**Intramolecular isotope effects during permanganate oxidation and acid hydrolysis of methyl tert-butyl ether**

Maxime Julien\*1,3, Didier Gori2, Patrick Höhener2, Richard J. Robins3, Gérald S. Remaud3

1 Department of Environmental Chemistry and Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa 226-8503, Japan.

2 University of Aix-Marseille-CNRS, Laboratoire Chimie Environnement – UMR 7376, place Victor Hugo 3, 13331 Marseille, France.

3 EBSI team, CEISAM, University of Nantes-CNRS UMR 6230, 2 rue de la Houssinière BP 92208, F-44322 Nantes, France.

*\*Correspondence: M. Julien; e-mail:* [*julien.m.aa@m.titech.ac.jp*](mailto:julien.m.aa@m.titech.ac.jp)

**Abstract**

Stable isotopes have been widely used to monitor remediation of environmental contaminants over the last decades. This approach gives a good mechanistic description of natural or assisted degradation of organic pollutants, such as methyl *tert*-butyl ether (MTBE). Since abiotic degradation seems to be the most promising assisted attenuation method, the isotopic fractionation associated with oxidation and hydrolysis processes need to be further investigated in order to understand better these processes and make their monitoring more efficient. In this study, position-specific isotope effects (PSIEs) associated with permanganate oxidation and acid hydrolysis of MTBE were determined using isotope ratio monitoring by 13C Nuclear Magnetic Resonance (irm-13C NMR) combined with isotope ratio monitoring Mass Spectrometry (irm-MS). The use of this Position-Specific Isotopic Analysis (PSIA) method makes it possible to observe a specific normal IE associated with each of these two abiotic degradation mechanisms. The present work demonstrates that the 13C isotope pattern of the main degradation product, *tert*-butyl alcohol (TBA), depends on the chemical reaction by which it is produced. Furthermore, this study also demonstrates that PSIA at natural abundance can give new insights into reaction mechanisms and that this methodology is very promising for the future of modeling the remediation of organic contaminants.

**Keywords**

Position-specific isotope effects – isotope enrichment factor – modeling – methyl *tert*-butyl ether (MTBE) – abiotic degradation – remediation

1. **Introduction**

Monitoring *in situ* degradation and bioremediation of groundwater pollutants is a major environmental challenge in which Compound Specific Isotope Analysis (CSIA) is today a routine technique (Hofstetter and Berg 2011). Measuring the isotopic signature of a molecule gives information pertinent to determining its origin and fate, as already demonstrated in many areas of research: geochemistry, forensics, pharmacology and environmental sciences (Aelion et al. 2010). In most cases, CSIA is performed using isotope ratio monitoring by Mass Spectrometry (irm-MS) targeting mostly 13C and 2H in organic contaminants (Elsner et al. 2012; Thullner et al. 2012). This analytical technique can be used routinely to determine isotopic compositions of a large range of compounds with a high precision (SD for 13C ≃ 0.3‰) and requires only a small amount of product (about 1 mg depending on the chemical composition and the analyzed element). Irm-MS can also be applied to complex mixtures when coupled to Gas Chromatography (irm-GC-MS) or High Performance Liquid Chromatography (irm-LC-MS), an interfacing that also has the advantage that sub-mg quantities can be analyzed (Meier-Augenstein 1999; Godin and McCullagh 2011).

The inconvenience of irm-MS is that it only allows a bulk 13C isotope composition (13Cbulk in the case of carbon) to be measured, thus averaging all carbon positions of the molecule. Hence, valuable information about the 13C isotopic fractionation within molecules with numerous carbon atoms is diluted or lost completely (Bouchard et al. 2008). Knowing only the average 13C isotopic composition value can be misleading when interpreting isotope effects (IEs); if bulk ≈ 0, does this mean that there is no fractionation or, rather, that there is a counteractive contribution of normal and inverse intramolecular IEs at different positions? This conundrum can be resolved by Position-Specific Isotope Analysis (PSIA), which makes possible the discrimination of those isotopomers preferentially involved in a process when bulk isotope analysis only allows discriminating isotopologues (Coplen 2011). Measuring position-specific isotopic composition by 13C Nuclear Magnetic Resonance (irm-13C NMR) is now a well-established technique (Jézéquel et al. 2017) employed over the last ten years to study plant metabolism (Gilbert et al. 2011, 2012), pharmaceutical origin (Silvestre et al. 2009) and, more recently, environmental contaminant remediation (Julien et al. 2015b, 2015a).

