FINAL REPORT

Quantitative Assessment of Long-term Abiotic Transformation Rates of Chlorinated Solvents – Phase I

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14. ABSTRACT

This limited scope study focused on developing a quantitative tool to assess the rates of long-term abiotic transformation occurring at sites contaminated by chlorinated solvents including tetrachloroethene (PCE) and trichloroethene (TCE). Abiotic dechlorination of PCE and TCE by naturally present or biogeochemically augmented iron minerals (e.g., mackinawite and green rust) is an important process during natural attenuation (NA) of chlorinated ethenes. Although abiotic reduction of TCE and PCE offers cleaner transformation pathways with less accumulation of undesirable intermediates, evidence supporting abiotic degradation in field studies has been difficult to establish. Acetylene is a major product of PCE and TCE reduction by reactive minerals and is an indicator molecule of abiotic degradation. However, the potentially slow rate of acetylene generation and its rapid loss due to mineral adsorption or biological assimilation make it hard to detect at sites undergoing natural attenuation. In this project, the team aimed to develop a sensitive and robust device that can be deployed in situ to selectively accumulate acetylene over a field relevant time scale. The acetylene collected was quantified using standard instrumental analyses after the sampling activity, and the results were expected to inform the rates and extents of abiotic transformation of chlorinated ethenes.

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ACRONYMS AND ABBREVIATIONS

6AHA	6-azido-hexanoic acid
BA	benzyl azide
BIRD	biogeochemical reductive dechlorination
BTA	1-benzyl-1H-1,2,3-triazole
CEM	cation exchange membrane
CV	cyclic voltammetry
cis-DCE	cis-dichloroethene
CuAAC	Cu-catalyzed alkyne-azide cycloaddition
DDI	distilled deionized water
DGT	diffusion gradient thin film
DOD	department of defense
6HA-triazole	6-[1H-1,2,3-triazole-1-yl] hexanoic acid
IC	ion chromatography
IR	Infrared
GC	gas chromatography
GC-FID	gas chromatography with flame ionization detector
GC-MS	gas chromatography mass spectrometry
LC-MS	liquid chromatography mass spectrometry
LPZ	Low-permeability zone
MMO	Methane monooxygenase
NA	natural attenuation
NOM	natural organic matter
PCE	tetrachloroethene
PDMS	polydimethylsiloxane
RHE	Reversible hydrogen electrode
S-ZVI	sulfur-amended ZVI
TCE	trichloroethene
TDS	total dissolved solids
UV-Vis	Ultraviolet-visible spectrometry
VC	vinyl chloride

VOC	volatile organic carbon
ZVI	zerovalent iron

ACKNOWLEGEMENT

This project is supported by the DOD SERDP/ESTCP program (ER19-1411). The project work was performed by two collaborative teams at the University of Massachusetts Lowell (UML) and Princeton Unviersity (PU). The team at UML, consisting of the lead investigator and a graduate student, Lingfei Fan, designed and evaluated the azide-based acetylene sampler. The team at PU, including the co-investigator (Dr. Bruce E. Koel) and a research associate (Dr. Wenhan Niu) designed and conducted electrochemical analyses. The authors appreciate asssitance from Wendy Gavin and Seamus O'brien at UML Core Research Faclities for LC-MS method development. The authors thank SERDP reviewers, who provided valuable comments about the project presentations and the final report.

ABSTRACT

INTRODUCTION AND OBJECTIVES

This limited scope study focused on developing a quantitative tool to assess the rates of long-term abiotic transformation occurring at sites contaminated by chlorinated solvents including tetrachloroethene (PCE) and trichloroethene (TCE). Abiotic dechlorination of PCE and TCE by naturally present or biogeochemically augmented iron minerals (*e.g.*, mackinawite and green rust) is an important process during natural attenuation (NA) of chlorinated ethenes. Although abiotic reduction of TCE and PCE offers cleaner transformation pathways with less accumulation of undesirable intermediates, evidence supporting abiotic degradation in field studies has been difficult to establish. Acetylene is a major product of PCE and TCE reduction by reactive minerals and is an indicator molecule of abiotic degradation. However, the potentially slow rate of acetylene generation and its rapid loss due to mineral adsorption or biological assimilation make it hard to detect at sites undergoing natural attenuation. In this project, the team aimed to develop a sensitive and robust device that can be deployed *in situ* to selectively accumulate acetylene over a field relevant time scale. The acetylene collected was quantified using standard instrumental analyses after the sampling activity, and the results were expected to inform the rates and extents of abiotic transformation of chlorinated ethenes.

TECHNICAL APPROACH

In this limited-scope project, the team explored two independent approaches to detect and quantify acetylene in water. The original (and the first) approach involved the use of Au and other metal-based electrodes to detect and quantify acetylene via electrochemical measurements. The choice of Au was based on its strong interactions with acetylene and its application in the past for gaseous acetylene quantification. In the second approach, the use of an azide compound as a highly specific molecular probe for aqueous acetylene was proposed. This approach is based on the Cu-catalyzed alkyne-azide cycloaddition reaction (or click reaction), in which acetylene reacts with an azide chemical to form a triazole, and quantification of the latter informs the amount of acetylene present. Once the concept of using click chemistry to quantify acetylene was established, the team designed and constructed an acetylene passive sampler containing an azide chemical immobilized in PDMS thin films. The performance of the acetylene passive sampler was verified in laboratory microcosm reactors containing trichloroethene (TCE) and sulfur-amended Fe(0) materials.

RESULTS

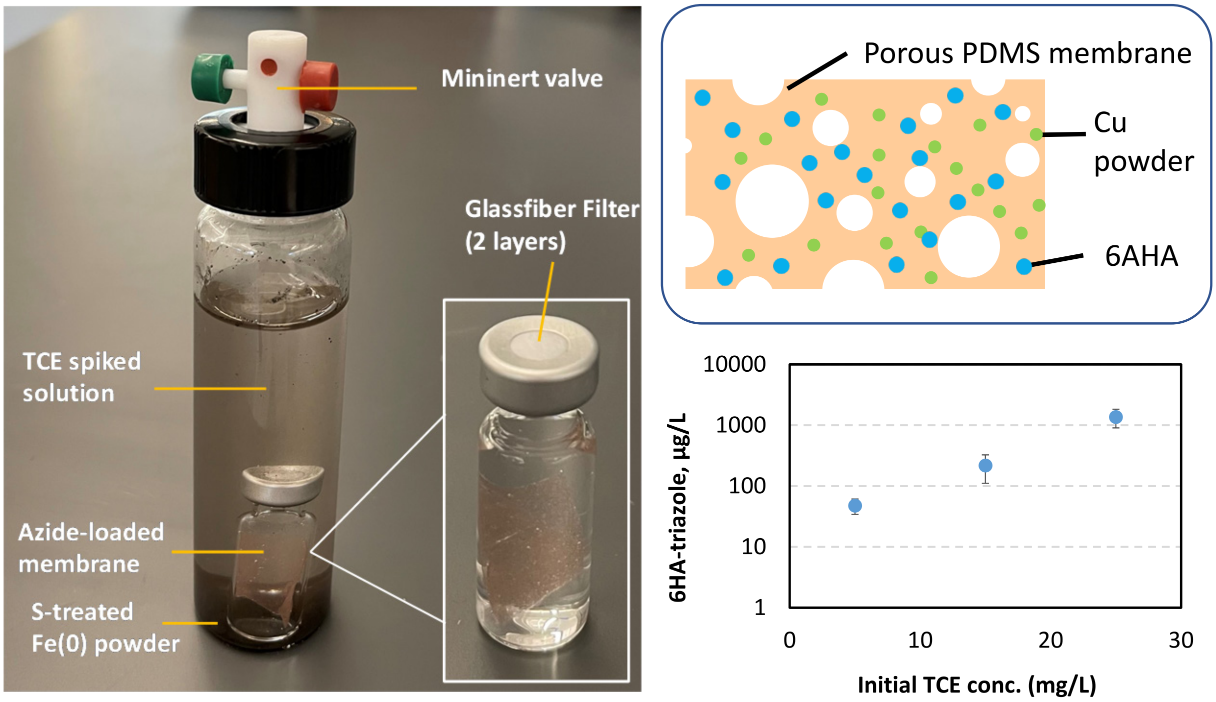
The original approach using an electrochemical method to quantify acetylene was found to pose significant technical and practical challenges including irreversible poisoning of the Au electrode surface by acetylene oxidation products, interference caused by ethene on a Pt electrode, and the practical difficulty associated with deploying an electrochemical sensing platform in a subsurface environment for long-term monitoring. The team pivoted to the second approach of developing a highly specific acetylene sampler based on the Cu-catalyzed acetylene-azide click reaction. The triazole product of the click reaction was stable and easily quantifiable. Further, the reaction was not affected by variations in groundwater chemistry or the presence of organic and inorganic solutes.

The design of the sampler was realized in a simple device containing lab prepared azide-impregnated PDMS membrane. Microcosm study confirms the ability of this device to capture acetylene generated during abiotic attenuation of TCE. The amounts of triazole formed correlate quantitatively with the extents of TCE degradation, confirming the validity of the azide-based passive sampler as a simple and inexpensive molecular tool to diagnose the occurrence of abiotic transformation of chlorinated solvents at contamination sites.

BENEFITS

The proposed acetylene monitoring device responds directly to a critical research need "*to improve the understanding and quantification of natural attenuation mechanisms*" at many Department of Defense (DOD) chlorinated solvent sites. If successful, the device and method proposed herein are expected to provide an independent line of evidence for the occurrence of abiotic degradation of chlorinated ethenes and to generate quantitative estimates of abiotic degradation rates and the long-term effectiveness of natural attenuation. Compared to direct water or soil gas sampling, the proposed approach is particularly attractive for sites with low but nontrivial abiotic reduction activity (*i.e.*, having small but non-negligible acetylene generation rates) and for characterizing abiotic attenuation in low-permeability regions that are difficult to acquire samples and investigate. The outcomes fill in critical data gaps in measuring the effectiveness of remedial actions, facilitate investigations into the roles of reactive minerals in natural attenuation of chlorinated solvents, and assist in formulating data-supported long-term site management decisions.

GRAPHIC DEPICTION



EXECUTIVE SUMMARY

INTRODUCTION

This limited scope study focused on developing a quantitative tool to assess the rates of long-term abiotic transformation occurring at sites contaminated by common chlorinated solvents including tetrachloroethene (PCE) and trichloroethene (TCE). Abiotic dechlorination of PCE and TCE by naturally present or biogeochemically augmented iron minerals (*e.g.*, mackinawite and green rust) is an important process during the natural attenuation of chlorinated ethenes. Although abiotic reduction of TCE and PCE offers cleaner transformation pathways, evidence supporting abiotic degradation in field studies has been difficult to establish. Acetylene is a major product of PCE and TCE reduction by reactive minerals and is an indicator molecule of abiotic degradation. However, the potentially slow rate of acetylene generation and its rapid loss due to background adsorption or biological assimilation make it hard to detect at sites undergoing natural attenuation.

OBJECTIVES

The overarching objective of this project is to develop a method to quantify the extents of abiotic reduction of chlorinated ethenes at complex contamination sites, where biotic and abiotic transformation may occur concurrently and slowly during long-term natural attenuation processes. Within this limited scope explorative project, the specific goal is to develop and evaluate two independent approaches to detect and quantify a characteristic product of abiotic reduction of chlorinated ethene, acetylene, which can inform the rates and extents of abiotic transformation of chlorinated ethenes.

TECHNICAL APPROACH

The original approach of the proposed project was to quantify aqueous acetylene using noble metal-based electrochemical measurements. Specifically, Au and Pt were evaluated as working electrodes in a 2-chamber electrochemical cell. The current responses of the system at varying aqueous acetylene concentrations were measured with cyclic voltametric and amperometric tests (Task 1). In the original design, the electrode was to be enclosed by protection materials to reduce interference by common groundwater solutes. Various materials, including PTFE membrane filters and strongly acidic cation exchange membrane, were evaluated for their efficiency in blockading particulates and solutes from contacting the electrode surface (Task 2).

Significant drawbacks revealed in the original approach prompted the team to pivot to the second approach using a specific chemical probe to detect and quantify acetylene in water. The approach is built upon Cu-catalyzed alkyne-azide cycloaddition (or click reaction). An azide compound was used as a chemical probe to convert acetylene to a triazole product, the latter can be reliably quantified. In this approach, the team evaluated the kinetics with different Cu catalysts, product yield, and the effects of groundwater chemistry (pH and organic or inorganic solutes) using acetylene solutions of known concentrations (Task 3). Following that, the team immobilized an azide chemical in PDMS membranes and assembled a passive simpler to evaluate the performance of the molecular sensing method in TCE-Fe(0) microcosm experiments (Task 4).

RESULTS AND DISCUSSION

Au and Pt electrodes were evaluated, and each was able to generate quantitative current signals proportional to acetylene concentration within a limited concentration range (upto 4000 and 350 µg/L for Au and Pt electrode, respectively) in simple electrolyte media. However, poisoning of Au by acetylene oxidation products and interference caused by background organics during acetylene sensing by Pt electrode were observed. The practical limitations of an electrochemical sensing method including difficulty to deploy in subsurface for *in situ* measurements and inability to monitor dynamic mass flux over a period of time were recognized during the investigations.

