

Compound Specific Isotope Analysis:
The Science, Technology and
Selected Examples from the Literature
with Application to Fuel Oxygenates
and Chlorinated Solvents

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Introduction

Compound Specific Isotope Analysis (CSIA) is an evolving analytical technique which is used generate isotopic characterization of individual compounds. Incorporation of isotopic characterization in addition to concentration and kinetic data can be used to more definitively characterize processes in groundwater which degrade contaminants of concern (COC's) such as BTEX, MTBE and CVOC's. The isotopic data provided by CSIA can be used to unambiguously determine that biodegradation of COC's is occurring; may be able to identify the process of degradation as aerobic or anaerobic; and in some cases determine the rate and extent of degradation. Further, and perhaps most importantly, when isotopic constraints are integrated into reactive transport models, these may become much more powerful predictive tools for assessing the extent and duration of contaminant plumes, thus decreasing monitoring and remediation costs.

Many processes which affect COC's in groundwater such as dilution, sorption and volatilization have either very small or no isotopic effects, however processes like biotic and abiotic degradation are associated with significant isotopic effects as shown in Figure 1, (Schmidt, et al. 2004, after Meckenstock, et al., 1999) which illustrates the anaerobic degradation of toluene under sulfate reducing conditions. Notice that, as the concentration of toluene remaining in solution decreases, $\delta^{13}\text{C}$, the measure of its isotopic composition, increases.

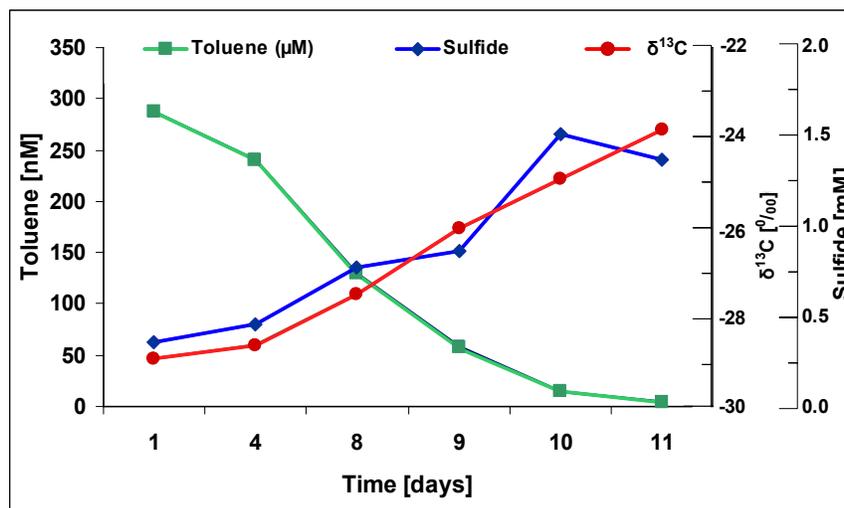


Figure 1

The study of isotopes in groundwater plumes of fuel oxygenates like MTBE, as shown on Figure 2, has provided unequivocal proof of its degradation, revealed the mechanism of its degradation and provided an in-situ measurement of the rate of degradation.

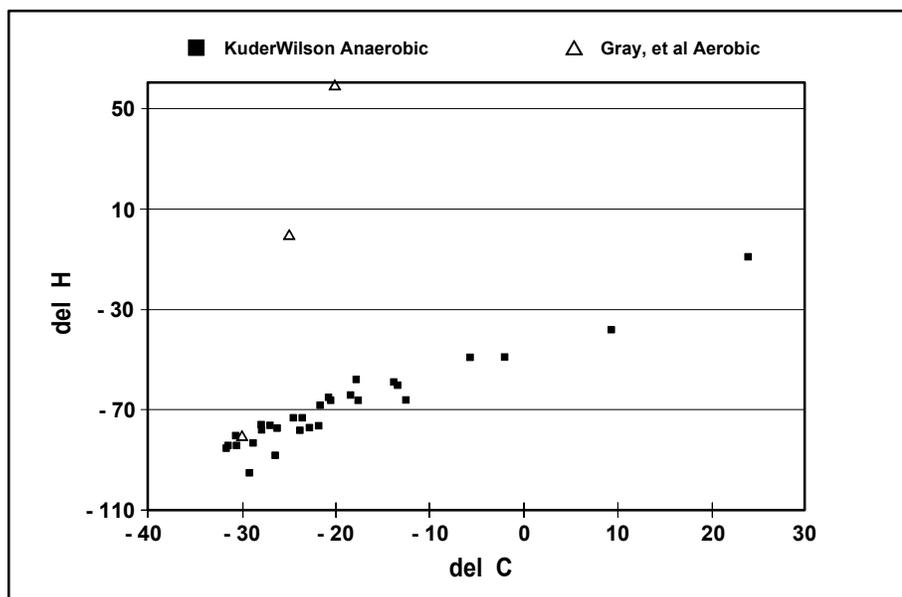


Figure 2

We will define $\delta^{13}\text{C}$ later, but let us just say here that isotopic studies such as are possible with CSIA can be a powerful tool in evaluating the progress of in-situ degradation and their use is likely to increase as the availability and reliability of this data increases.

The Analytical Technology

CSIA is developing at this time as the result of research over the last 30 years. Of great significance is new instrumentation which has recently become commercially available which allows the analysis of carbon and hydrogen isotopic content of compounds of interest in a continuous flow mode at concentrations of interest in environmental remediation.

We are all familiar with the GC/MS analysis of groundwater samples using a standard methodology such as SW846-8260. A typical set up is shown in Figure 3, which shows that a groundwater sample is purged of its volatile organic compounds, which are temporarily trapped, then thermally desorbed onto the analytical column of a modern gas chromatograph. The column separates the

individual compounds of interest (i.e. benzene, toluene, ethyl benzene) such that they exit the column in well defined packets which are then analyzed by a mass spectrometer as molecular ion fragments characteristic of each compound.

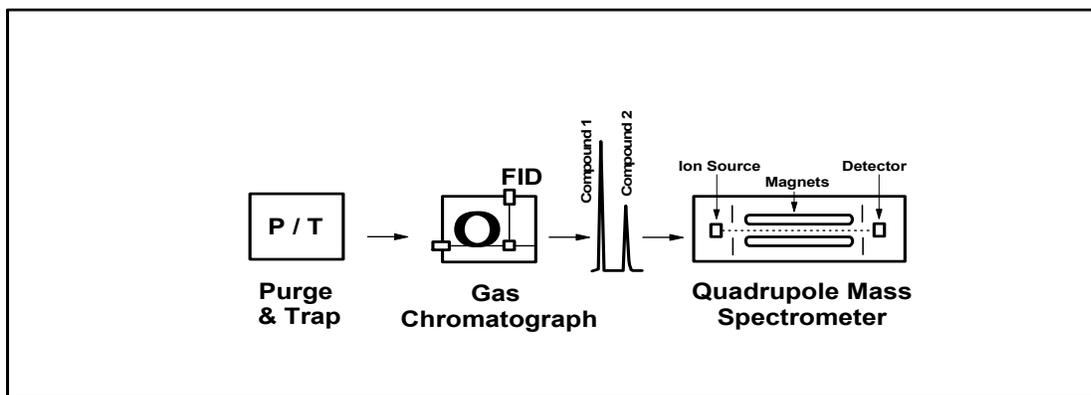


Figure 3

In the most useful application of CSIA for VOC's of interest in environmental remediation, the instrumentation is formally similar to that for SW846-8260. The instrumentation, shown in Figures 4 and 5, is now known as GC/C/IRMS where IRMS stands for Isotope Ratio Mass Spectrometer and "C" stands for a combustion (oxidation) or pyrolysis (reduction) chamber which has been positioned between the GC and the IRMS.

