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Assessing the Contribution of Vinyl Chloride-Oxidizing Bacteria to In Situ Bioremediation Performance

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Background/Objectives. Generation of vinyl chloride (VC) plumes during in situ bioremediation of chloroethene-contaminated groundwater is a common problem. Common VC removal strategies include adding electron donor and/or bioaugmenting with commercial dechlorinating cultures. However, an alternative strategy is to recognize and harness the abilities of VC-oxidizing bacteria to support an overall reductive dechlorination scheme. Because methane and ethene typically co-occur in a VC plume originating from anaerobic reductive dechlorination of higher chloroethenes, the two most important VC-oxidizing groups are likely to be the methanotrophs and the etheneotrophs (i.e., ethene-oxidizing bacteria). We hypothesize that aerobic VC oxidation processes are significant at many sites with VC plumes that are considered anoxic or anaerobic. This is partially because sources of low level oxygen flux could exist at these sites and etheneotrophs are known to operate at oxygen concentrations well below the detection limit of most field DO probes. Our objective is to determine to what extent VC oxidizers contribute to the performance of in situ chloroethene bioremediation schemes.

Approach/Activities. We investigate the abundance and activity of VC-degrading bacteria by collecting groundwater samples, extracting DNA and RNA, and targeting key VC biodegradation functional genes (and their transcripts) via quantitative PCR. Geochemical parameters (e.g., methane, ethene, dissolved oxygen [DO], and chloroethenes) are measured concurrently. Etheneotroph functional genes include *etnC*, which encodes the alkene monooxygenase (AkMO) alpha subunit and *etnE*, which encodes the epoxyalkane:coenzyme M transferase (EaCoMT). Methanotrophic functional genes include *pmoA* (encodes a particulate methane monooxygenase subunit) and *mmoX* (encodes a soluble methane monooxygenase subunit). We also estimate the abundance and activity of VC reductive dehalogenase genes *bvcA* and *vcrA*. We investigated correlations between gene and transcript abundance, VC (and other chloroethene) concentrations, and DO and ORP in groundwater using a multi-level model approach. In addition, we performed similar analyses on cryo-core samples taken from a contaminated site in 2016.

Results/Lessons Learned. A total of 95 groundwater samples were collected and analyzed from VC plumes at six contaminated sites. Aerobic and anaerobic VC-degrading bacteria co-occurred in 98.9% of the samples and were co-active in 54.7% of the samples. This suggests that simultaneous aerobic and anaerobic VC biodegradation is a widespread phenomenon. VC concentrations were positively correlated to etheneotroph functional gene abundance (*etnC* and *etnE*, $p<0.001$) and their transcripts ($p<0.01$). Strong positive correlations were also noted between VC concentrations and VC reductive dehalogenase genes and their transcripts *bvcA* ($p<0.001$) and *vcrA* ($p<0.01$). No correlation was observed between VC concentration and abundance of methanotroph functional genes (*mmoX* and *pmoA*) or their transcripts ($p>0.05$). Interestingly, the abundance of *etnC* and *etnE* was negatively correlated to DO concentration ($p<0.01$). These correlations suggest that etheneotrophs could contribute significantly to VC biodegradation in anoxic groundwater experiencing a low-level DO flux. Initial analyses of cryo-core sediment samples confirm that etheneotrophs and anaerobic VC dechlorinating bacteria spatially co-exist. Overall, our data indicate that strategies for mitigating groundwater VC contamination should incorporate methods that track the abundance and activity of etheneotrophs as an integral feature of their remediation strategy performance assessment.