In the alternative approach, the reactions of an azide compound with acetylene were conducted using different forms of Cu catalysts. Metallic copper powder was deemed the most suitable catalyst for its comparable activity as the classical Cu(I) catalyst and its amenability to impregnation in a solid medium to function over a long time. A strong linear relationship was observed between triazole formation and the concentration of aqueous acetylene, and the product yield was unaffected by variations in pH, salinity, and the presence of common inorganic solutes or small aliphatic hydrocarbons, demonstrating a sensitive and robust mechanism to measure acetylene concentration in complex environmental matrices.

Based on these results, the team developed an acetylene passive sampler in which an azide-impregnated porous silicone membrane served as a reactive receiving phase of the sampler (Figure ES-1). The product yield varies linearly with acetylene concentration in the aqueous phase down to approximately 1 mg/L and the yield was unaffected by groundwater chemistry. Since the lab tests were performed in small batch reactors (5 mL), lower levels of acetylene may be detected in actual implementation when the sampler is exposed to a large volume of water. The performance of the passive sampler was verified in a microcosm study, where the acetylene sampler was installed in a batch reactor containing TCE and sulfur-treated iron powder. Acetylene generated during abiotic dechlorination of TCE was effectively captured by the azide-loaded membrane, and the quantity of the product recovered from the sampler correlates with the amount of TCE degraded. This result confirms the capability of the azide-based acetylene passive sampler as a new diagnostic tool for monitoring abiotic transformation of chlorinated ethenes in the subsurface environment.

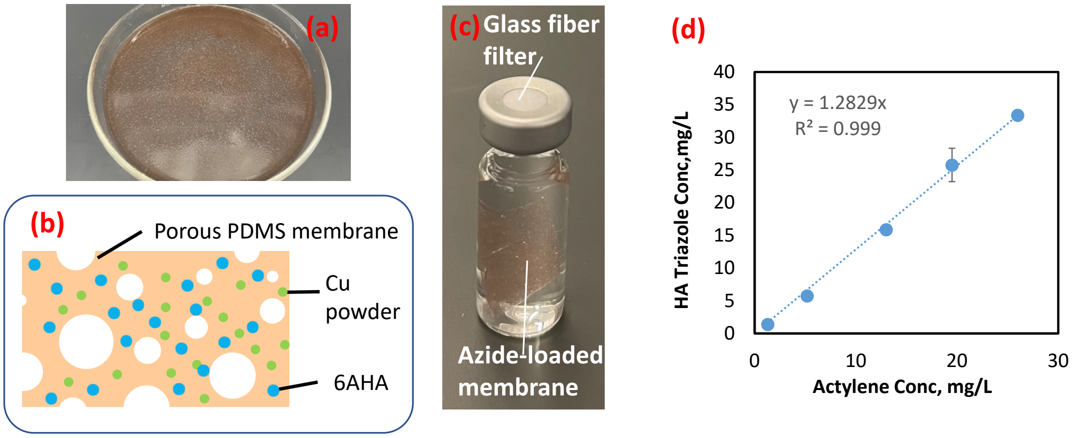


Figure ES-1. Photo (a) and Schematics (b) of an Azide-impregnated PDMS Membrane for Acetylene Detection and Quantification. (c) A Passive Sampler with the azide-loaded Membrane Inside. (d) Linear Relationship between a Triazole Product Recovered from the System and Acetylene Concentration.

IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

The outcomes of the project demonstrate that the project goal, that is, to develop a new method to quantify abiotic transformation of chlorinated ethenes, is attainable by capturing the characteristic reaction product, acetylene, with azide-loaded reactive media in a passive sampler.

There are several follow-ups questions to address before implementation of this method at contamination sites. First, the long term stability of the sampler (> 3 months) needs to be assessed in laboratory or simulated field conditions. Second, the quantitative relationship between the sampler responses (*i.e.*, quantity of triazole recovered) and the dynamic mass flux of acetylene needs to be assessed carefully in column experiments. Thus far, the evaluations were all conducted in batch reactors, which did not allow the investigations of the effects of flow rates and temporal changes in acetylene flux on the sampler response. Whether the sampler is able to capture cumulative generation of acetylene over a period of time of field relevance and based on that estimate averaged acetylene mass flux are important questions to be answered in column flow-through experiments. Lastly, the passive sampler should be directly tested at a pilot scale at a model site, where abiotic and biotic dechlorination activities are well characterized, to confirm its sensitivity and stability before deployment of this technology at complex sites.

1.0 OBJECTIVE

This limited-scope study aimed to develop a technique for detecting and quantifying acetylene, a characteristic product of abiotic reductive dechlorination of chloroethenes, in an aqueous environment so as to provide an independent means to confirm the occurrence and extents of abiotic attenuation of chlorinated solvents at complex contamination sites.

This project was motivated by the rationale that acetylene is a major and distinct product of abiotic reduction of trichloroethene (TCE) and perchloroethene (PCE) during natural attenuation or engineered remediation processes. The natural occurrence of acetylene in a clean subsurface environment is very low, thus an elevated presence of acetylene at a chlorinated solvent-impacted site is a strong indication of the occurrence of abiotic reductive dechlorination activities.

Acetylene, the smallest alkyne molecule, has several unique properties that can be exploited for its detection and quantitation in various environmental matrices. Acetylene binds strongly and irreversibly with transition metals such as platinum (Pt), palladium (Pd), or gold (Au)[1-6], which provides the basis for sensing acetylene using electrochemical means. Furthermore, acetylene readily and quantitatively reacts with an azide-terminated molecule in a Cu-catalyzed cycloaddition reaction to form a stable product that can be harvested and quantified[7, 8]. The specificity of the reaction and the high yield of the stable product under a diverse range of conditions suggest the feasibility of a passive sampler to capture and quantify trace levels of acetylene in the underground environment. The project therefore explored two independent approaches, electrochemical measurements and molecular detection via an azide-based chemical probe, to attain the project objective. The project consists of the following specific tasks:

Task 1. Evaluating electrochemical sensing of acetylene in aqueous media;

Task 2. Evaluating electrode-protection layers to reduce interference caused by common groundwater solutes;

Task 3. Evaluating the feasibility of detecting and quantifying acetylene using an azide probe; and

Task 4. Constructing a passive sampler for acetylene detection and assessing its performance in microcosm reactors.

2.0 BACKGROUND

Chlorinated ethenes, including PCE and TCE, are common groundwater pollutants and their efficient removal from contamination sites remains a challenge due to their widespread industrial and commercial uses as solvents and dry cleaning agents[9, 10], which led to sigificant accumulation in the envirnoment, particularly at sites containing complex hydrogeological features such as fracturued bedrocks or low-permeability formation[11, 12]. Chlorinated solvents undergo natural attenuation, albeit at slow rates [9,13]. The pathways of chlorinated solvent transformation have been extensively investigated. Abiotic reduction of TCE proceeds via the dichloro-elimination pathway to form chloroacetylene in the presence of green rust, iron sulfides, and other reduced iron minerals, and chloroacetylene quickly decomposes to acetylene (C_2H_2) in water[14-19]. Abiotic reactions of PCE with iron minerals such as mackinawite[16] proceed via either the hydrogenolysis pathway to give rise to TCE or (di)chloro-elimination to form acetylene[20]. Either pathway involves the formation of acetylene (Figure 2.1). **Therefore, acetylene is an important reaction product arising during reactions between chlorinated ethene parent compounds and reductive minerals**. Concurrent with chemical reduction, anaerobic biotic transformation often occurs at natural attenuation sites and can lead to the accumulation of *cis*-dichloroethene (*cis*-DCE) and vinyl chloride (VC), which are more toxic and persistent in the environment than the parent compounds[21, 22]. For this reason, methods to promote abiotic decomposition of chlorinated solvents has been a topic of intense research in the past two decades, including the more recent proposition of using biological means to induce the formation of reduced Fe minerals (*e.g.*, "*in situ* biogeochemical transformation" and "biogeochemical reductive dechlorination", or BIRD) [21, 23, 24].

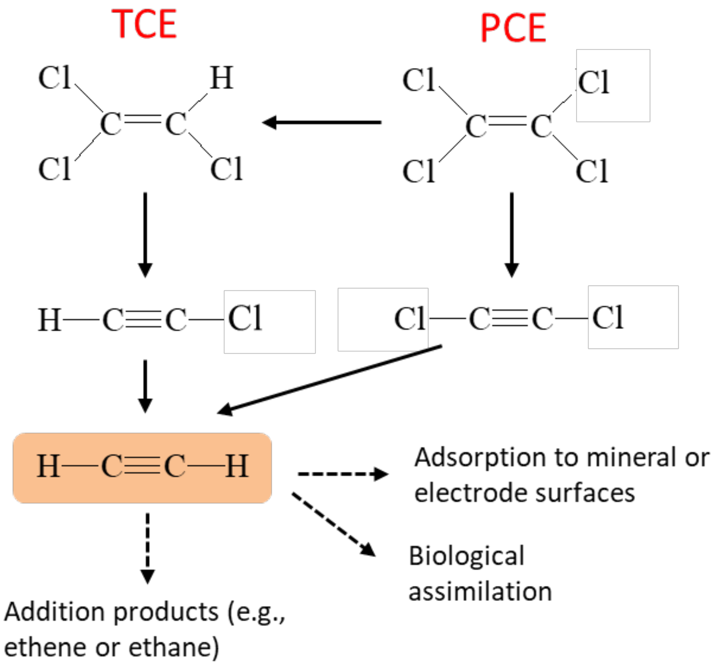


Figure 2.1. Abiotic Reductive Dechlorination Pathways of Chloroethene Parent Compounds.

Although the degradation mechanisms and kinetics of chlorinated ethenes have been carefully examined in controlled laboratory systems, measuring the progress of transformation under field conditions is difficult due to potentially very slow degradation rates[11], coupling between biological and abiotic processes [18, 23, 24], and the confounding role of hydrological effects. From scientific as well as practical site management points of view, it is important to distinguish abiotic and biotic transformation processes, however this has proven difficult especially at complex contamination sites[11, 21]. As a result, the kinetic info and the relative extent of abiotic reduction during biogeochemical transformation of the target contaminants are poorly charaterized.

Herein the team proposed to monitor the occurrence of abiotic dechlorination by designing a device that can accumulate and quantify acetylene in groundwater. Acetylene is not naturally occurring and its presence is uniquely associated with abiotic dechlroination by Fe(II)-containing minerals [14-18]. However, a survey of recent literature on field studies of TCE or PCE-contaminated sites indicates that most of the groundwater or soil gas samples did not find acetylene above the detection limit (~ 0.5 µg/L) [25, 26]. The elusive nature of acetylene in the field samples was attributed to its high biodegradation rates [27, 28]. A handful sites have documented the observation of actylene in groundwater samples at concentrations ranging from just above the detection limit to several hundreds of µg/L (see Table 2.1). In genearal, the detection of acetylene was accompanied by other hydrocarbon gases such as ethene and ethane at concentrations higher than acetylene. Considering the relatively low levels of acetylene encountered at chlorinated solvent-impacted sites and its rapid dissipation in the environment, direct groundater sampling at discrete time points tend to generate negative sampling bias[29]. Therefore, a good dignostic tool should allow acetylene to cumulate over a period of time and permit quantification of the cumulative amount of acetylene accured on the sampling device.

Table 2.1. Concentration Range of Acetylene and Other VOCs Detected in Field Samples

Site description and reference	Sampling and analysis method	VOC concentrations
A TCE-contaminated fractured sandstone site in California; Parker et al., 2018 [[29]]	Groundwater samples; GC-ECD/FID;	Acetylene: 0.5 -17 ug/L VC: 3.5 – 74 ug/L Ethene: 0.5 – 8.9 ug/L Ethane: 0.4 – 7.2 ug/L
A PCE site in South Carolina receiving EZVI treatment; Su et al., 2012 [[30]]	Groundwater samples; GC-MS (EPA 8260B);	Acetylene < 120 ug/L Conc of gases in the order of: Acetylene < ethane < ethene
A DOE TCE-contaminated clay site in Kentucky subject to Lasagna treatment Ho et al., 1999 [[31]]	Groundwater samples; GC-ECD/FID;	Acetylene: 15 - 426 ug/L Ethene: 66 – 385 ug/L Ethane: 38 – 816 ug/L

Among the short-chain aliphatics present in the environment, acetylene possesses some unique chemical properties. Acetylene interacts strongly with late transition metal surfaces, including Pd, Pt, Ni, and Au [1, 32-35]. Acetylene may adsorb on the metals reversibly, or it may undergo surface reactions leading to the formation of dissociated carbon and hydrogen species or forming dimerized or trimerized products [2, 3, 36]. The nature of these reactions varies with temperature, metal surface chemistry, and the underlying support materials. Owing to the strong and specific interactions, metal electrodes made of Pt or Au have been developed for acetylene detection in the gas phase[33, 37]. Compared to Pt, the adsorption of acetylene onto Au electrode is irreversible and is highly selective over short-chain alkenes and alkanes, H_2, and O_2 [33, 38], with the reported detection limit below 100 ppb for acetylene in the gas phase[39]. Direct electrochemical sensing of acetylene in the aqueous phase has not been studied, nonetheless, the extension to aqueous phase sensing is plausible for several reasons. Compared to other gaseous hydrocarbons, acetylene has a relatively high water solubility (~ 1200 mg/L), which is not affected by major water chemistry parameters[40]. Furthermore, measurement of acetylene gas as described in previous studies relies on its diffusion into an electrolyte solution, adsorption onto the electrode, and oxidation at a fixed potential, all of these are expected to take place similarly in gaseous and aqueous environments.

