



## **Butane Biostimulation Technology™ Applications**

### **Chlorinated Aliphatic Hydrocarbons**

The production and use of chlorinated aliphatic hydrocarbons (CAHs) has been widespread in the United States since the early 1900s. In addition to their extensive use in industrial processes, they have been used as solvents for cleaning and degreasing of mechanical and electronic components, as well as for dry cleaning of clothing in the military and commercial sectors. They are, perhaps, the most ubiquitous of groundwater contaminants. Due to their chemical and physical properties, CAHs are difficult to remediate. **Butane Biostimulation Technologies™ (BBT) provide a flexible and cost effective tool for *in situ* remediation of dissolved phase CAH plumes and CAHs in soil.**

#### **Butane Biostimulation Technology™ Description and Benefits**

Butane has been shown to be an effective growth substrate for the aerobic cometabolic degradation of CAHs (10, 11, 12, 13, 20, 21, 22, 23, 24, 25, 30, 32, 41, 42, 43, 46, 48, and 49). In the presence of butane and oxygen CAHs are oxidized fortuitously by enzymes that initiate the oxidation of the primary growth substrate, butane (13). Although other alkane gases can also serve as substrates for cometabolic degradation of CAHs, studies indicate that butane provides a superior substrate, perhaps because butane is the heaviest of the alkane gases, having 4 carbon atoms (11, 12, 19, and 20). The relative aqueous solubility of butane may also be a factor. Butane, with a solubility four times that of methane, is also the most soluble of the alkane gases. Studies show that butane-oxidizing bacteria express a diversity of butane monooxygenases (BMOs) that initiate degradation of CVOCs and that different bacteria utilize distinctly different BMOs (4, 13, 14, 15, 20 and 21).

During the past decade anaerobic bacteria have also been shown to grow with alkanes as organic substrates. Denitrifying bacteria and sulfate-reducing bacteria with the capacity for anaerobic alkane degradation have been isolated (7, 8 and 46). Investigation of the pathways of anaerobic alkane degradation and its applicability to bioremediation is only beginning, however.

For in-situ remediation of CAHs butane and air, or an alternative source of oxygen, 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 (52), or butane injection may be combined with the introduction of a slow-release source of oxygen. Butane Biosparging™ is typically combined with Butane Bioventing™ to oxygenate the soils resulting in the enhanced microbial degradation of target contaminants in the capillary fringe and vadose zone. The offgas from the bioventing system is piped back into the treatment zone, reducing O&M costs 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.

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One of the unique advantages of BBT is the ability to treat contaminants which degrade preferentially in either aerobic or anaerobic environments. Using Global BioSciences' (GBI's) patented control panel it is easy to switch from aerobic to anaerobic conditions within a short timeframe at most sites, or to establish zones of aerobic and anaerobic treatment.

Butane and air injection rates must be carefully managed to stimulate the growth of the indigenous microbial communities capable of degrading CAHs while controlling potential competitive inhibition (repression of target contaminant degradation by butane metabolism) (11, 12, 23, and 24). Using BBT the process is optimized by injecting controlled concentrations of butane into the subsurface cyclically to sustain a healthy microbial ecosystem capable of completely mineralizing target contaminants.

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 a wide variety of CAHs. BBT typically relies on indigenous bacteria. Bioaugmentation has not been required at any site. Studies confirm that butane is an effective growth substrate for bacteria capable of rapidly degrading high concentrations of CAHs (11 and 12). Because the growth of the butane-utilizing bacteria is dependent on supplied butane and not on the contaminant of concern, BBT can be used for sites with high or low concentrations of contaminants.

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 (47). 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 that can be managed to stimulate the growth of indigenous microbial communities. This is essential for complete mineralization of target contaminants. When organic carbon is not present in sufficient concentrations to act as the primary metabolic substrate an alternative electron donor is necessary to provide the energy necessary to support microbial growth (3 and 52). 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 (3). 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. 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

