PhD Dissertation Defense

DEVELOPMENT AND APPLICATION OF A RAPID, USER-FRIENDLY AND INEXPENSIVE METHOD TO DETECT AND QUANTIFY DEHALOCOCCOIDE McCARTYI GENES IN GROUNDWATER SAMPLES

By

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Abstract

Tetrachloroethene (PCE) and trichloroethene (TCE) are the major contaminants at hazardous waste sites in the United States. The remediation of these chlorinated solvents frequently involves bioremediation approaches such as biostimulation or bioaugmentation. Both approaches aim at increasing the population of Dehalococcoides mccartyi cells in the subsurface. It has become common to quantify the population of these microorganisms both before and during the remediation process. TaqMan probe based quantitative polymerase chain reaction (qPCR) specific to the biomarker reductive dehalogenase (RDase) genes, such as vcrA, bvcA, and tceA, is now a widely accepted molecular biological tool for this task. However, alternate molecular methods that are faster and cheaper may make quantification significantly easier.

In this research, loop mediated isothermal amplification (LAMP) assays were developed for the rapid and specific quantification of the RDase genes, vcrA, tceA, and bvcA in groundwater samples. As a first step, the developed LAMP assays were validated using DNA from commercially available bioaugmenting cultures (SDC-9 and KB-1) and groundwater samples. To do this, the concentrations of Dehalococcoides mccartyi RDase genes with DNA templates obtained using LAMP were compared to concentrations obtained using qPCR on a real time thermal cycler. Additionally, the use of direct amplification was investigated. LAMP assays were then adapted for the development a field deployable kit. Here, an approach that requires only low cost laboratory equipment (a bench top centrifuge and a water bath) and significantly less time and resources compared to qPCR was developed. The method involves the concentration of biomass from groundwater (without DNA extraction) and LAMP of the cell templates. The amplification products are detected by a simple visual color change (orange/green). Finally, the most probable number (MPN) technique was incorporated into the LAMP method for the quantitative estimation of RDase gene concentrations in groundwater samples. Overall, quantification with LAMP on a real time thermal cycler was comparable to quantification with qPCR when DNA extracts prepared from SDC-9 and KB-1 or bioaugmented groundwater samples were used as templates for amplification. The LAMP assays to visually detect RDase genes, without DNA extraction or a thermal cycler, was successful to 1.8 X 10^5 gene copies per L for vcrA and 1.3 X 10^5 gene copies per L for tceA. Both values are below the threshold recommended for effective in situ dechlorination. Quantification with the MPN-LAMP assay using cell templates underestimated the concentration of RDase genes in groundwater samples compared to quantification with DNA templates and qPCR assay. Based on these results, response factors to correlate the MPN-LAMP data to estimated concentrations of RDase genes in groundwater samples were developed.