

## **Annual Report (2006)**

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**Title:** Utilization of the carbon and hydrogen isotopic composition of individual compounds in refined hydrocarbon products to monitor their fate in the environment

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**Project Period:** 9-1-04 to 8-31-06 (project extended to 8-31-07)

**Project Amount:**

**Research Category:** Petroleum Environmental Technology - Natural attenuation

### **Introduction**

Progress of the last year of the project will be discussed using the framework of objectives defined in the proposal.

1. Verification of the hypothesis that degradation is the primary factor leading to isotopic fractionation in the field.
2. Verification of the hypothesis that stable isotopes provide both positive and negative evidence of biodegradation.
3. A database of isotope fractionation by available microbial cultures, primarily of MTBE degraders (and as applicable – TBA and other gasoline compounds).
4. A database of stable isotopic composition (carbon and hydrogen) of MTBE, TBA and other gasoline compounds (reference for data interpretation).
5. A set of case studies of contaminated sites, primarily on MTBE and TBA, but ultimately on other gasoline-range contaminants.
6. Development of a commercially viable method for site characterization based on the stable isotope values and trends observed in the results obtained from this study.

### **Summary**

Activities in year 2006 were primarily focused on analyzing carbon and hydrogen isotope composition of gasoline components (Objective 4) and on the study of isotope fractionation (determination of enrichment factors) in MTBE-degrading anaerobic cultures (Objective 3). A number of field sites, with oxygenate and benzene contaminant plumes has been sampled and evaluated by CSIA. Finally, experiments of abiotic (non-degradative) fractionation have been initiated to determine the potential of isotopic interferences from volatilization-related attenuation processes.

**Objective 1 – Verification of the hypothesis that degradation is the primary factor leading to isotopic fractionation in the field**

As described in our proposal, the two non-degradation factors potentially leading to isotope effects are sorption and volatilization. A recent publication in the field (Carbon Isotope Fractionation of Organic Contaminants Due to Retardation on Humic Substances: Implications for Natural Attenuation Studies in Aquifers; Frank-Dieter Kopinke, Anett Georgi, Michael Voskamp, and Hans H. Richnow, 2005, Environmental Sci. Tech., 39, 6052 - 6062) provides a good base to understand the significance of sorption in isotope fractionation of contaminants. The basic conclusion from their work is that the contribution of sorption to the net isotope effect is negligible.

A lab study on the volatilization-related isotope effects has been initiated in the second year of the project. A conceptual model of volatilization of MTBE involves a cumulative effect of phase equilibria and of diffusive/advective venting of the gas phase. Volatilization-related isotope effects are similarly complex and experiments have been set up to measure both the individual components and the net fractionation. Equilibrium fractionation of carbon and hydrogen isotopes has been measured for simple 2-phase systems: water-air, water-NAPL and NAPL-air. Kinetic fractionation associated with diffusion of MTBE vapor through a porous medium has been also determined. Sediment column experiments on passive volatilization of MTBE from aqueous and NAPL phase have been completed. The latter involve complex isotope effects, resulting from a combination of phase equilibria and vapor diffusion.

### **Objective 2 – Verification of the hypothesis that stable isotopes provide both positive and negative evidence of biodegradation**

In continuation of the early work on the effects of well purging on the obtained CSIA results, an experiment was performed to evaluate the effects of sampling strategy on the outcome of CSIA. While MTBE biodegradation may locally reduce MTBE concentration and cause a corresponding isotopic effect, the water recovered from the radius of a monitoring well can be dominated by the parts of the plume with limited or no biodegradation. CSIA performed on such a sample would be inconclusive. Pre-sampling purging of well volume can magnify this effect. On the other hand, sampling of discrete narrow intervals of a plume, with no purging, should increase the chance of detection of undiluted biodegraded material. A site with independent evidence of in-situ biodegradation potential has been selected based on the following criteria: 1) there has been historical presence of TBA in excess of MTBE in parts of the plume, suggesting a possibility of in-situ MTBE biodegradation; 2) Bio-Sep in-situ microcosms indicated that there is microbiological potential for anaerobic degradation of MTBE (the Bio-Sep experiments were performed by a different research group and were not part of the current project). On the other hand, previous round of CSIA showed isotope values for MTBE within the normal range of undegraded material. Samples from four monitoring wells (selected for high TBA/MTBE concentration ratios) were collected from two discrete depth intervals without well purging and subjected to CSIA. The results were negative/inconclusive. All of the analyzed samples were essentially identical in their isotope ratios. At this site, either the plume heterogeneity was not picked up at the spatial resolution of sampling or the isotope fractionation had not occurred.

