Environ. Sci. Technol. 1994, 28, 1727-1732

59063

Anaerobic Biodegradation of Gasoline Oxygenates: Extrapolation of Information to Multiple Sites and Redox Conditions

Melanie R. Mormile, Shi Liu, and Joseph M. Suflita*

Department of Botany and Microbiology, The University of Oklahoma, Norman, Oklahoma 73019

A series of alcohol, ketone, ester, and ether oxygenates were tested for their susceptibility to anaerobic decay in samples from four chronically contaminated sedimentary environments. The effect of various electron acceptors on oxygenate biodegradation was also evaluated with a single inoculum source. In addition, two acetogenic bacteria were tested for their ability to metabolize selected oxygenate compounds. The susceptibility of the test oxygenates to anaerobic decay could be related to their chemical structure. That is, compounds other than the ethers that possessed primary or secondary substituted carbon atoms were readily degraded under all conditions tested while compounds that had tertiary substituted carbon atoms resisted biodegradation. The ether oxygenates were generally not degraded when incubated with various inocula, regardless of the electron acceptor status. The exceptions included methyl butyl ether, which was depleted in both sulfate-reducing and methanogenic incubations, and the partial transformation of methyl *tert*-butyl ether to *tert*-butanol after a 152-day acclimation period in a single replicate from a river sediment chronically contaminated with fuel. Heat-inactivated control incubations suggested that the latter transformation was biologically catalyzed. This study provides a basis for assessing the environmental fate characteristics of the frequently used gasoline oxygenates as well as several potential alternate fuel additives.

Introduction

Oxygenates are organic compounds designed to increase the oxygen and octane content of gasoline. In an effort to reduce the environmental consequences associated with fuel combustion, provisions of the 1990 Clean Air Act Amendments mandate that oxygenates be seasonally added to gasoline in specific parts of the country (*1*, *2*). Currently, the most commonly used oxygenates are methyl *tert*-butyl ether (MTBE) and ethanol. Others in use include ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), isopropyl ether, and *tert*-butanol, although only relatively minor amounts of these are used in gasoline. MTBE is by far the most popular ether oxygenate due to its low-cost, ease of production, and transfer and blending characteristics. The domestic production of MTBE in 1993 was 8.98 billion kg (*3*), second in rank among all organic chemicals manufactured in the United States.

Questions have been raised about the environmental effectiveness of oxygenate blending programs (*4*). Moreover, the atmosphere and the internal combustion engine are not the only compartments where additives are released. Because of the large volumes of gasoline used daily in the United States (~300 million gal (*5*)) and the huge storage capacity and transportation system required to provide this fuel to end users, surface and subsurface releases are likely to occur, and the impact of oxygenates in recipient environments needs to be assessed. As oxygenates are much more water soluble than other gasoline components, they easily migrate to groundwater reserves when spilled. Once contaminated with MTBE, groundwater treatment is more complex and expensive (*6*).

As anaerobic conditions rapidly develop in hydrocarbon-contaminated aquifers (*7*), we investigated the fate of oxygenates under anaerobic conditions. Previously, we found that most of the common oxygenates resisted anaerobic decay in methanogenic aquifer samples taken from a single location (*8*). A few studies attest to the persistence of MTBE under aerobic conditions (*9*–*11*). We questioned whether our earlier findings could be extrapolated to other ecosystems with a history of exposure to fuel hydrocarbons and other contaminants and whether oxygenates could be anaerobically biodegraded when alternate terminal electron acceptors were made available to the resident microflora. We also examined the ability of two acetogens, *Acetobacterium woodii* and *Eubacterium limosum*, known to metabolize a variety of methyl ethers (*12*, *13*) to catalyze the destruction of several oxygenates. Our current study indicates that our previous results can largely be extrapolated to other environments and redox conditions.

Materials and Methods

Sample Collection. Sediment and groundwater were collected from a landfill leachate impacted aquifer as previously described (*14*) and from a chronically gasoline-polluted aquifer in Empire, MI (*15*). Sediment and surface water were also collected from the Ohio River and Mill Creek, Cincinnati, OH, using an Ekman dredge. The sampling location on the Ohio River was impacted by a number of oil storage and barge loading facilities, while the Mill Creek samples received both industrial waste and domestic sewage sludge (*16*).