Another strategy to detect and quantify acetylene relies on the well-established Cu-catalyzed alkyne-azide cycloaddition (CuAAC, or click chemistry in short). Click chemistry, first reported in 2001 by Sharpless, Meldal, and other researchers, is a bioconjugation method to attach two molecules with a terminal aizde and alkyne on each[7, 41]. The classic click reaction involves an azide reacting with an alkyne in the presence of Cu(I) to form a triazole ring.[41, 42] The reaction is robust and the product yield is quantitative. For this reason, click reactions have been used in many biochemical ligation reactions; however, its application in environmental monitoring is new. In the case of molecular sensing of acetylene in groundwater environment, the distinctive characteristics of CuAAC click reaction offers several notable advantages. The chemistry is specific for alkynes and is not subject to interference by common aliphatic hydrocarbons present in the soil environment. The reaction proceeds rapidly in aqueous solutions at room temperature and is not affected by the mixture of solutes present in groundwater matrix. The reaction produces chemically and biologically stable products (*i.e.*, substituted-1,2,3-triazoles) that can be reliably quantified using common analytical instruments. Most importantly, an azide-based molecular probe may allow acetylene to be captured and retained irreversibly in the sensing media over a time frame relevant to remediation or natural attenuation projects, thus enabling estimation of time-averaged mass flux of acetylene. This information is not attainable with conventional electrochemical measurements, which are typically used to generate instantaneous read-outs.

This project investigated both strategies, compared their advantages and limitations, and selected one approach to proceed with designing and constructing an acetylene sampler. The prototype sampler was evaluated in microcosm experiments to verify its performance as a new diagnostic tool to monitor the progress of abiotic reductive attenuation of chlorinated ethenes.

3.0 MATERIALS AND METHODS

3.1 CHEMICALS AND MATERIALS

The list of materials and chemicals used during the course of this project is provided in Appendix A. Ultrapure water (< 18 MΩ.cm, denoted as DDI water) was used to prepare all stock and working solutions.

3.2 ELECTROCHEMICAL SENSING OF ACETYLENE IN AQUEOUS MEDIA

The detection of acetylene in aqueous media was conducted in a customized two-chamber glass electrochemical cell (AF01CKT1008, Pine Research, Durham, NC, shown in Figure 3.1.) at room temperature. The working electrode was a piece of 5 mm x 5 mm polycrystalline Au foil. The counter electrode was graphite, and Ag/AgCl was the reference electrode. The two chambers were separated by a piece of Nafion^{TM} 117 membrane. The default electrolyte was 0.5 M H_2SO_4. In a typical electrochemical test, the working electrode was cleaned by a polishing step with aluminum oxide slurry, and the electrode was sonicated in 0.5 M H_2SO_4 for 10s and thoroughly rinsed with ethanol and distilled deionised water (DDI). The clean electrode was conditioned by CV cycling in the electrolyte solution in the range of 1.0 to 1.7 V (vs. RHE) for 20 cycles before experimental use. At the beginning of an experiment, a small aliquot of acetylene stock solution (in methanol) was spiked into the chamber of the working electrode. The solution was vigorously stirred using a magnetic stirring bar. Cyclic voltametric (CV) scans in the range of 1.0 to 1.7 V (vs. RHE) were collected, followed by setting the potential at 1.3 V (vs. RHE) for 1500 s and monitoring the amperometric response. This test was repeated with an ascending concentration of acetylene in the range of 0.5 to 5 mg/L.

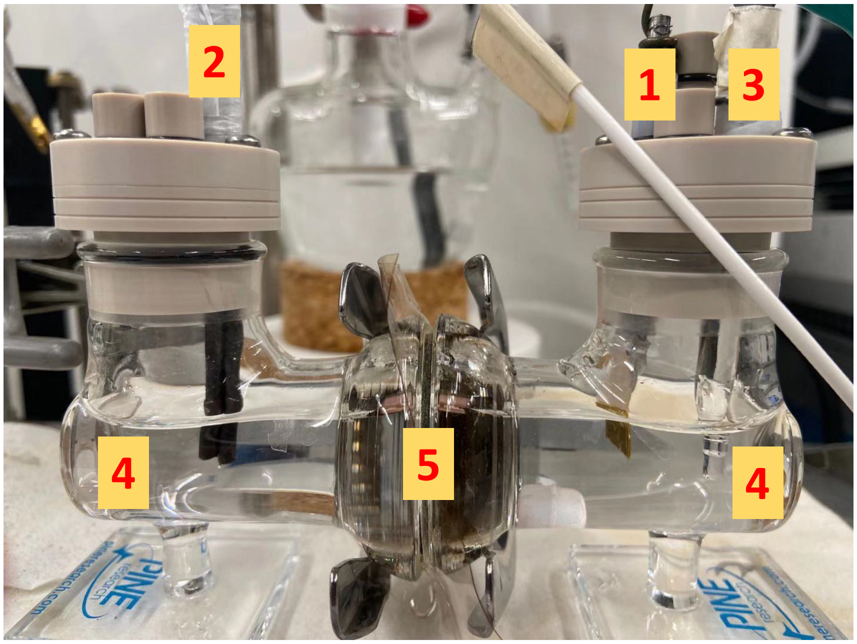


Figure 3.1. Experimental Set-up of the Electrochemical Cell for Acetylene Sensing.

Labels indicate the working electrode (1), counter electrode (2), reference electrode (3), electrolyte solution (4), and Nafion^{TM} 117 membrane (5) separating the two chambers.

3.3 EVALUATION OF ELECTRODE PROTECTION LAYERS

A two-chamber reactor was used to evaluate whether protection material may effectively block the passage of interfering solutes. The two chambers, each 100 ml in volume, were connected by flange joints, where the protection material of interest was inserted and securely clamped between the flange joints (Figure 3.2). Prior to a test, one chamber was filled with DDI while the other with equal volume of a solution containing colloidal particles or a solute of interest (Cl^- or HS^-). Colloidal suspension was prepared by dispersing 50 mg of bentonite in 100 mL DDI. Chloride and sulfide solution was prepared by dissolving the respective sodium salt in 100 mL DDI to reach an initial concentration of 3.4 mM and 1.2 mM, respectively. The reactor was gently agitated on a shaking platform for upto 72 hours, and periodically a small aliquot of solution from each chamber was withdrawn and analyzed. Due to facile oxidation of sulfide in air, the experiments involving sulfide were conducted in a glove bag under flowing N_2. The amount of colloidal particles in water was measured by absorbance at 450 nm. Chloride and sulfide analyses were performed using the HACH mercuric thiocyanate chloride reagent kit (HACH # 2319800) and methylene blue sulfide reagent set (HACH #2244500), respectively.

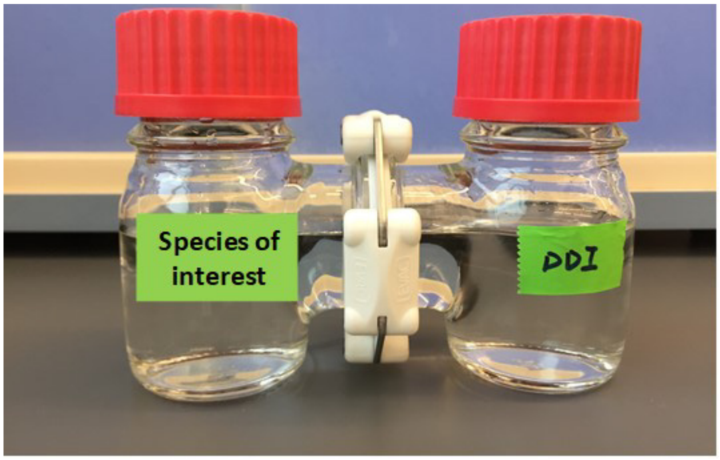


Figure 3.2. Experimental Set-up of a Two-chamber Reactor to Evaluate the Effectiveness of Protection Layers in Blocking Interfering Solutes.

3.4 SENSING OF ACETYLENE IN AQUEOUS MEDIA USING AZIDE-BASED MOLECULAR PROBES

Poisoning of electrodes by common groundwater solutes and by acetylene oxidation products in the earlier part of the project prompted a search for an alternative acetylene monitoring method. Based on an extensive literature survey, the team identified a more specific and sensitive molecular sensing mechanism based on the copper-catalyzed azide-alkyne cycloaddition reaction (or CuAAC click reaction) [7, 42, 43]. A request to revise the project approach was raised in Mar 2021 and the experimental work was carried out soon after. We first sought to establish proof-of-concept, ascertained the quantitation range, and confirmed the absence of significant background interference using a simple azide compound, benzyl azide. Subsequently, we evaluated the performance of immobilized azide probes, which can be installed in passive samplers for field deployment.

3.4.1 Proof-of-Concept Study: Acetylene Quantification with Benzyl Azide

Benzyl azide was chosen as the molecular probe for acetylene to establish proof-of-concept because of its commercial availability and the product with acetylene, 1-benzyl-1H-1,2,3-triazole, can be easily identified. The cycloaddition reactions were conducted in 8-mL glass vials at room temperature. The reaction solution in each vial was 5 mL unless otherwise noted. The reaction solution was amended with a known amount of benzyl azide to give a concentration 0.193 mM and an appropriate dose of a copper catalyst. The glass vials were capped with mininert^{TM} valves and a reaction was initiated with the spiking of a small aliquot of acetylene stock solution through the valve using a gas-tight syringe. The reaction vials were mixed on a platform shaker at 400 rpm for upto 4 days. Periodically, a reactor was sacrificed and the reaction mixture was filtered through a glassfiber filter (Whatman, GF/C). 2.8 mL of toluene was added into the filtrate. The mixture was agitated for 2 hours, allowed to settle, and 2 mL of the toluene phase was extracted for GC-MS analysis. The following variations in experimentation were carried out to assess the effects of pertinent factors.

Effect of Cu catalyst. Various forms of Cu catalysts were evaluated, including aqueous Cu(I), Cu(0) nanopowder, and Cu(0) powder [44]. Aqueous Cu(I) was amended by adding $CuSO_4$ and sodium ascorbate so that copper ion and ascorbate were at 0.2 and 0.6 equivalents of benzyl azide, respectively. The ratios of Cu(II) and adsorbate relative to benzyl azide were evaluated separately and the aforementioned ratios were found to be the optimal. Note that fresh stock solution of ascorbate was used in all applicable experiments since the chemical was prone to oxidation in air over time. Metallic copper powder (*i.e.*, Cu(0)) in the form of 20 mg of nanoparticles (60-80 nm, Sigma Aldrich, Catalogue #774103) or 100 mg micron-scale metal powder (< 45 µm, Sigma Aldrich, Catalogue #357456) in 5 mL of the reaction mixture was also evaluated. The yields of triazole were similar between the nanoscale and micron-scale Cu(0) particles, and therefore, the micron-scale Cu(0) powder was used in subsequent experiments for the ease of handling and solids/water separation.

Effect of temperature. Raising the reaction temperature from room temperature to 50 °C was found to increase the rate of triazole formation, however, since temperature is not adjustable in field experimentation, further tests were conducted at the room temperature.

Effect of pH. The initial pH of the reaction mixture was adjusted using dilute NaOH or HCl to 5, 7, and 9, respectively to assess the pH effect.

Effect of groundwater solutes. To assess the effect of common background solutes on the yield of acetylene-benzyl azide reaction, the solution phase was amended with 1 mM Cl^-, HCO_3^-, and SO_4^2. All anions were added in the form of their sodium salts.

Effect of low molecular-weight hydrocarbons. It was reported that small-molecule aliphatics such as ethene may interfere with the sensing of acetylene by electrochemical methods[38]. Although such interference is not expected in the case of acetylene-azide click reaction, experiments were conducted in which the reaction mixture was spiked with ethene, ethane, or methane separately at 5 mg/L in addition to acetylene to confirm whether the azide molecule binds specifically with acetylene only and it does not interact with other gaseous hydrocarbons. In these experiments, the solutions contained 1 mM Cl^- to simulate natural salinity.