### **Objective 3 – A database of isotope fractionation by available microbial cultures**

The microcosms used in biodegradation experiments have been set up under supervision of Dr. Davidova (Dr. Suflita's group at OU Microbiology Department) as described in the previous annual report. Concentrations of MTBE and TBA have been determined using a purge and trap method at the Geochemistry Lab at OU, methane concentrations have been determined through headspace-GC method at the Geochemistry Lab and sulfate have been determined by Dr. Davidova at OU Microbiology Department.

Eight sets of replicate anaerobic microcosms for MTBE degradation were set up using sediment from six contaminated gas station sites in California obtained from BP partner (including three separate experiments set up using three spatially different sediment samples from the same contaminated site). The sites have been previously studied for isotope effects in groundwater and evidence of MTBE biodegradation was detected. MTBE degradation (conversion to TBA) was observed in seven of the experiments in all or at least some of the "live" replicates, while no MTBE-TBA conversion was observed in sterile replicates. MTBE degradation was observed after repeated amendment with fresh substrate, and corresponding near-stoichiometric accumulation of TBA occurred. Degradation of MTBE resulted with strong isotope fractionation, measured by GCIRMS. One of the data sets had to be discarded, because isotope effects caused by biodegradation were obscured by the presence of  $^{13}\text{C}$ -labeled MTBE used in-situ by a different research group. No evidence of activity was found to date in microcosms developed with one of the sediment samples. Carbon isotope composition of MTBE and TBA was measured to obtain the objective value of enrichment factors characteristic of anaerobic MTBE bioconversion. The values of carbon isotope enrichment factor ( $\epsilon$ ) for MTBE degradation calculated for the data sets from all of the active microcosms are virtually identical and cluster around -18. This value implies an even stronger isotope effect for this type of reaction than the values reported earlier. The difference is likely due to different experimental designs. Unlike in the previous studies, all of the microcosms discussed here were kept homogenous (culture bottles set on a shaker or turned over daily) to minimize heterogeneity of the medium and avoid underestimation of isotope effects. A similar range of isotope effect was found in the original sulfate reducing or methanogenic specimens, in the enrichment cultures transferred from one of the original microcosms and in microcosms where the terminal acceptor status have been forced from methanogenic to sulfate reducing (with an addition of sulfate and BES, an inhibitor of methanogenesis). The high consistency of the  $\epsilon$  values between the individual data sets from different sediment samples, for the methanogenic and the sulfate reducing conditions, gives strong support for using the obtained value of  $\epsilon$  in evaluation of MTBE in-situ degradation. This phase of the project can be considered successfully completed. The remaining inactive MTBE microcosms are being monitored on a monthly basis and if activity develops, they may be possibly included in a later report.

In a follow-up experiment, carbon and hydrogen isotope ratios were measured for degradation of MTBE amendment in selected 13 individual microcosms from 6 different sediment sets (including two samples with BES inhibitor). The hydrogen fractionation was identical for all of the studied cultures, with the hydrogen enrichment factor of -25. The 2-dimensional trend from the microcosm experiment is identical to that reported by Kuder et al. (2005) from anaerobic MTBE plumes. The consistent pattern of the 2D trends for different cultures implies a common degradation mechanism and supports the use of 2D CSIA for validation of anaerobic biodegradation of MTBE.

Three sets of anaerobic microcosms with TBA amendment (two methanogenic, one sulfate reducing) were set up to study TBA biodegradation. Additionally, control samples of the MTBE experiments contain ppm-level concentrations of TBA and were also monitored for potential TBA-degrading activity. In one of the two methanogenic experiments, significant methane generation in TBA-amended microcosms has been observed (up to 1% methane in headspace). At present, concentration and isotope composition of TBA do not offer confirm anaerobic degradation.