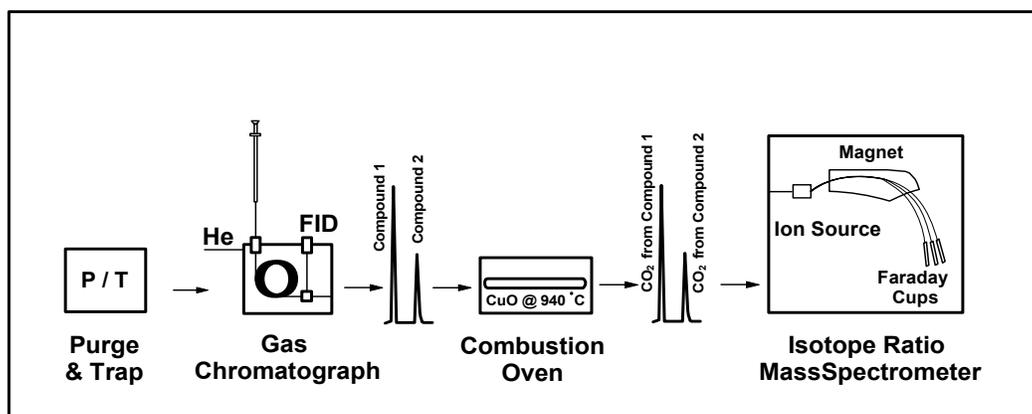


Figure 4

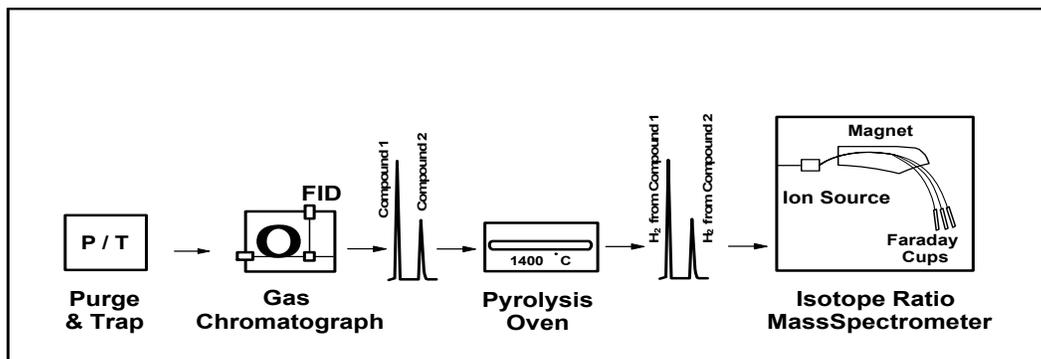


Figure 5

Groundwater samples are purged, trapped and desorbed onto a modern GC analytical column where they are separated into discrete packets before exiting the column. At this point instead of directly entering a mass spectrometer to be broken up into molecular ion fragments for analysis as in SW846-8260, each packet is passed through a chamber where the compound is thermally converted to carbon dioxide when it is desired to investigate the isotopes of carbon, as shown in Figure 4; or reduced to molecular hydrogen when it is desired to investigate the isotopes of hydrogen as shown in Figure 5. Upon exiting the oven, the carbon dioxide or hydrogen enters the IRMS for isotope analysis. Although the mass spectrometer for isotope measurements differs from the one used in SW846-8260, the process is formally similar.

Fundamentals of Isotopes

Now let's review some fundamentals of isotopes and we will focus on the isotopes of carbon and hydrogen as shown on Table 1.

Table 1			
<u>Carbon and Hydrogen Isotope Data</u>			
Stable Isotopes	Abundance of Heavy Isotope (%)	Gas Measured	Measured m/z
^2H and ^1H	0.015	H_2	2, 3
^{13}C and ^{12}C	1.11	CO_2	44, 45, 46

First, we will limit our discussions to stable isotopes, ones which are not subject to radioactive decay. Stable isotopes of other elements including oxygen, nitrogen, sulfur, chlorine, etc can be determined, however we will limit our discussion here to stable isotopes of carbon and hydrogen. There are two stable isotopes of carbon, ^{12}C and ^{13}C , and two stable isotopes of hydrogen, ^1H and ^2H (^2H is sometimes called deuterium and written as D). The lighter isotopes, ^{12}C and ^1H are the most common isotopes of each element. The heavier isotope ^{13}C is only about 1% of the total carbon and ^2H is 0.015% of the total hydrogen. When we determine hydrogen isotopes we analyze ions of molecular hydrogen with m/z (mass to charge ratio) of 2 and 3; and for carbon we analyze ions of carbon dioxide with m/z of 44, 45, and 46 as shown on Table 2.

<u>Table 2</u>		
<u>Measured Species</u>		
$^{16}\text{O} - ^{12}\text{C} - ^{16}\text{O}$	$^{16}\text{O} - ^{13}\text{C} - ^{16}\text{O}$	$^{16}\text{O} - ^{12}\text{C} - ^{18}\text{O}$
44	45	46
	$^1\text{H} - ^1\text{H}$	$^1\text{H} - ^2\text{H}$
	2	3

Therefore we could write the following for the concentrations of the isotopes of carbon and hydrogen:

$$[^{12}\text{C}] = [^{16}\text{O} - ^{12}\text{C} - ^{16}\text{O}] + [^{16}\text{O} - ^{12}\text{C} - ^{18}\text{O}] = M_{44} + M_{46}$$

$$[^{13}\text{C}] = [^{16}\text{O} - ^{13}\text{C} - ^{16}\text{O}] = M_{45}$$

$$[^1\text{H}] = 2 [^1\text{H} - ^1\text{H}] + [^1\text{H} - ^2\text{H}] = 2M_2 + M_3$$

$$[^2\text{H}] = [^1\text{H} - ^2\text{H}] = M_3$$

It is useful to get a bit of perspective here. Since the natural abundance of ^{13}C is only ~1% of the total carbon, if we could inspect toluene molecules which have 7 carbons per molecule, we would need on average to look at about 14 toluene molecules before we found one with a heavy atom of carbon, ^{13}C ; and since ^2H is only 0.015% of the total hydrogen we would need on average to look at ~ 833 toluene molecules before we found a heavy atom of hydrogen, ^2H . This means that in most molecules we would not expect to find any heavy atoms and virtually never more than one heavy atom per molecule, and that includes carbon and hydrogen (and oxygen)... i.e. in a molecule with a heavy carbon atom, it would be very unlikely to also find a heavy hydrogen atom. That is why, as shown on Table

2, we can concern ourselves with the measurement of species which have only one heavy atom of any kind.

So the picture is that all carbon containing compounds derived from natural products (most of them ultimately are derived from petroleum) and in particular the compounds of interest to us in environmental remediation such as BTEX, MTBE, CVOC's, have a small content of the stable heavy isotopes of carbon and hydrogen and with IRMS instruments we can determine the isotopic content with great sensitivity and precision.

The parameter that is measured on an IRMS is the concentration of each of the common stable isotopes of both carbon and hydrogen in each molecule chosen to study. These concentrations are usually reported as the concentration ratio of the heavy isotope to the light isotope, i.e. $[^{13}\text{C}]/[^{12}\text{C}]$ and $[^2\text{H}]/[^1\text{H}]$. In order to be able to compare data from one instrument or one laboratory to another, there have been adopted international standards for both carbon and hydrogen and these standards are analyzed contemporaneously. The results are reported in terms of a comparison of the sample to the standard, thus small differences in the response of individual instruments is not a factor as long as results are reported with reference to a standard which is also analyzed on the same instrument. For carbon, the standard is Vienna Peedee Belemnite, or VPDB, and for hydrogen the standard is "Vienna Standard Mean Ocean Water" or VSMOW.

Let's focus on carbon isotopes and define the isotope ratio R as the ratio of the concentration of compound with ^{13}C to the concentration of the compound with ^{12}C as shown in equation (1).

$$(1) \quad R = ([^{13}\text{C}]/[^{12}\text{C}])$$

We could define R for compound "x" in the sample and the standard,

$$(2) \quad R_x = ([^{13}\text{C}]/[^{12}\text{C}])_x \text{ and } R_{\text{std}} = ([^{13}\text{C}]/[^{12}\text{C}])_{\text{std}}$$

and, if we were studying hydrogen, we could define

$$(3) \quad R_x = ([^2\text{H}]/[^1\text{H}])_x \text{ and } R_{\text{std}} = ([^2\text{H}]/[^1\text{H}])_{\text{std}}$$

As we have said, the data is reported in terms of R_x relative to R_{std} , as defined by the parameter δ_x where for carbon in compound x,

$$(4) \quad \delta_x^{13}\text{C} = \left\{ \frac{R_x - R_{\text{std}}}{R_{\text{std}}} \right\} \times 1000$$

and the units are parts per thousand or “per mil” usually denoted with the symbol ‰.

A $\delta_x^{13}\text{C}$ value of 0 ‰ then corresponds to a sample with an isotope ratio that is equal to that of the standard. A $\delta_x^{13}\text{C}$ value of + 10 ‰ corresponds to a sample with an isotope ratio that is 10 parts per thousand or 1 % higher than that of the standard. For the international standard for carbon, VPDB, the ratio $[^{13}\text{C}] / [^{12}\text{C}]$ has been reported to be 0.011180, which means that $\delta_x^{13}\text{C} = + 10$ ‰ for the sample then corresponds to a $[^{13}\text{C}] / [^{12}\text{C}]$ ratio of 0.011292. This demonstrates the very subtle changes that need to be measured. The major difference between measurements made using quadrupole mass spectrometers in methods like SW846-8260 and those made with an IRMS instrument is the very high precision that is achieved in the latter instruments due to the simultaneous measurement of the ions on fixed collectors. Standard deviations of the order of 4 to 6 significant figures (Meier-Augenstein, 1999) can be achieved and are a requirement to measure the small changes in isotopic composition at the natural abundance level of the heavier isotopes of carbon and hydrogen.

Application to In-Situ Processes

So, why are we interested that there are very minor amounts of these stable heavy isotopes distributed in compounds made from naturally occurring materials? The fact is that when one of these heavy isotopes is a part of a compound, its bond to adjacent atoms is ever so slightly stronger than the equivalent bond of the lighter isotope when it is in the same position in another molecule of the same compound. When molecules of this compound enter into chemical or biologically mediated reactions, the molecules with the lighter isotopes react a little faster than the ones with the equivalent heavier isotopes. This means that as the reaction proceeds, the reactant that remains has a progressively higher content of the heavy isotope since the molecules containing light isotopes have reacted to form product faster than those containing heavier isotopes. This process is called fractionation.

The degradation processes of interest in environmental remediation are for the most part irreversible kinetic reactions. The magnitude of a kinetic isotope

fractionation depends on the reaction pathway or mechanism, the reaction rate, and the relative bond energies of the bonds being severed or formed by the reaction.