Incubation Conditions. Slurries were prepared by placing 50 g of sediment and 75 mL of water into sterile 160-mL serum bottles as previously described (*8*). Groundwater or surface water was amended with sodium sulfide (1 mM) and resazurin (0.0002%) to serve as reductant and redox indicator, respectively. The bottles were sealed with composite stoppers constructed by fusing the bottom portions of gray Teflon-coated septa (West Co., Phoenixville, PA) to the top portions of 1-cm-thick black rubber stoppers (GeoMicrobial Technologies, Ochelata, OK). The headspace above the slurries was adjusted to 80% N_2: 20% CO_2 (1 atm). Either sodium sulfate (5 mM) or sodium nitrate (8 mM) was added to the landfill leachate impacted samples in order to assess potential oxygenate decay coupled with the consumption of these electron acceptors. These incubations are referred to as sulfate-reducing or nitrate-reducing incubations, respectively. Each oxygenate was added to the slurries to give an initial concentration

* Corresponding author.

0013-936X/94/0928-1727$04.50/0

Table 1. Anaerobic Biodegradation of Select Oxygenates under Sulfate- and Nitrate-Reducing Conditions[a]

		sulfate-reducing[c]			nitrate-reducing[d]		
oxygenate	RON[b]	substrate loss (%)	SO_4 consumed (% theoretical)	rate ± SD[e] (μmol of SO_4/day)	substrate loss (%)	NO_3 consumed (% theoretical)	rate ± SD[e] (μmol of NO_3/day)
methanol	133	100	17	1.15 ± 0.12	100	120	71.2 ± 4.9
methyl ethyl ketone	116	100	101	1.44 ± 0.48	100	133	38.6 ± 20.7
acetone	115	100	76	6.72 ± 9.6	100	121	60.9 ± 2.4
methyl isobutyl ketone	108	100	4	1.64 ± 0.15	87	60	5.0 ± 6.0
ethyl acetate	117	100	104	3.35 ± 0.90	100	153	82.7 ± 41.8
methyl butyrate	110	100	85	1.05 ± 0.07	100	126	29.6 ± 15.5
methyl *tert*-butyl ether	118	0	4	0.04 ± 0.19	7	2	1.7 ± 0.3
methyl *tert*-amyl ether	111	0	0	0	0	0	0
ethyl *tert*-butyl ether	118	0	0	0	0	0	0
ethyl ether	UNKN[f]	0	0	0	0	17	1.3 ± 1.5
propyl ether	UNKN	0	0	0	0	0	0
butyl ether	UNKN	0	0	0	0	0	0
butyl ethyl ether	UNKN	0	0	0	0	5	1.0 ± 2.0
methyl butyl ether	UNKN	87	0	0	0	15	1.7 ± 0.6

[a] The values reported reflect the average measured in triplicate incubations. [b] Research octane number from ref 8. [c] Incubated for 244 days. [d] Incubated for 85 days. [e] Corrected for the rate of anion depletion in oxygenated-unamended controls. [f] UNKN = unknown.

of 50 ppm C. The rates of methane production, sulfate reduction, and nitrate depletion were monitored in slurries receiving oxygenates and compared to oxygenate-free controls. All slurries were incubated in the dark at 24 °C.

Pure Culture Studies. Stock cultures of *A. woodii* (ATCC 29683) and *E. limosum* (ATCC 8486) were grown in defined medium (*17*) with syringate as the sole organic carbon source under a 80% H_2:20% CO_2 (2 atm) headspace. The syringate-grown cells were harvested and suspended in 15 mL of *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (10 mM, pH 7.8) containing a select oxygenate (2 mM) under 11 mL of a 80% H_2:20% CO_2 or 80% N_2:20% CO_2 (2 atm) headspace. The initial protein concentrations of the cell suspensions (BioRad DC protein assay, BioRad Laboratories, Richmond, CA) were 1630 and 870 μg of protein/mL for *A. woodii* and *E. limosum*, respectively. The cell suspensions were incubated for 55 days in the dark at 30 °C.

