FIELD & LABORATORY PROTOCOLS REPORT

Field Assessment of Abiotic Attenuation Rates using Chemical Reactivity Probes and Cryogenic Core Collection

SERDP Project ER-2621

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14. ABSTRACT

During the execution of ER 2621, a number of new protocols were developed. The purpose of this document is to describe those new protocols. For completeness, several protocols that were developed concurrent with this project will be briefly outlined as well.

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ACRONYMS AND ABBREVIATIONS

CT	Carbon tetrachloride
ES	Electron shuttle
ESTCP	Environmental Security Technology Certification Program
SERDP	Strategic Environmental Research and Development Program
TCE	Trichloroethylene
TCP	1,2,3-Trichloropropane

1. Overview

During the execution of ER 2621, a number of new protocols were developed. The purpose of this document is to describe those new protocols. For completeness, several protocols that were developed concurrent with this project will be briefly outlined as well.

The protocols are summarized schematically in the flow diagram below (**Figure 1.1**). Each box is described in the sections below and the numbers in each box refer to the section number where the description is found.

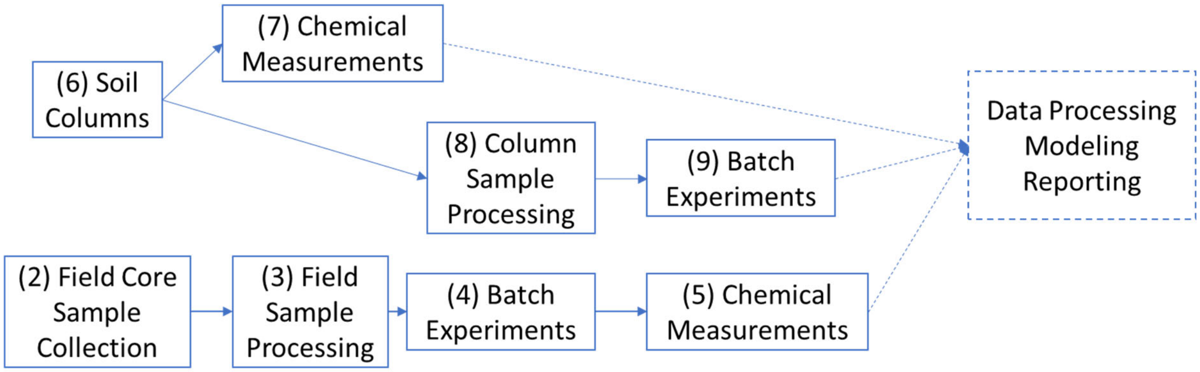

Figure 1.1. Flow diagram of sample handling for laboratory column experiments and field core samples. The numbers in parenthesis represent the section numbers of this report.

2. Field Core Sample Collection

Sample Collection and Preservation in the Field.

All field samples for this project were collected using one of two approaches and both utilize standard hollow-stem auger techniques. Samples from one site (St. Louis Ordinance Plant, MO) were collected using continuous coring and conventional hollow-stem sample recovery (**Figure 2.1**). Those samples were immediately frozen at ground surface by packing them in dry ice.

Most samples were collected using cryogenic core collection. The cryogenic core collection method used in this study was co-developed during this project and an overlapping Strategic Environmental Research and Development Program (SERDP) project (ER-1740) and was described in detail previously (Sale et al., 2016, Kiaalhosseeni et al., 2016; Johnson et al., 2012; Brow et al., 2010).

Figure 2.1. Photograph of the hollow-stem auger used for most of the core sampling (Here at Indian Head, MD).

In this study, cryogenic core collection was performed by Drilling Engineers Inc. (Ft. Collins. CO) using a truck mounted hollow stem auger and continuous sample tube system modified to allow for injection of liquid nitrogen into a dual-walled cooling cylinder. Rapid circulation of liquid nitrogen ensured fast-freezing in the polyvinyl-chloride sleeves, which resulted in greater than 95% recovery in all media, thereby enabling high throughput and high-resolution core analysis for multiple analytes. Cryogenic core collection and preservation has been previously shown to provide preservation for microbial analytes (Johnson et al., 2012; Brow et al., 2010), and the rapid freezing prevents redistribution of analytes within the sample matrix, providing the highest quality preservation available for ex situ analysis (Wilkin, 2006). Samples from the Indian Head, MD (IH) and SLOP sites were collected by collaborators through ESTCP grant numbers ER-201589 (Olson et al., 2017) and ER-201587 (Popovic et al., 2018). Samples from the Parris Island, SC (PI) site were collected in collaboration with Environmental Security Technology Certification Program (ESTCP) Project ER-201425 (Mattes, 2019). F.W. Warren AFB, WY (FEW) site samples were collected at the same location reported by Sale et al. (2016).

