# Bioaugmentation to Enhance Biodegradation of 1,4-Dioxane

***Rebecca Mora*** (Rebecca.Mora@aecom.com) and Holly Holbrook (AECOM, Orange, California), Dora Chiang Ph.D., P.E. (AECOM, Atlanta, Georgia), Shaily Mahendra, Ph.D., and Yu Maio (University of California, Los Angeles, California), Sandra Dworatzek (SiREM, Guelph, Ontario, Canada), Kerry Sublette (University of Tulsa, OK), Adria Bodour, Ph.D. and Hunter Anderson, Ph.D. (Air Force Civil Engineering Center, San Antonio, Texas)

**Background/Objectives.** 1,4-Dioxane is a probable human carcinogen and an emerging contaminant in groundwater at many military and industrial contaminated sites. Numerous studies provide evidence that 1,4-dioxane can be biodegraded aerobically and several cases have documented both metabolic and cometabolic 1,4-dioxane biodegradation since the early 1990s. However, enhanced in situ biodegradation efforts have been limited by inconsistent microbial performance in the field. *Pseudonocardia dioxanivorans* CB1190 (CB1190) is a monooxygenase-expressing microorganism that has been shown to metabolically degrade 1,4-dioxane as a source of carbon and energy in the laboratory. This technology field demonstration project will evaluate bioaugmentation with CB1190 as a means to enhance in situ biodegradation of 1,4-dioxane.

**Approach/Activities.** Specific technical objectives for this project include evaluation of: 1) growth and distribution of CB1190 in the subsurface, 2) the effect of CB1190 addition on in situ 1,4-dioxane biodegradation, 3) the effect of oxygen and nutrient addition (e.g., nitrogen, phosphorus, and trace elements) on in situ 1,4-dioxane biodegradation kinetics, and 4) the ability of in situ biodegradation of 1,4-dioxane to reduce treatment timeframes and (O&M) costs compared to other remediation technologies currently applied to treat this emerging contaminant. Based on results of the site selection process, the project is being performed in Area of Concern (AOC) 1 at former Air Force Plant 3 (AFP), in Tulsa, Oklahoma. Following site assessment, a microcosm study was performed to optimize the CB1190 culture dosage and nutrient requirements for bioaugmentation in the field. After confirming these requirements, larger volumes of CB1190 were grown for field application. In February 2016, CB1190 was injected into an injection well followed by a robust direct-push sampling event to evaluate distribution of CB1190 in the subsurface. In September 2016, CB1190 was injected into an injection well as well as in four direct push injection points. Groundwater sampling in surrounding wells was performed following bioaugmentation to evaluate in situ biodegradation of 1,4-dioxane. In late 2017, an in situ bioreactor (ISBR) approach will be evaluated. Control and CB1190-baited ISBRs will be deployed in up to two wells followed by a monitoring program to evaluate the CB1190 population in the ISBR and 1,4-dioxane degradation over time.

**Results/Lessons Learned.** Three different doses of CB1190 were tested in the microcosm study, both with and without nutrients. 1,4-Dioxane biodegradation was most effective in the mid and high-dose CB1190 bottles that included nutrients. Culture growth to larger volumes was performed by SiREM for field application. Results of the first bioaugmentation event in February 2016 indicated CB1190 was distributed in the subsurface at least 10 feet away from the injection well; however, cell counts were low. Based on these results, SiREM grew a larger volume of culture and maximized the CB1190 cell density to the extent possible for the next injection event. This larger injection volume was injected in early September 2016 and monitoring results showed that CB1190 survived in situ, but the population was still too low to degrade 1,4-dioxane. In late 2017, the ISBR approach will be employed to evaluate whether it can help sustain an adequate in situ population of CB1190 and increase effectiveness of in situ biodegradation of 1,4-dioxane. ISBR monitoring results will be presented at the conference.