IV. Project Summary

Title: Monitoring: Evaluation of the Abundance, Diversity, and Activity of Methanotroph Populations in Groundwater

Project I.D.: UW-WRI #99-SAM-6

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Contract: July 1, 1998 through June 30, 2000

Background/Need: Groundwater monitoring

Objectives:

The objectives of this work are to develop methods that can be applied to the assessment of bioremediation by methanotrophs. These methods are for the detection of methanotrophs, evaluation of methanotroph activity, estimation of the size of the methanotroph population, assessment of population diversity in methanotrophic bacteria.

Methods:

Methods based on the polymerase chain reaction (PCR) were developed. Primer design and PCR conditions were optimized. Direct PCR (DPCR), in which environmental samples were used directly as a template without DNA extraction, was employed.

Results and Discussion:

Primers and PCR conditions were optimized to achieve specific amplification of the *pmoA*, which encodes a protein of the particulate methane monooxygenase. The presence of methanotrophs was detected by PCR amplification of *pmoA* using DNA extracted from groundwater as the template. The activity of methanotrophs in groundwater was indicated by detection of *pmoA* RNA by reverse transcriptase PCR. Methanotrophs were quantified in groundwater samples by DPCR in conjunction with Most Probably Number analysis or Competitive PCR. Methods to distinguish among methanotroph populations were evaluated.

Conclusions and Recommendations:

PCR-based methods for the detection of methanotrophs and methanotroph activity in groundwater have been developed. Two quantitative methods have been developed and applied to groundwater samples. Both of these used DPCR which is a rapid and simplified approach to sample preparation for PCR that allows detection and quantification of particular bacterial groups without isolation of DNA.

DPCR should be applicable to the detection of other bacteria in groundwater, including pathogens. This application of DPCR should be explored. DPCR should also be applicable for PCR-based methods for examination of methanotroph populations. This will require further investigation and empiric optimization.

Related Publications:

Cheng, Y. S., J. L. Halsey, K. A. Fode, C. C. Remsen and M. L. P. Collins. 1999. Detection of methanotrophs in groundwater by PCR. Appl. Environ. Microbiol. *65:* 648-651.

Drought, J. F., E. A. Buc, T. J. Grundl, K. A. Fode, M. L. P. Collins. 1999. Fate of tetrachloroethene and benzene at a dry cleaning facility. Proceedings of the 5th In Situ and On-site bioremediation symposium. Battelle Press, pp. 253-258.

Key words: bioremediation, TCE, MMO, methanotrophs, PCR