Development and Evaluation of Stable Isotope and Fluorescent Labeling and Detection Methodologies for Tracking Injected Bacteria During In-Situ Bioremediation

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The goal of this research is to develop new methods for tracking bacteria in the subsurface. Methods to label bacteria with a variety of fluorescent dyes will be tested. The effects of each dye on cell culturability and adhesion to sediment, as well as the stability and longevity of the fluorescently-labeled bacteria in microcosms, will be assessed. The level of detection for fluorescently-labeled cells using microplate spectrofluorimetry will be determined. The fluorescent tracking method will be evaluated during both transport experiments using intact sediment cores and in the field, and compared with several other detection/enumeration methods.

Additionally, tracking methods involving the analysis of stable carbon isotopes incorporated into cells will be examined. Bacteria will be grown on ^{13}C-only substrates to achieve very highly ^{13}C-enriched cells. Standardization between the number of cells and the amount of ^{13}C in membrane fatty acids, determined by either gas chromatography/chemical reaction interface mass spectrometry (GC-CRIMS) or high performance liquid chromatography/electrospray ionization/mass spectrometry (HPLC/ESI/MS), will be performed. The stable isotope tracking method will be evaluated during transport experiments using intact sediment cores and in the field, and compared with several other detection/enumeration methods.

Once developed, these methods will allow the movement of bacteria injected during in situ bioremediation to be accurately and easily assessed, thus improving the overall effectiveness of bioaugmentation efforts.

During the past year, the fluorescent tracking method has undergone extensive evaluation. A green fluorescent compound, 5-(and-6-)-carboxyfluorescein diacetate, succinimidyl ester (CFDA/SE), was shown to stain bacteria without significant effects on cell viability or adhesion to sediment. The stained cells remained fluorescent for at least 21 days in both groundwater and sediment microcosms. CFDA-stained cells were quantifiable by epifluorescent microscopy, flow cytometry and microplate spectrofluorimetry. Optimization of the microplate enumeration method resulted in a lower detection limit of approximately 10^5 CFDA-stained cells per well. Cell concentrations in the effluent of intact cores during bacterial transport experiments, as determined by viable plate counts, scintillation counting, direct microscopic counts of CFDA-stained cells, and microplate enumeration, were all similar. Evaluation of the fluorescent tracking method during a field-scale bacterial transport experiment was also performed, with near real-time measurement of the cell concentrations in individual samples using the microplate reader.

Work on the CRIMS stable isotope tracking method has been limited by difficulties with the prototype instrument. However, an alternative stable isotope method using HPLC/ESI/MS is being pursued in collaboration with D.C. White (Univ. of Tennessee). Intact core and field-scale bacterial transport experiments using ^{13}C-labeled cells have been performed, and samples are currently being analyzed.