Microbial Magic: Method Development for Anaerobic Biodegradation of TCE

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Introduction

Environmental contamination from chlorinated volatile organic compounds (Cl-VOCs) is the result of numerous manufacturing processes, from metal degreasing to electronics manufacturing. Although emissions to soil and groundwater are most common, due to their volatility, Cl-VOCs easily evaporate allowing their dispersal to air. Exposure to these Cl-VOCs poses numerous health related risks such as drowsiness, vision loss, tinnitus, memory impairment, depression, kidney, liver and lymphoid tissue cancers, nerve damage, and cognitive impairment. Their chlorine atoms make these toxic compounds, such as tetrachloroethylene (PCE), trichloroethylene (TCE), dichloroethylene (DCE) and vinyl chloride (VC), very stable and resistant degradation making them persistent in the environment.

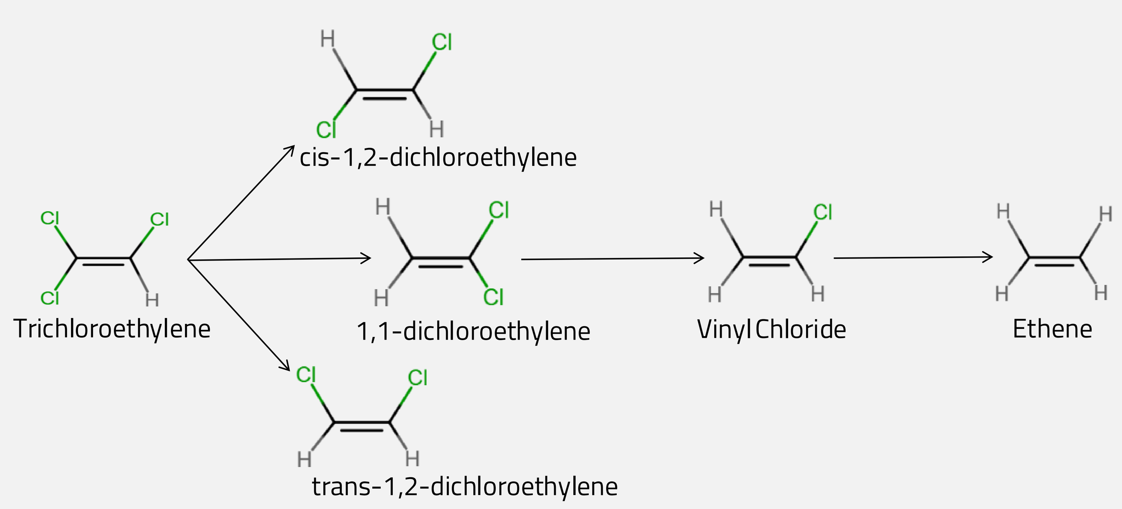

Figure 1. Dechlorination pathway of TCE. Displaying biodegradation products.

Current groundwater remediation is typically done ex-situ and uses expensive, highly disruptive water pumping methods which are not always accessible. Chlorinated compounds have been shown to be biodegradable by bacteria under anaerobic conditions. The use of microorganisms for the bioremediation of Cl-VOCs is a relatively new environmental remediation method, useful in scenarios where ex-situ treatment cannot be accomplished or, where the contamination is difficult to access. The bacteria work by replacing chlorine atoms with hydrogen atoms to produce degradation products and eventually fully degraded non-toxic compounds (Figure 1). For example, the bacterium *Dehalococcoides* is well known for its ability to completely reduce the toxic chlorinated TCE

Objective

The project focus was to develop a low-volume, cost effective platform to investigate anaerobic bioremediation at a bench scale. This was accomplished by developing an experimental platform and procedure using readily available, small-scale equipment. A secondary objective of this study was to evaluate the anaerobic bioremediation potential of KB-1®, in TCE contaminated groundwater. This was accomplished by inoculating a groundwater surrogate containing trichloroethylene (TCE) with a proprietary consortia of bacterium, KB-1® acquired from SiREM lab (Guelph, Ontario).

Methodology

Experimental Platform

Assembly

A sterile luer lock system with a three-port adapter connected via 0.2" tubing, and a rubber stopper were attached to a 500-mL Erlenmeyer flask (Figure 2). The system was assembled to provide an airtight injection port for material transfer and sampling without compromising the anaerobic environment within the flask. The tubing was pre-filled with DI water via syringe to further prevent air transfer into the system. The stopper and flask junction were sealed with parafilm to maintain anaerobic integrity. During sampling the tubing was flushed via the luer lock system from a side port syringe, while the sample was taken from the main port with a separate syringe. This system allowed for a clean sample to be taken from the vessel without contamination from the outside environment.

Figure 2. Luer lock system for flasks using 0.2" tubing, 3-port lure lock with adapter, rubber stopper with one hole and a sampling syringe.

