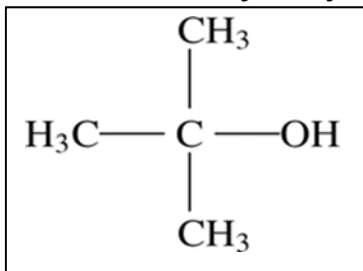


## Tertiary Butyl Alcohol (TBA) Biodegradation

### Some Frequently Asked Questions

**1: What is *tertiary* butyl alcohol (TBA)?** *Tertiary* butyl alcohol is a simple organic



compound that contains only carbon (C), hydrogen (H) and oxygen (O). The chemical formula for TBA is  $\text{C}_4\text{H}_{10}\text{O}$ . As its name suggests, this chemical contains a *tertiary* butyl group that consists of three methyl groups ( $-\text{CH}_3$ ), each separately attached to a central (*tertiary*) carbon. TBA also contains an alcohol group ( $-\text{OH}$ ) that is also attached to the central *tertiary* carbon (Figure 1).

Organic chemicals often have several different names. Some of the other commonly used names for TBA are *tert*-butanol, *tert*-butyl alcohol, *t*-butanol, 2-methyl-2-propanol, 2-methylpropan-2-ol, trimethylcarbinol, and trimethyl methanol. Each chemical has a unique CAS (Chemical Abstracts Service) Registry Number. The CAS number for TBA is 75-65-0. This number can be used to obtain specific information about TBA from a wide variety of sources.

**2: What are the main sources of TBA in ground water?** Ultimately, gasoline is likely to be the main source of TBA found in ground water. To contaminate ground water, gasoline must enter the subsurface. This can occur through a variety of routes involving, among others, gasoline distribution and end use (*e.g.*, storage system releases and pipe lines) and consumers (*e.g.*, refueling spills, automobile accidents, poor consumer disposal practices). One important reason why gasoline is an important source of TBA is because biodegradation of other gasoline components such as ether oxygenates (*e.g.*, methyl- and ethyl *tertiary* butyl ether [MTBE and ETBE]) can generate TBA.

**3: Why is only “older” gasoline a potential source of TBA?** Historically, TBA is most closely associated with gasoline that contained fuel oxygenates. By definition, all oxygenates contain oxygen (O). This oxygen can increase the efficiency of fuel combustion and decrease automobile emissions of air pollutants such as carbon monoxide. In the United States, the most widely used ether-based oxygenate, MTBE, was added to gasoline from approximately 1980 to 2006. From the early 1990’s onwards, many urban areas of the United States were required to use fuel oxygenates. MTBE and ethanol were most frequently used to conform to this federal requirement. In other areas of the country, MTBE was also included in gasoline to increase the fuel octane rating. MTBE-containing gasoline is considered a potential source of TBA for the following reasons:

- a. **TBA as an oxygenate:** Beginning in 1979 and into the early 1980’s, and in a small proportion of the gasoline supply, TBA itself was sometimes added to gasoline as an octane-enhancer, with methanol as a cosolvent.

- b. **TBA in fuel grade ether oxygenates:** As a byproduct of the manufacturing process, low concentrations of TBA (~2 wt%) were common in the “fuel grade” ether oxygenates such as MTBE.
- c. **TBA as a product of ether oxygenate biodegradation:** TBA is well-established as a product of MTBE biodegradation processes catalyzed by microorganisms in the environment.

**4: Is gasoline always a source of TBA?** Gasoline currently sold in the United States is not considered to be a source of TBA while older gasoline already present in the subsurface may be a potential source of TBA. The reason for this is the composition of gasoline changes over time due to technological advances, environmental regulations, and other factors. For example, gasoline sold in the United States before the mid 1980's often contained significant amounts of lead whereas gasoline sold after the mid 1980's did not. The composition of gasoline also varies regionally. For example, currently, much of the gasoline sold in the United State contains approximately 10% ethanol. Ten years ago, ethanol use in gasoline was largely restricted to the Midwest where it was produced.