As long as we are dealing with the low natural abundance of heavy isotopes, as opposed to labeled compounds in which the content of heavy isotopes may be 10% or greater, the fractionation due to typical kinetic reactions, either biological or abiotic, may be described by the classical equation presented by Lord Rayleigh (1896) for the separation of mixed gases.

$$(5) \quad R_t/R_o = f^{(\alpha-1)}$$

Applied to isotopic fractionation, R_t and R_o are the isotopic ratios at time $t = 0$, t and “ f ” is the fraction of reactant remaining at time “ t ” as compared to time $t = 0$, i.e.

$$(6) \quad f = [\text{reactant}]_t / [\text{reactant}]_o$$

and α is the fractionation factor. It is sometimes useful to transform α in terms of an enrichment factor ϵ , where,

$$(7) \quad \epsilon = (\alpha - 1) \times 1000$$

Transforming equation (5), the Rayleigh equation becomes,

$$(8) \quad \ln(R_t / R_o) = (\alpha - 1) \ln f = (\epsilon / 1000) \ln f$$

Instead of using the ratio's of R directly, equation (8) is often written in terms of the δ notation as

$$(9) \quad 1000 \ln((10^{-3} \delta_{r,t} + 1)/(10^{-3} \delta_{r,o} + 1)) = \epsilon \ln f$$

For typical enrichment factors ($-20 \text{ ‰} < \epsilon < 20 \text{ ‰}$), $\ln(10^{-3} \delta_r + 1) \sim 10^{-3} \delta_r$, then (9) may be simplified to:

$$(10) \quad \delta_{r,t} - \delta_{r,o} = \epsilon \ln f$$

This simplified version of the Rayleigh equation, originally developed by Mariotti, et. al. (1981) is commonly used to relate the extent of biodegradation to the isotopic ratio, although various forms including equations (8) and (9) are also used. It should also be said that these equations are not applicable to species which are simultaneously being formed and degraded such as cis-DCE or VC in the sequential degradation of TCE, although they would be applicable to the parent TCE species. This does not mean that isotopic fractionation is not useful in evaluating the more complex sequential degradations, however its evaluation will differ from less complex pathways where the parent molecule is the primary concern such as the degradation of benzene or toluene. The Rayleigh model may also be applicable when the rates of the parent species and its primary degradation product degrade at significantly different rates as may be the case with MTBE and TBA. We will first examine the case of the degradation of MTBE where the focus will initially center on MTBE without regard to TBA and proceed to the more complex degradation pathways later.

If we rearrange equation (10),

$$(11) \quad \delta_{r,t} = \epsilon \ln f + \delta_{r,0}$$

it is easily recognized to be the equation of a straight line of the form $y = mx + b$. Thus plots of $\delta_{r,t}$ vs $\ln f$ should be linear (if the assumptions outlined above are valid for the case in question) with slope ϵ and y intercept $\delta_{r,0}$. Enrichment factors, ϵ are often first determined in the laboratory by monitoring the degradation of a chosen contaminant compound using pure cultures or enrichments. Data from such experiments are plotted as $\ln (R_t / R_0)$ (or $\delta_{r,t}$) vs $\ln f$ and the enrichment factor, ϵ , is determined from the slope of a linear regression line.

Application to MTBE and TBA

Kuder, et. al. (2005) studied the degradation of MTBE in anaerobic microcosms and enrichment cultures from a site in Parsippany, NJ. The isotopic enrichment of carbon is shown in Figure 6a and the isotopic enrichment of hydrogen is shown in Figure 6b, both as a function of the fraction of MTBE remaining (after equation 8).

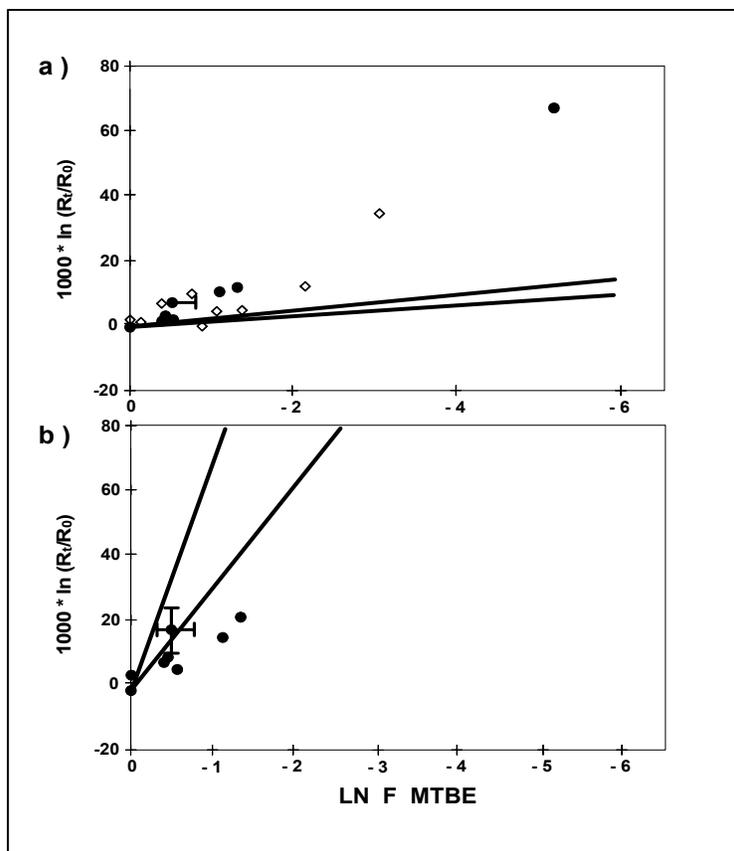


Figure 6

The diamonds are data from microcosms using sediment from the Parsippany NJ site and the circles are enrichment cultures developed from the microcosms (Kolhatkar, et.al., 2002). The solid lines are for carbon and hydrogen representing similar data from the aerobic degradation of MTBE(Gray, et. al., 2002).

The slope of the best fit line to the data from these experiments is the enrichment factor which differs significantly from those reported for aerobic degradation. This suggests that these two pathways likely have differing reaction mechanisms.

A more sensitive means to observe this apparent difference in mechanism of degradation is to plot the observed $\delta^2\text{H}$ vs $\delta^{13}\text{C}$ as shown in Figure 7.

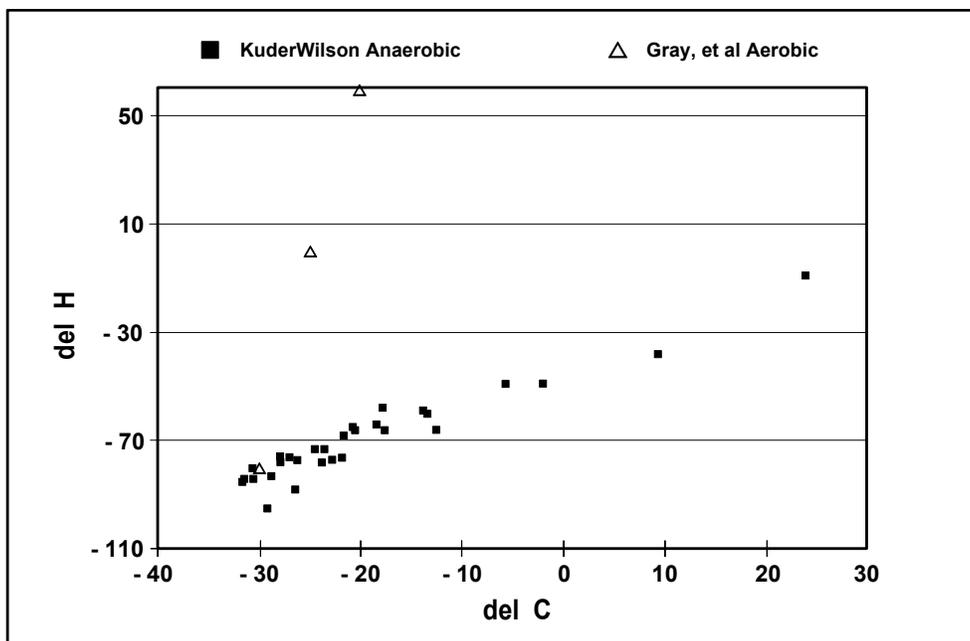


Figure 7

The slope of the best fit to this data is the ratio ϵ_H/ϵ_C . Open triangular data points in Figure 7 represent the reported data trend for the aerobic degradation of MTBE (Gray, et. al. ,2002) while the solid data points result from the anaerobic degradation (Kuder, et. al., 2002). Clearly the enrichment factors for hydrogen relative to carbon are significantly different in the two processes.

Carbon enrichment factors, ϵ_C , for the aerobic degradation of MTBE are reported in the range -1.4 ‰ to -2.4 ‰ by Hunkeler et al., (2001) and Gray et al. (2002). Values of ϵ_H reported for the aerobic process varied from -30 ‰ to -69 ‰ (Gray et al., 2002).

Enrichment factors, ϵ_C , for the anaerobic process have been reported in the range -8.2 ‰(Kolhatkar et al. ,2002) to -13 ‰ (Kuder et al. , 2005). The enrichment factor, ϵ_H , for the anaerobic process has been reported as -16 ‰ (Kuder et al. , 2005).

Zwank, et. al. (2005) have suggested that the mechanism of the aerobic degradation first involves the breaking of a carbon-hydrogen bond of the methyl group. This mechanism would be expected to have a larger ϵ_H/ϵ_C than the mechanism proposed for the anaerobic degradation which is suggested to involve breaking of an oxygen-carbon bond, specifically the bond of the ether oxygen to the methyl group carbon. The data of Figure 7 follow the order suggested, e.g. aerobic $\epsilon_H/\epsilon_C \gg$ anaerobic ϵ_H/ϵ_C .