Chemical Analysis. In methanogenic incubations, increases in headspace pressure were routinely monitored with a pressure transducer system (*18*). However, parent compound depletion and the formation of methane were always confirmed by gas chromatography as previously described (*8*). The net amount of methane formed over oxygenate-free controls was compared to that theoretically expected based on Buswell's equation (*19*). The net amount of sulfate and nitrate depletion over oxygenate-free controls was monitored by periodically withdrawing 1-mL aliquots of the slurries and storing them at −20 °C until analysis by high-pressure liquid chromatography (HPLC) as previously described (*14*). The amount of sulfate or nitrate depleted in the slurries was compared to that theoretically required to completely oxidize the compounds to CO_2 with the formation of hydrogen sulfide or N_2 gas. The acclimation period was estimated as the amount of time where no significant pressure or anion concentration differences were measured between substrate-amended and unamended controls. The linear rate of substrate or anion depletion was calculated from the time period immediately following the acclimation period. The identity of the MTBE degradation product, *tert*-butanol, was confirmed using a Hewlett Packard 5890A GC equipped with a 19251A/B split/splitless capillary inlet, Carbograph VOC capillary column, and a flame ionization detector. Helium served as the carrier gas with a flow rate of 1 mL/min. The split ratio was 1:50. The temperature profile was 250 °C for both the injection port and detector, and the column temperature was held at 30 °C.

Results

Biodegradation of Oxygenates with Sulfate or Nitrate Available as Electron Acceptors. We previously studied the biodegradation of numerous oxygenates using samples obtained from a landfill leachate impacted aquifer under methanogenic conditions (*8*). In this study, we examined the fate of selected oxygenates using comparable aquifer slurries amended with sulfate or nitrate. The results of this assay are summarized in Table 1.

Methanol has a relatively high research octane number and was biodegraded in the sulfate-amended incubation as evidenced by the removal of the alcohol to below detection limits following a 244-day incubation period. However, this loss was not coupled with sulfate reduction (Table 1). Instead, approximately 70% of the theoretically expected amount of methane was recovered from these incubations (data not shown), consistent with previous findings (*14*). In contrast, in nitrate-amended aquifer slurries, electron acceptor loss was accompanied by the depletion of methanol from the incubation mixture (Table 1). Methane generation was not evident in these experiments. Based on these criteria, methanol was mineralized under both incubation conditions.

The ketones assayed are not used as fuel additives but are common groundwater contaminants (*20*). Each ketone studied has an octane rating that is comparable to the more common oxygenates (Table 1). In methyl ethyl ketone-amended and acetone-amended aquifer slurries, 100% and 76% of the theoretically expected amount of sulfate was depleted, respectively, and each substrate was not detected after 244 days of incubation (Table 1). However, while methyl isobutyl ketone was removed to below detection limits in a comparable time period, only 4% of the theoretically expected amount of sulfate was utilized. Further, there was no conclusive evidence for increased methane production in these incubations. Since

Table 2. Anaerobic Biodegradation of Select Oxygenates under Methanogenic Conditions[a]

oxygenate	acclimation period (days)			rate ± SD (ppm C/day)			methane recovery (% expected)[b]		
	G[c]	R	IS	G	R	IS	G	R	IS
2-propanol	nt[d]	7–14	0–7	nt	2.4 ± 0.3	3.0 ± 0.1	nt	82	91
tert-butanol	>230	>180	>180	0	0	0	0	0	0
methyl ethyl ketone	nt	7–14	0–7	nt	2.1 ± 0.4	2.9 ± 0.2	nt	73	67
methyl *tert*-butyl ether	>230	>180[e]	>180	0	0	0	0	3	8
methyl butyl ether	105	14–32	14–32	1.0 ± 0.1	1.2 ± 0.8	0.9 ± 0.3	71	66	59
methyl *tert*-amyl ether	>230	>180	>180	0	0	0	0	1	0
ethyl *tert*-butyl ether	>230	>180	>180	0	0	0	0	0	0
isopropyl ether	nt	>180	>180	nt	0	0	nt	0	6
diethyl ether	nt	>180	>180	nt	0	0	nt	0	0

[a] The data reported reflect the average measured in triplicate incubations. [b] Based on Buswell equation. [c] G denotes gasoline impacted aquifer, R denotes fuel impacted river sediment, IS denotes industrial and sewage impacted creek sediments. [d] Not tested. [e] One replicate started to degrade MTBE at day 152 at a rate of 0.51 ppm C/day.

we could not correlate the consumption of methyl isobutyl ketone with the depletion of an electron acceptor or the formation of an end product, evidence for the biodegradation of this compound under sulfate-reducing conditions must be considered preliminary, and further experimentation is required to clarify its fate. In contrast, methyl ethyl ketone and acetone were completely depleted in nitrate-amended slurries, and >100% of the theoretically expected amounts of nitrate was consumed after 85 days of incubation. However, 60% of the theoretically expected amount of nitrate was depleted in aquifer slurries amended with methyl isobutyl ketone (Table 1), and the rate of nitrate depletion was an order of magnitude slower than the simpler ketones while trace amounts of the secondary ketone remained after 85 days of incubation.