Methyl *tert*-butyl ether (MTBE) is a fuel oxygenate used as octane enhancer since the 1970s to replace tetraethyl lead which is a Persistent organic pollutant (POP), toxic for living organisms. Despite its numerous advantages compared with lead derivatives, MTBE is soluble in water, meaning that it can travel faster and farther through soil and groundwater than other gasoline components (Johnson et al. 2000). For this reason it is one of the most encountered pollutants met in groundwater and, even if natural attenuation can be a solution, the corresponding accumulation of the metabolite *tert*-butyl alcohol (TBA) could be a more problematic pollution (see Figure 1 for the chemical structures of these compounds) (Kuder et al. 2005).

According to the literature, only a few position-specific isotopic fractionation studies have been performed on organic soil pollutants. Gauchotte *et al*. studied MTBE using Pyrolysis coupled with irm-MS (Py-GC-C-irm-MS) generating methanol and isobutene, thus losing a part of intramolecular information on the isobutene moiety (Gauchotte et al. 2009). Some other partial intramolecular 13C isotopic composition determinations of soil pollutants have been performed using a chemical or enzymatic degradation before irm-MS analysis (Huang et al. 1999). Pollutant removal has also been studied using isotopic 2H NMR, but working on hydrogen or oxygen, which are exchangeable, is very different from observing 13C distribution which observes the skeleton of the organic analyte. Irm-13C NMR appears to be the only method currently able to directly determine the total intramolecular isotopic composition of Volatile Organic Compounds (VOCs) such as MTBE. In its initial development, it requires a larger amount of compound (around 200 mg) but unlike (bio)chemical degradation and Py-GC-C-irm-MS, NMR it is not a destructive method so samples can be recovered.

MTBE is naturally removed from contaminated soil and groundwater through oxidation and acid hydrolysis reaction occurring in both soil and water. Different phenomena are involved in MTBE remediation and they all induce 13C and/or 2H isotopic fractionation. Kuder *et al*. demonstrated that 13C and 2H isotopic fractionation are induced by passive evaporation, with an inverse 13C IE appearing during the process (Kuder et al. 2009). Moreover, volatilization of VOCs has been proven to be associated with 13C position-specific isotopic fractionation (Julien et al. 2015b, 2015a). Isotopic fractionation in both 2H and 13C have also been detected during MTBE biodegradation (Gray et al. 2002). However, as natural MTBE degradation is not effective enough for a fast total remediation, different technologies have been developed for the removal of MTBE (Levchuk et al. 2014), such as air stripping (Sutherland et al. 2004), adsorption (Hung and Lin 2006), assisted biodegradation (Steffan et al. 1997) or electrochemical oxidation (Wu 2011). Advanced Oxidation Processes (AOPs) seem to be the most promising method to obtain a total mineralization of MTBE even if they can generate by-products which are themselves potentially toxic. Many studies have been done using Fenton reagent (Burbano et al. 2005, 2008), persulfate (Huang et al. 2002; Huling et al. 2011) or acid hydrolysis (Elsner et al. 2007; Rosell et al. 2012) but oxidation of MTBE using permanganate seems to be the most promising *in situ* method to eliminate this contaminant from groundwater.

Oxidation of MTBE using potassium permanganate has been proven as a means to rapidly clean up groundwater (Damm et al. 2002). As with other processes, MTBE oxidation induces significant bulk normal 13C and 2H IEs (Elsner et al. 2007). The bulk isotopic fractionation (13Cbulk) obtained during this reaction is similar to fractionation observed with bioremediation with some aerobic bacterial strains such as *Methylibium petroleiphylum* PM1 (Rosell et al. 2012). In this context, as the bulk isotope analysis does not allow the (bio)degradation reaction(s) involved in the remediation to be identified, PSIA using irm-13C NMR could be a valuable tool to determine which process(es) are involved by interpreting the measured changes in 13C distribution between the carbon positions. Furthermore, PSIA should be helpful in estimating the degree of MTBE remediation occurring by chemical oxidation in contaminated sites, since the position-specific isotopic fractionation is related directly to the mechanism of the process involved.