**5: Can TBA in ground water come from non-gasoline sources?** Several potential non-gasoline-related sources of TBA exist. For example, *tertiary* butyl acetate (TBAC) is an ester that is widely used as a low volatility solvent. TBAC degrades both biologically and chemically to TBA. TBA is also generated from the chemical decomposition of *tertiary* butyl hydroperoxide, a component of some hydraulic fracturing solutions. TBA is also a co-product of commercial propylene oxide production. Microorganisms that grow on volatile *n*-alkanes (methane, propane etc.) can generate TBA from isobutane and so can bacteria that actually grow on isobutane. Low concentrations of isobutane occur in gasoline but the major sources of isobutane in the environment are sources such as natural gas seeps.

**6: What are the properties of TBA in ground water and how do they compare to MTBE?** Like many other alcohols such as ethanol, TBA is fully miscible with water. Consequently, TBA can rapidly dissolve out of gasoline and high dissolved concentrations of TBA can occur in gasoline-impacted ground water. In contrast, the aqueous solubility of MTBE is ~50g L<sup>-1</sup> while that of benzene is less than 2 g/L<sup>-1</sup>. TBA also has a low vapor pressure (~40 mm Hg @ 25° C) compared to MTBE (~250 mm Hg @ 25° C). TBA also has a much lower Henry's Law Constant than MTBE, so while MTBE can be volatilized from contaminated ground water using air stripping, TBA does not strip and remains in solution. Neither MTBE nor TBA sorb strongly to organic matter which limits the remedial efficacy of activated carbon and also means that both compounds travel at the rate of ground water flow.

**7: What are microorganisms and how can they impact contaminants like MTBE and TBA in ground water?** Microorganisms are typically single-celled organisms that are too small to be seen with the naked eye. These include, among others, bacteria, archaea and fungi. Bacteria are the most metabolically versatile microorganisms and in many instances individual bacterial species or microbial communities containing multiple bacterial species can grow on and degrade organic compounds in ground water. In the case of gasoline, these

compounds include both MTBE and TBA, as well as normal and branched alkanes and alkenes, alicyclics and monoaromatic (*e.g.* BTEX) hydrocarbons. Microbial growth on these compounds removes these compounds from contaminated environments by converting them to innocuous products such as carbon dioxide (CO<sub>2</sub>).

**8: What compounds do bacteria in ground water need to be active?** To grow and be active, bacteria require a carbon source (to generate new biomolecules or cells), an energy source known as an electron donor, and an electron acceptor. Like many other bacteria that grow on gasoline components, bacteria that can grow on MTBE and TBA also use these compounds as both a carbon source and electron donor. Some bacteria can use oxygen (O<sub>2</sub>) as an electron acceptor. These organisms are called aerobes and these bacteria can only function and grow if oxygen is present in their immediate environment. Many of the bacteria that can biodegrade MTBE and TBA are aerobes. Many types of bacteria can also use compounds other than oxygen as an electron acceptor. These organisms are called anaerobes. Anaerobic electron acceptors include many compounds that can be present at in ground water such as nitrate, ferric iron, sulfate and others. In ground water systems the type of electron acceptors that are present strongly impacts the type of biodegradation processes that can occur. Both MTBE and TBA can be biodegraded under anaerobic conditions.

**9: How are contaminants biodegraded?** To be able to grow on contaminants such as MTBE and TBA, bacteria have to breakdown (biodegrade) these compounds. The biochemical mechanisms bacteria use to degrade contaminants is strongly impacted by the available electron acceptors. The most significant differences in biodegradation processes are dictated by whether or not the process occurs under aerobic or anaerobic conditions.