#### **Objective 4 – A database of stable isotopic composition (carbon and hydrogen) of MTBE, TBA and other gasoline compounds**

A collection of 50 samples of commercial gasolines from Midwestern, East Coast and Southeastern states has been obtained from Dr. Graham Rankin (Marshall University, WV). Selected specimens of the set were screened by direct injection onto GCIRMS (carbon and hydrogen mode). To obtain carbon and hydrogen isotope values of the oxygenates (MTBE and TBA), aliquots of gasolines have been equilibrated with water and analyzed by purge and trap-GCIRMS method. This pretreatment step was necessary to eliminate low molecular weight hydrocarbons of gasoline causing GC coelution upon GCIRMS analysis and assure accurate determination of  $\delta^{13}\text{C}$  and  $\delta\text{D}$ . In particular, adequate GC separation of TBA from gasoline matrix is impractical in direct injection. Carbon isotope ratios of MTBE ( $\delta^{13}\text{C}$  of -27.9 to -30.3) agree with the values published elsewhere. TBA carbon isotope compositions have been also measured, but the results have been discarded due to subsequent demonstration of an analytical artifact limiting the application of purge and trap method to TBA analysis in the presence of high-concentration MTBE matrix. The TBA isotope ratios will be determined again after the problem is resolved. Hydrogen isotope ratios of MTBE fall between  $\delta\text{D}$  of -80 and -106. Carbon and hydrogen isotope ratios of benzene, toluene, ethylbenzene, xylenes and 1,2,4-trimethylbenzene have been measured for 25 samples of gasolines. Carbon isotope ratios only of additional aromatic compounds (a number of tri- and tetramethylbenzenes and naphthalene) were measured for an additional 25 gasolines (these samples did not contain detectable amounts of MTBE). All samples have been analyzed by purge and trap-GCIRMS after equilibration with water to improve GC separation of target analytes and to increase the precision of isotope ratio determination (the effective concentration of a number of interfering compounds, e.g., of the aliphatic fraction of gasoline, was greatly reduced by this treatment). Carbon isotope ratios of gasoline aromatics (22 different compounds) range from  $\delta^{13}\text{C}$  of -23 to -28. Hydrogen isotope ratios of gasoline aromatics range from  $\delta\text{D}$  of -35 to -100.

#### **Objective 5 – A set of case studies of contaminated sites, primarily on MTBE and TBA, but ultimately on other gasoline-range contaminants**

As a part of this study, CSIA method has been applied to study benzene contamination at three separate locations. Two-dimensional CSIA analysis (carbon and hydrogen) visualized a fractionation pattern consistent with benzene biodegradation. On the other hand, due to relatively low magnitude of isotope fractionation in the case of benzene, carbon CSIA alone seems to be insufficient to confirm in-situ biodegradation, in particular with old or complex contaminant

plumes where it is difficult to determine the accurate value of the initial isotope composition of the contaminant.

A plume of MTBE and TBA (Illinois) has been studied over a period of time (also see Section 2). While at this site CSIA does not provide evidence of anaerobic biodegradation, we have observed changes of isotope ratios (carbon and hydrogen) in an area treated by air sparging. The observed pattern of isotope fractionation is consistent with aerobic biodegradation, suggesting the air sparging process stimulates biodegradation at this location. This part of the project is linked to the experimental work on the effects of volatilization on the isotope ratios of MTBE in the field (see Section 1).

Additionally, a site with TAME contamination was studied. While no evidence of isotope fractionation was detected, the analytical utility of PT-GCIRMS was demonstrated for this compound.

### **Publications/ Presentations:**

1. Stable Isotope Analysis in Remediation of Gasoline Oxygenates and Hydrocarbons. Presented at 13th Annual International Petroleum Environmental Conference, October 17-20, 2006, San Antonio, TX
2. A manuscript related to the scope of this project (“Modern geochemical and molecular tools for monitoring in-situ biodegradation of MTBE and TBA”) is currently in review for publication in Reviews in Environmental Science and Bio/Technology.

### **Future activities:**

The following activities are planned for the next season:

1. It is planned to continue experiments to simulate VOC volatilization in the conditions of porous medium in contact with contaminated groundwater. Experimental simulation of air sparging and soil vapor extraction will be performed.
2. Continuation of the work with the existing microcosms is planned, in particular, monitoring of TBA microcosms for evidence of biodegradation will continue.
3. Finishing the database of isotope ratios of the commercial gasoline samples. TBA will be re-analyzed. Currently the analytical method is optimized to allow analysis of TBA in the presence of high-concentration MTBE matrix.
4. It is planned to continue screening of the incoming field samples for carbon and hydrogen stable isotope effects indicative of monoaromatic compound degradation (benzene in particular). Continuation of work on an MTBE and TBA plume

**Supplemental Keywords:** Water, groundwater, sediments, bioavailability, metabolism, VOC, organics, bioremediation, cleanup, environmental chemistry, analytical, EPA Regions (1 through 10), petroleum industry

**Relevant Web Sites:** None