In this study, permanganate oxidation and acid hydrolysis of MTBE have been performed and the resulting TBA has been purified and the 13C distribution analyzed using irm-MS and irm-13C NMR. This data has been used to calculate position-specific enrichment factors (i) so as to obtain more precise information on the mechanism of these processes. Determined IEs associated with both acid hydrolysis and permanganate oxidation were then used to design models to observe the evolution of 13C intramolecular isotopic composition of MTBE and generated products and their concentration during these two abiotic degradation processes (see attached Excel worksheets).

1. **Materials and Methods**
   1. Chemicals

Methyl *tert*-butyl ether (99.8%), potassium permanganate (≥99.8%) and sulfuric acid (96%) were obtained from Sigma-Aldrich, sodium thiosulfate (≥97%) and tris(2,4-pentadionato)chromium(III) [Cr(Acac)3] were purchased from Merck, and DMSO-d6 and acetonitrile-d3 from Eurisotop.

* 1. Degradation experiments

Both abiotic degradations were performed in a 1 L round bottom flask. MTBE was oxidized in 600 mL of an aqueous solution of 100 mM potassium permanganate or hydrolysis was carried out using 500 mL of 250 mM aqueous sulfuric acid. The round bottom flask was closed with a turn-over flange stopper (in order to avoid MTBE evaporation) and 20 mL of pure MTBE were injected. Reactions were performed at room temperature (20-25°C) under stirring. Then, reactions were stopped by adding one equivalent (of the initial oxidant or acid) of sodium thiosulfate (100 mL at 0.6 M) or sodium hydroxide (100 mL at 1.25 M) to the oxidation or hydrolysis mixture, respectively. Afterwards, mixtures were distilled using a spinning band distillation column to purify the TBA generated by degradation, the remaining MTBE (and the generated methanol in the case of hydrolysis). A small amount (50 µL) of the distillate was employed to determine the reaction yield using 1H NMR; reaction yields of 13.8% and 29.8% were measured for permanganate oxidation and acid hydrolysis, respectively. Then, remaining MTBE (and methanol) were eliminated from the distillate by nitrogen purging. Finally, TBA samples (containing about 35% of water) were analyzed by irm-EA-MS and irm-13C NMR.

To measure the bulk isotope fractionation with irm-MS in MTBE, both permanganate oxidation and acid hydrolysis were also performed under supposedly equal conditions but on a smaller scale than the large-volume experiments described above. Oxidation was done in a 20 mL vial where 0.3 mL of MTBE was dissolved in 15 mL of water. After equilibration of the mixture (under stirring), 1.33 g of KMnO4 were added. Acid hydrolysis was also performed in a 20 mL vial in which 0.6 mL MTBE and 0.21 mL concentrated sulfuric acid were dissolved in 15 mL water (final pH = 0.48). The two reactions were performed at room temperature. Headspace gas (0.1 mL) was sampled every hour after heating the vials at 60° C for 7 min, in order to determine 13C of the remaining MTBE.

* 1. Isotope ratio monitoring by Mass Spectrometry (irm-MS)

The bulk 13C isotopic composition (δ13Cbulk) was determined by irm-MS as described previously (Julien et al. 2015a). Determination of δ13Cbulk was performed using an Integra2 spectrometer (Sercon Instruments, Crewe, UK) linked to a Sercon elemental analyzer (EA) (Sercon Instruments, Crewe, UK) for large scale experiments. About 1 mg of each sample was weighed into tin capsules (2x5 mm, Thermo Fisher scientific) using a 10-6 g precision balance (Ohaus Discovery DV215CD) in order to ensure analysis of 0.2-0.8 mg of carbon. Bulk 13C isotope analysis of MTBE samples from headspace of 20 mL vial reactions was performed using a Delta V advantage (Thermo Fisher Scientific) connected to a Gas Chromatography (Trace 1310, Thermo Fisher Scientific) equipped with a TG-5MS column (60 m x 0.25 mm i.d., 0.25 m film thickness, Thermo Fisher Scientific) via a CONFLOW-II interface. 13Cbulk (‰) values were expressed relative to the international reference (Vienna-Pee Dee Belemnite, V-PDB) using the equation 1:

(1)

For 13Cbulk determination, the laboratory standard of glutamic acid used for irm-EA-MS and the CO2 reference gas for irm-GC-MS analyses were calibrated against the international reference material.

