



Butane Biostimulation Technology™ Applications

MTBE and other Fuel Oxygenates

Methyl tertiary butyl ether (MTBE or MtBE) has been blended with gasoline in the United States since 1979, initially as an octane booster and later as a fuel oxygenate (11, 36, and 44). Until recently its use and production in the US continued to increase with concentrations up to 15% by volume. MTBE-oxygenated gasoline has been phased out of most US markets due to environmental concerns. It continues to be used in various international markets, however (44). Tertiary butyl alcohol (TBA or tBA), another oxygenate, has been used less extensively with gasoline but is also a common degradation product of MTBE and is therefore often found in association with MTBE.

MTBE is poorly adsorbed, chemically and biologically stable, and very soluble in water, making it very mobile and persistent in the environment (11, 16, and 44). The solubility of MTBE in water is approximately 30 times higher than benzene. The Henry's law constant of MTBE is approximately one tenth that of benzene. As a result of these factors MTBE has a tendency to migrate faster and further than other fuel constituents in the subsurface environment.

Butane Biostimulation Technology™ Description and Benefits

Butane Biostimulation Technologies™ (BBT) provide fast and effective in-situ soil and groundwater treatment for remediation of MTBE and TBA. Numerous studies have demonstrated that where appropriate environmental conditions are met MTBE, TBA and other fuel oxygenates can degrade naturally in the subsurface environment through direct metabolism or cometabolism by indigenous bacteria (3, 9, 11, 36, 43 and 44). Although MTBE can biodegrade slowly under anaerobic conditions, the fastest rates of MTBE biodegradation occur under aerobic conditions (14, 31, 11, 16, 36, and 44). Most bacteria cannot utilize MTBE or TBA as a sole growth substrate but can cometabolically degrade these compounds by an oxygenase enzyme in a fortuitous side reaction (13, 16, 31 and 44). Efforts to identify microorganisms suitable for bioremediation of MTBE have demonstrated the recalcitrance of this compound. A relatively small number of pure and mixed bacterial cultures that are able to biodegrade MTBE have been identified (16). These organisms include bacteria that grow on butane as well as hydrocarbon-utilizing bacteria grown on *n*-pentane, cyclohexane, and aromatics (13, 16, 31, 33 and 44). Although some of these organisms also grow on other alkane gases, studies indicate that butane is a superior growth substrate (14). It is noteworthy that all of the *n*-alkane-utilizing bacteria known to cometabolically oxidize MTBE also oxidize TBA. Furthermore, there is clear evidence that concentrations of butane result in a substantial increase in the biomass of cells that use other fuel constituents such as liquid *n*-alkanes as their primary growth substrates that are also capable of oxidizing MTBE and TBA, even though butane alone cannot support the growth of these microbial strains (31).



For in-situ remediation of MTBE or other fuel oxygenates butane and air are typically injected into the soil and groundwater using a biosparging or bioventing configuration to stimulate the cometabolism of these contaminants. Alternatively both gases may be introduced via gas diffusion or infusion (43) or butane injection may be combined with the introduction of a slow-release source of oxygen. **BBT has been shown to promote rapid destruction of MTBE through cometabolism under aerobic conditions.** Butane provides an easily metabolized carbon source which leads to the rapid development of a robust and diverse biomass and consequently, a healthy microbial ecosystem capable of providing the enzymes and cofactors necessary for complete and rapid degradation of MTBE, TBA and other fuel oxygenates. BBT typically relies on indigenous bacteria. Bioaugmentation has not been required at any site.

Butane and air injection rates are carefully managed using GBI's patented control panel. This is critical since butane acts as a competitive alternative substrate when remediating MTBE or TBA. Butane injection rates must be controlled to stimulate the growth of MTBE-degrading microorganisms without creating competitive inhibition (repression of target contaminant degradation due to the supply of an alternate substrate). The process is optimized by injecting butane cyclically to sustain a healthy microbial ecosystem capable of completely mineralizing MTBE and TBA.