Figure 2.2. Liquid nitrogen injection and vending during the freezing process.

Core Collection

The core collection process begins by advancing the auger down to the desired sampling interval. At that point, a specially designed dual-walled core barrel, connected to insulated stainless steel tubes was lowered into the auger and the auger and core barrel are advanced to fill the core barrel. One of the stainless steel tubes running from the core barrel to ground surface was then connected to a high-pressure liquid nitrogen (LN) tank and LN was injected through the core barrel. After passing through the core barrel the LN vapor and liquid were vented to the atmosphere (**Figure 2.2**).

This freezing process operates for ~5 minutes, is stopped, and the core sample is immediately brought to ground surface. The frozen core sample, still in it's clear PVC sleeve, is capped and taped before being placed in a cooler with dry ice for overnight shipment to the lab (**Figures 2.3 and 2.4.**).

Figure 2.3. Capping and sealing the frozen core section prior to placing on dry ice.

Figure 2.4. Packing frozen soil cores and dry ice into coolers for overnight shipment.

3. Laboratory Processing of Field Samples

Sample Processing in the Laboratory.

To preserve in situ conditions as much as possible, the cryogenically collected cores were stored intact in core sleeves in a -20^{o} C freezer until laboratory experiments were initiated. Processing of the field samples outside of an anoxic chamber was limited to 5 min in a walk-in refrigerator at 15^{o} C. This processing consisted of cutting the core into 1 inch “hockey pucks with a diamond blade cutoff saw (**Figure 3.1**) and cutting the frozen puck into pieces for batch reaction testing (**Figure 3.2**). All subsequent processing of samples for experiments was conducted inside an anoxic chamber (high purity N_2, <0.2 ppm O_2).

Figure 3.1. Schematic drawing of the batch reactivity experiment process used for field samples.

4. Batch Reactivity Experiments with Field Samples

The overall process for batch reactivity experiments using field samples is shown in **Figure 4.1**.

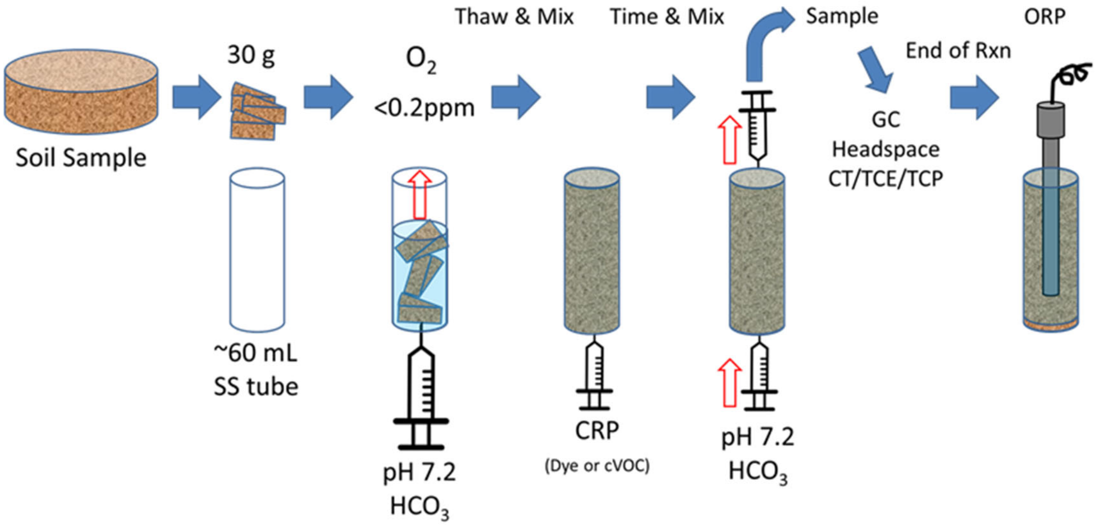

Figure 4.1. Schematic drawing of the batch reactivity experiment process used for field samples.

Briefly, the process is initiated by adding frozen soil samples to the reactor vessel, filling the vessel with carbonate-buffered water, adding the chemical reactivity probe (and a non-reactive tracer) and then slowly rolling the samples between periodic sampling intervals. The sampling and analysis steps were repeated as often as needed, and then a final measurement of reduction potential and pH were made.

Design of the "Rolling Tube Reactors" (RTRs)

An important innovation of this project was the development of specialized reactors for batch reactivity experiments. The design criteria for these reactors included:

- No septa or other porous materials (to allow long-term sampling)
- Accommodating high soil:water ratios
- Allowing testing at elevated temperatures
- Anoxic sampling
- The ability to re-sample the same experimental system
- Wide reactor opening to facilitate loading frozen samples.