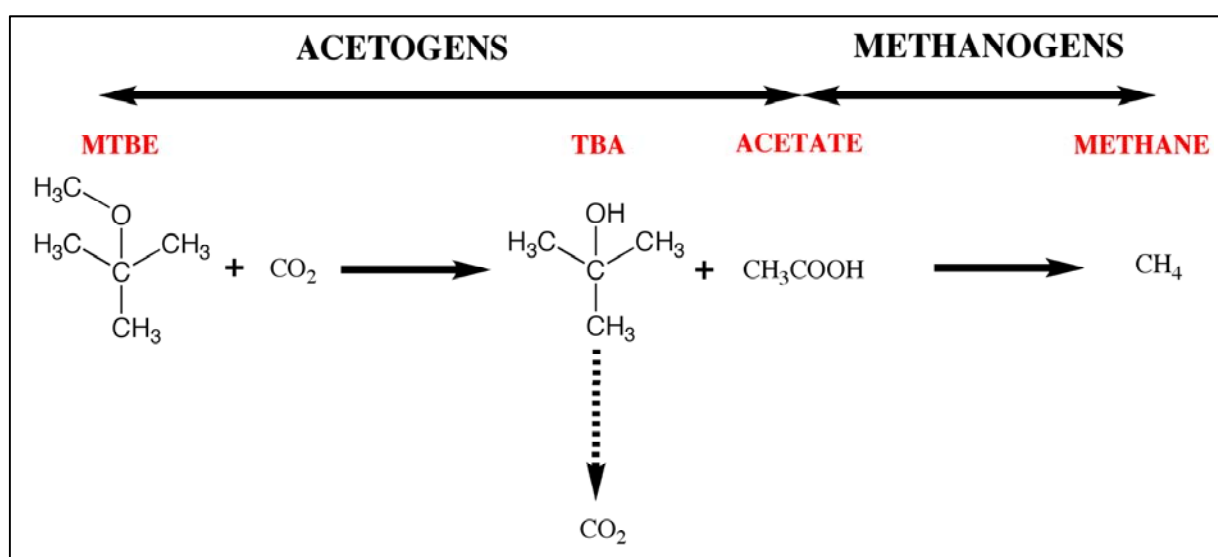
To characterize biodegradation processes, microbiologists typically use pure cultures of microorganisms. These cultures are pure as they only contain one type of microorganisms. To date, many pure cultures of microorganisms have been identified that biodegrade MTBE and TBA in the presence of oxygen. Biodegradation of MTBE and TBA can also be enhanced in contaminated environments through the addition of oxygen to stimulate the activities of these types of organisms. In contrast, no pure cultures of microorganisms have been identified that biodegrade MTBE or TBA in the absence of oxygen. However, this lack of pure cultures does not mean that biodegradation of these compounds only occurs in the presence of oxygen. Anaerobic biodegradation studies using a wide variety of environmental samples (ground water, sediments, aquifer solids and activated sludge) have demonstrated that MTBE, and to a lesser extent TBA, can biodegrade in the presence of all of the electron acceptors that support anaerobic microbial growth in ground water. These include nitrate, ferrous iron, sulfate and carbon dioxide.

Our current understanding of MTBE and TBA biodegradation is very similar to what we know about benzene, another important gasoline-derived ground water contaminant. Numerous strains of aerobic benzene-biodegrading bacteria have been characterized and aerobic biotreatment is widely recognized as an effective treatment option for this compound. In contrast, and despite many years of research, very few pure cultures of anaerobic benzene-degrading microorganisms have been identified. Current evidence

suggests that benzene, like many other contaminants, is biodegraded through the combined activities of diverse anaerobic bacteria operating as a microbial community. Identification of individual organisms capable of catalyzing all of the activities required for anaerobic benzene biodegradation may therefore be unrealistic. However, anaerobic benzene biodegradation frequently occurs in gasoline-impacted environments and this process is recognized in monitored natural attenuation (MNA) protocols for gasoline-impacted sites.

**10: What are the characteristics of anaerobic MTBE biodegradation?** Gasoline contains many readily biodegradable compounds and when this mixture is introduced into the subsurface rapid biodegradation can occur and deplete oxygen and other available electron acceptors. Consequently, it is important to understand the biodegradation process that impact MTBE and TBA under anaerobic conditions as these conditions are frequently encountered at gasoline-impacted sites.