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1. Arp, Daniel J. 1999. Butane metabolism by butane-grown 'Pseudomonas butanovora'. *Microbiology* 145:1173-1180.
2. Aziz, C. E., G. Georgiou, and G. E. Speitel, Jr. 1999. Cometabolism of chlorinated solvents and binary chlorinated solvent mixtures using *M. trichosporium* OB3b PP358. *Biotechnol. Bioeng.* 65:100–107.
3. 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*.
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. Doughty, D. M., E.G. Kurth, L.A. Sayavedra-Soto, D.J. Arp, D. J. and P.J. Bottomley. 2008. Evidence for Involvement of Copper Ions and Redox State in Regulation of Butane Monooxygenase in *Pseudomonas butanovora*. *J. Bacteriol.* 190: 2933-2938
7. Ehrenreich, P., A. Behrends, J. Harder and F. Widdel. 2000. Anaerobic Oxidation of Alkanes by Newly Isolated Denitrifying Bacteria. *Arch. Microbiol.* 173:58–64
8. Evans, P.J., and M.M. Trute. 2006. In situ bioremediation of nitrate and perchlorate in vadose zone soil for groundwater protection using gaseous electron donor injection technology. *Water Environ Res.* 78(13):2436-46
9. Folsom, B.R., P.J. Chapman, and P.H. Pritchard. 1990. Phenol and Trichloroethylene Degradation by *Pseudomonas cepacia* G4: Kinetics and Interactions Between Substrates. *Appl. Environ. Microbiol.* 56(5):1279-1285.



10. Frascari D., D. Pinelli, and M. Nocentini. 2002. Aerobic cometabolic degradation of chloroform with butane: influence of system features on biomass adaptation. In: Gavaskar A.R. and A.S.C. Chen (Eds). Proceedings of the Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Paper 2B-32.
11. Frascari, D., A. Zannoni, S. Fedi, Y Pii, D. Pinelli, M. Nocentini. 2005. Aerobic Cometabolism of chloroform by butane-grown microorganisms: long-term monitoring of depletion rates and isolation of a high-performing strain. *Biodegradation*. 16: 147-158
12. Frascari, D., D. Pinelli, M. Nocentini, S. Fedi, Y. Pii, and D. Zannoni. 2006. Chloroform degradation by butane-grown cells of *Rhodococcus aetherovorans* BCP1. *Appl Microbial Biotechnology*. 73: 421-428.
13. Hamamura N., C. Page, T. Long, L. Semprini, and D. J. Arp. 1997. Chloroform Cometabolism by Butane-Grown CF8, *Pseudomonas butanovora*, and *Mycobacterium vaccae* JOB5 and Methane-Grown *Methylosinus trichosporium* OB3b. *Appl. Environ. Microbiol.* 63(9):3607-3613.
14. 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.
15. Hamamura, N., C.M. Yeager, 1 and D.J. Arp. 2001. Two distinct monooxygenases for alkane oxidation in *Nocardioideis* sp. strain CF8. *Appl. Environ. Microbiol.* 67(11):4992-4998.
16. Hartmans, S., and J.A.M. de Bont. 1992. Aerobic Vinyl Chloride Metabolism in *Mycobacterium aurum* L1. *Appl. Environ. Microbiol.* 58(4):1220-1226.
17. ITRC (Interstate Technology & Regulatory Council). Vapor Intrusion Team. 2007. Vapor Intrusion Pathway: A Practical Guide. On the Internet at <http://www.itrcweb.org>
18. 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.
19. Johnson, E.L., and M. Hyman. 2006. Propane and n-Butane Oxidation by *Pseudomonas putida* GPo1. *Appl. Environ. Microbiol.* 72(1): 950-952.
20. Kim, Y., L. Semprini, and D. J. Arp. 1997. Aerobic Cometabolism of Chloroform and 1,1,1-Trichloroethane by Butane-Grown Microorganisms. *Biorem. Journal.* 1(2):135-148.
21. Kim, Y., L. Semprini, and D. J. Arp. 1997. Aerobic Cometabolism of Chloroform, 1,1,1-Trichloroethane, 1,1-Dichloroethylene, and the other Chlorinated Aliphatic Hydrocarbons by Butane-Utilizing Microorganisms. *In Situ and On-site Bioremediation: Volume 3.* 107-112.