Rearranging equation (11) and solving for the fraction remaining,

$$(12) \quad f = [\text{reactant}]_t / [\text{reactant}]_o = C_t / C_o = \exp ((\delta_{r,t} - \delta_{r,o}) / \epsilon)$$

The extent of biodegradation is of course equal to $(1 - f)$ and we can calculate “ f ” if we know ϵ for the process (from a laboratory study), C_o the initial concentration (usually taken as the highest concentration source zone well), and $\delta_{r,o}$, the isotopic ratio of the reactant in the source zone well. If we are dealing with a product like MTBE and know the range of δ_r from measurement of pure product in gasoline we might choose to take one of these measured values as $\delta_{r,o}$. Given these knowns, the expected concentration in the well if biodegradation is the only degradation process may be calculated from equation (12) using the value of $\delta_{r,t}$ measured in each well.

In practice, measured concentrations at monitoring wells are generally found to be smaller than the calculated concentration at least in part because of processes like dilution, volatilization or sorption that cause concentrations to decrease, but do not affect the isotopic ratio or affect it only slightly. Such an observation is shown in Figure 8 (Kuder et al. , 2005) where the observed concentrations are plotted on the ordinate and the calculated concentrations from the isotopic observations are plotted on the abscissa. Almost without exception, the observed data are equal to or less than the value calculated from the isotopic data. So the fraction of material degraded based on isotopic data is conservative.

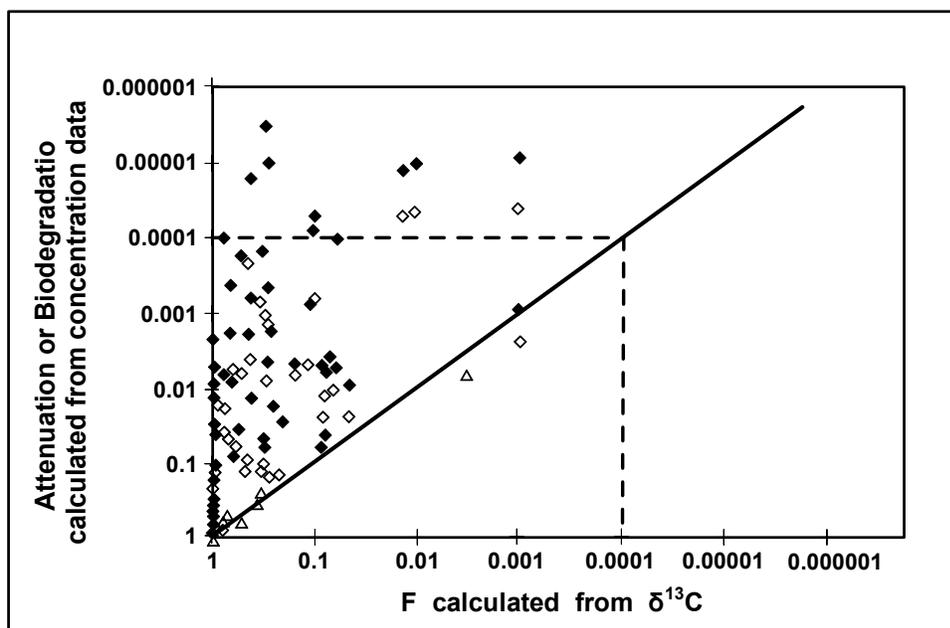


Figure 8

Wilson (2005) has demonstrated the use of these relationships at a gasoline spill site in Dana Point, CA. Concentrations of MTBE (ug/l) are shown on the site map on Figure 9. Concentration and isotopic data for MTBE and TBA are shown on Table 3. Also shown is the fraction of MTBE remaining, calculated from the isotopic ratio's by equation (12) using the conservative values: $\epsilon_C = -12\text{‰}$; and $\delta_{\text{MTBE},0} = -27.4\text{‰}$ which is the highest value reported for MTBE in gasoline.

The contemporaneously determined concentration of tert-butyl alcohol (TBA) is also shown on Table 3. The monitor well MW-14 is the most contaminated well and the concentrations of MTBE and TBA were approximately equal when measured in May of 2003, however the concentration of TBA was found to be nearly 4 times that of MTBE in August of 2004. Over this time δ_{MTBE} has become slightly more positive suggesting that biodegradation may be responsible for the generation of the TBA. Further along the flow path, δ_{MTBE} is much more positive and ratio's of TBA to MTBE are much higher suggesting that biodegradation is responsible for the generation of TBA. However in wells MW-7 and MW-11, where the concentration of MTBE is very low and TBA is relatively high, the δ_{MTBE} is even lower than in well MW-14, and is in fact within the range reported ($\delta_{\text{MTBE}} -27.5$ to -33‰) for MTBE in gasoline. Therefore there is no isotopic evidence for biodegradation of MTBE in these wells.

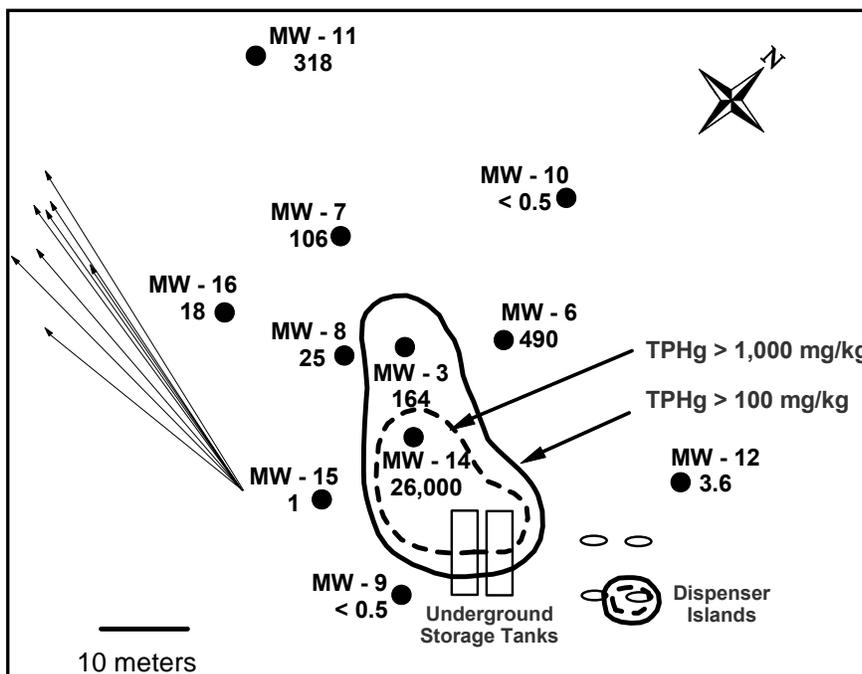


Figure 9

Table 3

Well	Date	TBA Measured (µg/L)	MTBE Measured (µg/L)	$\delta^{13}\text{C}$ MTBE (‰)	MTBE Fraction Remaining (C/Co)
MW-14	5/20/03	13,000	11,000	-23.88	0.75
	8/18/04	107,000	26,000	-21.58	0.62
MW-3	5/20/03	20,000	870	6.84	0.058
	8/18/04	32,000	164	8.53	0.050
MW-8	5/20/03	10,000	19	18.11	0.023
	8/18/04	32,000	25	37.99	0.0043
MW-6	5/20/03	3,600	47	9.83	0.045
	8/18/04	19,200	490	-1.58	0.116
MW-7	8/18/04	1,220	106	-27.33	0.994
MW-11	5/20/03	< 10	1	-31.5 *	1.41
	8/18/04	135	318	-28.92	1.14

Since the isotopic ratio is related to the fraction of reactant remaining, it can also be used to estimate the rate of biodegradation along a flow path in the plume. If we rearrange equation (12) as follows,

$$(13) \quad C_t = C_o \exp ((\delta_{r,t} - \delta_{r,o})/\epsilon)$$

we see that it has the form of the equation which describes a first order biodegradation process in groundwater, i.e.

$$(14) \quad C_t = C_o \exp (-k_t t)$$

or

$$(15) \quad C_d = C_o \exp (-k_d d)$$

Equation (14) describes the changes in concentration in a particular monitoring well with time and equation (15) describes the change in concentration along a

defined flow path with distance between the up-gradient or source well with concentration C_o and the monitoring well in question. Therefore we can write,

$$(16) \quad -k_t t = (\delta_{r,t} - \delta_{r,o})/\epsilon$$

or

$$(17) \quad k_t = (\delta_{r,o} - \delta_{r,t})/\epsilon t$$

where k_t is the rate constant for biodegradation in terms of time. This can be derived from multiple sets of data at a single well, or, if one knows the groundwater velocity, can be estimated from a single set of measurements at a well since time = (distance from source well)/(groundwater velocity), i.e. $t = d/v$, and substituting we get,

$$(18) \quad k_t = (\delta_{r,o} - \delta_{r,t}) v/(\epsilon d)$$

The rate constant with distance may be defined by,

$$(19) \quad -k_d d = (\delta_{r,d} - \delta_{r,o})/\epsilon$$

or

$$(20) \quad k_d = (\delta_{r,o} - \delta_{r,d})/\epsilon d$$

where k_d is the first order rate constant in terms of distance.

Wilson (2005) has calculated rate constants for degradation of MTBE along flow paths, both in terms of distance and time using equations 18 and 20. The data are shown in Table 4. In wells MW - 3 and MW- 8 (see Figure 9) the rate constants are 0.3 per meter of travel or 10 per year of residence time. In well MW – 7, the rate is much slower and in MW – 11 it is not detected at all. These projected rates along flow paths may also be used to predict the possible extent of plumes.