3.4.2 Acetylene Reactions with Azide-functionalized Agarose Beads

Section 3.4.1. employed a dissolved azide probe to collect preliminary proof-of-concept data. However, in field deployment, the use of an immobilized probe is highly preferred as it enables the construction of a passive sampler for measuring *in situ* flux of trace-level analytes over a large span of time (weeks to months) instead of *ex situ* instantaneous measurements afforded by electrochemical methods. To this end, a commercially available azide reagent immobilized on agarose beads was evaluated. The reagent contains a terminal azide group on one end and the other end covalently bond to agarose beads (Click Chemistry Tools, catalogue #1139, particle size 50 – 150 µm). The middle section of reagent is a cleavable Dde linker, which may be cleaved in the presence of hydrazine to release the triazole product formed during acetylene-azide reaction for quantitation. The beads in the original slurry were concentrated with centrifugation (at 5000 rpm for 10 min) and dispersed in methanol to make a stock solution of ca. 355 mg/L. Coupling reactions were performed in 8-mL glass vials, each added with 10 mg/L Dde Azide Agarose beads, 0.01 mM Cu catalysts (from $CuSO_4$ stock), 0.06 mM sodium ascorbate. The reactors were capped with Mininert^{TM} caps, and a small aliquot of acetylene stock solution (in methanol) was spiked to reach a concentration of ca. 0.1 mM. The reactors were mixed on a shaker at a speed of 400 rpm at room temperature for two days. Following that, 1 mL of 2% hydrazine stock solution was added to the reacted solution and was allowed to react for 1 hour. The mixture was centrifuged (5000 rpm for 10 min), and the supernatant was collected, diluted 1:1 with methanol, and analyzed with GC-MS.

3.4.3 Acetylene Reactions with Azide-loaded Polymer Membranes

6-azido-hexanoic acid (6AHA) was selected as the acetylene probe (to replace the previous choice of benzyl azide) because of its aqueous stability and non-volatile nature. The product of 6AHA with acetylene is 6-[1H-1,2,3-triazole-1-yl] hexanoic acid, or 6HA-triazole in short. To immobilize 6AHA in a solid phase, the probe was incorporated into polymer thin films as detailed below.

Preparation of Azide-loaded Membrane. In a 10-mm diameter glass petri dish, 6 g of polydimethylsiloxane (PDMS) and 0.3 g of a curing agent, both from Dow Sylgard® 184 Silicone kit, at a mass ratio of 20:1 were vigorously mixed with 100 mg of copper powder and 0.2 g of sodium bicarbonate. The addition of bicarbonate would later create a porous texture after curing, which increases the specific surface area of the membrane. The mixture was spiked with 46.8 µmole of 6AHA (in methanol) (equivalent to a concentration of 8.6 mM). The mixture was manually stirred vigorously with a spatula for 10 min. It was then transferred to another clean petri dish, which was gently shaken to ensure that the content formed a smooth and even layer in the dish. The membrane mixture was cured for one hour in an oven at 105 ºC. Figure 3.3 shows the resultant membrane after curing. The membrane was denoted as azide-loaded membrane in short. Fresh membrane was prepared and immediately used on the same day of preparation in various experiments.

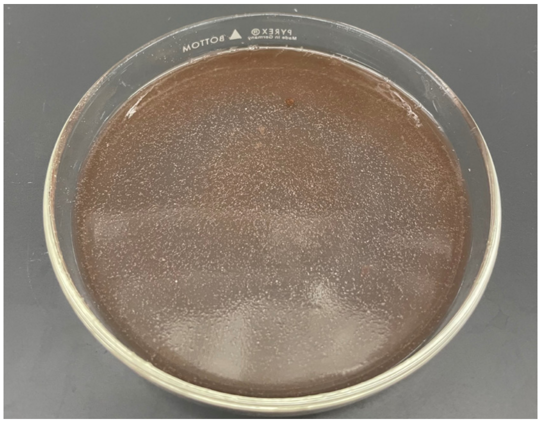


Figure 3.3. PDMS Membrane Impregnated with 6-azido-hexanoic Acid and Cu Catalyst.

The cycloaddition reactions were conducted in the same set-up as in previous experiments except that the azide probe and catalyst were added in the form of 100 mg of azide-loaded membrane into each reactor. The glass vials were capped with mininert^{TM} valves and a reaction was initiated with the spiking of a small aliquot of acetylene stock solution through the valve using a gas-tight syringe to yield a concentration between 0.01 and 1 mM. The reaction vials were mixed on a platform shaker at 400 rpm for up to 14 days. Periodically, a reactor was sacrificed and the membrane was recovered from the solution. The membrane was rinsed gently with a small amount of DDI water, and extracted in 20 mL of solvent for 24 hours. A small amount of the extractant was sampled for LC-MS analysis. We evaluated different solvents for extracting 6HA-triazole from the reacted membranes, including toluene, acetone, dichloromethane, methanol, and 1:1 toluene/methanol mixture. It was found that 1:1 toluene/methanol mixture gave the highest extraction efficiency and was used in subsequent experiments, although some early experiments used methanol as the extractant. This difference does not affect discussion as data was compared against that of the respective control runs, and the treatment and control groups always followed the same extraction procedure. Additional variations of the experiments were performed as follows to assess the effects of solution chemistry.

Effect of background solutes. Common background solutes were evaluated by adding 1 mM of Cl^-, HCO_3^-, SO_4^{2-} (all salts in sodium form) or 20 mg/L of humic acid into 5 mL of the reaction mixture separately.

Effect of low molecular-weight organics. The potential interfering effect of low-molecular-weight aliphatic hydrocarbons were evaluated by amending the reaction mixture with 5 mg/L methane, ethane, or ethene. These hydrocarbons were added in the form of the respective stock solution in methanol.

3.5 ACETYLENE PROBE VALIDATION IN TCE ABIOTIC DECHLORINATION MICROCOSMS

3.5.1 Preparation of Sulfur-treated Peerless^{TM} Iron

The performance of the azide-loaded membrane for detecting acetylene formed during abiotic reductive dechlorination of TCE was tested in a microcosm reactor. Sulfur-treated Peerless^{TM} iron (S-ZVI^{PLS}) was selected as the abiotic reductant because it can rapidly decompose TCE and is a common ingredient in abiotic remediation applications [45, 46]. The preparation of S-ZVI^{PLS} follows our previously published procedures[45, 47]. Briefly, 0.6 g Peerless™ iron powder (50D, Peerless Metal Powders and Abrasive) was acid-treated in 30-mL 0.1% HCl (v/v) solution for 20 min. After acid washing, a magnet was used to retain the iron powder in the vial. The acid solution was decanted, and the iron powder was collected after being thoroughly rinsed with DDI water. The iron powder was added to 30 mL of 8.96 mM sodium thiosulfate ($Na_2S_2O_3$) solution and the mixture agitated at 200 rpm for 20 min. The resultant iron powder was recovered using a magnet and utilized immediately in the Fe(0)-TCE microcosm experiments.

3.5.2 TCE Abiotic Dechlorination Microcosm Study

The microcosm reactor was constructed using a nested vial design (Figure 3.4), where a 2-mL vial containing azide-loaded membrane was inserted in a larger vial in which TCE-Fe(0) reaction took place. In essence, the inner vial acted as an acetylene passive sampler. When acetylene and other solutes passed through its permeable cap, acetylene would react with the membrane giving rise to the corresponding triazole product. The nested design of the microcosm ensures that the passive sampler captures acetylene without interfering with the reactions of TCE or other chlorinated ethene contaminants with reactive minerals or Fe(0) particles in the surrounding environment.

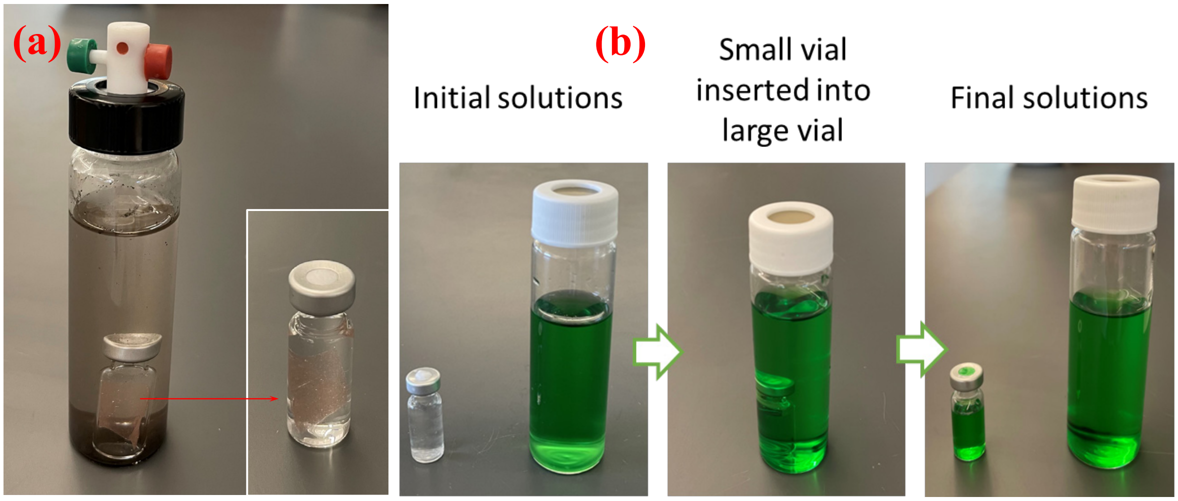


Figure 3.4. (a) Reactor Used in TCE Abiotic Dechlorination Microcosm Study. The Inset Shows an Enlarged View of the Acetylene Passive Sampler, Which Houses a Piece of Azide-loaded Membrane. (b) A Simple Test Demonstrating Facile Transport of Solutes from the Reactor (Outer Vial) into the Passive Sampler (Inner Vial).

Specifically, the acetylene passive sampler was constructed by placing 100-mg azide-loaded membrane in a 2-mL glass vial. The vial was filled with DDI water and sealed with a crimp cap where the septum of the cap was replaced with two layers of glass fiber filters (Whatman, GF/C filter). To verify that the cap of the small vial was permeable enough to allow facile transport of solutes between the small and large vials, a simple test was conducted (Figure 3.4b). The sampler, initially filled with DDI, was inserted into the large vial containing a dye solution. The rapid equilibration of the solution inside the inner and outer vials confirms that solutes including acetylene can readily move from the outer vial, where TCE dechlorination reaction takes place, into the passive sampler. Meantime, the ZVI particles and colloidal aquifer material are screened out by the filter layer of the sampler.

In a microcosm experiment, the passive sampler containing azide-loaded membrane was inserted into a 40-mL glass VOA vial, the latter contained 0.6 g S-ZVI^{PLS} dispersed in 30-mL DDI. The rim of the large vial was wrapped with PTFE for gas-tight sealing and capped with a Mininert^{TM} cap. The reaction started by injecting a small aliquot of TCE solution (in methanol). The reactor was placed on a platform shaker and mixed at a speed of 400 rpm at room temperature. Periodically, headspace samples were drawn for analysis of TCE and its daughter products including acetylene by GC-FID. After 8 days, the membrane was harvested and extracted in 20 mL of 1:1 methanol/toluene for 24 h. A small quantity of the extractant was sent for LC-MS analysis.

3.6 SAMPLE ANALYSES

GC-FID

Typically, 50 µL of headspace gas was withdrawn using a gastight syringe from the batch reactors. The samples were directly injected into a GC-FID system (Agilent 7890) equipped with an Agilent GS-GASPRO column (0.32 mm x 30 m) to analyze for the concentrations of acetylene and TCE. The headspace samples were injected in a splitless mode at 250 °C. The oven temperature was 35 °C for 5 min, ramped at 12 °C min^{-1} to 225 °C, and held at 225 °C for 2 min[1].

GC-MS

5 µL of the solvent extracted sample was injected into a GC-MS system (Agilent 8890 GC-5977 MS) equipped with an Agilent HP-5MS UI column (0.25 µm, 0.25 mm x 30 m) to analyze for the concentrations of benzyl triazole. The aqueous samples were injected in a split mode at ratio of 20:1 at 250 °C. The oven started at 105 °C, ramped at 20 °C min^{-1} to 320 °C, and held at 320 °C for 5 min.

LC-MS

LC-MS analysis was performed on a Shimadzu 8040 LC-Triple Quadrupole MS system equipped with a C18 analytical column (BEH, 1.7 µm). The column was maintained at 40 °C throughout the run. The eluent phase consisted of 0.1% formic acid (A) and acetonitrile (B). 50 µL sample was injected during the analysis. The eluent was maintained at 300 µL/min throughout the run. Eluent B was increased from 30% to 100% in the first 2 min, held at 100% for 4 min, and reduced to 30% and held there for 1 min. 6HA-triazole was monitored and quantified in a MRM mode at the transition of m/z 183.5 > 69.15.

Due to temporary instrument maintenance, LC-MS analysis was also conducted on another LC-MS system (Shimadzu HPLC and AB SCIEX API 4000 Triple Quad MS/MS). Analyte separation was performed on a C18 analytical column (Kinetex 2.6µm, 100 A, 100×4.6mm), which was maintained at 40 °C throughout the run. The eluent phase consisted of 0.1% formic acid in DDI (A) and 0.1% formic acid in methanol (B). 10 µL sample was injected and the eluent was maintained at 600 µL/min throughout the run. The pump program of eluent B consisted of 50% for the first 1 min, followed by a linear increase to 95% in 5 min, and holding at 10% for 2 min. 6HA-traizole was monitored and quantified in a MRM mode at the transition of m/z 182 > 68.0.