The first 4 criteria could be met using sealed glass ampules. However, re-sampling and the use of unbroken pieces of frozen core were key to our experiments and therefore ampules could not be used.

Our solution was to develop reactors made from 316L stainless steel, which could be polished to produce highly passive surfaces. An “exploded” view of the reactor is shown in **Figure 4.2**.

Figure 4.2. Photograph of disassembled Rolling Tube Reactor

The reactors consisted of 16.6 cm long, 2.54 cm OD, 2.2 cm ID tubes with Swagelok^{TM} caps on each end. The caps had been modified to add threaded Swagelok male connectors. The cap on one end contained a 1/8” tube x 1/8” male pipe thread (SS-200-1-2) and the other cap contained a 1/16” tube x 1/8” male pipe thread (SS-100-1-2). During normal operations, the male adapters were capped with Swagelok plugs.

<u>**Initiating batch experiments in the RTRs**</u>

Figure 4.3 illustrates further sample processing after the cores had been cut into frozen soil pucks. The pucks were then subdivided using a mallet and mason’s chisel on a cutting board (A and B), and the still-frozen sections were then placed into the RTR (C).

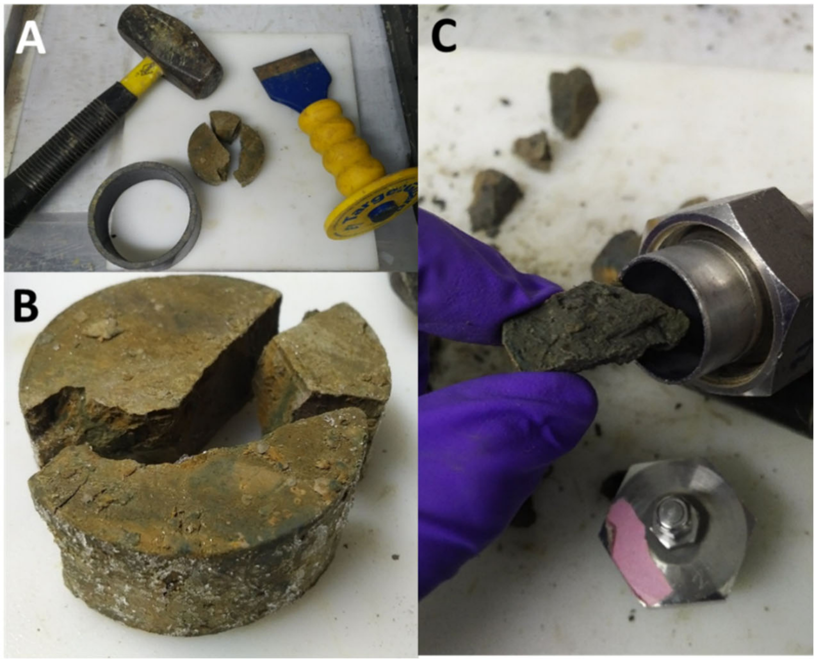

Figure 4.3. Photos of sample processing of cryogenically stored material and preparation of kinetic experiments. (A) Frozen material was split using a chisel and mallet and (B) subsampled from one inch. frozen puck sized pieces that are cut from a soil core. (C) Rotating Tube Reactors (RTRs) and electrochemical cells are loaded with frozen pieces of sample before being sealed. Processing time was kept under 2 mins of exposure to minimize passivation (oxidation) of mineral species. All of these steps were performed in a cold room at 4^{o} C.

Initiating the batch reactivity experiments

Once the cryogenic core material was loaded into an unsealed RTR, the RTR was capped and sealed except for one end plug It was then passed into an anoxic chamber by purging the anti-chamber 4 times with nitrogen. Once inside the chamber, the RTRs were flooded with deoxygenated 6.4 mM bicarbonate buffered solution to completely fill the tubes with water using a custom made Luer-to-1/16th Swaglock stainless steel adaptor (**Figure 4.4**). The reactor was then sealed completely and passed out of the anoxic chamber. It was allowed to mix overnight at 6 rpm using a custom-made roller (shown in **Figure 4.5**). The sample and fluid masses in the RTRs were determined gravimetrically at each step of the process.