Table 4
Rates of Natural Biodegradation of MTBE

Well	Date Sampled	Fraction MTBE Remaining (C/C ₀)	Distance from MW-14 (meters)	Projected Rate of Biodegradation with Distance (per meter)	Projected Rate of Biodegradation with Time (per year)
MW-3	May, 2003	0.058	9.6	0.30	10.9
MW-3	August, 2004	0.05	9.6	0.31	11.5
MW-8	May, 2003	0.023	11.7	0.32	11.9
MW-8	August, 2004	0.0043	11.7	0.46	17.1
MW-7	August, 2004	0.994	23.0	0.00025	0.0093
MW-11	August, 2004	1.0	44.1	0	0

Zwank, et al. (2005) have employed a two dimensional CSIA approach to describe biodegradation of MTBE and TBA in a groundwater plume at an industrial disposal site as shown in Figure 10. Both phenol and MTBE had been disposed at the site in open ponds over several years on a small hill, thus there is a radial groundwater flow pattern.

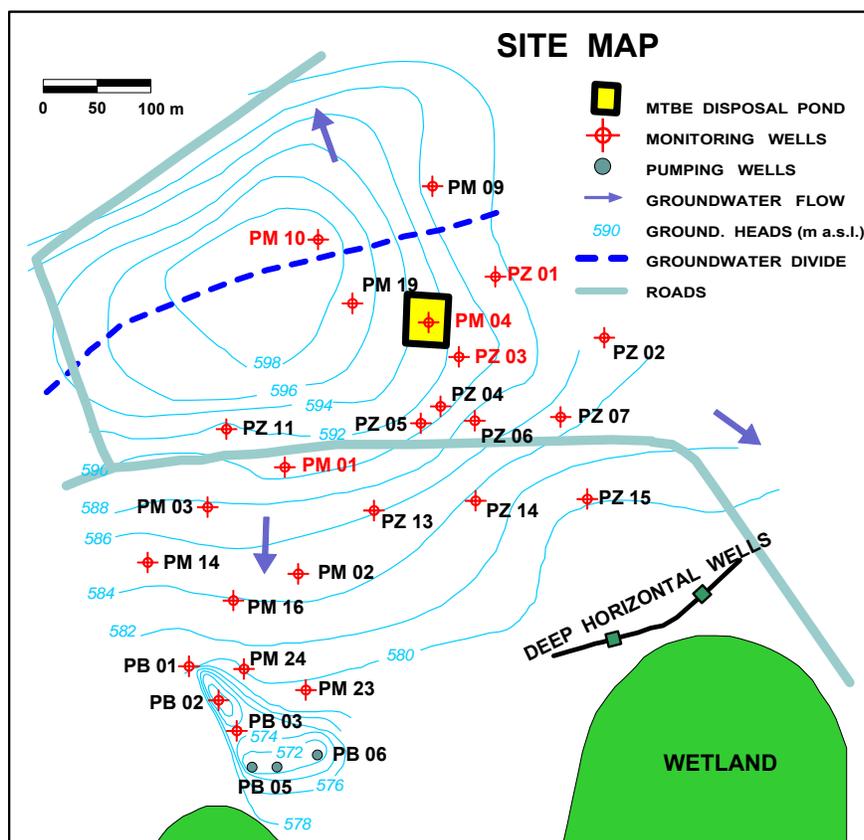


Figure 10

MTBE, which had been used as a solvent in chemical synthesis was, over several years, disposed in the pond near PM04. Minor amounts were also disposed near well PM01. A total of 13 ponds are located in the area between wells PM 10 and PM04 and were used for disposal of other organic production wastes.

As can be seen on Figure 11, the MTBE plume is extensive and has maximum concentrations in the range of 1.7 g/l at well PM04 (the aqueous solubility of MTBE has been reported to be 48 g/l). At PM04 the TBA concentration was below analytical detection levels suggesting that TBA was not a component of the MTBE disposed. Indeed, its presence as shown in Figure 12 results from in-situ degradation at the site.

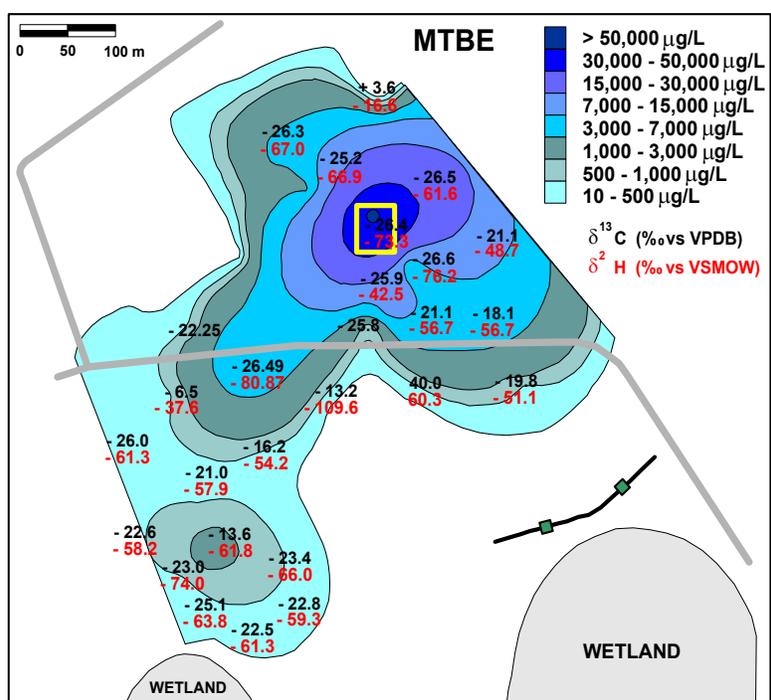


Figure 11

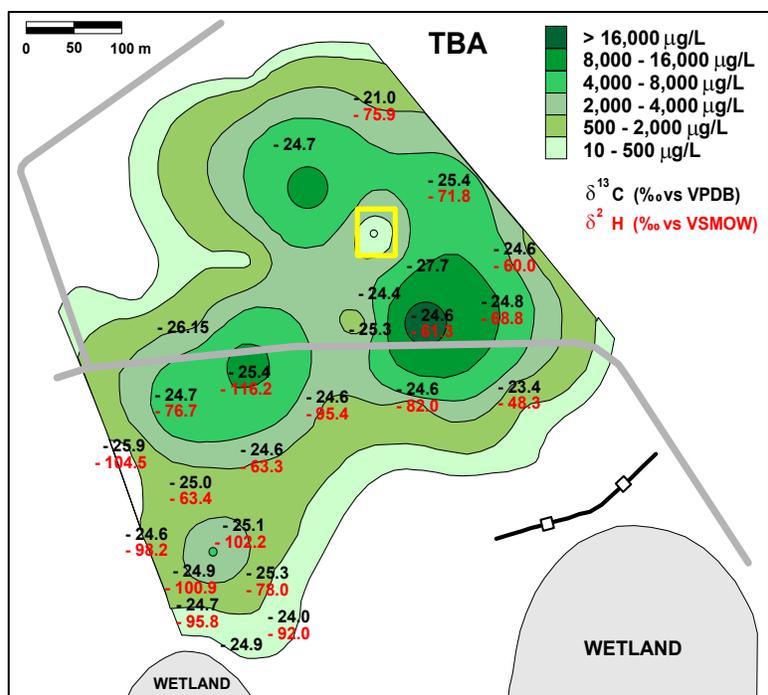


Figure 12

Although several wells near PM04 had near identical carbon and similar hydrogen isotopic signatures suggesting that biodegradation is not significant near the source, significant isotopic enrichment of MTBE with increasing distance from PM04 is evident with $\delta^{13}\text{C}$ up to $+40.0$ ‰ and $\delta^2\text{H}$ up to $+60.3$ ‰. With one exception, the carbon isotopic composition of TBA was relatively invariant, -25.02 ± 0.75 ‰ in the plume, however it was slightly enriched relative to the parent MTBE at the source well. This was explained as due to the fact that position specific carbon isotopic signature of the t-butyl group is not necessarily the same as the carbon isotopic signature of the methyl group. MTBE is made by the reaction of isobutene with methanol and the isotopic signatures of these two industrial chemicals are not necessarily the same, indeed it would be fortuitous if they were the same. Further, the isotopic signature of an MTBE standard and its associated t-butyl group were determined and it was found that $\delta^{13}\text{C}$ of the MTBE standard was -28.13 ± 0.15 ‰ while the $\delta^{13}\text{C}$ of the t-butyl alcohol obtained via complete acid hydrolysis was -25.49 ± 0.10 ‰.

Finally, Zwank et al. (2005) showed the power of using both the carbon and hydrogen isotopic signatures to enable verification of the mechanism of degradation (aerobic vs anaerobic) as shown in Figure 13.