The two test esters also possessed comparable octane ratings to the common oxygenates and were easily removed in the presence of either nitrate or sulfate (Table 1). Methyl butyrate-amended slurries exhibited substrate loss and net electron acceptor consumption at close to theoretically expected values while ethyl acetate-amended slurries consumed 100% and 150% of the theoretically expected amount of sulfate and nitrate, respectively. The reasons for the large consumption of nitrate over expected amounts when ethyl acetate was tested are unknown.

Unlike the other test oxygenates, there was no evidence for the degradation of the commonly used ether oxygenates (MTBE, ETBE, or TAME) under either nitrate- or sulfate-reducing conditions. The only ether to show any evidence of depletion was methyl butyl ether. An 87% loss of the parent substrate was observed when sulfate was available as an electron acceptor, but no loss was evident in the nitrate-amended incubations (Table 1). However, sulfate was not appreciably consumed above the oxygenate-unamended controls, and no significant increase in the net amount of methane was measured in the headspace of these incubations. Like the methyl isobutyl ketone, the ultimate fate of methyl butyl ether has yet to be determined.

Methanogenic Biodegradation of Oxygenates in Various Environmental Samples. We sought to determine if information on the methanogenic biodegradation of oxygenates established with samples from a landfill leachate impacted aquifer (*8*) could be extrapolated to other environments. An assay for the biodegradation of selected alcohol, ketone, and ether oxygenates was conducted with samples from several chronically contaminated ecosystems.

Results obtained were similar to the earlier findings (*8*) in that a simple alcohol, 2-propanol, was readily mineralized to CH_4 and CO_2 while no evidence for the biodegradation of the structurally more complex *tert*-butanol was evident (Table 2). Similarly, methyl ethyl ketone was degraded as evidenced by both substrate loss and the recovery of approximately 70% of the theoretically expected amount of methane.

As in our previous investigation (*8*), the ethers generally proved resistant to biodegradation under methanogenic conditions (Table 2). No evidence for substrate loss or methane formation over substrate-unamended controls could be obtained with five of the six ether oxygenates after 90 days of incubation. The exception was methyl butyl ether. With this compound, degradation was evidenced by both substrate loss and the recovery of approximately 65% of the theoretically expected amount of methane. The conversion of methyl butyl ether to methane is also consistent with our earlier findings (*8*). Shorter acclimation times were measured when methyl butyl ether was incubated in river or creek sediment slurries as compared to the gasoline-contaminated (Table 2) and landfill leachate impacted aquifer samples (*8*).

After 152 days of incubation, one of the triplicate slurries prepared from the Ohio River samples had a 26 ppm C decrease in the amount of MTBE (48 to 22 ppm C) (Figure 1). An additional peak that co-chromatographed with a known standard of *tert*-butanol was observed. The size of the *tert*-butanol peak accounted for the stoichiometric loss of the MTBE. The pH of this slurry was 7.0–7.2, and thus it is unlikely that the MTBE was abiotically transformed as has been reported for acidic solutions (*21*). With continued incubation, MTBE was further depleted and exhibited a concomitant increase in *tert*-butanol. To provide additional evidence that MTBE was transformed biotically, 5 mL of aquifer slurry was placed in two sterile anaerobic culture tubes. In an effort to inactivate biologically catalyzed reactions, one tube was placed in a boiling water bath for 5 min. The amount of MTBE was measured with time in each tube. After 21 days, the slurry that had been boiled showed no depletion of MTBE nor an increase in *tert*-butanol. The non-inactivated slurry showed a decrease in MTBE and an increase in the *tert*-butanol peak. The identification of *tert*-butanol from MTBE by two different chromatographic techniques suggested that the later was O-demethylated and converted to the corresponding alcohol by the microorganisms in this replicate. However, the fate of the methyl group of MTBE