* 1. Isotope ratio monitoring by 13C Nuclear Magnetic Resonance spectrometry (irm-13C NMR)

The position-specific 13C isotopic composition (13Ci, where i is the corresponding carbon position) was determined as described previously (See Supporting Information for more details) (Julien et al. 2015b). The TBA sample was prepared in a 4 mL vial in which 100 µL of the lock substance (DMSO-d6) containing 50 mM of the relaxing agent Cr(Acac)3 were introduced. DMSO-d6 was chosen because of its miscibility with TBA and water and to avoid peak overlapping on the 13C spectrum between the sample and the deuterated solvent. The amount of Cr(Acac)3 was adapted according to the T1 values (longitudinal relaxation). Then, 600 µL of sample are added and, after mixing, the sample was introduced into a 5 mm NMR tube. MTBE NMR measurements were performed in the same way but with the following sample preparation: 500 µL of MTBE mixed with 200 µL of acetonitrile-d3 containing 200 mM of Cr(Acac)3.

13C NMR spectra of both MTBE and TBA were recorded using a Bruker AVANCE I 400 MHz spectrometer fitted with a 5 mm i.d. 1H/13C dual+ probe, carefully tuned at the recording frequency of 100.61 MHz. Detailed explanation of the isotopic 13C NMR protocol and 13Ci calculation is available in Supporting Information.

* 1. Determination of enrichment factors ()

For large scale degradation experiments, 13C Isotope Effects associated with both permanganate oxidation and acid hydrolysis were calculated using the t0 value and a single data point. This method of calculating the enrichment factor has already been shown to provide very similar results to the classical Rayleigh plot method which requires multiple data points (Jeannottat and Hunkeler 2012). Moreover, a recently published study has demonstrated that this “two point calculation” (t0 and one experimental point) gives the same result as a Rayleigh plot when less than 30% of MTBE has reacted (Julien et al. 2018). In order to quantify bulk and position-specific enrichment factors, the equation 2, adapted from that work, has been used in the present work:

(2)

where *f* is the reaction yield, 13CMTBE is the bulk (13CBulk) or position-specific (13Ci) isotopic composition of MTBE used as reactant in abiotic degradation experiments and 13CTBA is the bulk (13CBulk) or position-specific (13Ci) isotopic composition of TBA. When i (position-specific enrichment factor) is calculated, the 13Ci of the corresponding carbon position is used for both MTBE and TBA. In this calculation, the bulk 13CMTBE cannot be used directly in the calculation but the average value of the quaternary carbon and the methyl groups of MTBE (corresponding to the carbon atoms of the reaction product, TBA see Figure 1) need to be used in order to calculate the bulk IE associated with the generation of TBA from MTBE. When  is negative, the IE is considered as normal (light isotopologues are preferentially used during the process) and when ε is positive, the IE is inverse (transformation is faster for heavy isotopologues).

In the case of smaller scale experiments, 13C of MTBE was monitored using irm-GC-IRMS. In this context, Rayleigh plots (1000\*ln(+1000)/(0+1000)) vs ln(*f*)) were drawn and  corresponds to the slope of the regression curve (see Figure S3 in the Supporting Information).

Finally, in order to determine the significance threshold of the measured enrichment factors, the expanded uncertainty (U) has been calculated. The calculation of U associated with  takes into account all sources of uncertainty generated by weighing of compounds or the measurement of the reaction yield. While most studies only use the standard deviation (SD) of the isotopic measurement, U gives a better assessment of accuracy. The calculation of U has been fully described in a previous study (Julien et al. 2018) and results are expressed as  ± U in this article (note that results from cited articles are expressed as  or 13C ± 1SD).