Anaerobic Conditions

The system was kept in an anaerobic chamber with an airtight sealable lid. Prior to sealing, to create the anaerobic environment, GasPak™ sachets were placed into the chamber. The GasPak™ sachets generated CO_2 within the headspace of the Chamber, where the CO_2 – enriched environment was maintained. Following sampling, when the lid was removed, the anaerobic environment was re-established with fresh sachets, and the lid was resealed.

Figure 3. Flasks in airtight chamber under anaerobic conditions.

Experimental Validation

Matrix Conditioning

Three 500 mL Erlenmeyer flasks (triplicate) and one control were prepared under aseptic conditions. Each flask contained 250 mL of DI water spiked with 10 µL of 99.5% TCE. 30 µL of methanol and 24µL ethanol were added as electron donors. 35mL of emulsified vegetable oil (EVO), consisting of Tween-20, DI water and Soybean oil (1:4:7), was added to enhance dichlorination of TCE.

Bacterial Inoculation

KB-1® culture (0.5 mL) was introduced into treatments #1-3, followed by 14 mL DI water to ensure uniform distribution. Treatments were maintained under anaerobic conditions using the developed experimental platform.

Sampling

On collection days 0, 2, 7 and 15, flask contents were homogenized on a stir plate at ~600 rpm for 5 minutes. A 5-mL aliquot was extracted from each flask, centrifuged, and supernatant was collected.

GC/MS Sample Analysis

Prior to analysis, 5 mL of hexane (1:1 v/v) was added to the sample to extract TCE, and1.5g of NaCl was added to disrupt the EVO emulsion. After vortexing and centrifugation, the hexane layer was transferred to GC-MS vials.

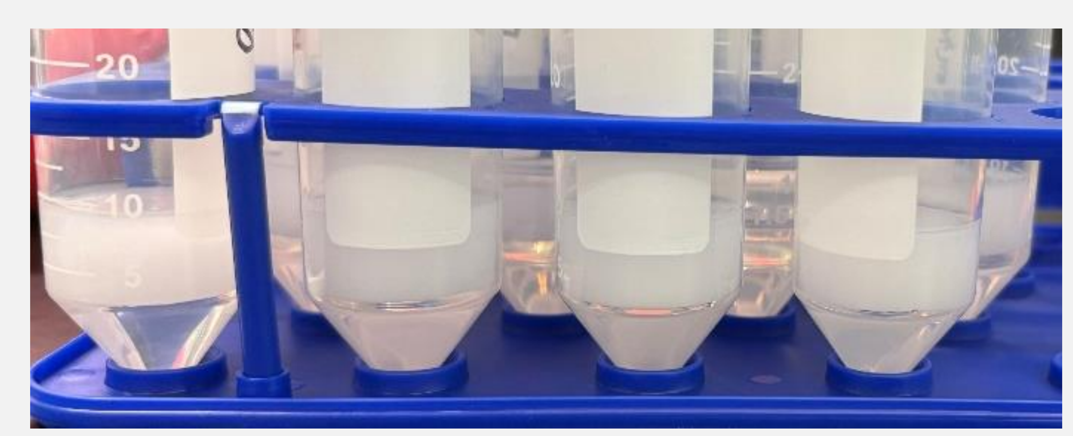

Figure 4 . Settled layers after samples with hexane are vortexed and centrifuged, prior to NaCl addition.

GC-MS Analysis

Analysis was performed on an Agilent 8890 GC-MS with a DB-624 UI column. The injection volume was 1µL at a flow rate of 1.0 L/min and a temperature run from 35°C to 160°C, with a run time of 6-minutes.

Results & Discussion

The intention of creating a cost-efficient bench-scale biodegradation process was achieved, as indicated by the remarkable ease to use and limited space requirements. The physical system maintained an anaerobic environment and provided ample opportunity for modularity and experimental modification for future investigations.

During experimental validation TCE degradation results were inconclusive. The complete degradation of TCE was expected to occur by day-3 according to SiREM, which was not observed herein. Bacterial viability throughout the investigation was potentially the main drawback. The emulsifier for the EVO solution, lack of adequate buffer to control pH shifts, lack of adequate ionic content in solution, may have contributed to a reduction in cell viability throughout the investigation. Nonetheless, the observed inconclusive results do not necessarily indicate error in the experimental platform but are more likely associated with error in the experimental validation methods.

Future Work

During prospective future experiments, the following may be implemented:

- Active monitoring of microbial viability.
- Adjustments to microcosm conditions, as presented in the Discussion, would ensure bacterial viability.
- TCE contamination with a saturated solution of TCE and equilibration to the microcosm may provide a more homogenous contaminant profile.
- Repeated injections of electron donors (EtOH and MeOH), ensure continuous degradation by the microbes throughout the treatments.

The challenges encountered during this procedure highlight the complexities of bioremediation effort, particularly with replication of environmental conditions.

Acknowledgements

Jennifer Webb, SiREM
Jordi Cain, Conestoga
Ian Jenkins, Conestoga
Anthony Bauer, Conestoga

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