Verification of Analytical and Amendment Approaches for an *In Situ* Microcosm Device for Testing Enhanced Remediation Processes

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Introduction

In situ **microcosm (ISM) devices are increasingly used to test remediation processes in groundwater though incubation in monitoring wells and retrieval, after which parameters relevant to the remediation processes are tested on the groundwater or solid matrices contained within the ISM (IRTC, 2011; Stroo et al., 2012).**

The functions of an ISM device include:

- **Acting as a retrievable passive sampler for microbial colonization and molecular testing;**
- **Providing a matrix/surfaces for biotic/abiotic reactions; and**
- **Delivery/assessment of remediation amendments including electron donors, trace nutrients, bioaugmentation cultures, chemical oxidants, zero-valent iron or other relevant amendments.**

To accomplish the above, a ISM prototype design and testing regime was developed by SIREM.

FIGURE 1: ISM Prototype is comprised of modified Snap Sampler™ with the addition of an aquifer matrix filled column that provides a simulated aquifer environment for in situ testing of remediation processes once filled with site groundwater-note sampler is shown in closed mode which creates an isolated microcosm for controlled testing of remediation parameters

In Situ Microcosm Design and Concept

The ISM design (Figure 1) includes modifications to the patented Snap Sampler™ (ProHydro www.snapsampler.com) including an interior mounted PVC column with porous stainless steel end caps. The PVC column can be filled with a matrix of choice (e.g., aquifer sand). The units can be deployed in sets to evaluate different amendment regimes including addition of electron donors, trace nutrients, bioaugmentation cultures, pH, ZVI and other solid amendments. The matrix provides an "aquifer like" environment once filled with groundwater, with surface areas for microbial colonization, sorption and abiotic reactions. The Snap Sampler™ can be left open to communicate with groundwater or be closed to isolate the microcosm.

ISM testing suites include quantification of the contaminants (e.g., chlorinated volatile organic compounds [CVOCs]), volatile fatty acids (VFA), total organic carbon, dissolved hydrocarbon gases (DHG), anions, molecular genetic tests for biodegradative organisms and functional genes, as well as overall microbial community composition and compound specific isotope analysis (CSIA) to confirm degradation processes.

Key questions in this study included:

- How well does the ISM column device communicate with groundwater?
- Does the device function effectively in terms of filling, closing and remaining sealed?
- Can the ISM effectively introduce electron donors?
- Are reductive dechlorination and related parameters measurable upon retrieval?
- How does the ISM data compare to that from standard microcosm studies?

Results

Filling of ISM and Communication with Groundwater

Using a dye diffusion experiment, the ISM column device was demonstrated to effectively fill and communicate with groundwater. This is important for its use as a passive sample matrix for microbial constituents and introduction of groundwater geochemistry to the ISM column.

FIGURE 2: Dye diffusion test indicated that aquifer matrix column containing bromophenol blue crystals **(A)** filled effectively when immersed and dye had mostly diffused into surrounding water in ~6 hours **(B)** 24 hours after immersion, dye had completely diffused from the column to the water, demonstrating effective communication of the column matrix with the surrounding "aquifer".

Mechanical Functioning of the ISM Unit

Experiments to determine the feasibility of this ISM approach in simulated groundwater wells were carried out using glass columns filled with groundwater or media (Figure 3). Under these test conditions the ISM was determined to fill, close and seal effectively.

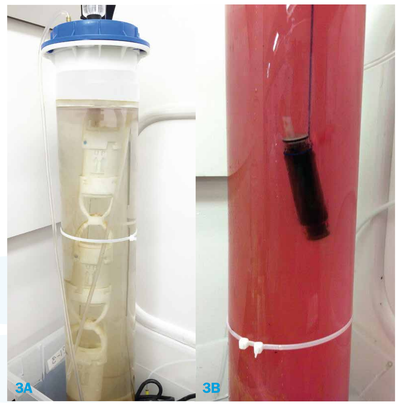

FIGURE 3: (A) Laboratory simulation of ISM deployment with groundwater in glass column simulating a 4" well. Three ISM devices were deployed and closed after a 1 hour equilibration. **(B)** Demonstrates the end-point of e-donor test (outlined in Figure 4). After 4 weeks of incubation the ISM was highly reducing (black with ORP -249 mV) while the bulk media in which it was incubated was oxidizing [pink redox indicator/ ORP =+113 mV]. This demonstrates that the ISM unit is an effective microcosm that remains isolated once closed *in situ*.

Electron Donor Test

This test was performed to determine if electron (e-) donors added to the ISM were effective for biostimulation and if the ISM could be effectively sampled to quantify the impact of e- donors. Sodium lactate, chitin (CHITOREM® [JRW Bioremediation]), 10 mg each, and emulsified vegetable oil (EVO) (EDS-ER™ [Tersus Enviromental]) at 0.1% v/v, representing an ~10X stoichiometric demand of TCE, were mixed with ISM column sand prior to deployment. The units were deployed into reduced (ORP -250 mV) anaerobic mineral salts media (KB-1® media) with 10 mg/L TCE media lacking e-donor. The media was inoculated with KB-1® to a target *Dehalococcoides (Dhc)* concentration of 5 x 10^8/L. The units were incubated open for ½ hour to allow equilibration with geochemistry/microbes prior to closing. Dechlorination was monitored using, identically amended, parallel microcosms to inform when to retrieve the ISM units and to compare performance of ISM to standard microcosms. ISMs were tested for CVOCs, DHG, VFAs, *Dhc*/total *Bacteria* as indicated in Figure 4.