2.6. Position-specific modeling of isotope ratiosA model for the temporal evolution of the isotope ratio at each specific position during the reaction of MTBE was developed based on equations which were published previously (Höhener et al, 2017). The model was modified in the sense that no transport in a groundwater system is included, but it was extended in order to include the isotope evolution of the formed products as well. The model is an Excel spreadsheet and is provided in Appendix A. A detailed description of all model equations is included in this spreadsheet.

1. **Results and discussion**
   1. Reaction rates and bulk isotope analysis

The change in concentration of MTBE for both abiotic transformation reactions followed a first-order type kinetics, with half-life times of 0.33 and 5.7 per day for permanganate oxidation and hydrolysis, respectively. These rates are similar to previously published rates (Elsner et al., 2007; Gauchotte et al., 2009). The monitoring of the bulk MTBE 13C isotope enrichment allowed  values of -4.9 ± 0.2‰ and -6.4 ± 0.6‰ (see Table 1) to be obtained for permanganate oxidation and acid hydrolysis, respectively, which is also in accordance with previous data found in the literature. In previous studies, Rosell et al. 2012 described  values of -5.5 ± 0.1‰ and -6.1 ± 0.1‰ for permanganate oxidation and acid hydrolysis respectively, Elsner et al. 2007 measured  of -4.9 ± 0.2‰ associated with acid hydrolysis and Gauchotte et al. 2010 determined  of -4.9 ± 0.6‰ in the case of permanganate oxidation (see Table 1).

In the present study, the bulk isotope effect was also monitored using the product of the reaction, TBA. The present study shows that large scale permanganate oxidation and acid hydrolysis are both associated with a normal bulk 13C IE according to their  values of -1.9 ± 0.7‰ and -3.8 ± 0.7‰ obtained from the product TBA, respectively (see Table 1). Note that most isotope fractionation studies found in literature are performed analyzing the remaining substrate, but in this study the reaction product was also analyzed. However, it must be noted that TBA is not the only product (the methyl carbon left) and therefore it is logical that the value for the isotope effect obtained from the product does not relate directly to the value obtained from the substrate.

Nonetheless, the results all agree that the abiotic degradation velocity is higher with light MTBE isotopologues (molecules containing 12C isotope) (Bigeleisen 1949), in accordance with the principles of reaction rate theory in the presence of heavy isotopes (Haring 1942). Since a 13C–12C bond is shorter than a 12C–12C bond and the vibrational energy of shorter bonds is smaller (lower zero point energy), the activation energy needed to break a 13C–12C bond is higher than for a 12C–12C bond. In the conditions studied, abiotic degradation of MTBE apparently preferentially uses light substrate, as normal IEs are detected.

* 1. Position-specific isotope analysis – oxidation

In the present work, PSIA performed using isotopic 13C NMR showed an unbalanced distribution of normal IEs associated with MTBE oxidation by potassium permanganate. Despite a relatively small 13CBulk it is found that this abiotic degradation process is associated with a strong normal IE on the quaternary carbon ( = -10.1 ± 0.7‰) and negligible IEs on the methyl groups ( = +0.8 ± 0.7‰, see Table 1). The degradation mechanism of oxidation of MTBE by potassium permanganate has been described previously (for a detailed description, see Figure 2) (Damm et al. 2002; Elsner et al. 2007) and it is considered that it is initiated by H abstraction from the methyl of the methoxy group, leading to oxidation and bond breakage via an SN2 reaction mechanism. This reaction scheme is compatible with both the observed high secondary normal IE on the quaternary carbon, which is in  position to the broken bond, and the absence of significant IE on the methyl groups, which are inert during the degradation mechanism. This theory is confirmed by results of the present study and those from Gauchotte *et al*. (2009) who determined the 13C isotopic fractionation (13C) occurring during MTBE oxidation using pyrolysis coupled with GC-C-irm-MS. This method allowed the observation of the isotopic composition of the methoxy group and an average value of the central carbon and methyl positions of MTBE. These experiments showed a large 13C enrichment in the methoxy group and no significant change in 13C isotopic composition of other carbon positions of the remaining MTBE. Results from these studies demonstrate that the bond breaking (between the oxygen atom and the methoxy group) occurring during permanganate oxidation of MTBE is associated with a primary normal 13C IE on the methoxy group and a strong secondary normal 13C IE on the quaternary carbon position.