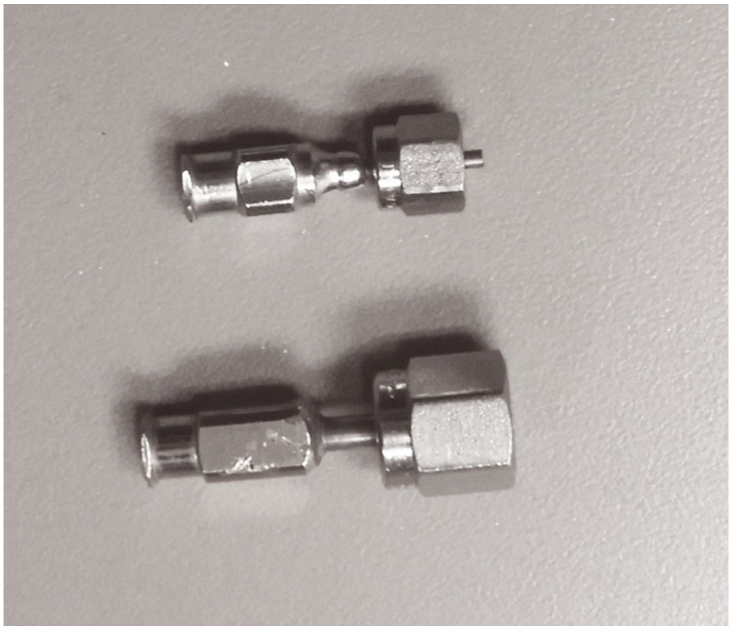

Figure 4.4. Luer to Swagelok adaptors. The 1/8" adaptor uses an 11-gauge needle, the 1/16" adaptor uses a 16 gauge needle.

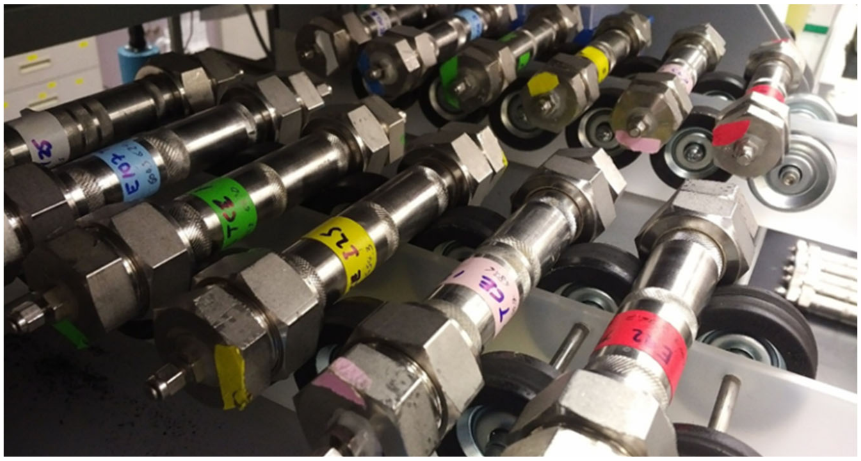

Figure 4.5. Rolling Tube Reactors (RTR) reactors used for the kinetic studies. Here they are shown on the roller system that rotated them at ~6 revolutions per minute.

Addition of reactants – To initiate the reactivity experiments, each RTR was hung vertically for 1 hour, then the 1/16” plug was removed from the bottom and a syringe containing 1 ml of saturated carbon tetrachloride (CT) solution and 1 ml of saturated 1,2,3-trichloropropane (TCP) solution was attached to the male connector using a Luer to Swagelok adapter. The upper 1/8” plug was then removed, a syringe attached and the solution was injected into the RTRs (**Figure 4.6**). The resulting initial concentrations of the CT and TCP are 84 and 190 uM, respectively.

Contaminant Degradation Rate Experiments. Preliminary experiments using RTRs and field sediment show no significant loss of TCP over 500 hours. (This was consistent with a wide range of other experiments in our lab). As a consequence, it was concluded that TCP was appropriate to use as an internal standard for subsequent experiments. Additionally, the TCP can be used to automatically account for dilution resulting from the sampling process.

Sampling – Prior to each sampling event, the RTRs are removed from the rollers and hung vertically to settle for an hour. After hanging, the 1/16th inch fitting at the bottom was removed and a 3 mL syringe with 2 mL of deoxygenated, carbonate-buffered water was attached. The 1/8th inch fitting at the top was then removed and an adaptor and a 1 mL syringe was connected to it. One mL of water is then injected from the bottom syringe into the tube and a sample was collected it in the top syringe.

The 1 mL of sample was then injected into a 20-mL crimp-sealed, anaerobic GCMS headspace vial for analysis. The Swagelok plugs were then replaced on the RTR and it was returned to the roller.