When treating MTBE, Butane Biosparging™ is typically combined with Butane Bioventing™ to oxygenate the soils resulting in the enhanced microbial degradation of MTBE and other fuel constituents in the capillary fringe and vadose zone. In addition, since the offgas from the bioventing system is piped back into the treatment zone, O&M costs are reduced by eliminating the need for carbon treatment or air stripping. The bioventing system also maximizes the radius of influence and controls the potential migration of hydrocarbon vapors from the treatment area into adjacent buildings

The gaseous nature of butane makes BBT uniquely suited to remediation in low-permeability soils, beneath structures, and even in fractured rock, reducing costs and improving the effectiveness of treatment. As a gas, butane is dispersed rapidly and broadly in the subsurface through advective and diffusive transport mechanisms. An added benefit is that contaminated soil in the unsaturated zone and capillary fringe is also treated by the advective and diffusive migration of butane above the water table. Diffusion is the mechanism by which soil gas moves from high concentration to low concentration due to a concentration gradient. Advection is the transport mechanism by which soil gas moves due to differences in pressure. As a result of the high diffusivity of butane gas, BBT overcomes the limitations of groundwater mixing seen with liquid chemical injection and solid phase release compound systems.

It is also noteworthy that butane-utilizing bacteria have been shown to fix nitrogen. Consequently they are able to produce their own nutrients. This capacity was not observed in comparative studies using propane (34). In nutrient-limited environments the ability to fix nitrogen can substantially improve the sustainability of the remedial process.



Injected butane gas provides a sustainable carbon source to stimulate the growth of indigenous microbial communities. This is essential for complete mineralization of target contaminants. If contaminants are actively transformed to innocuous by-products by biochemical processes that are present in natural systems and if energy is available to drive these processes to completion, then degradation is inherently sustainable (2). Butane provides an ideal carbon source to accelerate and ensure the sustainability of these processes.

GBI is the industry leader in the development and application of Butane Biostimulation Technologies™ (BBT). As a pioneer in the field, GBI holds many patents in the application of these technologies, and continues to develop new remedial solutions to complex soil and groundwater contamination problems.

Selected References

1. Arp, Daniel J. 1999. Butane metabolism by butane-grown 'Pseudomonas butanovora'. *Microbiology* 145:1173-1180.
2. Chapelle, F.H., J. Novak, J. Parker, B.G. Campell, and M.A. Widdowson. 2007. A Framework for Assessing the Sustainability of Monitored Natural Attenuation: *U.S. Geological Survey Circular 1303*.
3. Cowan, R. M., and K. Park. 1996. Biodegradation of the gasoline oxygenates MTBE, ETBE, TAME, TBA, and TAA by aerobic mixed cultures, p. 523–530. In *Hazardous & Industrial Waste. Proceedings of the Mid-Atlantic Industrial Waste Conference*. Technomic Publishing Co., Lancaster, Pa
4. Doughty., D.M., L.A. Sayavedra-Soto, D.J. Arp, P.J. Bottomley. 2006. Product Repression of Alkane Monooxygenase Expression in *Pseudomonas butanovora*. *J. Bacteriol.* 188(7): 2586-2592.
5. Doughty, D. M., K.H. Halsey, C.J. Vieville, L.A. Sayavedra-Soto, D.J. Arp, and P.J. Bottomley. 2007. Propionate inactivation of butane monooxygenase activity in 'Pseudomonas butanovora': biochemical and physiological implications. *Microbiology* 153: 3722-3729
6. Ehrenreich, P., A. Behrends, J. Harder and F. Widdel. 2000. · Anaerobic Oxidation of Alkanes by Newly Isolated Denitrifying Bacteria. *Arch. Microbiol.* 173:58–64
7. Hamamura N., R. Storfa, L. Semprini, and D. J. Arp. 1999. Diversity in Butane Monooxygenases among Butane-Grown Bacteria. *Appl. Environ. Microbiol.* 65(10):4586-4593.
8. Hamamura, N., C.M. Yeager,1 and D.J. Arp. 2001. Two distinct monooxygenases for alkane oxidation in *Nocardioides* sp. strain CF8. *Appl. Environ. Microbiol.* 67(11):4992-4998.



