Determining the Minimal Inhibitory Concentration of Arsenic for *Dehalococcoides ethenogenes* strain 195

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Introduction: Groundwater contaminated with hazardous substances and pollutants, poses a health risk to communities that rely on it. The Agency for Toxic Substances and Disease Registry ranks arsenic first on the Priority List of Hazardous Substances (Zhou et al., 2014). A known carcinogen, arsenic may cause lung, skin, and liver cancers in addition to severe nerve damage (Petrusevski et al. 2007). The primary mode of exposure to arsenic is through contaminated drinking water. Arsenic occurs naturally in sediment, from which it can leach into groundwater reservoirs. Industrial and agricultural runoff also contribute significantly to arsenic accumulation. The Superfund database lists 228 sites that contain arsenic and organic co-contaminant TCE (Lee et al., 2011). The toxin TCE is linked to neurological issues, as it depresses the central nervous system, and to severe cardiac defects (National Research Council, 2007).

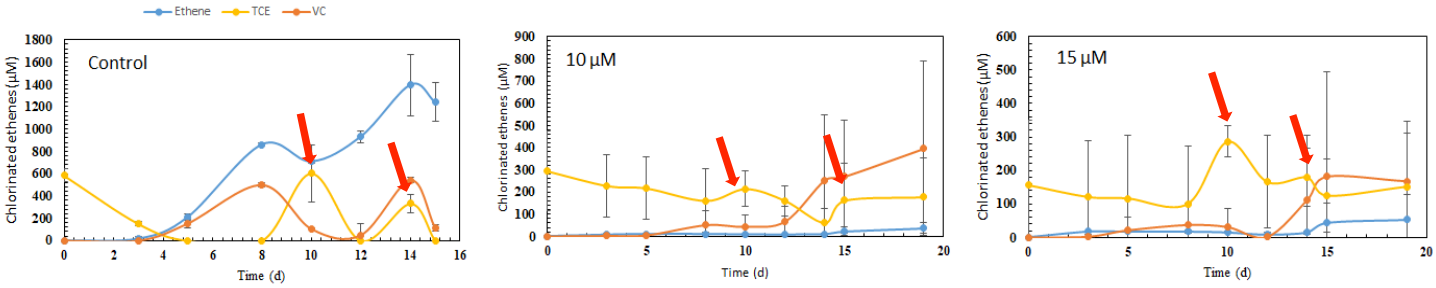
Current bioremediation strategies utilize subsurface microorganisms and mixed consortia able to survive in environments contaminated with heavy metals or metalloids like arsenic. These microorganisms employ survival mechanisms such as enzymatic oxidation/reduction, volatilization, precipitation, adsorption, and complexation (Nies, 1999). *In situ* bioremediation of chlorinated solvents using dechlorinating bacteria is well studied. These strategies rely on *Dehalococcoides* spp. because they utilize perchloroethene (PCE) and trichloroethene (TCE) as an energy source and thus have the metabolic pathways necessary to reduce these biohazards to nontoxic ethene (Men et al., 2012).

Research Objective: The primary research goal was to establish the minimal inhibitory concentration (MIC) of arsenic for *D. ethenogenes* strain 195 (DE195). The MIC quantifies the amount of arsenic DE195 can tolerate while reducing TCE. This research will provide an understanding of the *Dehalococcoides* found at co-contaminated Superfund sites, indicating if the native population will be viable for immediate TCE remediation, would need to be augmented, or would require decreasing of the arsenic concentration prior to TCE remediation.

Procedure: To determine the MIC of arsenic for pure DE195, it was grown in BAV1 medium (Maymó-Gatell et al. 1995), maintained at 34 °C. The medium was purged with H_2/CO_2 to maintain anaerobic conditions and provide the necessary electron donor for DE195. Cultures were initially fed 5 mM acetate as the carbon source and 7 µL TCE as the energy source, in addition to a vitamin solution (Wolin et al. 1963). Arsenic species were added before DE195 inoculation. Arsenite (As(III)) concentrations tested: 5 µM, 10 µM, and 15 µM. Arsenate (As(V)) concentrations tested: 300 µM, 400 µM, and 500 µM. All treatments were tested in biological triplicates, and the arsenic concentrations were determined from preliminary data.

To determine DE195 activity TCE reduction was monitored with gas chromatography. When no TCE/cDCE peaks were observed additional TCE was amended to ensure a sufficient energy source. To test for growth inhibition by the arsenic species, cell numbers were analyzed using quantitative polymerase chain reaction (qPCR). The tceA gene was amplified because it is specific to *Dehalococcoides*.