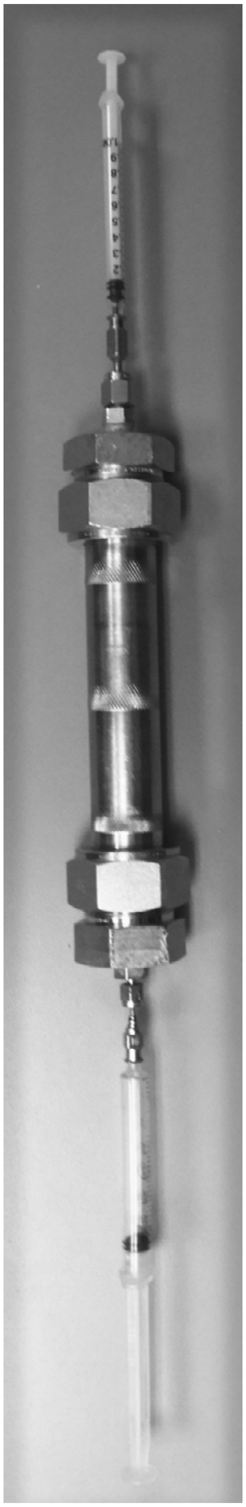

Figure 4.6. RTR with upper and lower syringes attached. (Process for injecting analytes and for sampling)

5. Chemical Measurements

Gas Chromatographic Analysis of Contaminants/Reactivity Probes.

GC/ECD Measurements –

For approximately the first half of this project, determination of trichloroethylene (TCE), CT and TCP concentrations, as well as chlorinated reduction products, was accomplished using a Hewlitt Packard 5790 gas chromatograph with an electron capture detector (GC/ECD). Calibration of the GC/ECD was via external standards prepared from a known stock solution. All calibration and sample analyses were performed by extracting a volume of headspace (~100 uL) out of the 20 mL sample vial that contained a 1 mL water sample.

The calibration stock solution was contained in a sealed 2-L bottle with a pair of Luer fittings on the top. The bottle contained DI water to which known masses of a suite of analytes had been added. One mL of the stock solution was displaced out of the bottle with 1 mL of DI water. The stock solution is depleted by ~0.05% in this process, resulting in a ~3% reduction over 60 events. Daily response factors were tracked and the stock solution was replaced as needed.

GC/MS/FID Measurements –

Midway through this project, with the support of SERDP, we obtained a custom-designed GC/MS/FID system. This instrument has the capability to analyze a broad range of contaminant compounds and reaction products in a single-injection analysis using a dual-column configuration. For this project, the list of available analytes is shown in Table 5.1. The system is automated with an RSI 85 autosampler (PAL Systems, Zwingen CH), which injects a 0.1 mL headspace sample into a customized Agilent 7890B GC. Initial separation of respiration gases from chlorinated and other analytes is performed on a DB-624 Ultra Inert column (0.25 mm ID, 30 m, Agilent, Santa Clara, CA) at 35 C. A switching valve directs the respiration gases to a carbonPLOT column (0.32 mm ID, 30 m, Agilent, Santa Clara, CA) for further separation. Compounds eluting from the carbonPLOT column pass through a nickel-catalyzed methanizer, where they are all converted to methane or ethane. Concurrent with activation of the switching valve, the oven temperature is increased from 35 C to 175 C at a rate of 20 C per min. Effluent from the DB-624 column was directed to the mass spectrometer (5977B MSD, Agilent, Santa Clara, CA).

FID analytes	MS analytes
CO	Chloromethane
CO2	Dichloromethane
CH4	Chloroform
Ethane	Carbon Tetrachloride
Ethene	Chloroethene
Acetylene	Dichrlorethene
	Trichlroethene
	Hexachloroethane

Table 5.1. Analytes used in this project detected with the GC/MS/FID method.

Reduction potential and pH measurement of RTR samples.

At the conclusion of each RTR experiment, the RTR was mounted vertically on a rack, the upper 1-inch cap removed, and a combination platinum electrode (MI-800-411B, Microelectrodes Inc, Bedford, NH) was used to determine the reduction potential. An electron shuttle (ES) was then introduced into the tube (20 µM indigo tetrasulfonate or indigo disulfonate, TCI America, Portland, OR), and the reduction potential was monitored until a stable value (E_{ES}) was reached. The performance of the platinum electrode was demonstrated by comparison to an electrochemistry-grade rotating disk electrode (Kocur et al., 2020). Concurrent with the E_{ES} measurements, the final pH of the experiment was determined using a combination pH electrode (Vernier Software and Technology, Beaverton, OR).