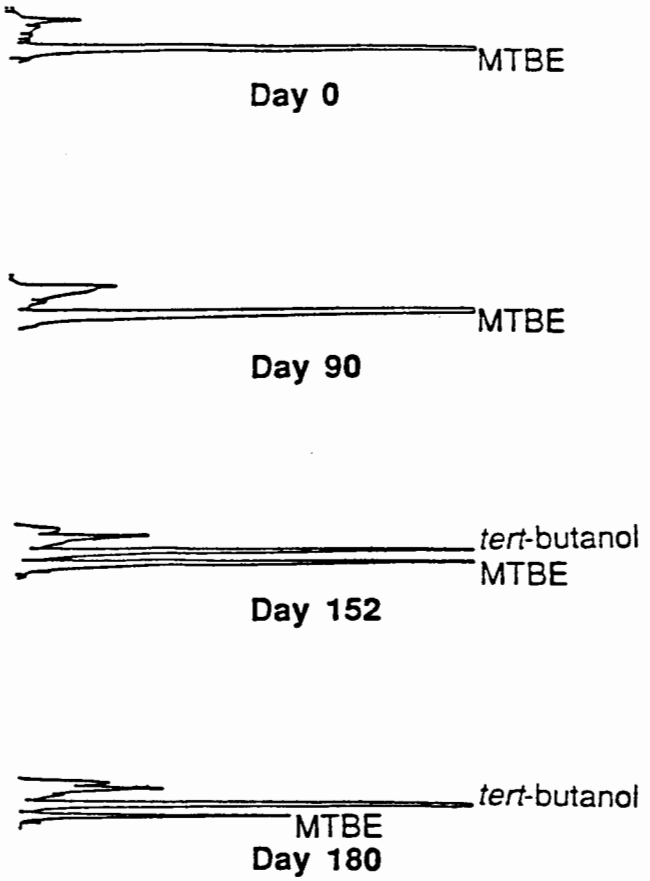

Figure 1. Series of gas chromatographic profiles showing the depletion of MTBE and the appearance of an additional peak, identified as *tert*-butanol, over a period of 180 days in an experimental replicate inoculated with a fuel-impacted river sediment and amended with the oxygenate.

Table 3. Rates of Oxygenate Depletion by Cultures of *Acetobacterium woodii* and *Eubacterium limosum*[a]

oxygenate	depletion rate [nmol h^{-1} (mg of protein)$^{-1}$] with *A. woodii*	*E. limosum*[b]
methyl acetate	86.5 ± 10.4	143.1 ± 12.3
methyl propionate	126.4 ± 9.6	115.3 ± 12.9
methyl butyrate	292.6 ± 12.0	213.6 ± 12.0
methyl isobutyrate	110.9 ± 44.9	107.5 ± 8.2
methyl *tert*-butyl ether	0[c]	0
methyl *tert*-amyl ether	0	0
methyl butyl ether	0	0

[a] Average ± SD of two duplicated treatments incubated under either a H_2/CO_2 or a N_2/CO_2 headspace (the differences between these two treatments were insignificant). [b] Cells were stored under N_2 at 4 °C for 16 days before incubation suspensions were made. [c] Zero (0) stands for no biodegradation when compared with abiotic controls.

remains obscure, and we are currently using ^{14}C-labeled MTBE to probe this question.

Susceptibility of Ester and Ether Oxygenates to Anaerobic Degradation by Acetogens. Pure culture studies were performed with representative acetogens *A. woodii* and *E. limosum*, because of the central role these organisms play in anaerobic biodegradative pathways of organic matter and their known ability to degrade phenyl methyl ethers (*12*, *13*, *22*). *A. woodii* and *E. limosum* were able to hydrolyze the methyl esters of acetate, propionate, butyrate, and isobutyrate (Table 3) to methanol and the corresponding carboxylic acids (*23*). Rates of depletion of the primary esters were positively correlated with the carbon chain length of the acyl group with the exception of methyl acetate in *E. limosum* incubations (Table 3). Both bacteria showed a reduced rate of methyl isobutyrate depletion in comparison to methyl butyrate. However, there was no evidence for ether biodegradation in these cultures even though the cells were consuming gaseous substrates from the headspace of the incubation system and producing formate and acetate with no apparent toxicity of the oxygenate substrates (data not shown).

