Research Brief 139: MTBE Biodegradation by *Methylibium petroleiphilum gen. nov, sp. nov.*

Superfund Basic Research Program

http://www.niehs.nih.gov/sbrp/

Background:

Methyl tert-butyl ether (MTBE) has been used in gasoline in the United Stated since 1979 – first as a replacement for tetra-ethyl lead to increase octane ratings and then, at higher concentrations, in response to the Clean Air Act Amendments of 1990 which required the use of oxygenated gasoline in air quality non-attainment regions. By the mid-1990's, MTBE was in the gasoline supplies in many major metropolitan areas, with California and New York accounting for 40% of U.S. MTBE consumption.

MTBE quickly became the second most commonly observed contaminant in ground waters and threatened public water supplies because of:

- Widespread use
- Releases from leaking underground storage tanks
- Distinctive physico-chemical properties compared to other gasoline components (e.g., greater solubility; lower Henry's partition coefficient from water to air; lower sorption partition coefficient; remarkably low taste and odor thresholds)
- Apparent recalcitrance to biodegradation

MTBE was listed on the CERCLA Priority List of Hazardous Substances in 1997. As of September 2005, use of MTBE has been banned in 25 states, yet site clean-up costs are estimated in the billion-dollar range (http://www.aehs.com/press/MTBECleanup.htm) and drinking water treatment costs in the tens of billions of dollars (http://www.awwa.org/Communications/news/index.cfm?ArticleID=459).

Over the past decade, SBRP-funded researchers at the University of California, Davis led by Drs. Kate Scow, Krassi Hristova, Doug Mackay, Dan Chang and Ed Schroeder have cultured and studied a novel, robust, naturally occurring strain of bacteria that is capable of aerobic mineralization of MTBE, its degradation byproduct tertiary butyl alcohol (TBA) and other gasoline components. The researchers discovered a mixed culture that serendipitously degraded air contaminated with MTBE in a biofilter located at the County Sanitation Districts of Los Angeles' Joint Water Pollution Control Plant (JWPCP) in Carson, CA in 1995. (As a side note, the biofiltration technology was introduced at the JWPCP in 1991 for VOC control by UC-Davis SBRP-funded graduate student Sarina Ergas). Soon, Juana Eweis from the Chang/Schroeder lab found conditions suitable for growing the MTBE-degrading mixed culture from the compost within the biofilter, and Jessica Hanson from the Scow lab subsequently isolated a single strain known as PM-1.

Advances:

Focusing on use of the mixed culture containing the PM-1, the Chang/Schroeder group concentrated on applications of technologies that could be deployed quickly for *ex situ* pump-and-treat applications or contaminated air from air-stripping or soil vapor extraction treatments. Teaming with a small business concern, the Chang/Schroeder group tested a pilot-scale unit in the field treating MTBE-contaminated water at a site in Healdsburg, CA by 1999. Subsequently, they conducted a USEPA Phase I SBIR effort with Environmental Resolutions, Inc. (ERI) to design a fluidized sand-bed bioreactor to retain mixed cultures including the relatively slow-growing PM-1 organism in the reactor. They have over 30 successful bioreactors in California and in New England and now deliver units for operation either by ERI or by the site-owner.

Concurrently, the UC-Davis researchers conducted *in situ* biostimulation and bioaugmentation studies at Port Hueneme and Vandenberg Air Force Base. With the development of molecular tools for identifying the PM-1 organism, they determined that the organism's occurrence is more widespread than was initially imagined. In fact biostimulation (e.g., providing sufficient dissolved oxygen in the groundwater) enabled the native PM-1 organisms in groundwater aquifers to biodegrade MTBE in several cases. Bioaugmentation with PM-1 appears to be justified only when the strain is not already present, which can now readily be determined. The Regenesis Company, which manufactures an oxygen-release compound, has licensed use of the organism for *in situ* bioremediation.

Researchers working with Drs. Hristova and Scow, in collaboration with other research groups, have focused on a establishing a better scientific understanding of the PM-1 organism itself, a necessary step for verification of the success of *in situ* applications. The research team recently determined the entire genome sequence of PM-1, now officially a new species of Betaproteobacteria named *Methylibium petroleiphilum* PM1.

Significance:

Knowledge of the genome will allow researchers to determine the activity of the laboratory strain of *M. petroleiphilum* or a naturally occurring close relative present at field sites and responsible for MTBE and TBA biodegradation. Knowledge of the genome will ultimately permit identification of the genes responsible for the enzymatic activity of the organism on MTBE, possibly leading to a better understanding of the environmental controls on the process. Knowledge of the MTBE degradation pathway and its regulation will allow for optimization of MTBE bioremediation and the ability to monitor this unique process *in situ* using state-of-the-art molecular tools.

Substantial cost savings for site clean-ups have already been made possible for *ex situ* pump-and-treat operations. The technology is considered innovative in EPA Region I.
See: http://www.epa.gov/region1/assistance/ceit_iti/tech_cos/ERIBioreactor.html

Although the microorganism is non-pathogenic, its potential to reduce drinking water treatment costs has not yet been fully exploited. Industry acceptance has been slow, in part because of reluctance to employ biological treatment of drinking water supplies although microorganisms are known to colonize granular activated carbon, which is already often used for treating drinking water supplies.

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To learn more about this research, please refer to:

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