9. Hristova, K., B. Gebreyesus, D. Mackay, and K. M. Scow. 2003. Naturally occurring bacteria similar to methyl tert-butyl ether (MTBE)-degrading strain PM-1 are present in MTBE-contaminated groundwater. *Appl. Environ. Microbiol.* 69:2616–2623.
10. Hyman, M., P. K. Won, K. Williamson, and M. O'Reilly. 1998. Cometabolism of MTBE by alkane-utilizing microorganisms, p. 321–326. In G. B. Wickramanayake and R. E. Hinchee (ed.), *Natural attenuation: chlorinated and recalcitrant compounds*. Battelle Press, Columbus, Ohio.
11. ITRC (Interstate Technology & Regulatory Council). 2005. Overview of Groundwater Remediation Technologies for MTBE and TBA. Available on the Internet at <http://www.itrcweb.org>.
12. ITRC (Interstate Technology & Regulatory Council). Vapor Intrusion Team. 2007. Vapor Intrusion Pathway: A Practical Guide. On the Internet at <http://www.itrcweb.org>
13. Johnson, E.L., C.A. Smith, K.T. O'Reilly, and M.R. Hyman. 2004. Induction of Methyl tertiary Butyl Ether (MTBE)-Oxidizing Activity in *Mycobacterium vaccae* JOB5 by MTBE. . *Appl Environ. Microbiol.* 70(2):1023-1030.
14. Johnson, E.L., and M. Hyman. 2006. Propane and n-Butane Oxidation by *Pseudomonas putida* GPo1. *Appl. Environ. Microbiol.* 72(1): 950-952.
15. Koch, D.J., M. M. Chen, J. B. van Beilen, and F.H. Arnold. 2008. In Vivo Evolution of Butane Oxidation by Terminal Alkane Hydroxylases AlkB and CYP153A6 *Appl. Environ. Microbiol* 75(2): 337-344.
16. Liu, C.Y., G.E. Speitel, Jr., and G. Georgiou. 2001. Kinetics of Methyl t-Butyl Ether Cometabolism at low Concentrations by Pure Cultures of Butane-Degrading Bacteria. *Appl. Environ. Microbiol.* 67(5):2197-2201
17. McLee, A.G., A.C. Kormendy, and M Wayman. 1972. Isolation and characterization of n-butane utilizing microorganisms. *Canadian J Microbiol.* 18:1191-1195.
18. NJDEP. 2005. Vapor Intrusion Guidance. Site Remediation and Waste Management Program. Available on the internet at: www.nj.gov/dep/srp/guidance/vaporintrusion .
19. Patel. R.N., C.T. Hou, C.T. Laskin, A. Felix, and P. Derelanko. 1983. Oxidation of alkanes by organisms grown on C2-C4 alkanes. *J Appl. Biochem.* 5:107-120.
20. Perriello, F.A. 1999. U.S. Patent No. 5,888,396, The Bioremediation of Pollutants with Butane-utilizing Bacteria