Results and Discussion:



TCE reduction decreased upon exposure to As(III) above 10 µM (Figure 1). The control (not supplemented with arsenic) accumulated ethene whereas the cultures containing 10 and 15 µM of As(III) did not. DE195 reduces TCE to vinyl chloride (VC) metabolically and VC to ethene co-metabolically. The lack of ethene accumulation could indicate diversion of energy to compensate for enzymes inhibited by AS(III). These results show a As(III) MIC range between 10 and 15 µM. As(V) has similar inhibitory effect, but at concentrations fiftyfold higher. It is not expected for groundwater to reach the As(V) MIC.

Within the experiment time frame, cell growth did not significantly change (Figure 2). The cell measurements may be an over estimate, since targeting DNA with qPCR can also amplify sequences from dead bacteria (RNA amplification is recommended for future work). It is expected long term exposure to MIC levels of arsenic will lead to increased cell death since TCE reduction is inhibited.

Conclusion: Data corresponds to the trend that increasing As concentration inhibits TCE reduction

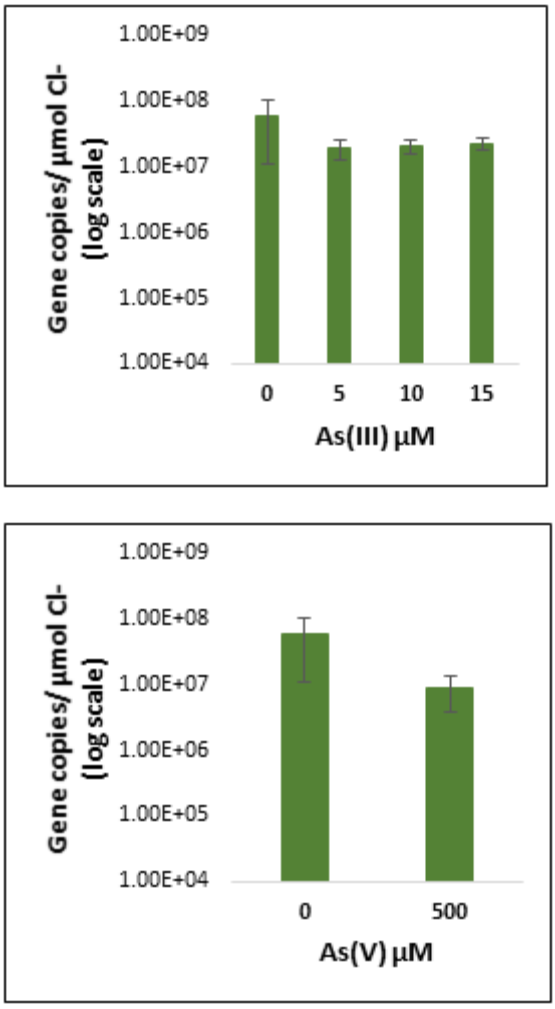


Figure 2. Gene copies/ µmole Cl^- for varying arsenic concentrations.

faster than it causes cell death. Since the inhibition of TCE is indicative of the ability of the bacteria to receive energy, it is a more important parameter to follow. Of the two arsenic species, As(III) is of more concern since the observed MIC for As(V) is above environmental values. The results of this experiment will help guide future experiments looking at co-remediation of arsenic and TCE.

References

Lee, P. K. H., D. Cheng, P. Hu, K. A. West, G. J. Dick, E. L. Brodie, G. L. Andersen, S. H. Zinder, J. He, and L. Alvarez-Cohen. 2011. Comparative genomics of two newly isolated *Dehalococcoides* strains and an enrichment using a genus microarray. *ISME J.* **5**(6):1014-1024.

Maymó-Gatell, X., Tandoi, V., Gossett, J. M., & Zinder, S. H. (1995). Characterization of an H2- utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis. *Applied and Environmental Microbiology*, *61*(11), 3928-3933.

Men, Y., H. Feil, N. C. VerBerkmoes, M. B. Shah, D. R. Johnson, P. K. H. Lee, K. A. West, S. H. Zinder, G. L. Andersen, and L. Alvarez-Cohen. 2012. Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* Hildenborough and *Methanobacterium congolense*: global transcriptomic and proteomic analyses. *ISME J.* **6**(2):410-421.

National Research Council. (2007). *Assessing the human health risks of trichloroethylene: Key scientific issues*. National Academies Press.

Nies, D. H. 1999. Microbial heavy-metal resistance. *Appl. Microbial. Biotechnol.* **51**(6):730-750.

Petrusevski, B., Sharma, S., Schippers, J. C., & Shordt, K. (2007). Arsenic in drinking water. *Delft: IRC International Water and Sanitation Centre*, *17*(1), 36-44.

Wolin, E. A., Wolin, M., & Wolfe, R. S. (1963). Formation of methane by bacterial extracts. *Journal of Biological Chemistry*, *238*(8), 2882-2886.

Zhou, J., Z. He, Y. Yang, Y. Deng, S. G. Tringe, and L. Alvarez-Cohen. 2014. High-throughput metagenomic technologies for complex microbial community analysis. *mBio* **6**(1):e02288-14.