4.0 RESULTS AND DISCUSSION

4.1 ELECTROCHEMICAL SENSING OF ACETYLENE IN AQUEOUS MEDIA (TASK 1)

Figure 4.1 shows cyclic voltametric (CV) scans using polycrystalline Au or a glassy carbon electrode as the working electrode in the H-cell setup (Figure 3.1). In blank electrolyte solution without acetylene, no peak was observed with the glassy carbon electrode; however, Au foil electrode generated two anodic peaks at +1.25 and +1.50 V and two cathodic peaks at +1.18 and +1.0 V (all potential is w.r.t. RHE), respectively. The responses observed on Au but not glassy carbon indicate reversible Au oxidation within the range of the potential scan. Electrochemical studies of Au(111) and Au(100) surface observed adsorption of oxygen species on the electrode surface at ca. +1.40 V and the process is reversible in the reduction sweep of the scan[48]. The oxidation of Au surface is associated with surface reconstruction and the process takes place via two steps (R1 and R2)[48, 49], consistent with the twin oxidation peaks observed here.

$$Au + H_2O \rightarrow Au^{I}OH + H^+ + e^- \qquad R1$$

$$Au^{I}OH \rightarrow Au^{II}O + H^+ + e^- \qquad R2$$

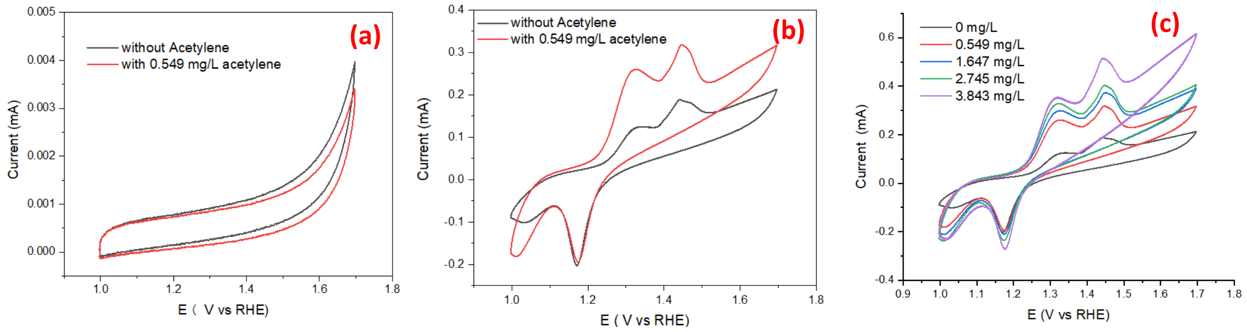


Figure 4.1. CV Scans of (a) Glass Carbon and (b) Au in Electrolyte Blank (0.5 M Sulfuric Acid) and Electrolyte with 0.55 mg/L Acetylene. (c) CV Response of Au Electrode at Varying Concentrations of Acetylene.

When 0.55 mg/L acetylene was introduced into the electrolyte solution, the glassy carbon electrode produced no discernable signals. In contrast, the oxidation and reduction twin-peaks of Au electrode became more pronounced compared to those in the blank electrolyte solution. Increasing the concentration of acetylene corresponds to an increase in the oxidation current (Figure 4.1c), indicating electrochemical oxidation of acetylene mediated by the gold surface. These results are in good agreement with prior studies in which Au was considered uniquely suited for acetylene sensing due to its strong chemical interactions with acetylene[38, 39]. The interactions were postulated as entailing chemisorption of acetylene followed by its oxidation[39].

With these results, amperometric i-t tests were performed to establish a calibration curve for acetylene at different concentrations. The procedure of the test was detailed in Section 3.2. Figure 4.2 shows the i-t curves at 1.25 V (vs. RHE) and an acetylene concentration between 1 to 5 mg/L.

The current stabilized within 100 s after the potential reached the set value and the stable current reading at 300 s was plotted as a function of the concentration. A linear current vs. acetylene concentration trend was observed as concentration reaches 4 mg/L. At higher concentrations, the electrode registered no further increases in the current density, implying saturation of the electrode surface. The saturated electrode could not regain its baseline when brought back to a low concentration or after a brief oxidative pulse, suggesting irreversible poisoning of the electrode by acetylene oxidation products.

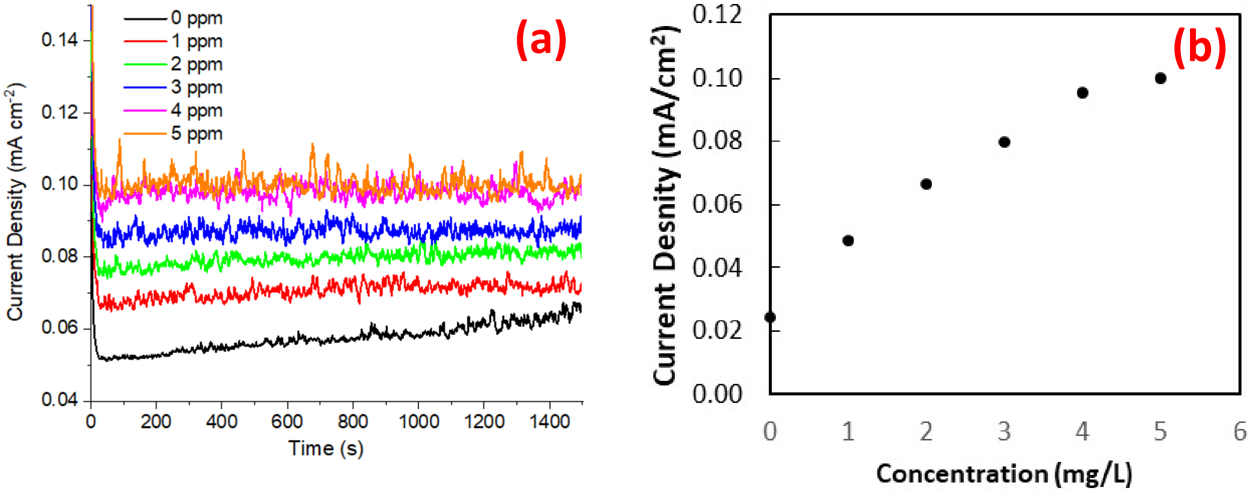


Figure 4.2. (a) i-t tests of Au Electrode at +1.3 V (vs. RHE) in Electrolyte Solution with Varying Concentrations of Acetylene. (b) Current Density as a Function of Acetylene Concentration.

Acetylene is a highly reactive molecule and it undergoes coupling or tri- or polymerization upon partial oxidation. These byproducts are known to be highly affinitive for Au and other transition metal surfaces[1, 32, 35, 36]. Similar observations related to the loss of electrode sensitivity at a higher acetylene concentration have been reported in prior studies employing Au-based electrodes for acetylene sensing. Jordan and Hauser noted plateauing of the current response when an Au-on-Nafion electrode was exposed to a gaseous acetylene concentration above 10 ppm (note that 1 ppm in the gas phase does not equate with 1 mg/L of dissolved acetylene in electrolyte solution)[39]. The authors suggested that pulsing at a high oxidizing potential was able to strip off the poisoning species[39], however, this method fails to restore electrode performance in the current study, possibly due to a higher concentration of acetylene accumulated on the surface or irreversible change of the electrode chemistry encountered in solution phase sensing applications (*e.g.*, adsorption of water molecules on surface). As noted in Section 4.2, poisoning by acetylene oxidation intermediates is not the only shortcoming of electrochemical sensing using noble metal electrodes. More significantly, the ubiquitous presence of groundwater solutes, particularly inorganic ions such as chloride (Cl^-) and sulfide (HS^-) and dissolved organic carbon, poses concerns to the stability and reproducibility of an electrochemical sensing platform for monitoring chemicals in an undergound environment.

A Pt wire electrode was evaluated and the results of amperometric tests are provided in Figure 4.3. The current responses stabilized within 300 s after the potential was raised to the set value. The current density obtained at 300 s was proportional to the concentration of acetylene at upto 350 μg/L. At a higher concentration, electrode saturation became evident. Although Pt electrode was able to attain a higher signal-to-noise at similar conditions than Au, the current response was not specific to acetylene, but received a large interference when a similar level of ethylene was present. The low selectivity of Pt electrode against aliphatic gases was noted in prior research[38].

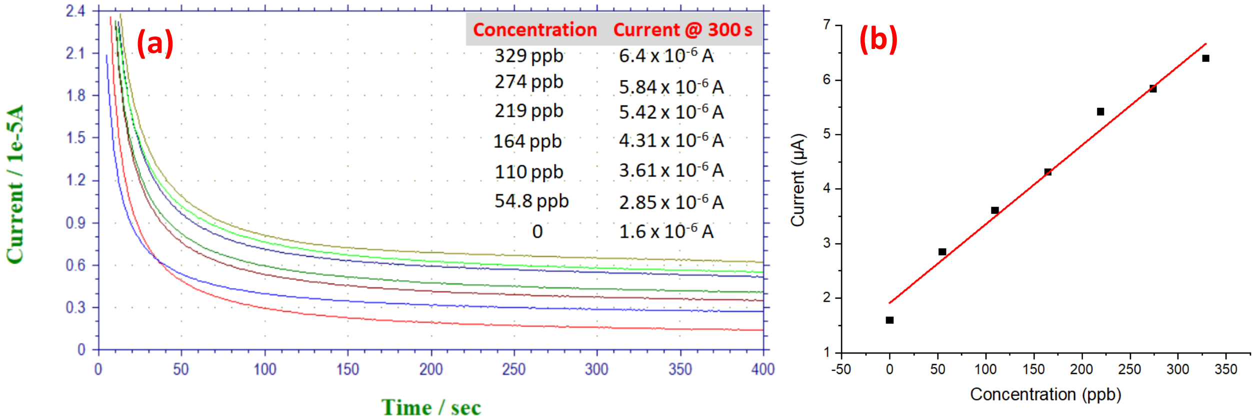


Figure 4.3. (a) i-t tests of a Pt Electrode at +1.25 V (vs. RHE) in Electrolyte Solution with Varying Concentration of Acetylene. (b) Current Density as a Function of Acetylene Concentration.

4.2 EVALUATION OF ELECTRODE PROTECTION LAYERS (TASK 2)

Apart from electrode poisoning by acetylene oxidation products and interference caused by background alkene or alkane gases, an important practical concern over the deployment of electrode-based sensing system in the subsurface environment is the potential interactions between the electrode surface and common groundwater solutes. Reduced sulfur (H_2S, HS^-) and chloride (Cl^-) are known to cast a poisoning effect on transition metals[50, 51], and accumulation of colloidal particles may result in surface fouling. To mitigate this issue, we propose in the original project plan to enclose the electrodes within several layers of protection covers. Ideally, the cover should remove colloidal particulates, anions (including Cl^- and sulfide), and organic matter, and at the same time, possess adequate permeability for acetylene to pass through.

In this task, a 0.2 μm PTFE membrane filter was tested for its ability to block colloidal particles. The colloidal particle suspension was 0.5 g/L bentonite suspension. The results, in Figure 4.3a, indicate that the membrane filter was effective for screening out most of the suspended particles in a short time span (< 1 h). Gradual penetration of fine particulates through the membrane filter was observed over an extended period.

To protect the electrodes against chloride and sulfide adsorption, a strongly acidic cation-exchange membrane (PCSK^{TM} from PCCell GmbH) was evaluated for its ability to impede the movement of anions. As shown in Figure 4.4b, the membrane was able to guard against the penetration of 20 mg/L chloride into the opposite side of the membrane (*i.e.*, the DDI solution) for 4 h. The membrane was similarly effective at holding off the migration of sulfide ion into the DDI solution (Figure 4.4c), and the gradual loss of sulfide in the source chamber was attributed to the volatile nature of hydrogen sulfide. These results confirm the effectiveness of the PCSK membrane to protect the electrode surface from interfering solutes for hours. However, the cation exchange membrane completely barricaded the movement of acetylene as well (Figure 4.4d). This unexpected result implies severe mass transport limitation if the electrodes were encapsulated in protection layers as originally proposed.

