Stable isotope ratios as a tool to assess biodegradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA)

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Abstract The aim of this study was to evaluate if compound-specific isotope analysis can be used to assess biodegradation of MTBE. The effect of aerobic biodegradation on carbon isotope ratios of MTBE was evaluated using microcosms with MTBE as the only substrate and cometabolic microcosms with 3-methylpentane as the primary substrate. In both types of microcosm, the δ13C of MTBE steadily increased as degradation proceeded while no change in δ13C occurred in control experiments. After >95% degradation, a shift in δ13C of 5.1 to 6.9% was observed. The δ13C of TBA, which accumulated transiently in the cometabolic microcosm, increased by 4.3% confirming that TBA was also degraded. This study suggests that carbon isotope analysis is a potential tool for tracing in situ biodegradation of MTBE and TBA, and thus to aid better understanding of the fate of these contaminants in the environment.

Key words biodegradation; isotope fractionation; MTBE; stable isotopes; TBA

INTRODUCTION

In North America and Europe, methyl tert-butyl ether (MTBE) has been added to gasoline as an octanol enhancer and/or to improve air quality. In the last decade, MTBE has increasingly been detected in urban air, surface water, and shallow groundwater (Squillace et al., 1996, 1999). MTBE affects the quality of drinking water due to its strong taste and odour and its possible carcinogenic effects. Since MTBE only sorbs weakly on solids and appears difficult to biodegrade (Squillace et al., 1999), it can travel nearly unretarded in groundwater and long plumes of dissolved MTBE are frequently observed (Einarson et al., 1999; Landmeyer et al., 1998). In several laboratory studies, biodegradation of MTBE by aerobic bacteria has been observed, while in others, MTBE was reported to be recalcitrant, in particular under anaerobic conditions. At field sites, MTBE degradation is often difficult to prove using concentration data or a mass balance approach since MTBE plumes are frequently long and degradation rates are low. The aim of this study was to evaluate if carbon isotope ratios of MTBE can be used as a tool to trace biodegradation of MTBE in aquifers. The isotope method is based on the frequent occurrence of differences in reaction rates between molecules with light and heavy isotopes of an element. As a result, the degradation products are usually depleted in the heavy isotopes while the substrate becomes increasingly enriched in the heavy isotopes (isotope fractionation). The method has previously been applied to investigate biodegradation of chlorinated solvents (Bloom et al., 2000; Hunkeler & Aravena, 2000; Hunkeler et al., 1999; Sherwood Lollar et al.,
Fig. 1 Left: microcosm enrichment with MTBE as the only substrate (▲ MTBE, △ TBA) and sterile control (● MTBE) Right: microcosm with MTBE and 3-methylpentane as co-substrate (▲ MTBE, △ TBA) and microcosm with MTBE as only substrate (● MTBE). Modified from Hunkeler et al., 2001b.

1999) and BTEX (Hunkeler et al., 2001a; Meckenstock et al., 1999; Ward et al., 2000). In this study, carbon isotope fractionation during aerobic biodegradation of MTBE was investigated. The biodegradation studies included microcosms amended with MTBE and microcosms with MTBE and 3-methylpentane as co-substrate.

MATERIAL AND METHODS

The biodegradation studies were performed using aerobic microcosm enrichments that degrade MTBE as only substrate and alkane-degrading microcosms that cometabolize MTBE. The microcosm enrichments were based on MTBE-degrading aquifer microcosms that had been prepared using aquifer sediments and groundwater from the Borden aquifer, a shallow, sandy unconfined aquifer (located near Alliston, Ontario, Canada), to assess the potential for MTBE biodegradation at the site. In some of the original microcosms that had been amended with MTBE only, MTBE degradation was observed after >200 days. The microcosms in which MTBE degradation occurred were split into second and third generation microcosms and replenished several times with MTBE. These microcosms are denoted as microcosm enrichments since repeated addition of MTBE likely increased the population of MTBE degraders. Cometabolic microcosms were prepared with Borden aquifer material never exposed to MTBE or 3-methylpentane, the alkane chosen for use as the primary substrate. To confirm that in these fresh microcosms degradation occurred only in the presence of a co-substrate, microcosms with MTBE and no co-substrate were also prepared. Data of one of each type of microcosm are shown since a similar behaviour was observed in repeat microcosms (Fig. 1). Concentrations and isotope ratios of MTBE and TBA were analysed as described in detail in Hunkeler et al. (2001b).
RESULTS
In the microcosm enrichment with MTBE as the only substrate, 12 mg l\(^{-1}\) MTBE were degraded within 10 days and no accumulation of TBA was observed (Fig. 1). The \(\delta^{13}C\) of MTBE steadily increased from \(-29.6\) to \(-23.6\)\%. In sterile controls, no change in the concentration and \(\delta^{13}C\) of MTBE occurred. In the cometabolic microcosm, degradation of MTBE took longer and TBA accumulated transiently (Fig. 1). After day 8, the sum of the MTBE and TBA concentration started to decrease, indicating that TBA consumption started. The \(\delta^{13}C\) of MTBE increased from \(-29.4\) to \(-22.8\)\%, similarly as in the microcosm enrichment. Initially, TBA was slightly enriched in \(^{13}C\) compared to MTBE. After day 15, the \(\delta^{13}C\) of TBA steadily increased confirming that TBA was degraded. In the co-substrate-free microcosm, no significant change in the concentration and \(\delta^{13}C\) of MTBE was observed.

