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technical BRIEF
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Aerobic Biodegradation of Chlorinated Volatile Organic Compounds and 1,4-Dioxane in Groundwater

Introduction

Biodegradation is the transformation of groundwater contaminants by microbial enzymes into less toxic compounds or minerals. It can occur given the correct conditions, but several transformations may be needed to achieve desired results. This brief summarizes a journal article by Clark and Rhea (2023) about cometabolic biodegradation of comingled chlorinated volatile organic compounds (CVOCs) and 1,4-dioxane (dioxane) groundwater plumes. Remediation approaches using aerobic cometabolic processes are presented, including the application of microbes (bioaugmentation) or substrates like propane or dextrose (biostimulation) to supplement the reactions.

Microbial biodegradation effectiveness and contaminant biodegradation pathways are dependent on subsurface geochemical conditions. The degree to which the environment is oxygenated (aerobic) or lacking oxygen (anaerobic) is important in predicting microbial presence and behavior. Generally, reducing environments are also anaerobic and oxidizing environments are aerobic. Other factors include the amount of "food" (substrate) and nutrients that are available for necessary microbial metabolism chemical reactions.

Historically, bioremediation of mixed groundwater plumes containing both CVOCs and dioxane focused only on CVOC destruction through a bacterial metabolic process known as reductive dechlorination. This process decreases the concentrations of CVOCs under reducing environmental conditions but rarely eliminates them. Today, many mixed plumes have contaminant concentrations less than 100 µg/L and occur with oxidizing conditions, which is a situation more appropriate for aerobic biodegradation.

Aerobic biodegradation of both CVOCs and dioxane can occur through the processes of metabolism or cometabolism. Cometabolism is the fortuitous degradation of contaminants when microbes produce enzymes to metabolize other materials. It is capable of degrading contaminants to lower concentrations than metabolism alone.

Although many remaining plumes are aerobic, they often lack microbial food (substrate), nutrients, and necessary microbes to support aerobic biodegradation. CVOCs and dioxane degrade slowly or not at all under these conditions. However, aerobic degradation can be induced or accelerated if bacteria that produce the necessary enzymes for metabolism or cometabolism of contaminants are present or introduced, and they are provided a suitable growth substrate and needed nutrients. When these conditions are met, bioremediation via aerobic cometabolism is a viable remedial alternative for some groundwater contaminant plumes containing CVOCs and dioxane.

Plume Constituents and Reductive Dechlorination

Many groundwater plumes of mixed CVOCs and dioxane originate from areas where either tetrachloroethene (PCE) or more commonly trichloroethene (TCE) was used as a solvent, prior to being replaced by the somewhat less toxic and less environmentally persistent chemical 1,1,1-trichloroethane (TCA). Dioxane often co-occurs with these CVOCs because it was commonly added to TCA as a stabilizer and is often less rapidly degraded than TCA, which is also true for chloroethenes. Dichloroethenes (DCEs) commonly occur as daughter products of TCE, formed by reductive dechlorination of TCE via microbial metabolism under sulfate or methanogenic reducing conditions.

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DCEs may accumulate if conditions are not conducive to further dehalogenation to vinyl chloride (VC) and eventually ethene, known as the "DCE stall". However, there are a few bacterial strains within genera *Dehalococcoides* and *Propionibacterium* that, if present, can dechlorinate DCEs and VC, as well as TCE and PCE. Nonetheless, bioremediation by reductive dechlorination is sometimes inadequate to reach target cleanup concentrations, particularly for the less heavily chlorinated DCEs and VC and those mixed with dioxane.

Reductive dechlorination does not significantly degrade dioxane. However, co-occurring DCEs, VC, and dioxane can all degrade to parts-per-trillion concentrations under aerobic conditions via co-metabolic bacteria (***Table 1***). Direct aerobic metabolism of dioxane is more effective at above cometabolism is more effective at concentrations

Table 1. List of bacterial strains and mixed cultures that cometabolize both 1,4-dioxane and CVOCs.

Microbe/Culture	Contaminant	Substrates
Azoacarus sp. Strain DD4	1,4-dioxane, 1,1 DCE	Propane, Toluene, propanol
Mycobacterium vaccae JOB5	1,4-dioxane, TCE, DCE, VC	Propane, butane, pentane, isobutane, isopentane, dextrose
Mixed culture CL-OUT®	1,4-dioxane, PCE, TCE, TCA, DCE, VC	Dextrose
Mycobacterium sphagni E NV482	1,4-dioxane, TCE	Ethane
Mycobacterium chubuense strain NBB4	1,4-dioxane, cis-DCE, 1,2-DCA, VC	C2-C4 alkenes, C2-C16 alkanes

about 10 mg/L and below about 7.5 mg/L (Barajas-Rodriguez et. al., 2019).

Plume Structure and Significance to Metabolic Inhibition of Dioxane Degraders

Separation in a mixed plume between the locations of CVOCs versus dioxane may be advantageous if the CVOC concentrations are sufficient to induce metabolic inhibition of dioxane degraders. Differing relative extents of dioxane versus CVOCs in plumes may result because dioxane is comparatively more water soluble, lower in transport retardation, and is metabolized by different microbes than CVOCs. The downgradient extents of CVOCs in plumes frequently increase in the order of PCE, TCE, DCEs, and VC, but the downgradient extent of comingled dioxane varies relative to these CVOCs. Data mining of more than 2,000 sites in California found that 62% of dioxane plume lengths were shorter than comingled CVOC plumes and 21% of dioxane plumes were longer than comingled CVOC plumes (Adamson et al., 2015).