* 1. Position-specific isotope analysis – hydrolysis

In the case of acid hydrolysis, irm-13C NMR analysis indicates a very different distribution of the 13Ci values contributing to the observed 13CBulk normal IE to that of permanganate oxidation. Acid hydrolysis catalyzed by sulfuric acid is associated with a normal IE distributed between the quaternary carbon ( = -3.2 ± 0.7‰) and the methyl groups ( = -4.0 ± 0.7‰), see Table 1. Measuring different PSIEs is not surprising, given that the mechanism of reaction is considered to be very different from that of permanganate oxidation (see Figure 2). In the hydrolysis reaction, the bond between the oxygen atom and the quaternary carbon is broken, which fits with the observed large primary 13C IE located at this carbon position. Elsner *et al*. (2007) demonstrated that acid hydrolysis is associated with a large normal IE and calculated an  value for the central carbon position ( = -24.3 ± 2.3‰), assuming that all other carbons do not enrich (Table 1) (Elsner et al. 2007). The most surprising result here is the presence of secondary normal IEs located on the methyl groups, however, because this part of the MTBE structure is supposed not to be involved in the initial bond breaking reaction. Our results show that the effect on the reactive position is smaller, as assumed by Elsner and co-workers. However, acid hydrolysis is an SN1 reaction, with a carbocation intermediate (see Figure 2) and the normal IE located on the methyl groups can be proposed as due to the stabilization process of this intermediate state. As a consequence, the presence of a 13C isotope on methyl groups could affect the stability of the carbocation intermediate and aid these isotopomers to react faster during bond breaking between the quaternary carbon and the oxygen atom.

As explained above (section 3.1.), 13C–12C bonds are more difficult to break than 12C–12C, because chemical bonds involving heavy isotopes have a lower zero-point energy and the activation energy required for bond breaking is greater, so heavy isotopomers are more stable than light ones. According to this convention, the presence of a 13C isotope at the methyl positions of MTBE molecules could play a role in the stabilization of the transition-state (carbocation) during the acid hydrolysis of MTBE (Figure 2). As a consequence, MTBE molecules with 12C on their methyl positions (light isotopomers) should react faster, while heavy isotopomers form more stable carbocation intermediates. This carbocation stability difference can explain the detection of a normal secondary isotope effect on these carbon positions. This precept has already been described in the case of enzyme catalytic activity in which the presence of heavy isotopes influences the stability of the transition-state and is directly responsible for the IEs associated with enzyme activity (Schramm 1998).

* 1. Modeling MTBE abiotic degradation

Bulk carbon isotopes are employed for (i) detecting the origin of organic contaminants, (ii) monitoring their natural or assisted remediation until their complete mineralization and (iii) understanding the mechanisms involved. The use of PSIA has already proved to be an efficient tool to determine the origin of organic contaminants such as VOCs (Julien et al. 2016) or pharmaceuticals (Silvestre et al. 2009). This method offers more parameters to identify the origin of detected pollutants compared with bulk isotope analysis, which only allows one 13C value to be determined. Moreover, PSIA has also proved its capability to detect unexpected intramolecular IEs associated with VOCs evaporation (Julien et al. 2015b, 2015a) and, more generally, effects caused by the presence of non-covalent interactions (Botosoa et al. 2008; Julien et al. 2017).

In the present study, PSIEs associated with two abiotic degradation reactions (oxidation and acid hydrolysis) of MTBE were measured. These new data strengthen our fundamental understanding of these processes by validating the two different mechanisms previously proposed in the literature (Elsner et al. 2007). A highlight of this study is the construction of a model capable of predicting isotope ratios of MTBE carbon positions as a function of time during abiotic degradation (here, acid hydrolysis or oxidation). This new model requires the measurement of the initial 13C distribution in MTBE and the PSIEs measured in this article (see blue boxes in Appendix A). The initial MTBE concentration, the degradation half time and the duration of reaction can be adjusted in the Excel worksheet. Figure 3 presents the evolution of 13C on both degraded MTBE and formed TBA carbon positions. The development of this model can help predicting the isotope changes in MTBE along a contaminant plume of gasoline in groundwater or soil using the 3-dimensional transport code BIOSCREEN-AT-ISO (Höhener et al. 2017).

