non-detect concentration was:

- No. 2 Ferric Chloride – 189 Days
- No. 3 Ferric Sulfate – 189 Days
- No. 7 Aerobic – 96 Days
- No. 8 Live Control – 189 Days

Given the goals of complete degradation of the MTBE and TBA following systems were viable options for field application:

- No. 2 Ferric Chloride
- No. 3 Ferric Sulfate
- No. 7 Aerobic

Nitrate reduction appeared to not be active at all for either MTBE or TBA. The degradation of MTBE and TBA by nitrate reducing bacteria has been reported in the literature (18), but it appears to be a relatively rare occurrence (another example of the spatial variability of anaerobic bio-oxidizing bacteria). The appropriate native nitrate reducing bacterial consortia do not appear to be present in the soils and groundwater underlying the Kinder Morgan facility.

Sulfate alone does not appear to stimulate degradation with great efficiency. The effect of humate is bi-modal. It appears to significantly accentuate the kinetics of the degradation of MTBE, but then inhibit the degradation of TBA. Since the live control did show MTBE and TBA degradation, it is possible that the presence of nitrate, and to a lesser degree sulfate without ferric iron, also served in an inhibitory fashion as well.

Results for Benzene and 1,2-DCA

Complete degradation of the benzene was seen in 124 days in No. 7 the Aerobic system and No. 4 the Ferric Chloride with soluble humate. Reduction of benzene concentrations in the remaining microcosms after 124 days were as follows:

- No. 1 Potassium Nitrate 0%
- No. 2 Ferric Chloride 23%
- No. 3 Ferric Sulfate 28%
- No. 5 Ferric Sulfate with Humate 25%
- No. 6 Potassium Sulfate 51%
- No. 8 Live Control 38%

After 124 days the greatest reduction of DCA concentration took place in No. 4 Ferric Chloride with soluble humate. The specific percent reductions in DCA concentrations after 124 days were:

- No. 1 Potassium Nitrate 0%
- No. 2 Ferric Chloride 25%
- No. 3 Ferric Sulfate 23%
- No. 4 Ferric Chloride with Humate 60%
- No. 5 Ferric Sulfate with Humate 13%
- No. 6 Potassium Sulfate 20%
- No. 7 Aerobic 37%
- No. 8 Live Control 9%

By the end of 190 days all of the systems except aerobic had demonstrated complete degradation of the 1,2-DCA.

Among the volatile organic carbons monitored during the benzene/DCA study acetone was detected in every microcosm except No. 7 Aerobic. The typical profile was acetone production at concentrations ranging from 30 to 90 ppb during the 30 to 60 day time frame. Concentrations then declined to near zero. The exception to this profile was the live control where acetone concentrations continued to climb out to 124 days to a concentration of over 2 ppm. This is attributed to methanogenic degradation of the benzene.

Given the goals of complete degradation of the benzene and DCA it would appear that the optimum system for field application is No. 4 Ferric Chloride catalyzed with soluble humate.

Initial Results of Field Pilot Testing of MTBE Degradation

A field scale pilot test in the MTBE release area has begun. The reagent for this application is ferric sulfate with the addition of a chelating reagent. Results are preliminary, but iron concentrations in MTBE impacted soil have been increased from 1 mg/kg to the range of 16 to 22 grams per kilogram range. There is a high degree of heterogeneity in what is an MTBE impacted source area with MTBE concentrations as high as 2 to 45 grams per kilogram, however, the initial ratio of MTBE to TBA has been shifted from 10 to 1 to 3 to 1, indicative of the production of the first stage TBA degradation product.

Conclusion

A broad range of hydrocarbons are biodegradable by oxidation. This includes petroleum hydrocarbons, di- and mono-substituted halogenated hydrocarbons, and other xenobiotic hydrocarbons such as MTBE. The dominant oxidation system under near-surface conditions is based on oxygen. However, other bio-oxidation processes also take place under anaerobic conditions using alternate electron acceptors such as nitrate, ferric iron, or sulfate. These biochemical degradation pathways date to the initial development

of microbial life early in the Precambrian making them ubiquitous, but with a high degree of spatial variation. The primary advantage to the use of stimulated anaerobic bio-oxidation for remediation is that the stimulating reagents are water soluble. Also, in many instances, these reagents are only reactive in the presence of impacting hydrocarbons, allowing for ready transport without consumption by abiotic or biological side reactions. Which is commonly not the case for oxygen.

Kinder Morgan and ARCADIS have designed and are implementing field scale anaerobic bio-oxidation systems at a large liquid storage terminal along the Houston Ship Channel. As part of the design process a site wide survey was made of the biochemical conditions and anaerobic bio-oxidation pathways available. A discrete plume of MTBE and a mixed plume of benzene and 1,2 DCA are the focus of a proactive remediation program. Each of the constituent systems were evaluated in two bench scale design studies in which the bio-oxidation systems tested included: oxygen; nitrate; ferric chloride (for ferric iron alone); ferric sulfate (with both components potential electron acceptors); sodium sulfate (for sulfate alone); and humate-catalyzed ferric chloride and ferric sulfate.

The MTBE and benzene were rapidly degraded by oxygen. MTBE was degraded by humate-catalyzed ferric iron almost as rapidly as with oxygen, however the MTBE daughter product tert-butyl alcohol (TBA) was not degraded with humate-catalyzed ferric iron. Ferric chloride alone degraded MTBE and TBA completely at a rate about 20% that of oxygen. Ferric sulfate, and sulfate also slowly degraded MTBE. The nitrate was completely ineffective. In the case of benzene and 1,2-DCA, the oxygen alone was effective for the degradation of benzene. Oxygen did not stimulate the degradation of 1,2-DCA. All of the anaerobic systems did degrade the 1,2-DCA within 190 days, with the humate-catalyzed ferric chloride the most rapid of the set.