Discussion

As in our previous study (*8*), straight-chain alcohol, ketone, or ester oxygenates were easily degraded in incubations prepared from different types of sediments and when held under different redox conditions (Tables 1 and 2). The inclusion of a highly branched moiety in these molecules imparts recalcitrance to these compounds regardless their functional groups (Table 2). The ether oxygenates were generally very difficult to degrade, even with sediments having a chronic history of contamination. The presence of alternate electron acceptors also did not facilitate the biodegradation of the ethers. The general recalcitrance of the ethers may reflect a fundamental lack of metabolic potential by the indigenous microflora under the selection conditions imposed by our assay.

Unlike the other ethers, methyl butyl ether was depleted under most assay conditions (Tables 1 and 2). The degradation of methyl butyl ether in methanogenic slurries prepared from four chronically contaminated sites was largely accountable by methane formation (Table 2, ref 8). However, the removal of this compound from sulfate-amended slurries was not accompanied by anion depletion or methane production over background levels. This phenomenon was also evident with sulfate-amended incubations of methyl isobutyl ketone. Work is continuing to determine the fate of both these compounds in the presence of sulfate.

Another exception to the generalized difficulty of anaerobic ether degradation was observed when MTBE was converted to *tert*-butanol in a single replicate of Ohio River sediment slurry (Figure 1). This is the first paper to suggest the degradation of MTBE under anaerobic and neutral pH conditions. Even though the degradation of MTBE is interesting, our studies would suggest that it is a rare occurrence, and the product formed, *tert*-butanol, also resists anaerobic decay.

The ease of degradation of a number of compounds assayed in our studies (Tables 1–3, ref 8) is not surprising in the light of previous research. The anaerobic biodegradation of methanol has been studied extensely, and there are at least 11 known species of methanogenic bacteria that can grow on methanol (*22*, *24*). Acetogenic bacteria have also been isolated that can grow on methanol when CO_2 is present (*22*, *25*). Generally, high concentrations of methanol are not used by sulfate-reducing bacteria to an ecologically significant extent. However, sulfate reducers were found to contribute to methanol depletion in some environments (*26*, *27*). Puhakka and co-workers (*28*) suggest that sulfate-reducing bacteria could metabolize methanol from wastewater systems when methanogens were inhibited with bromoethanesulfonic acid. Additionally, four species of sulfate-reducing bacteria have been

isolated that can utilize methanol, but the mechanism of this bioconversion is unknown (*29*). However, it does not appear that sulfate-reducing bacteria were utilizing methanol in our studies because no sulfate depletion over background levels was evident and methane was recovered from these incubations. Such a phenomenon has been shown with other environmental samples (*14*, *30*, *31*).

Studies have also shown that enrichment cultures from a littoral sediment formed CH_4, acetate, and acetone from 2-propanol (*32*). Further, pure cultures of methanogens able to use this alcohol were obtained from various environments (*33*, *34*). A *Desulfovibrio* sp. has also been found to oxidize 2-propanol to acetone (*35*), which is a substrate for a number of bacteria. A methanogenic enrichment culture from a creek sediment could form methane and CO_2 from acetone by interspecies acetate transfer (*36*). When acetone was supplied along with succinate or ethanol to slurries prepared from a littoral sediment, methane, acetate, propionate, and 2-propanol were found as products (*32*). *Desulfococcus biacutus* has also been found to degrade acetone via acetoacetyl-CoA (*37*), and a denitrifying bacterium can carboxylate acetone to acetoacetate and further metabolize this intermediate (*38*). Therefore, our observation of propanol and acetone degradation under anaerobic conditions is not without experimental precedence.

The biodegradation of various esters detected in our studies is not surprising considering that esterase activity is widely distributed in bacteria isolated from anaerobic environments (*39*, *40*). Work is currently underway toward the isolation of the organisms responsible for degrading these potential oxygenates.

While our investigations on the anaerobic fate of gasoline oxygenates are far from exhaustive, the general trends observed in an earlier study (*8*) can be extrapolated to other anaerobic environments and redox conditions. That is, the common ether oxygenates resist both anaerobic (*8*, this study) and aerobic (*9*, *10*) decay and must be considered recalcitrant chemicals. It may ultimately be possible for these chemicals to be metabolized by broad specificity oxygenases elaborated by aerobic microorganisms. However, no evidence to this effect is currently available in the literature. Similarly, the effects of branching on biodegradation activities have been widely documented (*41*–*47*). That is, the less branched compounds generally degrade more readily than the more structurally complex chemicals. Our studies confirm that structurally simple compounds such as the primary and secondary alcohols, ketones, and esters, while comparable to the ether oxygenates in their research octane ratings, are easier to degrade than most of the latter compounds and the structurally complex *tert*-butanol. This trend is also evident when secondarily branched and straight-chain compounds are compared. Methyl isobutyl ketone was degraded in our studies, but the rate of nitrate depletion was an order of magnitude lower than comparable primary ketones. In addition, methyl isobutyrate was degraded by resting cell suspensions of *A. woodii* and *E. limosum* at half the rate of methyl butyrate. Thus, when oxygenation of gasoline is necessary, the structurally simpler compounds might be superior candidate molecules in terms of their susceptibility to bacterial metabolism. We contend that this should be considered before further legislative mandates are made concerning the addition of these chemicals to gasoline.