1. **Conclusions**

PSIA performed using irm-13C NMR is still considered as difficult to apply for field investigations because of the amount of sample needed for each analysis. However, it has recently been shown that PSIA by NMR can be performed with less than 50 mg, and analysis of samples of the order of 10 mg is in advanced development (Joubert et al. 2018). Hence, PSIA by 13C NMR targeting TBA could be an excellent tool for mechanism elucidation and modeling of natural processes, such as environmental contaminant remediation. PSIA is shown to give a more detailed picture of the isotopic fractionation during abiotic remediation processes, distinguishing which isotopomers are preferentially involved in the reaction system. The determination of position-specific isotopic composition provides a more detailed dataset, crucially, one that indicates which mechanism of reaction was involved in the degradation. Thus, it can act as a useful indicator of which remediation processes may be dominant during the remediation of an organic pollutant, such as MTBE. As already explained, permanganate oxidation and acid hydrolysis of MTBE are both associated with a similar normal bulk IE, which does not give access to detail of the observed abiotic degradation process. In contrast, PSIA shows a large variation of the IE distribution between the two studied reactions, which means that the different processes involved in remediation can be distinguished by using the measured intramolecular 13C isotope signatures, thus offering a new tool to monitor contaminant removal. Intramolecular IEs measured *in vitro* using irm-13C NMR combined with designed models predicting the evolution of 13C distribution in MTBE and its degradation products (see attached Excel worksheet) is a new efficient tool for monitoring soil contamination by gasoline-derived pollutants.

Complementary investigations are now required to underpin the interpretation of these observed position-specific IEs in terms of the proposed reactions mechanisms. The determination of 18O IEs would be of interest, as these should differ considerably between the permanganate oxidation and acid hydrolysis, because the oxygen atom of TBA comes from MTBE in the case of oxidation while it is added from water during acid hydrolysis (Figure 2). This, however, requires purifying the TBA from water without introducing isotopic fractionation. Theoretical calculations should also help to relate the observed IEs to mechanism (Wolfsberg et al. 2009), but these have proved challenging and are beyond the scope of the present report. It is evident that PSIA gives new clues about environmental contaminant removal and can be used for modeling, as already shown in the case of tetrachloroethene (Höhener and Atteia 2014). The constant improvement in terms of sensitivity, precision and complexity of analyzed structures of PSIA techniques, including more sensitive irm-13C NMR (Jézéquel et al. 2017), Py-GC-C-irm-MS (Gilbert et al. 2016) and the recent proof of concept for high resolution MS (Eiler et al. 2017) assure that PSIA can be an excellent tool for the future of monitoring of organic contaminant remediation.

**Appendix A. Model of permanganate oxidation and acid hydrolysis of MTBE**

**Appendix B. Supplementary material**

**Acknowledgements**

This work is funded by the French National Research Agency ANR, project ISOTO-POL funded by the program CESA (no. 009 01). M.J. thanks the ANR for funding his PhD bursary through this project and JSPS for funding his postdoctoral fellowship. We thank Dr. Martin Elsner for his help in understanding reaction mechanisms.

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**Figures and Tables**



**Figure 1:** The molecular structure of Methyl *tert*-Butyl Ether and *tert*-Butanol with the carbon atoms numbered in relation to decreasing chemical shift in the 13C NMR spectrum and their corresponding chemical functions.



**Table 1:** Comparison of enrichment factors () associated with permanganate oxidation and acid hydrolysis of MTBE determined in this study and values found in literature (C(IV) corresponds to the quaternary carbon of MTBE). Position-specific isotope data from Gauchotte et al. 201033 are expressed as isotopic fractionation (13C), so they are detailed in a separated table below.

a: Calculated through mass balance.

b: Theoretical value if no secondary isotope effects on other carbons.



**Figure 2:** Proposed reaction mechanisms of MTBE degradation during (a) potassium permanganate oxidation and (b) acid hydrolysis. The symbol “-“ indicates the carbon positions where a significant normal isotope effect was measured.



**Figure 3:** Evolution of 13C as a function of time during permanganate oxidation (a: MTBE; b: reaction products) and acid hydrolysis (c: MTBE; d: reaction products). The calculations were carried out using the model developed in the present study.