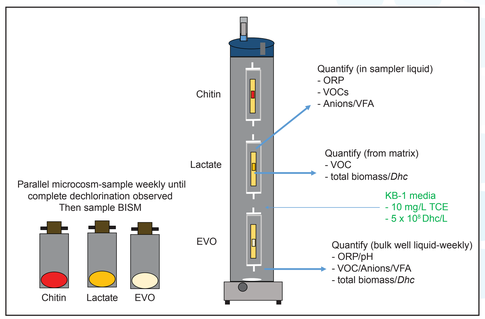

Figure 4: Overview of electron donor experiment to test dechlorination with addition of lactate, EVO and chitin to ISM units in media containing *Dehalococcoides* and TCE. The same treatments were set-up as parallel standard microcosms (left) for performance comparison.

Parallel microcosm testing indicated that chitin treatment promoted the most rapid dechlorination and the corresponding ISM unit was retrieved after 8 days of incubation. It contained no TCE, cDCE was predominant, some vinyl chloride (VC) was observed, and ethene was not detected (Table 1A). The lactate and EVO treatments were retrieved after 28 days and VC concentrations were 0.47-0.62 mg/L with low concentrations of ethene quantified at 0.02-0.04 mg/L. Comparison of dechlorination rates between the ISM and the parallel microcosms suggested that dechlorination rates were substantially slower in the ISM. A previous study (Dworatzek et al, 2011) reported that dechlorination in standard microcosms may be faster than corresponding field rates. Whether slower dechlorination compared to standard microcosms is more, or less representative of *in situ* remediation results will require further study.

Interestingly, methane was detected in the ISM at higher concentrations than in the parallel microcosms. In the ISM EVO treatment, methane was observed at 7 mg/L compared to only 0.29 mg/L in the parallel microcosms which may indicate increased methanogenesis in the ISM. Gene-Trac® tests for *Dhc* and total *Bacteria* performed on DNA extracted from the ISM column sand were moderately positive indicating that *Dhc* and other microorganisms had entered the columns and colonized the matrix. The abundance of *Dhc* (10^4-10^5 *Dhc*/g) and total *Bacteria* observed in the chitin, lactate and EVO ISMs was similar, however, the proportion of *Dhc*, relative to other bacteria, was higher in both the EVO and lactate treatments possibly due to the longer incubation time. Fermentation of the lactate and EVO donors was observed in the EVO and lactate treatments. Table 1B summarizes the VFAs quantified within these ISMs, with acetate and propionate both detected while other VFAs were not detected. Additionally, the pH was neutral and ORP was strongly reducing which are optimal for reductive dechlorination in all the ISM units.

Table 1A CVOC and Related Parameters in Electron Donors Test

Electron Donor Tested	Incubation Time (Days)	TCE	cDCE	VC	ethene	methane	*Dhc*/g	Total *Bacteria* /gram	% *Dhc*
		mg/L	mg/L	mg/L	mg/L	mg/L			%
Chitin									
ISM bulk liquid	8	<0.02	3.8	0.07	0.01 U	0.26	--	--	--
ISM Column	8	<0.02	3.41	0.04	0.02	0.2	1.00E+05	5.00E+07	0.20
Parallel Microcosm	8	<0.02	1.52	2.35	0.44	0.11	--	--	--
Lactate									
ISM Bulk liquid	28	0	2.82	0.62	0.02	0.59			
ISM Column	28	0.01	1.77	0.48	0.02	1.24	2.00E+05	3.00E+06	6.67
Parallel Microcosm	28	0.01	0.00	0.01	0.35	0.94	--	--	--
EVO									
ISM bulk liquid	28	0.00	2.67	0.52	0.02	2.23			
ISM Column	28	0.01	2.35	0.47	0.04	7.00	7.00E+04	3.00E+06	2.33
Parallel Microcosm	28	0.00	0.00	0.01	0.53	0.29	--	--	--

Table 1B Detection of VFAs in ISM amended with Electron Donors

Electron Donor Added	Lactate	Acetate	Propionate	Pyruvate	Butyrate	Formate	pH	ORP
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L		mV
Lactate (28 days)								
ISM Bulk liquid	<0.39	307	177	<0.69	<0.41	<0.22	7.06	-249
EVO (28 days)								
ISM Bulk liquid	<0.39	122	64	<0.69	<0.41	<0.22	7.36	-180
Chitin (8 days)								
ISM Bulk liquid	<0.39	<0.54	<0.31	<0.69	<0.41	<0.22	7.29	NM

Conclusions/Future Work

A novel ISM approach has several advantages over other commonly used ISM or passive sampling matrix designs including:

- **Ease of use, should not require substantial assembly at the site of deployment.**
- **Sufficiently small so that several units are deployable within a short (2-foot) interval.**
- **Columns can be filled with geologic materials including (sand/limestone/clay/silt) with microbial attachment and geochemical properties similar to aquifer matrices at subject sites.**
- **They can be sealed *in-situ*, allowing retrieval and shipment to lab without interrupting internal conditions.**

Initial Testing Results

- **Upon immersion in water, the ISM columns filled effectively and communicated with groundwater allowing them to be colonized and exposed to aquifer geochemistry.**
- **Samplers effectively closed and produced a true microcosm isolated from bulk well conditions.**
- **The liquid and the column aquifer matrix in the ISM were successfully sampled for a variety of parameters including pH, ORP, CVOCs, VFAs, dissolved gases and molecular genetic tests.**
- **Electron donors can be added to the ISM column, effectively fermented and promote biostimulation**

Future Work

- **Testing of ISM unit as a platform for delivery of anaerobic bioaugmentation cultures.**
- **Determining additional amendment and testing regimes for different contaminant classes and remediation scenarios (e.g., anaerobic BTEX, ammonium/nitrate, perchlorate) remediation.**
- **Field deployment and comparison of field biodegradation rates to ISM half-lives to better understand the use of this approach as a predictor of field performance.**

References

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