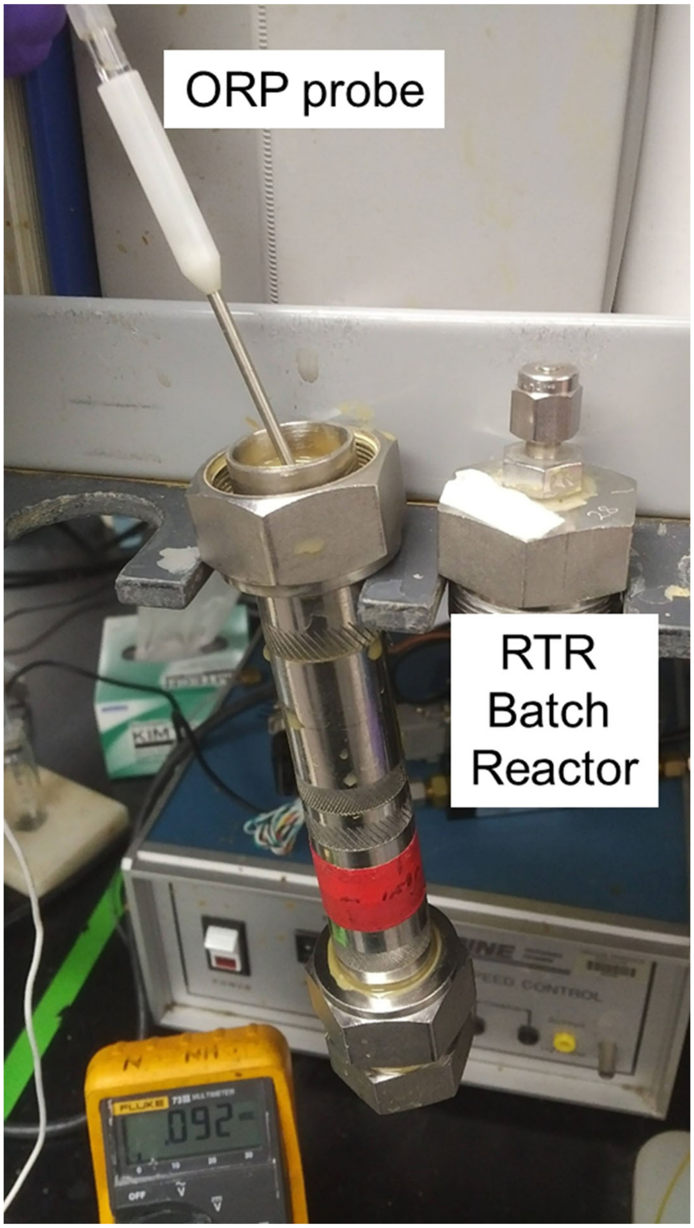

Figure 5.1. Photograph showing the RTR with upper cap removed and the platinum ORP probe inserted.

6. Soil Columns

The soil columns used in Objectives 1 and 3 are shown in **Figure 4.1**. They were constructed out of polycarbonate and had dimensions of 42 cm long and 5 cm inside diameter. They were filled with naturally-sourced Columbia River sand, to which had been added 1% by weight 60-80 mesh magnetite (Prospectors Choice, Surprise, AZ). The final porosity of the columns was about 40%. The columns were mounted vertically and were operated in an up-flow mode.

Four sampling ports were located 6, 16, 26, and 36 cm along the flow path. Each of those sampling ports was composed of a nylon pipe reducer, a 1/16” tube to 1/16” pipe Swagelok connector and a #16 hypodermic needle (see insert in **Figure 4.2**).