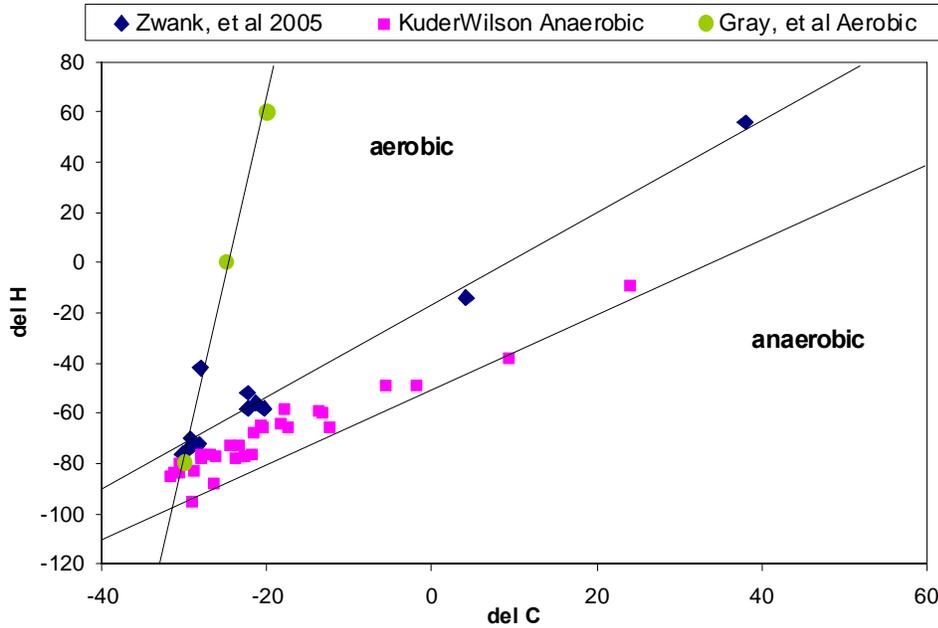


Figure 13

Once the mechanism of degradation is determined, as in this case, to be anaerobic, then appropriate values of ϵ can be used to calculate the fraction of MTBE degraded (Wilson et al., 2005).

Day et al. (2003) and Gulliver (2003) have shown that in a plume of parent TBA (no MTBE) at a manufacturing site in Texas, TBA is apparently degraded under sulfate reducing conditions. Not only is the carbon of the remaining TBA enriched in ^{13}C as shown in Figure 14, but the sulfur in the residual SO_4^- is simultaneously enriched in ^{34}S as shown in Figure 15.

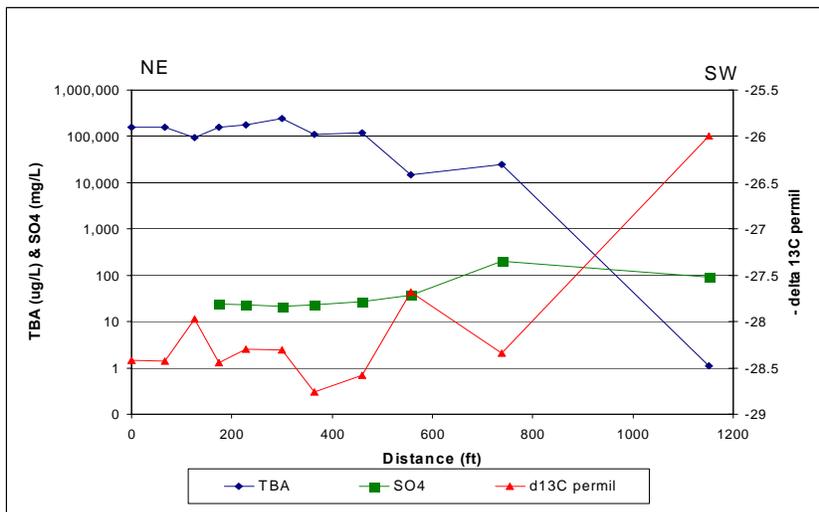


Figure 14

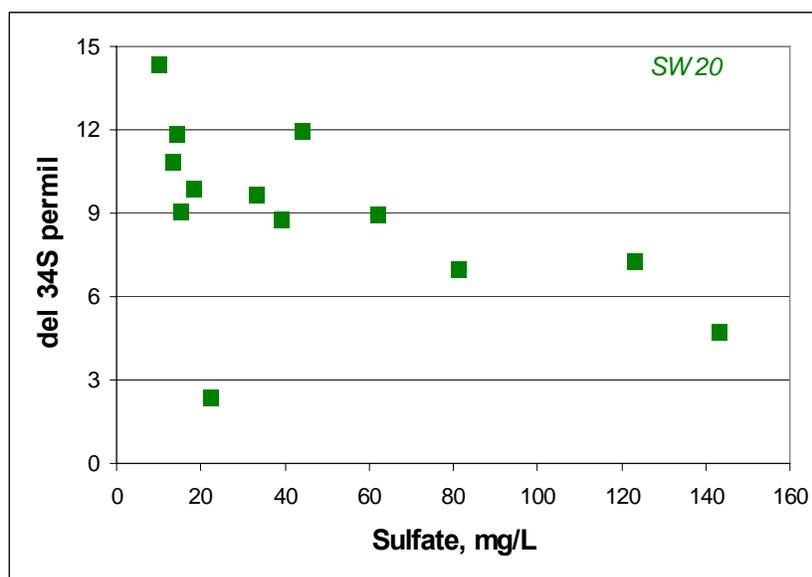


Figure 15

As shown in Figure 16, in wells where TBA concentrations are significantly reduced the carbon isotope ratio shows moderate, but unmistakable fractionation suggestive of biodegradation. This is also shown in Figure 17 for 6 specific wells where the carbon isotopic signature is plotted vs the natural log of the ratio of TBA concentration in 1992 vs the concentration in 2002. As that ratio increases due to the decrease in concentration over the 10 year interval, the carbon isotopic signature of TBA increases as would be expected if the concentration decrease were due to biological or abiotic degradative processes.

This data provides unequivocal proof that TBA can and will biodegrade under naturally occurring conditions in groundwater and CSIA may be the most definitive means to identify its occurrence. Consideration of the possible mechanism of TBA degradation, leads one to suggest that the first step is likely hydrogen abstraction rather than a nucleophilic attack on carbon. If this is the case, then it would be expected that the isotopic fractionation of hydrogen would be more significant than that of carbon and thus δH could be a more sensitive indicator of in-situ TBA degradation than δC . Unfortunately, δH was not determined in this study so we have yet to observe this effect.

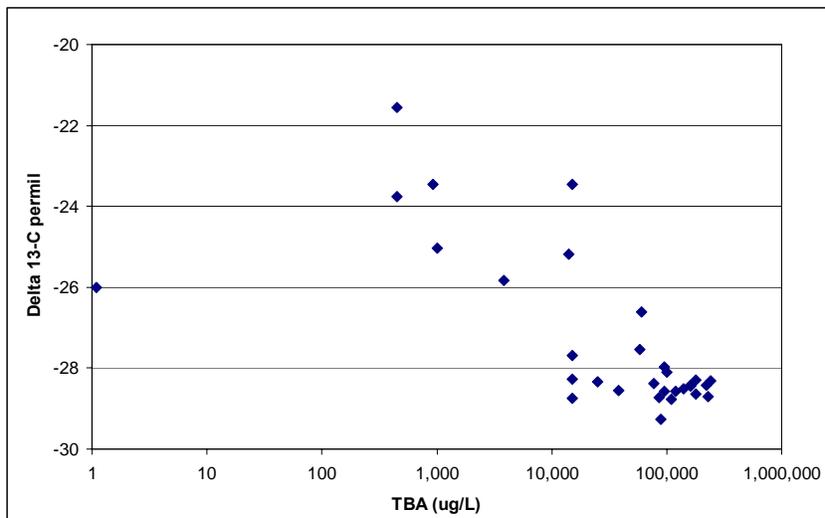


Figure 16

The degradation of TBA seems to be redox potential sensitive in that while in some limited cases such as described above it does degrade under sulfate reducing conditions there are numerous reports in the literature that it accumulates under methanogenic conditions. It is clear that much further study into the in-situ degradation of TBA is needed and stable isotope analyses will no doubt play an important role in elucidating the nuances of this process.

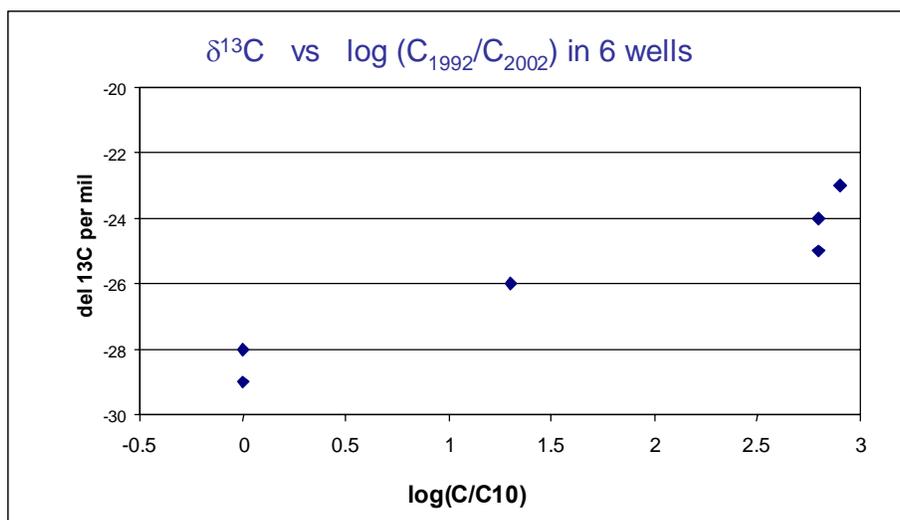


Figure 17

So in summary, compound specific isotope analysis is a powerful technique to evaluate the characteristics and progress of MTBE plumes in groundwater. Isotopic fractionation of the residual MTBE is the only incontrovertible evidence

of degradation and it can also be applied to determine the degradative mechanism. Given the mechanism of degradation and thus the applicable fractionation factors, one can then calculate several characteristics of the process, including the fraction of MTBE degraded and the in-situ rate of degradation. In-situ rates can be used to estimate the extent of the plume under the existing conditions thus enabling the determination of the potential impact to sensitive receptors in its path.

