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DEVELOPMENT AND EVALUATION OF STABLE ISOTOPE AND FLUORESCENT LABELING AND DETECTION METHODOLOGIES FOR TRACKING INJECTED BACTERIA DURING IN SITU BIOREMEDIATION

FINAL TECHNICAL REPORT

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Development and evaluation of stable isotope and fluorescent labeling and detection methodologies for tracking injected bacteria during *in situ* bioremediation.

ABSTRACT

The goal of this research project was to develop new methods to label bacterial cells so that they could be tracked and enumerated as they move in the subsurface after they are introduced into the groundwater (i.e., during bioaugmentation). Labeling methods based on stable isotopes of carbon (^{13}C) and vital fluorescent stains were developed. Both approaches proved successful with regards to the ability to effectively label bacterial cells. Several methods for enumeration of fluorescently-labeled cells were developed and validated, including near-real time microplate spectrofluorometry that could be performed in the field. However, the development of a novel enumeration method for the ^{13}C-enriched cells, chemical reaction interface/mass spectrometry (CRIMS), was not successful due to difficulties with the proposed instrumentation. Both labeling methodologies were successfully evaluated and validated during laboratory- and field-scale bacterial transport experiments. The methods developed during this research should be useful for future bacterial transport work as well as other microbial ecology research in a variety of environments.

SUMMARY OF RESULTS

The procedures and results of this research project have been detailed in manuscripts and meeting presentations over the duration of the project. The core of the methods that was developed during this research project is presented in an *Applied and Environmental Microbiology* article published in October 2000, which is available for download at http://aem.asm.org/cgi/reprint/66/10/4486.pdf. The reader is directed to the PUBLICATIONS AND PRESENTATIONS section of this report for specific details regarding the other various aspects of this project.

A summary of the major results that were obtained during this project is as follows:

1) A yellow-green stain, CFDA/SE (5-(and-6-)-carboxyfluorescein diacetate, succinimidyl ester) was found to be able to label bacterial cells with no undesirable effects on viability or transport. Stained cells remained

fluorescent for at least five months. A red-orange stain, TAMRA/SE (5-(and-6)-carboxytetramethylrhodamine, succinimidyl ester) was found that could also efficiently label cells for an extended duration.

2) A sequential culturing method was developed which resulted in cells that were highly isotopically enriched for ^{13}C. These cells were detectable using both isotope ratio mass spectrometry and high performance liquid chromatography/electrospray/mass spectrometry.

3) Detection and enumeration methods for CFDA/SE-stained and TAMRA/SE-stained cells were developed, including epifluorescence microscopy and high throughput microplate spectrofluorometry. Two additional detection methods, flow cytometry and ferrographic capture, were found to be effective for enumerating CFDA/SE-stained cells, but flow cytometry was not effective for enumerating TAMRA/SE-stained cells with the standard configuration of the flow cytometer.

4) A reproducible protocol for staining cells with CFDA/SE and TAMRA/SE has been developed, and successfully scaled-up to allow staining of 10 L of bacterial cells.

5) The microplate spectrofluorometry method has been optimized for detection of both CFDA/SE-stained and TAMRA/SE-stained in the same sample under both laboratory and field conditions. Analysis time for 24 samples (4 replicates per sample) was approximately two minutes.

6) The use of cells enriched in stable carbon isotope (^{13}C) was successfully evaluated during a field injection at the NABIR bacterial transport field site in Oyster, VA.

7) The use of the fluorescent staining and enumeration procedures were successfully evaluated during three field injections at the NABIR Bacterial Transport field site in Oyster, VA.

8) Multiple manuscripts related to this research have been published and/or submitted for publication. Multiple posters and platform sessions at national and international scientific meetings related to this research have been presented (see below).

PUBLICATIONS AND PRESENTATIONS

Peer Reviewed Journal Articles

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Hall J. A., Mailloux B. J., Long K., Onstott T. C., Fuller M., and DeFlaun M. Physical versus chemical effects on bacterial transport as determined during in situ short-core pulse experiments. *Water Resources Research* (In preparation):

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Book Chapters

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Fuller M. E., DeFlaun M. F., and Onstott T. C. (1999) Development and evaluation of fluorescent labeling techniques for tracking bacteria injected into subsurface environments. ASM 99th Annual Meeting. Chicago, IL, USA.

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