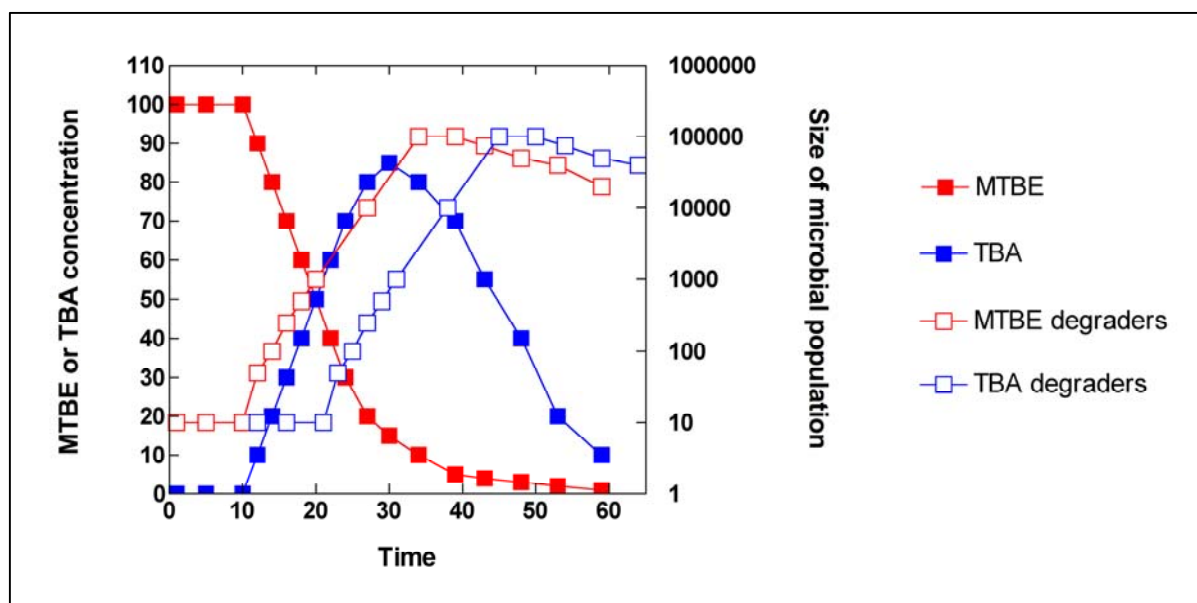
When MTBE biodegradation occurs under anaerobic conditions, TBA frequently accumulates as a byproduct. As we currently do not have pure cultures of anaerobic MTBE-degrading microorganisms, the biochemical reactions involved in this process have not been fully established. However, there is strong evidence that CO<sub>2</sub>-utilizing anaerobic microorganisms known as acetogens play an important role in this process. Acetogens are a diverse group of widely distributed bacteria that generate acetate (CH<sub>3</sub>COOH) as a product of their biodegradation activities. Based on what is known about the biodegradation of other ether-containing compounds by acetogens, these organisms use the methoxy methyl group of MTBE as their electron donor and CO<sub>2</sub> as their electron acceptor. The carbon from the methyl group is incorporated into acetate and both acetate and TBA are excreted as byproducts (Figure 2).



Acetate excreted by acetogens can be biodegraded by other common soil microorganisms called methanogens that often grow under the same environmental conditions as acetogens. Methanogens excrete methane (CH<sub>4</sub>) as the terminal product of their own

distinctive metabolic activities. A similar pathway of MTBE biodegradation to TBA may also occur with other anaerobic microorganisms.

**11: What is the fate of TBA under anaerobic conditions?** In MTBE-degrading laboratory cultures the biodegradation of MTBE is often accompanied by a near stoichiometric accumulation of TBA. In these cultures TBA is typically stable and does not undergo further biodegradation. A substantially similar effect has been observed in field studies of MTBE biodegradation. However, in the environment there is also evidence that TBA can often undergo further biodegradation. Based on the time course of these reactions, it is likely that the further biodegradation of TBA is brought about by a separate group of microorganisms whose activities are only stimulated once sufficient TBA has accumulated to support their growth and activities. A highly stylized model illustrating this potential interaction is shown in Figure 3.