Application to Chlorinated Solvents

As we have mentioned, the Rayleigh model cannot be applied to sequential degradation processes such as the well known biodegradation of PCE to ethene. It can be applied to the parent compound of the sequential process, but not to its daughter products because they are being simultaneously formed and consumed. The Rayleigh model has been used to determine enrichment factors for each of the members of this sequence by constructing microcosms starting with each member to determine the enrichment factor based on isotopic ratios vs fraction remaining. In general it has been found that the enrichment factors increase with each step in the sequence from PCE to TCE, TCE to c-DCE, c-DCE to VC and VC to ethene as shown in Table 5.

	PCE	TCE	cDCE	VC
Lollar et al.(1999)		-7.1		
Bloom et al.(2000)		-4.6	-15.1	-24.1
Slater et al.(2001)	-5.5	-13.8	-20.4	-22.4

The differing enrichment factors for each compound, as shown on Table 5, are attributable to the precise conditions under which they are determined. It is suggested (Slater et al., 2001) that enrichment factors differ for different microbial consortia, for replicate degradations by the same consortia and for differing electron donors. Even with the observed variations in enrichment factors reported to date, there are consistent trends in the isotopic fractionation observed during reductive dechlorination of chlorinated ethenes. The fact that enrichment factors fall into distinct ranges allows one to estimate the relative extent of degradation of a chlorinated ethene. The consistent observation of isotopic fractionation during

reductive dechlorination suggests that CSIA is a useful tool to identify the occurrence of biodegradation.

Song, et. al. (2002) have presented a detailed, time-series isotope study of a dynamic system undergoing enhanced bioremediation at the Idaho National Engineering and Environmental Laboratory (INEEL) Test Area North (TAN). The bioremediation of TCE and daughter products was significantly stimulated by the addition of lactate to the source area. Concentrations of the chlorinated ethene species varied greatly during this pilot study, making it impossible to use concentration data to determine the extent of degradation. The isotope data were used to clearly show that all of the TCE that was degraded was fully converted to ethene.

The site plan is shown in Figure 18. The source well is TSF-05 and wells TAN-25 and TAN-26 are used to illustrate the sequential degradation using both concentration and isotopic data. Wells TAN 29 and TAN 31 are used as background wells. Well TAN-29 was not affected by the pilot study and TAN 31 was only minimally affected.

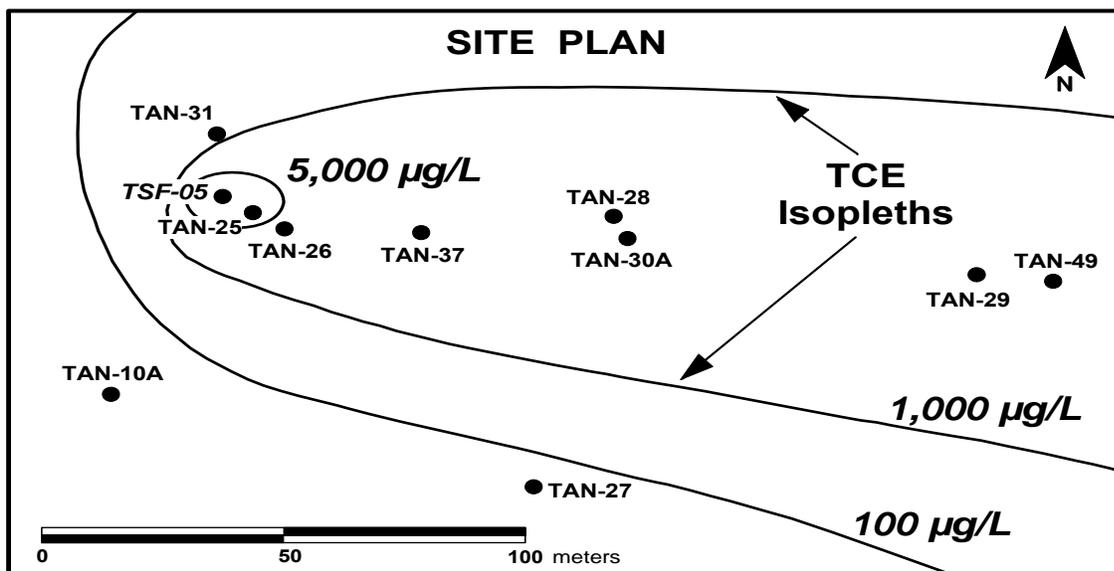


Figure 18

The pilot study began with the injection of clean water into well TSF-05. Lactate injection through the same well began some 25 days after the clean water injection was ended. Although lactic acid was injected as a stimulant for reductive dechlorination, it was rapidly converted to acetic, propionic, and butyric acids via

fermentation. Concentrations of total acids at the four wells discussed above are shown in Figure 19.

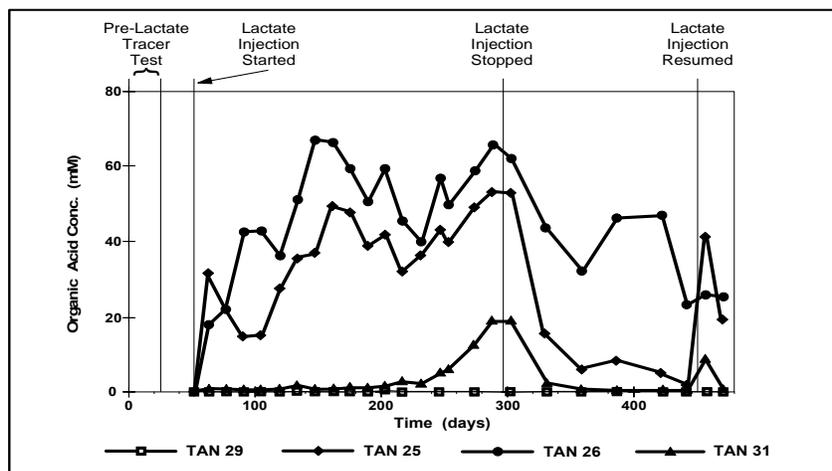


Figure 19

It is clear that significant concentrations of organic acids are present at wells TAN-25 and TAN-26 throughout the lactate injection period. Significant concentrations of organic acids were never observed in TAN-29 and only toward the end of the injection period in TAN-31.

The chlorinated ethene concentration and isotopic data are plotted on Figure 20, where the effects of the clean water injection can be seen to reduce the concentration of TCE and other chlorinated species present during the first 25 days of the pilot test. Concentrations rebounded when clean water injection was ended and leveled out during the first month of lactate injection. TCE concentrations then decreased sharply with a corresponding increase in cis-DCE. It is interesting that the sharp drop in TCE concentration just prior to day 100 was not accompanied by an increase in its $\delta^{13}\text{C}$ as would be expected as a result of the obvious degradation of the TCE to cis-DCE. This is likely due to mobilization of fresh TCE from the source by the water associated with lactate injection. The volume of water injected was increased on day 106 and again on day 204. The concentration of TCE began to increase on day 133 and its $\delta^{13}\text{C}$ decreased to less than -30‰ , which is likely near the ratio in the undegraded TCE. Concentration and isotopic ratio for t-DCE followed a similar path which may indicate that it was also mobilized from the source area.

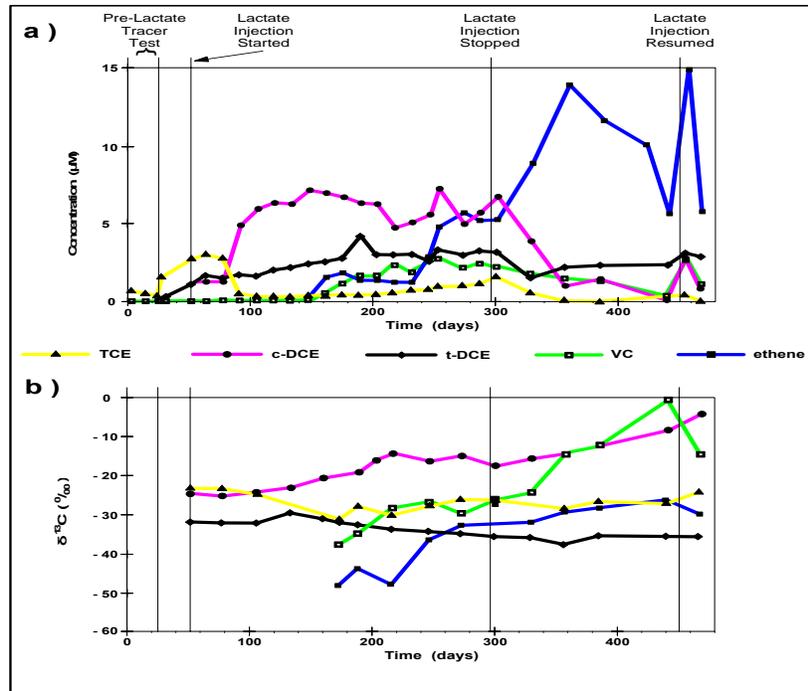


Figure 20

It is notable that the $\delta^{13}\text{C}$ of the cis-DCE began to increase almost immediately after its concentration increased due to degradation of the TCE. This almost certainly indicates that it too is being degraded and the isotopic evidence of degradation appears significantly before the first observations of VC or ethene. This suggests that isotopic evidence of degradation may be used to indicate the activity of degradation processes in plumes where daughter products of reductive dechlorination are not observable or where indeed they are not being formed as would be the case in anaerobic oxidation or abiotic degradation of cis-DCE and VC.

The $\delta^{13}\text{C}$ of both VC and ethene were initially very low, but increased rapidly as degradation proceeded. Eventually the $\delta^{13}\text{C}$ of ethene, in all wells where there was significant organic acid concentrations, reached the $\delta^{13}\text{C}$ of the original TCE indicating complete reductive dechlorination was taking place. It is clear that such a conclusion based on concentration or mass balance data alone would not have been possible at this complex field site. This dataset also demonstrates that the isotopic fractionation data can be used to distinguish between changes in concentration due to physical processes such as groundwater transport or dilution from those associated with biotic or abiotic degradation processes.