21. Perriello, F.A. 1999. U.S. Patent No. 6,051,130, Bioreactor for Remediation of Pollutants with Butane-utilizing Bacteria
22. Perriello, F.A. 2000. U.S. Patent No. 6,110,372, The Bioremediation of Petroleum Pollutants with Butane-utilizing Bacteria
23. Perriello, F.A. 2001. U.S. Patent No. 6,210,579, The Bioremediation of Pollutants with Butane-utilizing Bacteria
24. Perriello, F.A. 2001. U.S. Patent No. 6,245,235, System and Method of In-Situ Bioremediation with Butane-utilizing Bacteria
25. Perriello, F.A. 2002. U.S. Patent No. 6,488,850, Method and Apparatus for Anaerobically Degrading Pollutants with Alkanes
26. Perry, J.J., 1980. Propane utilization by microorganisms. *Adv. Appl. Microbiol.* 26:89-115
27. Phillips, W.E., and J.J. Perry. 1974. Metabolism of n-butane and 2-butanone by *Mycobacterium vaccae*. *J Bacteriol.* 120:987-989.
28. Sayavedra-Soto, L.A., C.M. Byrd, and D.J. Arp. 2001. Induction of butane consumption in *Pseudomonas butanovora*. *Arch. Microbiology* 176:114-120.
29. Sayavedra-Soto, L.A., D.M. Doughty, E.G. Kurth, P.J. Bottommely, and D.J. Arp. 2005. Product and product-independent induction of butane oxidation in *Pseudomonas butanovora*. *FEMS Microbiol. Lett.* 250:111-116.
30. Smith, C. A., K. T. O'Reilly, and M. R. Hyman. 2003. Characterization of the initial reactions during the cometabolic degradation of methyl tert-butyl ether (MTBE) by propane-grown *Mycobacterium vaccae* JOB5. *Appl. Environ. Microbiol.* 69:796–804.
31. Smith, C.A., K.T. O'Reilly, and M.R. Hyman. 2003. Cometabolism of Methyl tertiary Butyl Ether and Gaseous n-Alkanes by *Pseudomonas mendocina* KR-1 Grown on C5 to C8 n-Alkanes. *Appl Environ. Microbiol.* 69(12):7385-7394.
32. Spormann, A.M., and F. Widdel. 2000. Metabolism of Alkylbenzenes, Alkanes, and Other Hydrocarbons in Anaerobic Bacteria. *Biodegradation.* 11:85-105.
33. Steffan, R. J., K. McClay, S. Vainberg, C. W. Condee, and D. Zhang. 1997. Biodegradation of the gasoline oxygenates methyl tert-butyl ether, ethyltert-butyl ether, and tert-amyl methyl ether by propane-oxidizing bacteria. *Appl. Environ. Microbiol.* 63:4216–4222.



34. Toccalino, P.L., R. L. Johnson and D. R. Boone. 1993. Nitrogen Limitation and Nitrogen Fixation during Alkane Biodegradation in a Sandy Soil. *Appl. Environ. Microbiol* 9(9):2977-2983.
35. U.S. Environmental Protection Agency. Office of Solid Waste and Emergency Response. 2000. Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications. EPA 542-R-00-008.
36. U.S. Environmental Protection Agency. Office of Solid Waste and Emergency Response. Office of Superfund Remediation and Technology Innovation. 2004. Technologies for Treating MtBE and other Fuel Oxygenates.
37. U.S. Environmental Protection Agency. National Risk Management Laboratory Research Report. 2002. Envirogen Propane Biostimulation Technology for In-Situ Treatment of MTBE-Contaminated Ground Water. EPA/600/R-02/092.
38. van Beilen, J. B., D. Penninga, and B. Witholt. 1992. Topology of the membrane-bound alkane hydroxylase of *Pseudomonas oleovorans*. *J. Biol. Chem.* 267:9194–9201.
39. Vangnai, A., and D. J. Arp. 2001. An inducible 1-butanol dehydrogenase, a quinohemoprotein, is involved in the oxidation of butane by *Pseudomonas butanovora*. *Microbiol.* 147:745-756.
40. Vangnai, A., L. Sayavedra-Soto, and D. J. Arp. 2002. Roles for the Two 1-Butanol Dehydrogenases of *Pseudomonas butanovora* in Butane and 1-Butanol Metabolism. *Journal of Bacteriology.* 184 (16) 4343-4350.
41. Vangnai, A., D. J. Arp and L. Sayavedra-Soto. 2002. Two Distinct Alcohol Dehydrogenases Participate in Butane Metabolism by *Pseudomonas butanovora*. *Journal of Bacteriology.* 184 (7) 1916-1924.
42. Wackett, L.P., G.A. Brusseau, S.R. Householder, and R.S. Hanson. 1989. Survey of Microbial Oxygenases: Trichloroethylene Degradation by Propane-Oxidizing Bacteria. *Appl. Environ. Microbiol.* 55(11):2960-2964.
43. Wilson, R. D., K. M. Scow, and D. Mackay. 2002. In situ MTBE biodegradation supported by diffusive oxygen release. *Environ. Sci. Technol.* 36:190–199
44. Zeeb, P., and T.H. Weidemeier. 2007. Technical Protocol for Evaluating the Natural Attenuation of MtBE. API Publication 4761