Microbial Metabolism of the Priority Pollutant Trichloromethane in Subsurface Environments

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Background/Objectives. Trichloromethane, or chloroform (CF), is a common groundwater priority pollutant, ranked 11th on the US EPA's list of hazardous chemicals. CF is a naturally occurring compound, however human activity has introduced CF en masse into subsurface environments in all industrialized countries. In addition to its acute and chronic toxicity to humans, CF is problematic in that it is a powerful inhibitor of most subsurface microbial processes including organohalide respiration. Therefore, where CF exists as a co-pollutant with other organohalides, remediation using organohalide respiring bacteria is not feasible.

Approach/Activities. Recent discoveries of CF respiring dichloromethane (DCM) fermenting strains have the potential to open up CF co-polluted sites to bioremediation strategies. *Dehalobacter* strain UNSWDHB, which was discovered in subsurface soil (5 m depth) from the Botany Sands Aquifer, can tolerate up to 500 mg/L of CF (more than 500 times the inhibitory concentration for most bacteria) and dechlorinate it at a maximal rate of ~50 mg/L per day. The reductive dehalogenase (TmrA) responsible for CF dechlorination was identified and shown also to dechlorinate a range of chlorinated ethanes. Compound specific isotope analysis (CSIA) of CF during respiration showed vastly different carbon isotope enrichment factors in closely related *Dehalobacter* strains CF (-27.2‰) and UNSWDHB (-4.3‰). These CSIA data, coupled with comparison of physiological and enzyme kinetics, shows that strain UNSWDHB is highly adapted to metabolizing CF. Further, dual isotope analysis (C, Cl) of CF during respiration by strain UNSWDHB gave uncharacteristic inverse chlorine isotope effect (+2.5‰) that can be used to good effect in tracking the in situ degradation of CF by this strain.

The product of microbial CF respiration is DCM. While less toxic and less problematic than CF, DCM is still an undesirable substance in subsurface environments and is in fact ranked 88th on the US EPA's list of hazardous chemicals. Fortunately, recent discoveries of DCM fermenting microorganisms (two of) from the Botany Sands Aquifer, co-existing with CF respiring *Dehalobacter* UNSWDHB, were found to transform DCM to acetate and/or hydrogen. The combination of CF respiration with DCM fermentation in essence results in complete detoxification of chloroform. Of the two DCM fermenting organisms discovered, one belongs to the *Dehalobacter* genus, the other represents a new genus in the family and has been isolated and characterized both physiologically and genomically, and tentatively named strain DCMF.

Results/Lessons Learned. Collectively, data will be presented in terms of how our understanding of CF degradation in subsurface environments has recently advanced, and how CF co-contaminated sites may now be remediated using microorganisms.