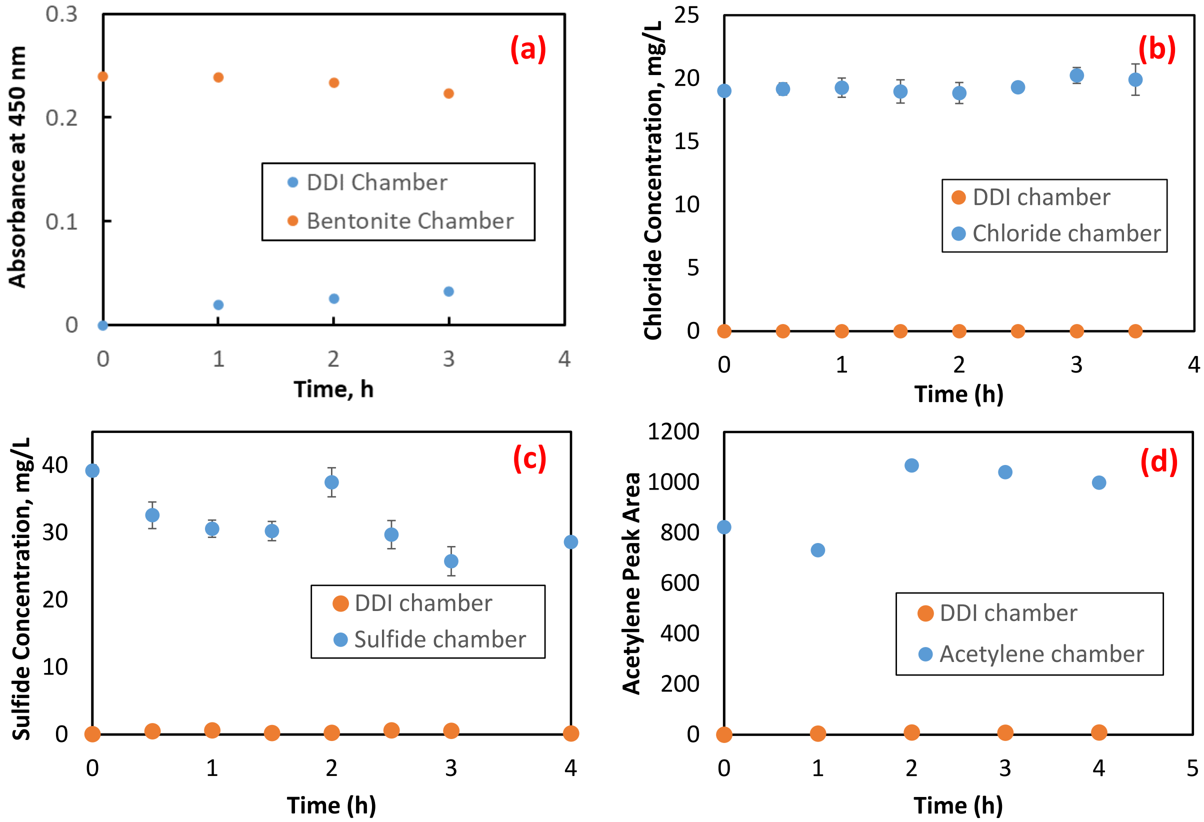


Figure 4.4. Results of Two-chamber Tests to Evaluate the Ability of (a) 0.2 µm PTFE Membrane and (b-d) Strongly Acidic Cation Exchange Membrane to Block the Movement of Colloidal Particles or Specific Solutes.

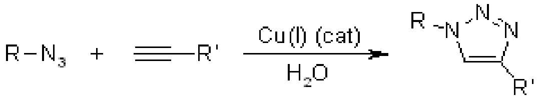
Lessons from Tasks 1 and 2. In summary, although electrochemical measurements using Au and Pt electrodes generate strong and quantitative responses in simple dilute acetylene solutions, irreversible poisoning of Au electrode when exposed to an elevated level of acetylene and interference caused by alkene gases on Pt electrode were observed. Another concern arises from the susceptibility of the metal electrodes to poisoning by groundwater solutes. Although encapsulating electrodes in protection layers may mitigate this concern, one protection material evaluated was found to block the transport of acetylene from bulk water to the electrode surface.

Pivoting. Apart from the practical issues noted in the above discussion, the most significant drawback of deploying an electrochemical sensing system for subsurface monitoring lies with the instantaneous nature of electrochemical tests. Electrochemical sensing provides real-time 'snapshot' estimates of the analytes of interest. It is not able to gauge the dynamic flux of a molecule over a broad timescale relevant to remediation projects. Moreover, the instrumentation requirements of electrochemical tests dictate that the analysis can only be performed at a shallow depth or above ground with extracted samples rather than sensing under *in situ* conditions. Reflecting on these limitations, the team proposed a change in experimental approach during the course of project. The alternative approach focused on designing a molecular probe that binds specifically with acetylene and can be deployed in a construct similar to a passive sampler at different depths or in various biogeochemical environments. These efforts are documented in the following sections.

4.3 CUAAC CLICK CHEMISTRY FOR ACETYLENE QUANTIFICATION – PROOF-OF-CONCEPT EXPERIMENTS (TASK 3)

The results from Tasks 1 and 2 suggest that a chemical probe that is stable in groundwater, highly specific for acetylene, and capable of accumulating low levels of acetylene over an extended time is more aligned with the mission of the project than what can be offered by real-time electrochemical measurements. To this end, the team proposed to create an alternative acetylene sampling and quantitation method based on the well-established alkyne-azide click chemistry.

The copper-catalyzed 1,3-dipolar cycloaddition of organics with terminal alkyne and azide groups, also known as **CuAAC click chemistry**, is a robust, high-yield reaction that takes place in a broad range of environments including aqueous solutions at ambient temperature[7, 43]. CuAAC click reaction produces 1,2,3-triazole (scheme 1), which is chemically inert against oxidation, reduction, and hydrolysis[7]. In addition, triazole is known to have antimicrobial activities[52] and is therefore biologically stable as well. The stability of the product permits a sampling scheme in which gradual accumulation of acetylene reaction product over an extended period of time is feasible, thereby enabling the estimation of time-averaged concentration or mass flux. The click chemistry is highly specific (thus has low background interference), efficient (both from kinetics and chemical utilization perspectives), and its product yield is not affected by solvent environment, pH, and complex solutes present in the reaction media. For these reasons, it has found important uses in organic synthesis and bio-conjugation studies, the latter has led to a broad range of diagnostic applications[53, 54].



Scheme 1. Cu(I) catalyzed alkyne-azide cycloaddition to produce 1,2,3-triazole

Since the application of click chemistry in remediation research is new, the team sought to establish proof-of-concept using a simple commercially available azide compound, benzyl azide. The reaction of benzyl azide and acetylene gives benzyl-1,2,3-triazole (BTA), which is readily identified and quantified with the GC-MS at the PI's lab.

First, various forms of copper catalysts were evaluated. The classic CuAAC reaction employs dissolved Cu(I) or Cu(II) with ascorbic acid[7]. More recent studies suggest that other forms of Cu including Cu metal may serve the catalytic function well[8, 44, 55]. At room temperature and with Cu(II) and ascorbic acid, the reaction between aqueous acetylene and benzyl azide took approximately 4 days to complete. 0.2 equivalent of Cu(II) (w.r.t. acetylene) gave the highest product yield (Figure 4.5a). In comparison, the use of Cu powder achieved a similar reaction rate (reaction product peaked at approximately 4 days, as shown in Figure 4.5b). Within the range of Cu powder dosed, no appreciable difference in BTA yield was observed. We evaluated Cu nanoparticles as catalysts, and the results were comparable to that of Cu powder (data not shown). Overall, Cu powder stood out as the most desirable form of catalyst and was used in subsequent experiments owing to multiple considerations: i) unlike cupric ion, metallic Cu does not require ascorbic acid as a co-catalyst; ii) Cu powder is more amenable to impregnation in a polymer thin film compared to dissolved or nanosized Cu catalysts; iii) Cu powder is stable and remain catalytically active over a long period of time; and iv) the antimicrobial property of Cu powder may continually protect the sensing medium against biofouling, a side benefit that has much practical value in remediation applications.

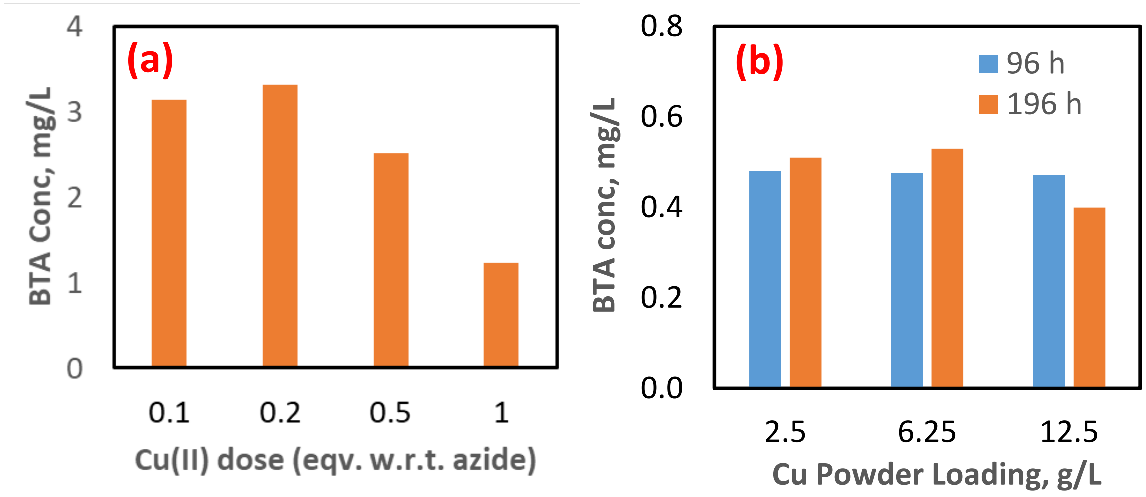


Figure 4.5. Reaction Between Acetylene and Benzyl azide at Room Temperature in the Presence of (a) Cu(I) and Ascorbic Acid and (b) Cu(0) Powder.

C_o of benzyl azide and acetylene was 0.385 mM each. Reaction time was 96 h in (a).

When a sufficient amount of benzyl azide was present, the quantity of triazole (BTA) produced was found to increase linearly with the concentration of acetylene in the solution (Figure 4.6) This linear response provides the basis of acetylene quantification and established proof-of-concept of the proposed molecular sensing mechanism based on Cu-catalyzed alkyne-azide click chemistry.

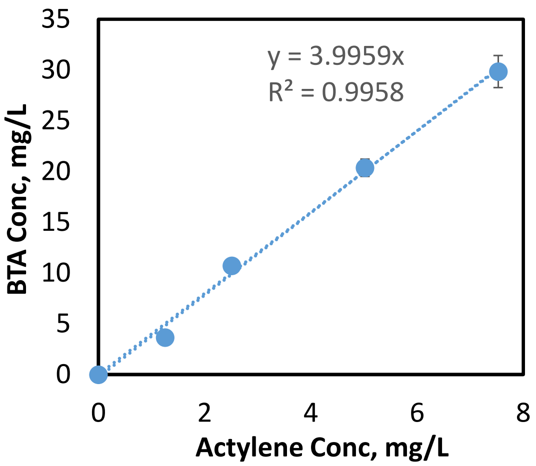


Figure 4.6. **Formation of BTA as a Function of Acetylene Concentration. C_o of Benzyl Azide Was 0.385 mM.**

Dose of Cu powder was 2.5 g/L. Reaction time was 96 h.

The effects of pertinent environmental factors on triazole product yield were investigated. Figure 4.7a confirms that reaction between acetylene and the azide probe was independent of solution pH within a pH range of 5 - 9. In another set of experiments, common groundwater solutes including1 mM of Cl^-, HCO_3^-, and SO_4^{2-} were amended into the reaction mixture. Chloride and bicarbonate ion did not affect triazole product yield (Figure 4.7b). Interestingly, the presence of sulfate slightly increased the reaction yield by ca. 10%. While the reason for this observation was unclear, it is possibly related to the tendency of sulfate to complex with dissolved Cu ions, thus promoting the activity of the catalyst (it is postulated that dissolved Cu is the active catalyst, and Cu metal serves as a slow-release source of dissolved Cu). We also evaluated the effect of sulfide (as HS^-), and it did not cast an influence on the click reaction. Finally, the co-presence of small aliphatic hydrocarbons, such as methane, ethene, and ethane, had no appreciable effect on acetylene reaction with benzyl azide (Figure 4.7c).

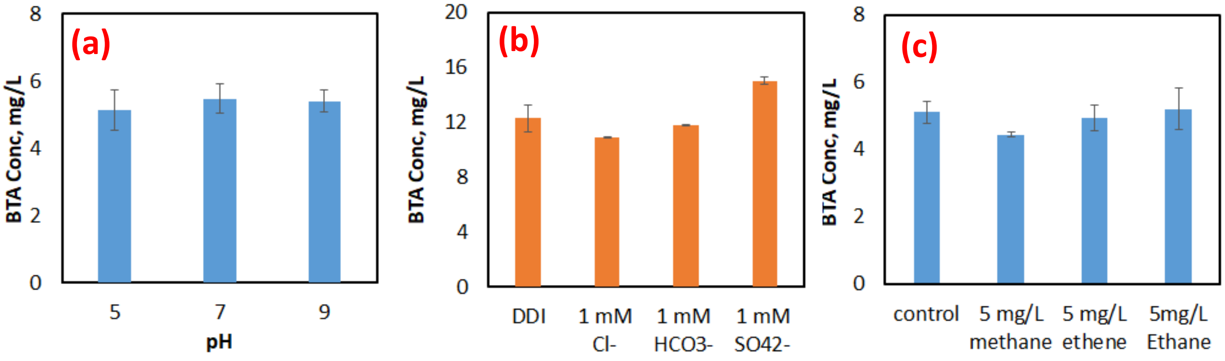


Figure 4.7. **Effects of (a) pH, (b) Common Solutes, and (c) Low Molecular-weight Alkanes and Alkenes on BTA Formation from Acetylene-azide Reaction.**

C_o of benzyl azide was 0.2 mM and that of acetylene was between 0.1 and 0.2 mM. Dose of Cu powder was 2.5 g/L. Reaction time was 96 h.

Lessons from Task 3. Overall, the results of aqueous acetylene-azide reactions are in agreement with the known characteristics of CuAAC click chemistry. The reaction is relatively facile at room temperature and the product yield is quantitative. Furthermore, the rate of this reaction is not affected by variations in groundwater chemistry in the typical ranges encountered in the remediation scenarios. These results establish the feasibility of using an azide molecule to trap and quantify acetylene in groundwater matrix.