Acknowledgments

We thank Michael C. Miller and Kevin Johnston of the University of Cincinnati for the help in collecting the Ohio River and Mill Creek sediment and water samples. This research was funded by the American Petroleum Institute. The opinions, findings, and conclusions expressed are those of the authors and not necessarily those of the American Petroleum Institute.

Literature Cited

(1) Haggin, J. *Chem. Eng. News* **1992**, *70*, 22.
(2) Anderson, E. V. *Chem. Eng. News* **1993**, *71*, 9–18.
(3) Reisch, M. S. *Chem. Eng. News* **1994**, *72*, 12–15.
(4) Calvert, J. G.; Heywood, J. B.; Sawyer, R. F.; Seinfeld, J. H. *Science* **1993**, *261*, 37–45.
(5) American Petroleum Institute. *Basic Petroleum Data Book*; American Petroleum Institute: Washington, DC, 1994; p 14.
(6) American Petroleum Institute. *Cost-Effective, Alternative Treatment Technologies for Reducing the Concentrations of Methyl Tertiary Butyl Ether and Methanol in Groundwater*; IT Corporation: Knoxville, TN, 1991.
(7) Suflita, J. M. *Transport and Fate of Contaminants in the Subsurface*; Technical Report No. EPA/625/4-89/019; U.S. EPA: Cincinnati, OH, and Ada, OK, 1989.
(8) Suflita, J. M.; Mormile, M. R. *Environ. Sci. Technol.* **1993**, *27*, 976–978.
(9) Barker, J. F.; Hubbard, C. E.; Lemon, L. A. In *Proceedings of the 1990 Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection, and Restoration*; Oct 31–Nov 2, 1990; American Petroleum Institute and Association of Ground Water Scientists and Engineers: Houston, TX, 1990; pp 113–127.
(10) Jensen, H. M.; Arvin, E. In *Contaminated Soil '90*; Arendt, F., Hinsenveld, M., van den Brink, W. J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988; p 445.
(11) Fujiwara, Y.; Kinoshita, T.; Sato, H.; Kojima, I. *Yukagatu* **1984**, *33*, 111.
(12) Bache, R.; Pfennig, N. *Arch. Microbiol.* **1981**, *130*, 255–261.
(13) Cocaign, M.; Wilberg, E.; Lindley, N. D. *Arch. Microbiol.* **1991**, *155*, 496–499.
(14) Beeman, R. E.; Suflita, J. M. *Microb. Ecol.* **1987**, *14*, 39–54.
(15) Barlaz, M. A.; Shafer, M. B.; Borden, R. C.; Wilson, J. T. In *Symposium on bioremediation of hazardous wastes: research, development, and field evaluations*, Dallas, TX, May 4–6, 1993; U.S. EPA: Washington, DC, 1993; pp 100–105.
(16) Barnhart, C. L. H.; Vestal, J. R. *Appl. Environ. Microbiol.* **1983**, *46*, 970–977.
(17) Tanaka, K.; Pfennig, N. *Arch. Microbiol.* **1988**, *149*, 181–187.
(18) DeWeerd, K. A.; Concannon, F.; Suflita, J. M. *Appl. Environ. Microbiol.* **1991**, *57*, 1929–1934.
(19) Symons, G. E.; Buswell, A. M. *J. Am. Chem. Soc.* **1933**, *55*, 2028–2036.
(20) Riley, R. G.; Zachara, J. M. *Chemical contaminants on DOE land and selection of contaminant mixtures for subsurface science research*; U.S. Department of Energy: Washington, DC, 1992.
(21) *The Merck Index*, 10th.; Merck and Co., Inc.: Rahway, NJ, 1983; p 865.
(22) Heijthuijsen, J. H. F. G.; Hansen, T. A. In *Autotrophic microbiology and one-carbon metabolism*; Codd, G. A., Dijkuizen, L., Tabita, F. R., Eds.; Kluwer Academic Publishers: Boston, 1990; pp 163–191.
(23) Liu, S.; Suflita, J. M. *J. Ind. Microbiol.* **1994**, in press.
(24) Vogels, G. D.; Keltjens, J. T.; van der Drift, C. In *Biology of anaerobic microorganisms*; Zehnder, A. J. B., Ed.; Wiley: New York, 1988; pp 707–770.
(25) Diekert, G. In *The prokaryotes*, 2nd ed.; Balows, A., Truper, H. G., Dworkin, M., Harder, W., Schleifer, K. H., Eds.; Springer-Verlag: New York, 1992; pp 517–533.