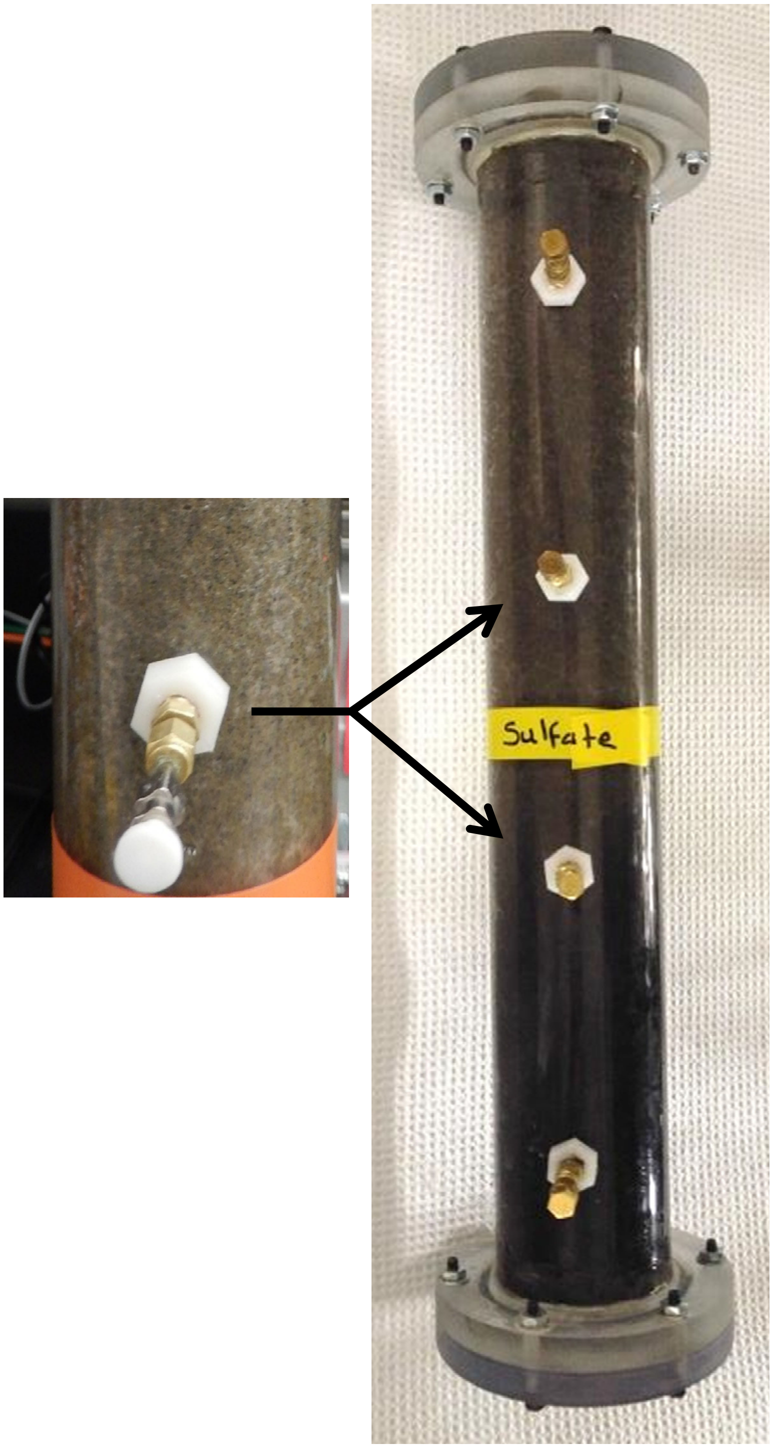

Figure 6.1. Photographs of one of the soil columns used for this project. The insert shows detail of the sample port construction, which is further described in the text.

7. Column Groundwater Chemistry

Groundwater chemistry – The synthetic groundwater flowing into the columns was buffered with 6.4 mM sodium bicarbonate that was adjusted to pH=7.2. Lactate was also added to the influent groundwater (10 mM). For the sulfate column, sodium sulfate was added to achieve a final concentration of 14 mM. Prior to injection, the groundwater was stripped of oxygen (and some carbonate) using a counter-current stripper with nitrogen gas.

Groundwater flow control – Flow in the column was controlled using ceramic-piston pumps (Fluid Metering, Inc. Model H0CKCLF pump heads.) The flow rate through the column was ~30 mL/hr. With an internal volume of ~830 mL and a pore volume of ~360 mL, that corresponds to a residence time of approximately 12 hours.

Geochemical sampling – For geochemical measurements, water was periodically removed from the columns via the Luer fitting ports at the 4 locations along each column (6, 16,26, and 36 cm down the column). For sampling of geochemical parameters, 1 to 10 mL of water (depending on the type of analysis) was removed from the column using a syringe, after letting ~0.5 mL flow out of the port to flush it.

Contaminant/reactivity probe introduction – Periodically, TCE or CT (along with TCP as an internal standard) was introduced into the column. This was accomplished using a syringe pump and a water reservoir containing a saturated solution of a 2:1 (by volume) of (CT or TCE): TCP. To deliver the RPs, a 60-mL syringe filled with water was connected to the reservoir the syringe pump was set at 0.5 mL/hr. Water flowing from the syringe displaced stock solution from the reservoir and delivered it to the influent line of the column. The resulting concentrations were ~450 uM for CT and ~87 uM for TCE.

Contaminant/Reactivity Probe sampling – As with the geochemical measurements, for contaminant reactivity analysis 1 mL of water was removed from the column ports with a syringe. In this case, the water was immediately injected into a sealed 20-mL headspace vial for analysis by GC/FID/MS (as discussed in Section 5).

8. Column Soil Processing

At the conclusion of the column experiments, the columns were disassembled and the soils used for subsequent analysis. To accomplish this, the columns were capped and cut into four sections, centered around each sampling port.

The first step in the sectioning process was to score the column with a circular saw. The depth of the blade was set such that the plastic was almost cut through (**Figure 8.1**). A chisel was then used to break the column at the score marks (**Figure 8.2**). Each time a section was cleaved from the column, it was capped with foil which was taped to the column and immediately transferred to the glove box. Once in the glove box, the soil was removed from a section and thoroughly mixed on a clean piece of aluminum foil. Then the homogenized soil was divided up into ~20 gram portion, which were wrapped in clean aluminum foil.