4.4 DESIGN AND VALIDATE AN AZIDE-BASED ACETYLENE PASSIVE SAMPLER (TASK 4)

To use an azide compound as a molecular probe for acetylene, the chemical needs to be immobilized into a solid phase, so that the latter can be incorporated in a passive sampler as the reactive medium (or "receiving phase"). A variety of passive samplers have been developed to monitor many inorganic and organic pollutants in environmental research and remediation studies[56-58]. Conceptually, a passive sampler consists of a receiving phase that irreversibly binds with the analytes, an optional gel film if diffusion-controlled dynamic monitoring is desired, and a gasket that holds different component together[59]. The receiving phase is typically a resin material containing high loads of chelating agents or chemical reagents specific for the analyte of interest. In the field, a passive sampler is deployed at a sampling location and left at the site for a predetermined amount of time. During this period, analytes in bulk groundwater diffuse into the passive sampler, react or bind with the receiving phase, and are retained inside the sampler until the device is later recovered for lab analysis. The methodology of environmental passive sampling is mature, and there are established mathematical models describing analyte uptake as a function of analyte properties, concentration, or flux [57, 60].

The objectives of this task are to design a strategy to immobilize an azide compound into an inert polymer phase and to validate the performance of a prototypical passive sampler in a microcosm reactor.

To attain the objectives, the team explored two different strategies:

1) using a commercial azide-tagged agarose beads as the receiving phase
2) developing a method to prepare an azide-impregnated PDMS membrane in house

Strategy 1 involved a commercial cleavable azide product (Product ID 1139, Click Chemistry Tools, CA). This reagent consists of three components, an azide end group connected to agarose beads via a cleavable Dde linker. As shown schematically in Figure 4.8, the beads may be deployed in a solution containing acetylene in the presence of Cu powder. Click reaction transforms the azide terminal to a triazole moiety. Subsequently, the addition of a cleaving reagent (ca. 0.3% hydrazine) releases the triazole-containing fragment for quantitation. The pursuit of this strategy was aborted for several reasons. We did not obtain quantitative yield of the expected product, possibly a result of inefficient cleaving or loss of agarose beads due to adherence to reaction vessels. Notwithstanding this result, this commercial azide-tagged probe has several drawbacks that renders it not suitable for the intended application. The patented reagent is of a high unit cost (ca. $180 for 40 μmole); it entails the use of hydrazine, an acutely toxic chemical; and agarose is prone to degradation, which raises the question of the long-term stability of the reagent in a biologically active environment.

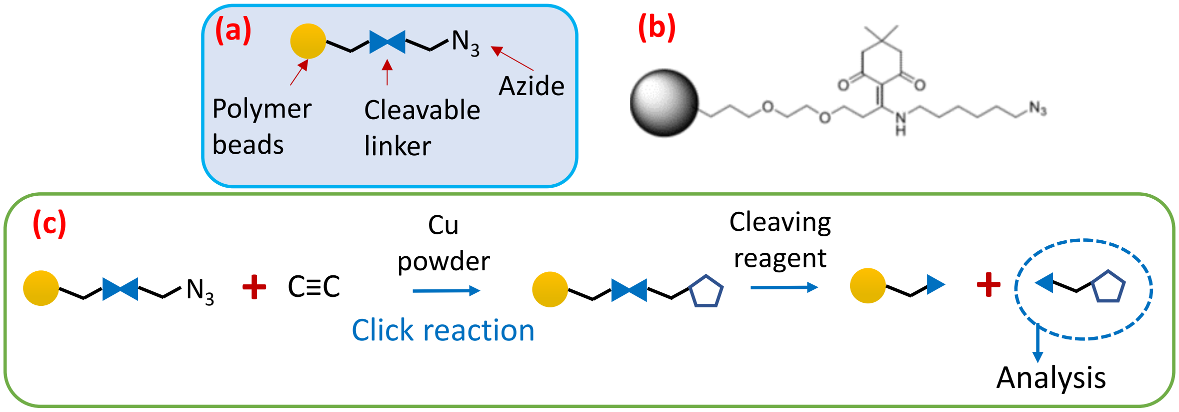


Figure 4.8. (a) Schematics of a Cleavable Azide-tagged and Agarose Bead-immobilized Reagent. (b) Molecular Structure of Product in (a). (c) Application of the Azide Probe to Acetylene Quantitation.

The alternative strategy focused on the development of lab-prepared azide-impregnated PDMS membrane. PDMS thin films are stable in water, biologically inert, and made of benign chemical ingredients. There is precedence of using PDMS films in environmental sampling including the Waterloo™ membrane filters[60]. As shown schematically in Figure 4.9, the membrane was prepared from Dow Sylgard™ 184 Silicone Kit following the procedure detailed in the Materials and Methods section. The azide compound selected for impregnation in PDMS matrix is 6-azido-hexanoic acid (6AHA) for its non-volatile nature. The membrane preparation procedure was optimized to ensure adequate mechanical strength, uniform loading of Cu powder, and consistent thickness. The optimized membrane synthesis and casting methods are detailed in Materials and Methods. The membrane was made to have a porous texture to increase surface area.

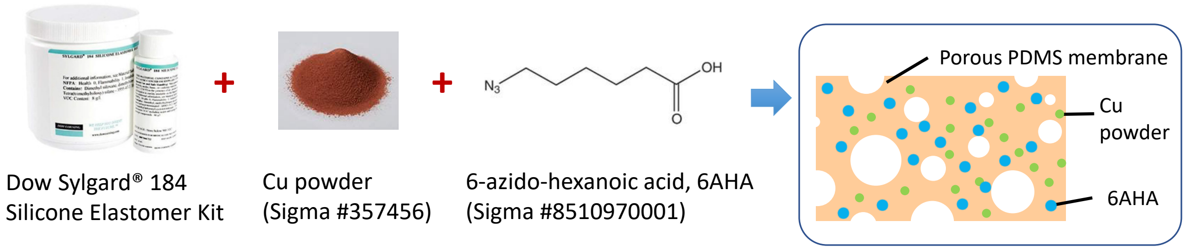


Figure 4.9. The Construction of Azide-impregnated PDMS Membrane.

To validate the performance of the azide-loaded membrane for acetylene detection, 100 mg of membrane was deployed in 5 mL solution containing varying concentrations of acetylene. After 96 h immersion in the reaction mixture, the membrane was harvested and the product of acetylene reaction with 6AHA, 6HA-triazole, was quantified with LC-MS. Figure 4.10 depicts a strong linear trend of the product yield as a function of aqueous acetylene concentration. The concept of using an azide-loaded membrane for quantitative measurement of aqueous acetylene is therefore validated. Note that the concentration of 6HA-triazole indicated on the figure was the equivalent concentration in the solution phase. The amount of product formed was approximately 16% of the ideal yield assuming a 1:1 stoichiometry between acetylene and 6AHA. This indicates that not all acetylene was reacted and/or not all products were recovered via extraction.

This observation itself does not affect the intended application of the membrane, but suggests the need to include reference compounds during field implementation to calibrate product formation against the amount of acetylene present. The concept of reference compounds is detailed in the last section about future research.

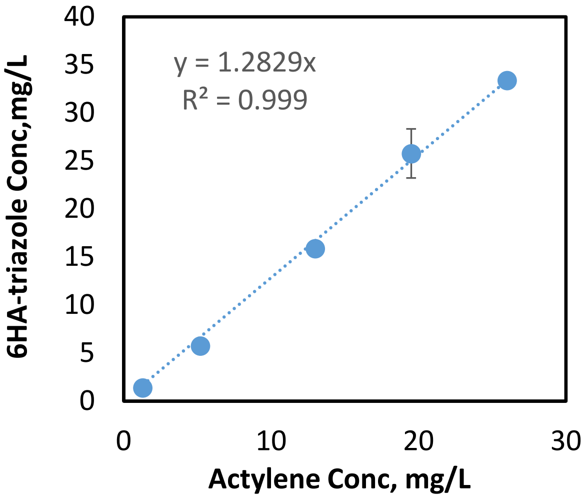


Figure 4.10. The Amount of 6HA-triazole Formed as a Function of Aqueous Acetylene Concentration.

100 mg of azide-impregnated PDMS membrane was applied in 5 ml of solution. Reaction time was 96 h.

It is noteworthy that, although Figure 4.10 shows that the lowest concentration of acetylene for the sampler to generate a quantifiable response is approximately 1 mg/L, this value should not be taken as the quantitation limit of the sampler. This is because during actual applications, the amount of triazole product formed is controlled by the *cumulative* amount of acetylene the sampler is exposed to. The data in Figure 4.10 was acquired in small batch reactors (5 mL), whereas in field deployments, the sampler is expected to interact with a much larger volume of solution and be able to pick up adequate responses for a more dilute acetylene flux. The lowest mass flux the sampler is capable of detecting should be assessed in column tests as discussed in the last section.

To confirm the robustness of the membrane performance in different groundwater matrices, the experiments were repeated in solutions amended with 1 mM of Cl^-, HCO_3^-, SO_4^{2-}, or 20 mg/L humic acid (Figure 4.11a). The difference in product yield relative to the control (in DDI) was within 15%. Experiments were also conducted in the presence of 5 mg/L methane, ethane, and ethene. These small hydrocarbons did not pose a noticeable effect (Figure 4.11b), demonstrating the high specificity of this molecular sensing method, a notable advantage over the more variable nature of electrochemical sensing.

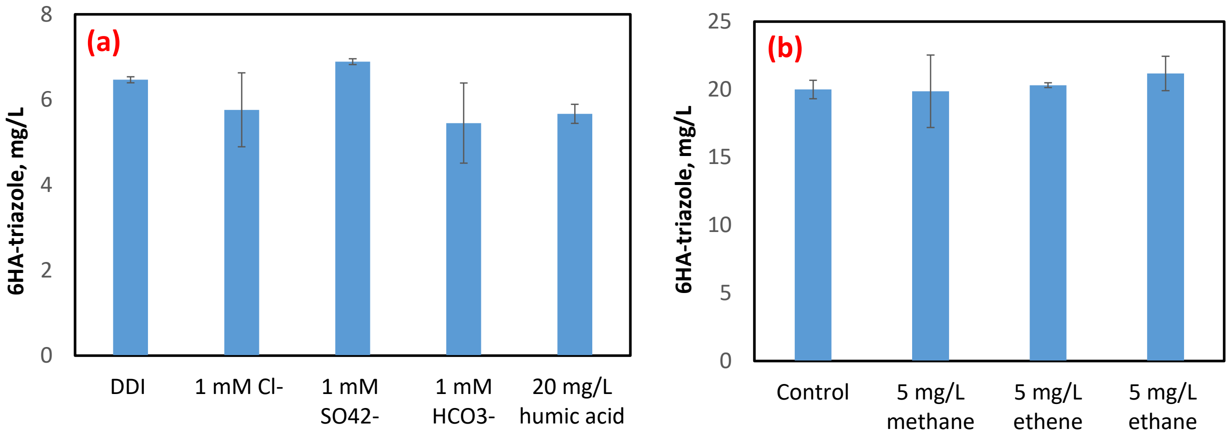


Figure 4.11. (a) Effects of Groundwater Solutes and Humic Acid and (b) Low Molecular-Weight Alkanes and Alkenes on Product Yield from Reaction between Acetylene and 6AHA-loaded PDMS Membrane.

C_o of acetylene was 0.5 mM. Membrane loading was 100 mg in 5 ml of solution. Reaction time was 96 h.

As a final step towards the project goal, a prototype of an acetylene passive sampler was constructed by placing 100 mg azide-loaded PDMS membrane in a 2-ml glass vial capped with glass fiber filters (Figure 3.4a). The glass fiber filters were to prevent the passage of colloidal particles into the sampler to clog up the pore space of the membrane. The passive sampler was placed in a reaction microcosm, in which TCE reacted with sulfur-treated Peerless Fe(0) powder (S-ZVI^{PLS}) and was reductively dechlorinated to form acetylene inside the reactor. The acetylene generated *in situ* was expected to migrate to the passive sampler and get captured by the azide compound immobilized in the PDMS membrane. The quantity of acetylene formed in the microcosm was adjusted by changing the initial concentration of TCE, since the Fe(0) powder was in far excess and TCE was the limiting reactant.

When the microcosm experiments were commenced, the concentrations of TCE and acetylene in the system were monitored via regular headspace sampling. Figure 4.12a portrays the trend of TCE disappearance during its abiotic degradation in the presence of S-ZVI^{PLS}. Over 90% of TCE was depleted in less than 5 days, and concomitantly, acetylene, the major product of TCE abiotic dechlorination, built up in the system. As revealed in Figure 4.12b, the amount of acetylene peaked at ca. 3 days. Afterwards, acetylene amount declined rapidly, reflecting it being captured in the azide-impregnated membrane in the passive sampler.