(26) Nanninga, H. J.; Gottschal, J. C. *FEMS Microbiol. Ecol.* 1986, *38*, 125–130.
(27) Esnault, G.; Caumette, P.; Garcia, J.-L. *System. Appl. Microbiol.* 1988, *10*, 147–151.
(28) Puhakka, J. A.; Ferguson, J. F.; Benjamin, M. M.; Salkinoja-Salonen, M. *System. Appl. Microbiol.* 1989, *11*, 202–206.
(29) Widdel, F.; Hansen, T. A. In *The prokaryotes*, 2nd ed.; Balows, A., Truper, H. G., Dworkin, M., Harder, W., Schleifer, K.-H., Eds.; Springer-Verlag: New York, 1992; pp 583–624.
(30) Oremland, R. S.; Marsh, L. M.; Polcin, S. *Nature* 1982, *296*, 143–145.
(31) Ward, D. M.; Winfrey, M. R. *Adv. Aquat. Microbiol.* 1985, *3*, 141–179.
(32) Rothfuss, F.; Conrad, R. *FEMS Microbiol. Ecol.* 1993, *12*, 265–276.
(33) Widdel, F. *Appl. Environ. Microbiol.* 1986, *51*, 1056–1062.
(34) Lalitha, K.; Krishnan, S. *Arch. Microbiol.* 1991, *155*, 402–408.
(35) Tanaka, K. *J. Ferment. Bioeng.* 1992, *73*, 362–365.
(36) Platen, H.; Schink, B. *Arch. Microbiol.* 1987, *149*, 136–141.
(37) Platen, H.; Temmes, A.; Schink, B. *Arch. Microbiol.* 1990, *154*, 355–361.
(38) Platen, H.; Schink, B. *J. Gen. Microbiol.* 1989, *135*, 883–891.
(39) Lanz, W. W.; Williams, P. P. *J. Bacteriol.* 1973, *113*, 1170–1176.
(40) Hespell, R. B.; O'Bryan-Shah, P. J. *Appl. Environ. Microbiol.* 1988, *54*, 1917–1922.
(41) Swisher, R. D. *Surfactant Biodegradation*, 2nd ed.; Marcel Dekker, Inc.: New York, 1987; p 1085.
(42) McKenna, E. J.; Kallio, R. E. In *Principles and applications in aquatic microbiology*; Heukelekian, H., Dondero, N. C., Eds.; Wiley: New York, 1964; pp 1–14.
(43) Alexander, M. *Adv. Appl. Microbiol.* 1965, *7*, 35–80.
(44) Alexander, M. *Biotechnol. Bioeng.* 1973, *15*, 611–647.
(45) Hutzinger, O.; Veerkamp, W. In *Microbial degradation of xenobiotics and recalcitrant compounds*; Leisinger, T., Hutter, R., Cook, A. M., Nuesch, J., Eds.; Academic Press: London, 1981; pp 3–45.
(46) Atlas, R. M.; Bartha, R. *Adv. Microbiol. Ecol.* 1992, *12*, 287–338.
(47) Muller, R. In *Microbial control of pollution*; Fry, J. C., Gadd, G. M., Herbert, R. A., Jones, C. W., Watson-Craik, I. A., Eds.; Cambridge University Press: Cambridge, 1992; pp 35–58.

Received for review March 20, 1994. Revised manuscript received June 6, 1994. Accepted June 10, 1994.•

• Abstract published in *Advance ACS Abstracts*, July 1, 1994.