Implementation of Cometabolic Bioremediation

Cometabolic bioremediation can naturally occur but frequently requires addition of a gaseous or liquid substrate like ethane, butane, butanol, tetrahydrofuran, propane, propanol, or toluene. Nitrogen and phosphorous are also needed and are typically added as diammonium phosphate brine. Oxygen concentrations need to be at least 4 mg/L and can be added in the form of compressed air or peroxide. The substrates can be introduced using methods such as biosparging or groundwater recirculation (***Figure 1***). Laboratory-grade substrate may be necessary, as consumer grade propane can contain up to 10% propylene which has been found to inhibit growth of propanotrophs. The effect of propylene on contaminant degrading microbes is dependent on whether alkene metabolizers are present to detoxify epoxides formed during propylene metabolism.

Some additional subsurface conditions can significantly affect bioremediation. Metals affect dioxane-degrading bacteria. Manganese (II) at concentrations of 0.001-0.1 mg/L can be stimulatory, while copper (II) and cobalt (II) can be

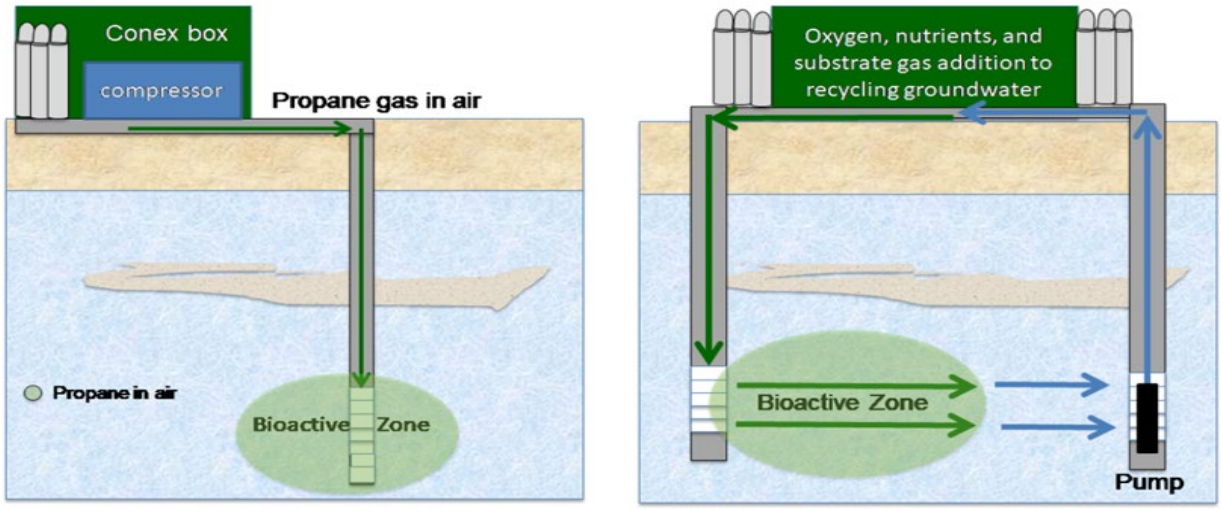

Figure 1. *Graphical representation of active biosparging (left) and groundwater recirculation (right).*

inhibitory. The optimal temperature for dioxane biodegradation is 20-30 degrees Celsius (°C), and temperatures below 4 °C are extremely inhibitory. Lastly, 1,1-DCE, TCE, and TCA have been found to inhibit dioxane degradation if present in concentrations higher than 2 mg/L.

Monitoring

Regulating authorities require a demonstration of the potential efficacy of bioremediation that is based on multiple lines of evidence. Bench-scale and pilot studies may be necessary. Testing that is typically necessary before, during, and after the treatment period is summarized in ***Table 2***.

New characterization tools have been developed since the adoption of bioremediation as a remedial method for groundwater. These include Environmental Molecular Diagnostic (EMD) tools, Compound-Specific Isotope Analysis (CSIA), and carbon-14 (C14) assays. The EMD tools are used to determine the presence, abundance, and expression of enzymes responsible for contaminant degradation. They include Quantitative Polymerase Chain Reaction (qPCR), Reverse Transcriptase qPCR (RT-qPCR), and microarrays. Metals and humic substances may affect EMD results, and microbes that grow attached to the aquifer matrix may underrepresented.

CSIA uses fractionation of stable isotopes to determine whether contaminant reduction in groundwater plumes is due to destruction or dilution. Many natural processes that involve microscale molecular movement or chemical reactions have characteristic propensities to preferentially transport lighter isotopes. CSIA uses the resulting ratios of isotopes to identify degradation processes and pathways. However, degradation rate constants obtained are often highly spatially variable throughout a plume and it can therefore be difficult to separate different degradation processes and contaminant sources.

A C-14 assay can be used to detect biotically mediated destruction in groundwater but requires both soil and groundwater samples to detect abiotic degradation. It can also be used to calculate bioremediation rate constants.

Summary

The biochemistry of microbial cometabolism is complex and depends on multiple parameters; however, suitable conditions for bioremediation via cometabolism are possible through biostimulation and bioaugmentation. Site-specific microcosm studies are needed to confirm what is needed to facilitate biodegradation at a given site. Fine tuning a bioremediation strategy on a smaller scale is less costly over the lifecycle of a site remediation.

Table 2. Bioremediation monitoring

Parameters	When to Measure	Purpose
Aquifer porosity, hydraulic conductivity, & groundwater flow gradients	Before treatment	Understand groundwater transport
Oxidation reduction potential, dissolved oxygen, carbon dioxide & pH	Before & during treatment	Assess the suitability of groundwater chemistry for bioremediation
Primary substrate(s)	Before & during treatment	Assess whether adequate substrate is available for metabolism of the target microbes
Concentrations of contaminants	Before, during & after treatment	Before treatment to support remedial design; during treatment to assess remedial performance; and after treatment to document whether contaminants have been adequately remediated

References

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