Hunkeler et al., (2005) have used stable carbon isotope analysis in conjunction with concentration data to clarify and confirm the active degradation pathways at a

former waste solvent disposal site where at least 14 different chlorinated hydrocarbons were present in groundwater. One of several issues which were resolved using carbon isotopic data was the observation of TCE at down gradient locations with $\delta^{13}\text{C}$ in the range of -41.9 to -45 ‰ which is well below the range of values known for pure-phase industrial TCE which has been determined in the range -24.3 to -31.9 ‰ (Hunkeler et al., 2004; Jendrzejske et al., 2001; Van Wierdam et al., 1995). Other sources of TCE included reductive dechlorination of PCE or dehydrohalogenation of 1,1,2,2-PCA. Although PCE was not a major contaminant at the site, it was present with $\delta^{13}\text{C}$ in the range -30.5 to -28.3 ‰. Enrichment factors for degradation of PCE to TCE (-2 to -5.5 ‰) suggest that the observed TCE was not a degradation product of PCE. Carbon isotopic values for 1,1,2,2-PCA in the source area were found to be $\delta^{13}\text{C} = -39.1$ ‰ which suggested that it was most likely the source of the TCE.

Shouakar-Stash et al. (2003) have characterized selected chlorinated solvents in terms of their hydrogen, carbon and chlorine isotopic composition. They have noted that $\delta^2\text{H}$ for a range of manufactured TCE varied between +466.9 ‰ and +681.9 ‰ whereas TCE generated as a dechlorination product of PCE was significantly depleted, $\delta^2\text{H} < -300$ ‰. This suggests that $\delta^2\text{H}$ of certain chlorinated solvents may be a powerful means of distinguishing between dechlorination products and manufactured solvents. At complex sites like the one described above (Hunkeler et al., 2005) the combination of carbon and hydrogen isotopic data together with concentration data will no doubt significantly enhance the ability to unravel the source of contaminants. Shouakar-Stash et al. (2003) suggested that the combination of carbon, hydrogen and chlorine isotopic data may even provide forensic evidence of the manufacturer of a particular solvent.

Isotopes and Groundwater Transport Modeling

While qualitative conclusions about the occurrence of biodegradation and its relative extent may be obtained and indeed be useful from sites as described above, these data will be potentially much more powerful and quantitative when stable isotope constraints are integrated into groundwater transport models. Indeed several such investigations have been reported (Beranger et al., 2005; van Breukelen et al., 2005; Morrill et al., 2006) with initial application to microcosm and column studies. Van Breukelen et al. (2005) verified their model for a single species by comparison to the Rayleigh model. They confirmed the model, as shown in Figure 21 for sequential degradation including sorption by simulation of a previously published experiment (Hunkeler et al., 1999) in which complete

reductive dechlorination of PCE to ethene occurred. This model was reportedly capable of addressing sources of mixed composition and also accounts for sorption. Isotopic enrichment factors and Monod kinetic parameters were obtained from the model through optimization using the nonlinear parameter optimization program PEST developed by Watermark Numerical Computing: www.sspa.com/pest.

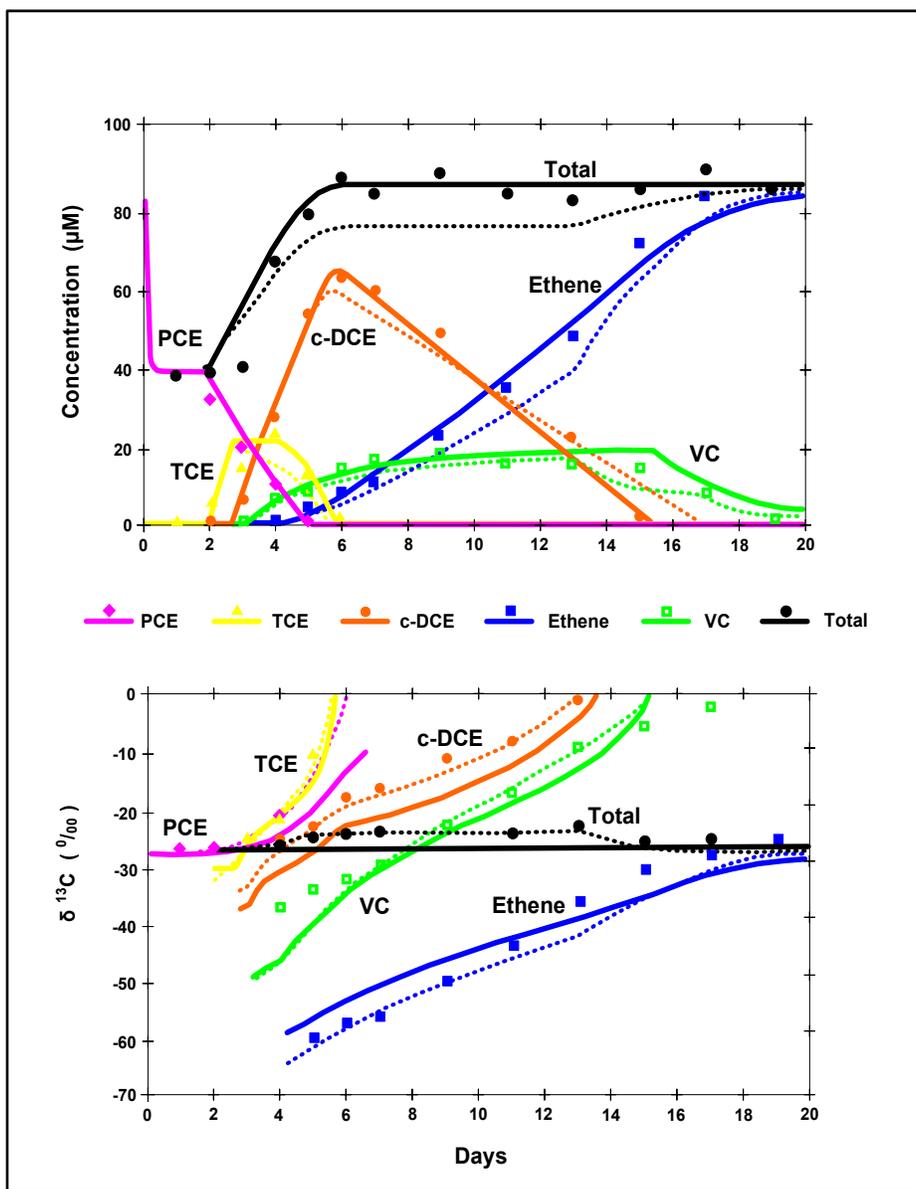


Figure 21. Lines are simulated values; symbols depict observations. Dashed lines are the results from the modified model.

In addition to sorption, van Breukelen et al. (2005) addressed the use of their model, which incorporates isotopic constraints, to demonstrate degradation of an

apparently accumulating intermediate degradation product, e.g. c-DCE; and the occurrence of mixed sources, i.e. both PCE and TCE parent compounds present. Neither of these situations can be quantified using the Rayleigh model.

Morrill et al., (2006) have developed a model to predict concentrations during sequential reactions such as the reductive dechlorination of chlorinated ethenes. Their model incorporates Rayleigh model isotopic principals and specified enrichment factors for each step of the process. The model was tested by attempting to predict concentration values in three experimental datasets of concentration and isotopic values which have been reported by Slater et al. (2001). The model was then coupled to a parameter estimation method to estimate the values for the isotopic enrichment factors of the intermediates TCE, cDCE and VC. The enrichment factors for the intermediates TCE and cDCE were found to either be within or near the published range determined when they were the parent compound.

In contrast, the enrichment factor for intermediate VC was significantly outside the published range for VC when it was a parent compound. This difference could be attributable to the presence of multiple chloroethene dechlorinating enzymes, each having a different affinity for the VC substrate and imparting a different isotopic fractionation. It is known that there are at least two, and possibly three differing VC reductive dehalogenase genes associated with the KB-1 consortium (Muller et al, 2004; Krajmalnik-Brown et al., 2004; Waller et al., 2005). Therefore it is plausible that a different set of dechlorinating enzymes are active depending on the conditions when the initial reactants are TCE and/or c-DCE, and VC is present as an intermediate compound, versus when VC is added to the culture directly. Other explanations also may be possible.

Presently there has been considerable success in applying models of contaminant transport in 3 dimensions which incorporate not only the physical characteristics of groundwater flow, but the reactive processes of biodegradation, both natural and enhanced, and the effects of redox geochemistry of the groundwater. These reactive processes of course uniquely affect both the light and heavy isotopes of carbon and hydrogen as we have discussed in the pages above. The way that the isotopes are affected is measured very precisely by the isotopic ratio and its change with time and location. The models we have discussed above have for the most part been applied in one dimension in batch or simple column flow experiments where concentrations can be measured very accurately. As such the models have been used to reveal other fundamental characteristics such as enrichment factors. As we begin to understand actual enrichment factors that occur at field sites, the

incorporation of isotopic constraints into 3 dimensional reactive transport models will make them even more powerful predictors of concentration. Will this plume stabilize and/or contract before impacting a receptor? Can we confidently reduce the long term monitoring efforts and costs by accurately modeling plume development? The incorporation of isotopic constraints into such models will certainly enhance our ability to answer these questions and may ultimately be the most useful aspect of CSIA.

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