The homogenized 20-gram samples were divided into 3 groups: 1) samples to be analyzed immediately in the RTRs; 2) samples frozen to -80C, stored over night before being placed in RTRs; and 3) samples frozen to -80C and stored for 60 days before being placed in RTRs for batch testing.

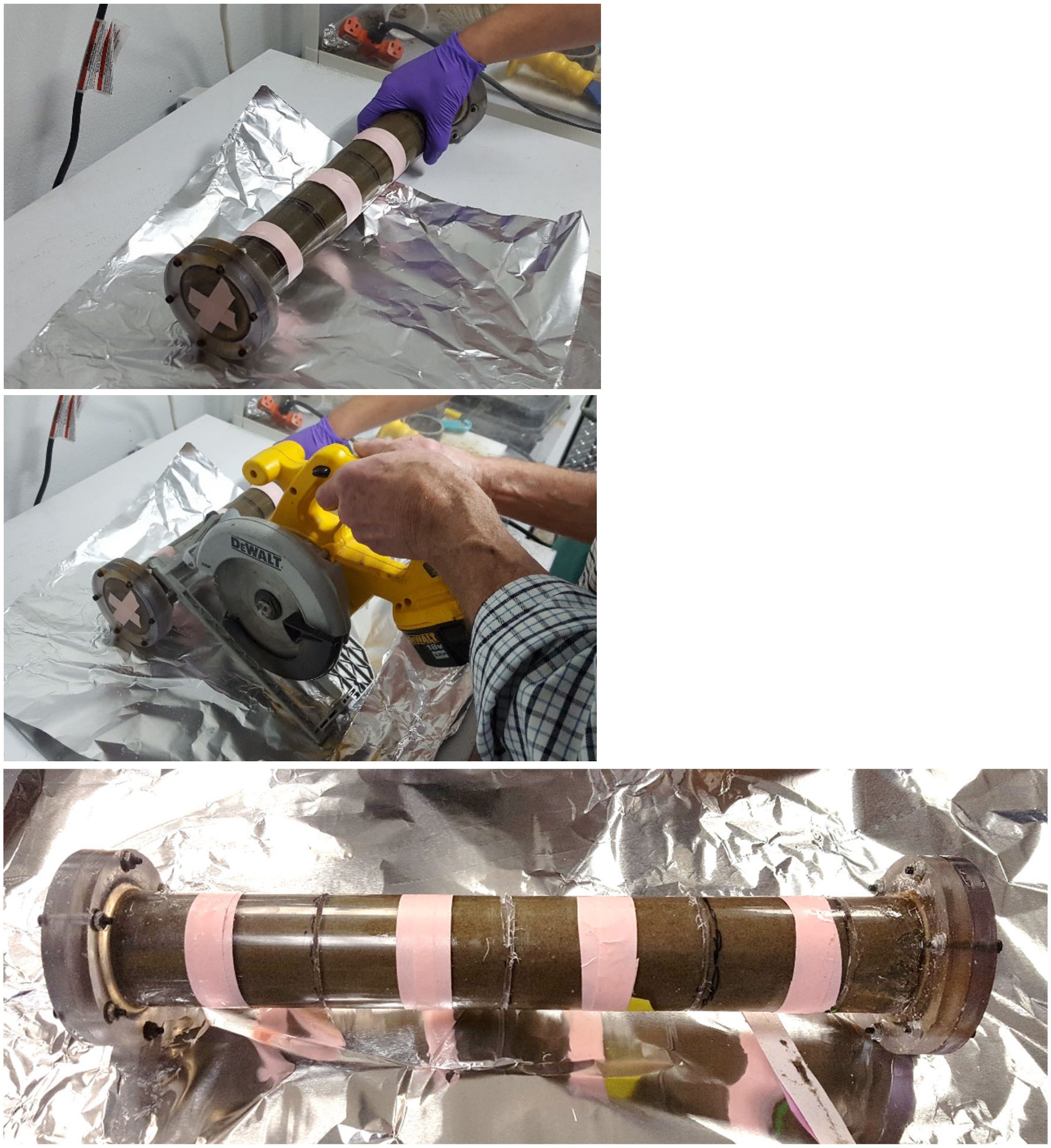

Figure 8.1. The columns werelaid horizontally and the plastic tube was scored with a circular saw, but the plastic was not cut all the way through.

Figure 8.2. Using a chisel to break the column at the scored locations.

9. Batch Experiments with Column Soils

The processes for batch analysis of the column samples in the RTRs were identical to the processes used for the field samples (Sections 4 and 5, above).

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