Galleries of horizontal screened pipes have been installed in trenches beneath newly installed storage tanks to deliver treatment fluids in-situ for remediation of the MTBE plume and field pilot scale applications of chelated ferric iron solutions are currently taking place.

REFERENCES CITED

1. Aronson, D. and Howard, P.H., "Anaerobic Biodegradation of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies", Environmental Science Center Report, Syracuse Research Corp., N. Syracuse, NY (1997).
2. Wiedemeier, T.H., Swanson, M.A., Moutoux, D.T., Gordon, E.K., Wilson, J.T., Wilson, B.H., Kampbell, D.H., Hass, P.E., Miller, R.N., Hansen, J.E., and Chapell, F.H., "Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water", USEPA, EPA/600/R-98/128 (1998).
3. Saleem, Z.A., "Anaerobic Biodegradation Rates of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies" (Draft), U.S.EPA, Office of Solid Waste, Wash. DC, Contract No. 68-W7-0035 (1999).
4. Azadpour-Keeley, A., Russel, H.H., and Sewell, G.W., "Microbial Processes Affecting Monitored Natural Attenuation of Contaminants in the Subsurface", USEPA, EPA/540/S-99/001, 18 pp., Sept. 1999.
5. Lovley, D.R., Woodward, J.C., and Chapelle, F.H., "Rapid Anaerobic Benzene Oxidation with a Variety of Chelated Fe(III) Forms", *Appl. Environ. Microbiol.*, Vol. 62, No 1, pp. 288-291 (1996).
6. Anderson, R.T., Rooney-Varga, J.N., Gaw, C.V., and Lovley, D.R., "Anaerobic Benzene Oxidation in the Fe(III) Reduction Zone of Petroleum-Contaminated Aquifers", *Environ. Sci. Technol.*, Vol. 32, No. 9, pp.1222-1229 (1998).
7. Rooney-Varga, J.N., Anderson, R.T., Fraga, J.L., Ringeleberg, D., and Lovley, D.R., 1999. "Microbial Communities Associated with Anaerobic Benzene Degradation in a Petroleum-Contaminated Aquifer", *Appl. Environ. Microbiol.*, Vol. 65, No 7, pp. 3056-3063 (1999).
8. Weiner, J.M. and Lovley, D.R., "Anaerobic Benzene Degradation in Petroleum-Contaminated Aquifer Sediments after Inoculation with a Benzene-Oxidizing Enrichment", *Appl. Environ. Microbiol.*, Vol. 64, No 2, pp. 775-778 (1998).
9. Anderson, R.T. and Lovley, D.R., "Anaerobic Bioremediation of Benzene Under Sulfate-Reducing Conditions in a Petroleum-Contaminated Aquifer", *Environ. Sci. Technol.*, Vol. 34, No. 11, pp.2261-2266 (2000).
10. Burland, S.M. and Edwards, E.A., "Anaerobic Benzene Biodegradation Linked to Nitrate Reduction", *Appl. Environ. Microbiol.*, Vol. 65, No 7, pp. 3056-3063 (1999).

11. Harwood, C.S. and Gibson, J., "Shedding Light on Anaerobic Benzene Ring Degradation: a Process Unique to Prokaryotes?", *J. Bact.*, Vol. 179, No. 2, pp. 301-309, Jan. 1997.
12. Hutchins, S.R., Sewell, G.W., Kovacs, D.A. and Smith, G.A., "Biodegradation of Aromatic Hydrocarbons by Aquifer Microorganisms Under Denitrifying Conditions", *Environ. Sci. Technol.*, Vol. 25, No. 1, pp.68-76 (1991).
13. Kazumi, J., Daldwell, M.E., Suflita, J.M., Lovley, D.R., and Young, L.Y., "Anaerobic Degradation of Benzene in Diverse Anoxic Environments", *Environ. Sci. Technol.*, Vol. 31, No. 3, pp.813-818 (1997).
14. Evans, P.J., Mang, D.T., Young, L.Y., 1991. "Degradation of Toluene and *m*-Xylene and Transformations of *o*-Xylene by Denitrifying Enrichment Cultures", *Appl. Environ. Microbiol.*, Vol. 57, No. 2, pp. 450-454 (1991).
15. Straub, K.L., Benz, M.S., Bernhard, and Widdel, F., "Anaerobic, Nitrate-Dependent Microbial Oxidation of Ferrous Iron", *Appl. Environ. Microbiol.*, Vol 62, No. 4, pp. 1458-1460 (1996).
16. Finneran, K.T. and Lovley, D.R., "Anaerobic Degradation of Methyl *tert*-Butyl Ether (MTBE) and *tert*-Butyl Alcohol (TBA)", *Environ. Sci. Technol.*, Vol 35, No. 9, pp. 1785-1790 (2001).
17. Lovley, D.R. and Blunt-Harris, E., "Role of Humic-Bound Iron as an Electron Transfer Agent in Dissimilatory Fe(III) Reduction", *Appl. Environ. Microbiol.*, Vol. 65, No. 9, pp 4252-4254, Sept. (1999).
18. Bradley, P.M., Chapelle, F.H., and Landmeyer, J.E., "Effect of Redox Conditions on MTBE Biodegradation in Surface Water Sediments", *Environ. Sci. Technol.*, Vol. 35, No. 23, pp. 4643-4647 (2001).