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Assessing ecological competition for electron donor within a groundwater microbial community that contains organohalide-respiring bacteria

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Introduction

In many remediation and engineering applications, groundwater microbial communities are treated as a single, black-box element of the biogeochemistry of a contaminated site. In reality, this interpretation simplifies what is often a complex microbial ecosystem. Understanding microbial community interactions in groundwater communities could be important to determine drivers or inhibitors to chlorinated solvent biodegradation (Meckenstock et al., 2015; Shani, 2012).

Organohalide-respiring bacteria (OHRB) are capable of sequentially dechlorinating the contaminants tetrachloroethene (PCE) and trichloroethene (TCE) to dichlorothene (DCE), vinyl chloride (VC), and ultimately ethene – the only compound in the series not harmful to human health. Biodegradation often stalls at the intermediate steps, even when conditions seem conducive to complete reductive dehalogenation (Shani, 2012). This stall could be due to competition for resources, namely for hydrogen, which other community members also use as an electron donor in conjunction with compounds such as iron(III) and sulfate as electron acceptors. Field investigations and modelling initiatives indicate that competition from iron- and/or sulfate-reducing bacteria inhibit OHRB (Shani et al., 2013; Malaguerra et al., 2011).

Understanding the ecological interplay in these microbial communities is essential to determine the fate of organic solvents in groundwater and design bioremediation systems for clean-up. Laboratory experiments to determine the effect of competition on the community function (i.e. reductive dehalogenation) will be conducted – the aim of this preliminary experiment is to show that iron reduction does impact dechlorination by an OHRB community when only hydrogen is available as an electron donor.

Material and Methods

A cell suspension experiment with an OHRB containing consortium and an iron-reducing organism, *Shewanella oneidensis*, was conducted. The cells were suspended in a buffer solution containing specific electron donors and electron acceptors.

S. oneidensis, a facultative anaerobe, was cultivated aerobically in lysogeny broth. The OHRB consortium was cultivated in anaerobic mineral medium (Holliger et al., 1993), given PCE in hexadecane as electron acceptor, and was sealed in an anaerobic vessel whose headspace was filled with hydrogen and carbon dioxide. The live cultures were centrifuged and washed with anaerobic Tris buffer in sealed, anaerobic vessels, then suspended in Tris buffer such that both concentrated inocula had equivalent optical density.

For the experiment, 20 mL anaerobic flasks each containing 5 mL of Tris buffer were prepared. The headspace in the flasks was filled with hydrogen. Each flask was prepared in

the combinations shown in Table 1. Headspace composition was measured with gas chromatography and iron(II) was measured via colorimetric ferrozine assay. Measurements were taken at time zero and once approximately every 24 h for a period of 115 h.

Table 1 Flask configuration for cell suspension experiment. 1 mL of approximately 250 mM L^{-1} naturally produced DCE in hexadecane was added. Ferric citrate was added to a final concentration of 5 mM L^{-1}.

Flask	Electron Acceptor	Bacteria	*S. oneidensis*:OHRB consortium ratio (by vol.)
1	DCE	OHRB consortium	0:100
2	Ferric citrate	*S. oneidensis*	100:0
3	DCE	*S. oneidensis*, OHRB consortium	50:50
4	DCE, Ferric citrate	*S. oneidensis*, OHRB consortium	50:50
5	DCE, Ferric citrate	*S. oneidensis*, OHRB consortium	10:90

Results and Conclusions

Iron(II) concentrations increased at the same rate in all flasks; within 45 h of beginning the experiment, all iron(III) was consumed in all flasks. However, at 115 h, the gaseous chlorinated compound composition of each flask varied, as indicated by the VC:DCE ratio shown in Figure 1.

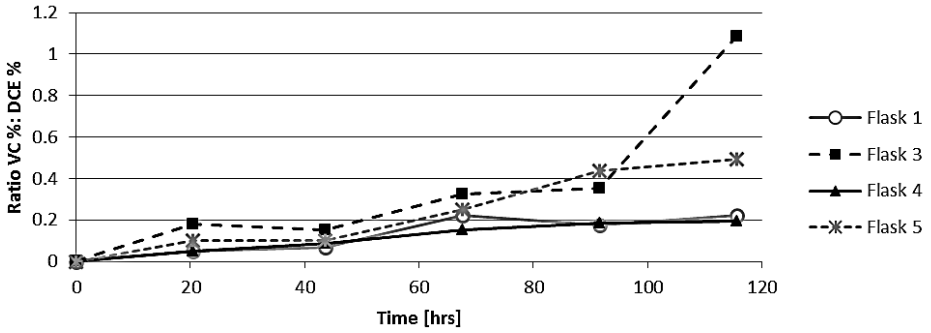

Figure 1 Gaseous chlorinated compound composition of each flask. Initial composition was 100% DCE in each.

The flask that experienced the least change included active iron reduction and a 50:50 inocula ratio. Interestingly, the flask with the most dechlorination was that with both inocula and only DCE – the presence of *S. oneidensis* may accelerate dechlorination. This preliminary study indicates that iron-reducing organisms do impact dechlorination when only hydrogen is available as electron donor. Future growth experiments will track the complete dechlorination pathway and include OHRB consortia that begin degradation with PCE.

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