22. Kim Y, D.J. Arp, and L. Semprini. 2000. Chlorinated solvent cometabolism by butane-grown mixed culture. *J Environ Eng* 126:934–942
23. Kim., Y., D.J. Arp. and L. Semprini. 2002. A combined method for determining inhibition type, kinetic parameters, and inhibition coefficients for aerobic cometabolism of 1,1,1-trichloroethane by a butane-grown mixed culture. *Biotechnol. Bioeng.*77: 564-576
24. Kim Y., D.J. Arp and L. Semprini. 2002. Kinetic and inhibition studies for the aerobic cometabolism of 1,1,1-trichloroethane, 1,1-dichloroethylene, and 1,1-dichloroethane by a butane-grown mixed culture. *Biotechnol Bioeng* 80:498–508
25. Kim Y, and L. Semprini. 2005. Cometabolic transformation of *cis*-1,2-dichloroethylene and *cis*-1,2-dichloroethylene epoxide by a butane-grown mixed culture. *Water Science & Technology* 52(8):125-131
26. 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.
27. McLee, A.G., A.C. Kormendy, and M Wayman. 1972. Isolation and characterization of n-butane utilizing microorganisms. *Canadian J Microbiol.* 18:1191-1195.
28. NJDEP. 2005. Vapor Intrusion Guidance. Site Remediation and Waste Management Program. Available on the internet at: [www.nj.gov/dep/srp/guidance/vaporintrusion](http://www.nj.gov/dep/srp/guidance/vaporintrusion) .
29. Oldenhuis, R., J. Y. Oedzes, J. J. Van Der Waarde, and D. B. Janssen. 1991. Kinetics of Chlorinated Hydrocarbon Degradation by *Methylosinus trichosporium* OB3b and Toxicity of Trichloroethylene. *Appl. Environ. Microbiol.* 57(1):7-14.
30. Pardi J., L.A. Sayavedra-Soto, and L. Semprini. 2001. Bioaugmentation of butane-utilizing microorganisms to promote cometabolism of 1,1,1-trichloroethane in groundwater microcosms. *Biodegradation* 12:11–22
31. 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.
32. Perriello, F.A. and S. Simkins. Biotransformation of Trichloroethylene Using Butane-Oxidizing Bacteria. *Journal of Soil Contamination.* 8(1):117-129.
33. Perriello, F.A. 1999. U.S. Patent No. 5,888,396, The Bioremediation of Pollutants with Butane-utilizing Bacteria
34. Perriello, F.A. 1999. U.S. Patent No. 6,051,130, Bioreactor for Remediation of Pollutants with Butane-utilizing Bacteria



35. Perriello, F.A. 2000. U.S. Patent No. 6,110,372, The Bioremediation of Petroleum Pollutants with Butane-utilizing Bacteria
36. Perriello, F.A. 2001. U.S. Patent No. 6,210,579, The Bioremediation of Pollutants with Butane-utilizing Bacteria
37. Perriello, F.A. 2001. U.S. Patent No. 6,245,235, System and Method of In-Situ Bioremediation with Butane-utilizing Bacteria
38. Perriello, F.A. 2001. U.S. Patent No. 6,244,346, Method and Apparatus for Reducing Fouling of Injection and Recovery Wells
39. Perriello, F.A. 2002. U.S. Patent No. 6,488,850, Method and Apparatus for Anaerobically Degrading Pollutants with Alkanes
40. Phillips, W.E., and J.J. Perry. 1974. Metabolism of n-butane and 2-butanone by *Mycobacterium vaccae*. *J Bacteriol.* 120:987-989.
41. Sayavedra-Soto, L.A., C.M. Byrd, and D.J. Arp. 2001. Induction of butane consumption in *Pseudomonas butanovora*. *Arch. Microbiology* 176:114-120.
42. 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.
43. Semprini, L., M.E. Dolan, G.D. Hopkins, and P.L. McCarty. 2009. Bioaugmentation with butane-utilizing microorganisms to promote in situ cometabolic treatment of 1,1,1-trichloroethane and 1,1-dichloroethene. *J Contamin. Hydrol.* 103: 157-167.
44. Sluis M.K., L.A. Sayavedra-Soto, D.J. Arp. 2002. Molecular analysis of the soluble butane monooxygenase from *Pseudomonas butanovora*. *Microbiology* 148:3617–3629
45. Spormann, A.M., and F. Widdel. 2000. Metabolism of Alkylbenzenes, Alkanes, and Other Hydrocarbons in Anaerobic Bacteria. *Biodegradation.* 11:85-105.
46. Tocalino, 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.
47. 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.



48. 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.
49. 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.
50. 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.
51. Weidemeir, T.H., J.T. Wilson, R.N. Miller and J.E. Hansen. 1999. Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Ground Water. Prepared for Air Force Center of Environmental Excellence Technology Transfer Division.
52. 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
53. Wilson, J.T, and B.H. Wilson. 1985. Biotransformation of Trichloroethylene in Soil. *Appl. Environ. Microbiol.* 49(1):242-243.
54. Zeeb, P., and T.H. Weidemeier. 2007. Technical Protocol for Evaluating the Natural Attenuation of MtBE. API Publication 4761