As is the case with MTBE, no pure cultures of anaerobic TBA-utilizing organisms are currently available. However, laboratory studies with mixed cultures have indicated that TBA can be biodegraded under nitrate, iron- and sulfate-reducing conditions.

**12: What are the characteristics of MTBE and TBA biodegradation under aerobic conditions?** Like many other ground water contaminants, both MTBE and TBA can be more rapidly biodegraded under aerobic conditions than anaerobic conditions. As many aerobic MTBE- and TBA-degrading microorganisms have been isolated and characterized, more is known about the biodegradation mechanisms and the organisms responsible for these activities than the equivalent anaerobic processes.

A number of aerobic bacteria have been isolated that can grow on MTBE and all of these organisms also have the ability to grow on TBA. Conversely, there are examples of bacteria

that can grow on TBA but do not appear to grow on MTBE. Despite this difference and to the extent this process has been analyzed, the pathway of TBA biodegradation appears to be very similar in all of these organisms. A key feature of many aerobic biodegradation processes is that oxygen is introduced into the contaminant as an early part of the biodegradation process. For examples, *n*-alkanes are generally unreactive compounds. The vast majority of aerobic *n*-alkane biodegradation processes are initiated by bacteria that use diverse oxygenase enzymes to insert O atoms from O<sub>2</sub> into these compounds. This has the effect of “activating” the inert *n*-alkanes by converting them into readily biodegradable alcohols. The same is also true of MTBE and TBA. The activation of these compounds by oxygenase enzymes is particularly important as both MTBE and TBA contain a branched hydrocarbon structure, the *tertiary* butyl group. As a general rule, branched structures are more resistant to biodegradation than their straight-chained hydrocarbon equivalent. In the case of MTBE, the presence of an ether-bonded methoxy group also contributes to the stability of this compound. Current research indicates aerobic microorganisms consistently use oxygenase enzymes to initiate the degradation of both MTBE and TBA. In the case of MTBE, an oxygen atom from O<sub>2</sub> is first added onto the methoxy carbon in a reaction that generates an unstable intermediate. This intermediate then decomposes to generate TBA. A second oxygenase enzyme is then used to initiate the further biodegradation of TBA to intermediates that the active organisms can subsequently use as a carbon and energy source. Since MTBE-degrading organisms do not obtain significant amounts of energy from the initial conversion of MTBE to TBA, the organisms technically grow on TBA rather than MTBE. So organisms that grow on MTBE rarely allow TBA to accumulate as it is rapidly consumed and converted to CO<sub>2</sub>.

Both MTBE and TBA can also be biodegraded by bacteria that grow on other compounds but cannot grow on either MTBE or TBA alone. This process is called cometabolism. Many research studies have shown alkane components of gasoline can support the growth of diverse bacterial strains that can cometabolically convert MTBE to TBA. In some instances TBA can also be further biodegraded by these organisms although TBA is often excreted as a byproduct of MTBE cometabolism. As aerobic TBA-utilizing microorganisms are widely distributed in the environment, the accumulation of TBA as a result of a cometabolic conversion of MTBE to TBA can stimulate the growth and activity of these separate TBA-utilizing microorganisms. This is another example of how the metabolic activities of separate groups of microorganisms can be combined and can result in the full biodegradation of a contaminant to CO<sub>2</sub>.

The rapid biodegradation of both MTBE and TBA under aerobic conditions has been exploited in several in situ and ex-situ bioremediation approaches for these compounds. For example, the introduction of oxygen into the sub surface using biobarriers has been shown to be very effective for the treatment of large dissolved plumes of MTBE. Likewise, various aerobic bioreactor configurations have been developed and commercialized for the ex-situ treatment of MTBE and TBA. Although less well studied, aerobic conditions can also be reasonably expected to promote the natural attenuation of both TBA and MTBE in contaminated ground water.