Isotope fractionation was quantified using the simplified Rayleigh equation which applies for enrichment factors \(|e|<20\%\) (Mariotti et al., 1981):

\[
\delta^{13}C_{S} = \delta^{13}C_{S,0} + e \cdot \ln f
\]

where \(\delta^{13}C_{S,0}\) is the initial carbon isotope ratio of the substrate, \(\delta^{13}C_{S}\) is the isotope ratio of a remaining fraction \(f\) of substrate, and \(e\) is the isotopic enrichment factor. For the microcosm enrichments with MTBE as only substrate, an enrichment factor of \(-1.84\%\) was found and for the cometabolic microcosm an enrichment factor of \(-1.52\%\).

DISCUSSION AND CONCLUSIONS
During biodegradation, the \(\delta^{13}C\) of MTBE and TBA significantly increased in all experiments, which demonstrates that reaction rates are slightly faster for molecules with \(^{12}C\) than for molecules with \(^{13}C\). A similar observation has previously been made for oxidation of dichloromethane (Heraty et al., 1999), toluene (Meckenstock et al., 1999), benzene (Hunkeler et al., 2001a) and 1,2-dichloroethane (Hunkeler & Aravena, 2000), and for reductive dechlorination of chlorinated ethenes (Bloom et al., 2000; Hunkeler et al., 1999). The enrichment factors for MTBE degradation were similar for microcosm enrichments with MTBE as the only substrate and the cometabolic microcosm, which may be due to a similarity in the enzymatic mechanism used by the bacteria for initial transformation of MTBE. They were in the same range as enrichment factors obtained for aerobic and anaerobic oxidation of benzene and toluene (Hunkeler et al., 2001a; Meckenstock et al., 1999), and smaller than the enrichment factors observed for biodegradation of chlorinated solvents (Bloom et al., 2000; Heraty et al., 1999; Hunkeler et al., 1999; Sherwood Lollar et al., 1999).

Stable isotope analysis can potentially be used to trace biodegradation of organic contaminants at field sites, if isotope fractionation associated with biodegradation is much larger than isotope fractionation associated with physical processes. Important physical processes that affect concentrations of MTBE in groundwater are dissolution of MTBE from gasoline, and advective and dispersive transport. Sorption of MTBE to aquifer solids and volatilization from the aqueous phase are of minor importance due to the low organic carbon/water partitioning and the Henry’s coefficient of MTBE (Pankow et al., 1997). For TBA originating from MTBE degradation, advective and dispersive transport are probably the most important physical processes influencing its concent-
ration. Advection and dispersion are not expected to significantly affect the isotope ratios of dissolved compounds. Previous studies have shown that dissolution of MTBE from an organic phase is not accompanied by carbon isotope fractionation, at least not under equilibrium conditions (Hunkeler et al., 2001b). Thus, physical processes are not expected to lead to significant changes of the $\delta^{13}C$ of dissolved MTBE and TBA. In contrast, biodegradation of MTBE and TBA is accompanied by a systematic shift of the $\delta^{13}C$ values. For MTBE a significant shift (2 times uncertainty of measurement) occurs after 33% degradation, for TBA after 13% degradation, based on the isotopic enrichment factors determined in this study. The sensitivity of the method may further be increased by a combined measurement of C and H isotopes. Isotope fractionation is often larger for H than for C isotopes due to the larger relative mass difference. Studies on hydrogen isotope fractionation during MTBE degradation and field studies are in progress.

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REFERENCES