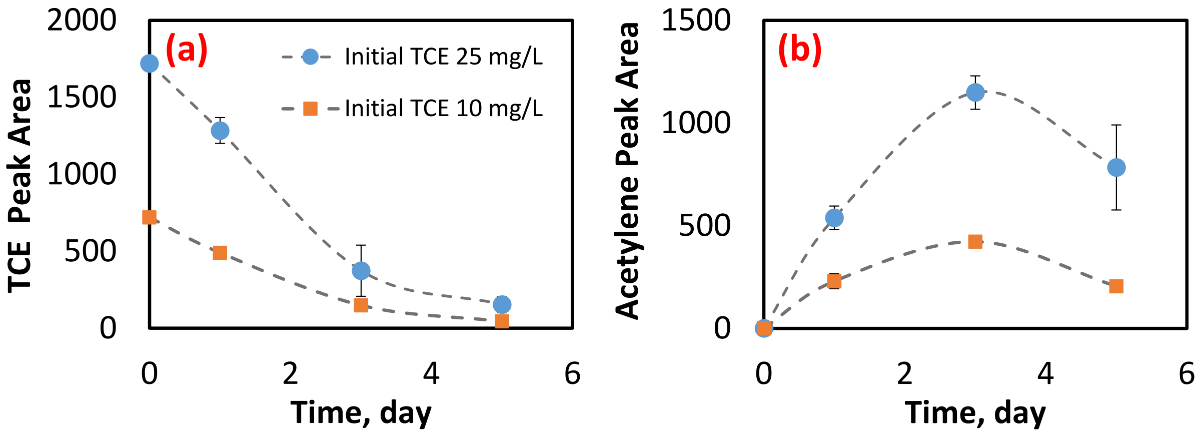


Figure 4.12. **(a) TCE Degradation by S-ZVI^{PLS} and (b) Formation of Acetylene During Microcosm Experiments.**

S-ZVI^{PLS} dose was 20 g/L.

The passive samplers were harvested from the microcosm reactors after 8 days. The membrane was extracted and analyzed for the quantity of 6HA-triazole with LC-MS. The data are plotted in Figure 4.13. A strong correlation exists between the triazole product recovered from the samplers and the initial concentration of TCE in the system. Since acetylene was being produced and consumed simultaneously in the microcosm, the absolute amount of acetylene formed in the microcosm cannot be directly measured. We cannot directly evaluate the correlation between the amount of product formed and acetylene generation. Nonetheless, since TCE reduction by S-ZVI^{PLS} is a well-characterized reaction with acetylene being the dominant product[45], the trend in Figure 4.13a **confirms the attainment of the project goal, that is, quantifying abiotic transformation of chlorinated ethenes by capturing the characteristic reaction product, acetylene, with azide-loaded reactive media in a passive sampler.**

We postulate that the acetylene concentration detected in the early stage of the reaction is a good proxy of the relative amount of acetylene produced in the microcosm system, since at this early stage, acetylene was rapidly formed, but it had not been consumed by the azide reaction to a large extent. Figure 4.13b plots 6HA-triazole formation against acetylene concentration at 48 h of the microcosm experiments. A strong positive correlation can be discerned when acetylene concentration exceeds a threshold value (~ 0.8 mg/L in the current system). When small amounts of acetylene were produced, the amounts of triazole recovered from the samplers were negligible. The occurrence of this low response regime is likely attributable to the background loss of acetylene including adsorption to membrane or the reaction vessel, which was exaggerated by the limited size of the reactor vial relative to the capacity of the passive sampler. In actual implementation in the field, the vast size of the aquifer reaction zone is expected to greatly reduce the effect of background loss. Column experiments are valuable to assess dynamic effects, including flow rate and flux of acetylene (as opposed to concentration of acetylene in a batch reactor), on the performance of the passive samplers. This is discussed in Section 5.

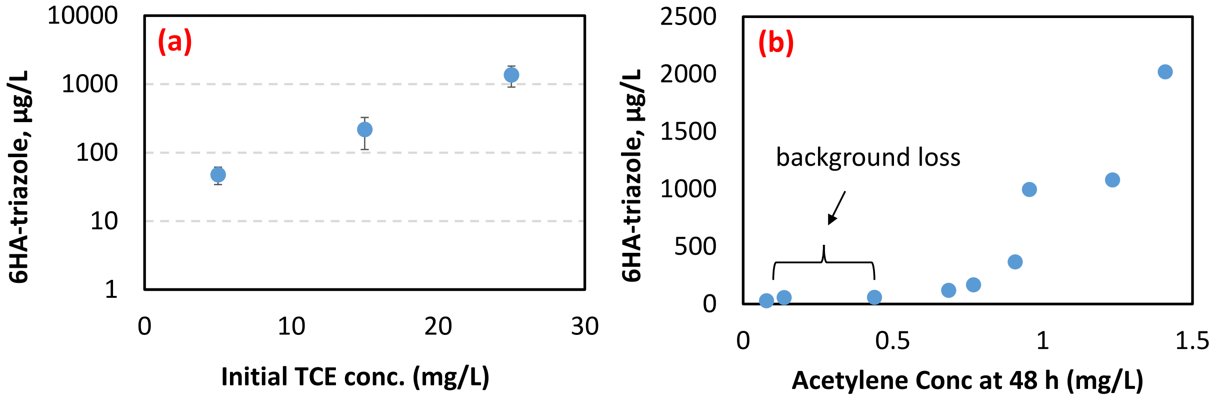


Figure 4.13. (a) 6HA-triazole Formed in Systems with Varying Initial Concentrations of TCE. (b) 6HA-triazole Formation vs. Acetylene Concentration at 48 h of the Microcosm Experiments.

5.0 CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

The overarching objective of this project is to develop a method to quantify the extents of abiotic reduction of chlorinated ethenes at complex contamination sites, where biotic and abiotic transformation may occur concurrently but slowly during long-term natural attenuation processes. Within this limited scope explorative project, the specific goal is to develop and evaluate multiple approaches to detect and quantify a characteristic product of abiotic reduction of chlorinated ethene, acetylene, which can inform the rates and extents of abiotic transformation of chlorinated ethenes.

The original approach of the proposed project was to quantify aqueous acetylene using noble metal-based electrochemical measurements. Au and Pt electrodes were evaluated and each was able to generate quantitative current signals proportional to acetylene concentration within a limited concentration range in simple electrolyte media. However, poisoning of Au by acetylene oxidation products and interference caused by background organics during acetylene sensing by Pt electrode were observed. The practical limitations of an electrochemical sensing method including difficulty to deploy in subsurface for *in situ* measurements and inability to monitor dynamic mass quantities over a period of time render the electrochemical approach unsuitable for attaining the project goal.

The team pivoted to the second approach of using a highly specific chemical probe to detect and quantify trace level of acetylene in groundwater. The approach is based on the Cu-catalyzed alkyne-azide cycloaddition reaction (or click reaction). The reaction between acetylene and an azide compound generates a biologically and chemically stable triazole product. A strong linear relationship was observed between the product formation and the concentration of aqueous acetylene, and the product yield was unaffected by variations in pH, salinity, and groundwater solutes, demonstrating a sensitive and robust mechanism to measure the presence of acetylene. Based on these results, the team developed an acetylene passive sampler in which an azide-impregnated porous silicone membrane served as a reactive receiving phase of the sampler. The performance of the passive sampler was verified in a microcosm study, where the acetylene sampler was installed in a batch reactor containing TCE and sulfur-treated iron powder. Acetylene production during abiotic dechlorination of TCE was effectively captured by the azide-loaded membrane, and the quantity of the product recovered from the sampler correlates with the amount of TCE degraded. This result confirms the suitability and capability of the azide-based acetylene passive sampler as a new diagnostic tool for monitoring abiotic transformation of chlorinated ethenes in the subsurface environment.

The conclusions obtained thus far are based solely on laboratory investigations using acetylene solutions or acetylene generated in small batch reactors. To enable this technology for field implementation, the team recommends additional work in the following areas.

1. Evaluate the azide-based acetylene passive sampler in 1-D column study to assess the sensitivity and responsiveness of the sampler in a flow-through system. A schematic of the column setup is shown in Figure 5.1. Reactive minerals such as mackinawite, $Fe(OH)_2$, green rust, or Fe(0) may be used[61-64]. Recent studies suggest that under appropriate conditions (often requiring specific forms of reactive Fe(II) species to be present), facile abiotic reduction of chlorinated ethenes may occur,[63, 64] highlighting the need to investigate

this subject further and the value of the proposed acetylene sampler to collect unambiguous evidence of abiotic transformation of chlorinated solvents. Pertinent parameters to be adjusted include the fluid flow rate, flow direction, and flux of acetylene (may be adjusted by the initial concentration of TCE, flow rate, and mineral loadings). As the sampler is primarily designed to measure the mass flux of acetylene, we propose to introduce a secondary alkyne compound as an internal reference compound embedded in slow-release capsules, which are placed in the vicinity of the azide-loaded membrane. This will create an internal calibration mechanism, where the ratio of the triazole products formed by acetylene and the internal reference will inform the mass flux of acetylene. This column study should also investigate the long-term stability (3-12 months) of the passive sampler, including its susceptibility to clogging, biofouling, and biodegradation. As mentioned earlier, we believe that the unique chemistry of the sampler, including the biocidal effect of the Cu catalyst and the bio-inert nature of the triazole product and PDMS, predicts good long-term stability. Lastly, the column study will be coupled with mathematical modeling to establish the quantitative relationship between acetylene flux and the sampler response.

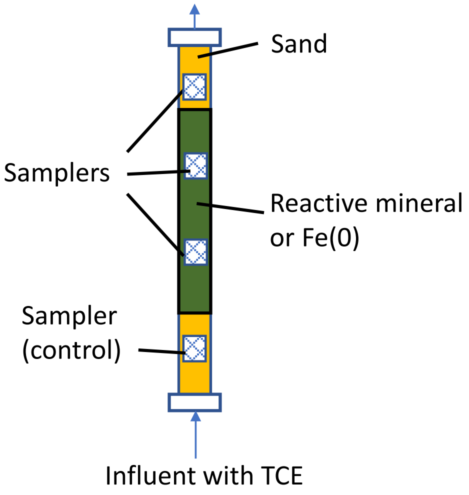


Figure 5.1. Schematics of Evaluating the Acetylene Passive Sampler in a 1-D Flow System.

2. Evaluate the performance of the acetylene passive sampler in a pilot scale at a complex contamination site. Once the long-term stability of the sampler is confirmed, deployment of the sampler at sites that have complex hydrogeological features such as NAPL, low-permeability lenses, or fractured bedrocks will be pursued to evaluate its potential to pick up abiotic degradation product *in situ* over months to years. The initial test should be conducted at a pilot scale at a well-investigate and well-documented site. The inclusion of an internal reference in the sampler and careful placement of field blanks and controls in the pilot test will differentiate faulty sampler (false negative) from the absence of analyte (true negative). Further, the quantitation method based on the product ratio as proposed here will be validated in the field test.

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APPENDIX A LIST OF CHEMICALS AND MATERIALS

Chemical Name	Chemical Formula	Manufacturer	Catalogue Number
Acetone	C_3H_6O	Fisher Scientific	A949SK-4
Acetylene (10%, balance N_2)	C_2H_2	Matheson Trigas	G2702776
6-azido-hexanoic acid	$C_6H_{11}N_3O_2$	Sigma Aldrich	8510970001
Benzyl Azide	$C_7H_7N_3$	Fisher Scientific	AA3263504
1-Benzyl-1H-1,2,3-triazole	$C_9H_9N_3$	Sigma	AMBH324AB896-250MG
Chloride Reagent Set, Mercuric Thiocyanate		HACH	2319800
Copper nanopowder, 60 – 80 nm	Cu(0)	Sigma Aldrich	774103-5G
Copper powder (< 45µm, 99.7%)	Cu(0)	Sigma Aldrich	357456-100g
Copper Sulfate Pentahydrate	$CuSO_4 . 5H_2O$	Fisher Scientific	AA1417836
Dde Azide Agarose		Click Chemistry Tools	1139-2
Dichloromethane	CH_2Cl_2	Fisher Scientific	AC364230025
Ethane (1000 µg/mL in methanol)	C_2H_6	Fisher Scientific	S-1880
Ethene (1000 µg/mL in methanol)	C_2H_4	Fisher Scientific	S-1895
Humic Acid	$C_9H_9NO_6$	Fisher Scientific	AA4174706
Hydrazine	N_2H_4	Fisher Scientific	AC196711000
Methane (1000 µg/mL in methanol)	CH_4	Fisher Scientific	S2379
Methanol	CH_3OH	Fisher Scientific	A4524
Peerless^{TM} ZVI	Fe (0)		
Polydimethylsiloxane, Dow Sylgard 184 Silicone Elastomer Kit		Fisher Scientific	NC9285739
Sodium Ascorbate	$C_6H_7NaO_6$	Fisher Scientific	18-606-309
Sodium Bicarbonate	$NaHCO_3$	Fisher Scientific	S233-500
Sodium Chloride	NaCl	Fisher Scientific	S2711
Sodium Sulfate	Na_2SO_4	Fisher Scientific	S415-500
Sodium Thiosulfate	$Na_2S_2O_3$	Fisher Scientific	S445-500
Sulfide Reagent Set, methylene blue		HACH	2244500
Toluene	C_7H_8	Fisher Scientific	T290-1
Trichloroethene (1000 µg/mL in methanol)	C_2HCl_3	Fisher Scientific	S-3615