EXECUTIVE SUMMARY

The Development of Anaerobic Bioremediation Approaches for Chlorinated Solvent and 1,4-Dioxane Co-Contaminated Sites

SERDP Project ER-2712

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ACRONYMS AND ABBREVIATIONS

CSIA	Compound specific isotope analysis
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
EDTA	Ethylenediaminetetraacetic acid
K_{OC}	Organic Carbon Water Partition Coefficient
PI	Principal Investigator
SDIMO	soluble di-iron monooxygenases

Keywords

1.4-dioxane, *Actinomycetales*, soluble di-iron monooxygenases

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1.0 INTRODUCTION

1,4-dioxane, a probable human carcinogen (DeRosa et al., 1996), was commonly used as a stabilizer in 1,1,1-trichloroethane formulations and is now frequently detected at sites where the chlorinated solvents are present (Adamson et al., 2015; Adamson et al., 2014; ATSDR, 2012; Mohr et al., 2010). No federal maximum contaminant level for 1,4-dioxane in drinking water has been established (EPA, 2017), however, several states have set low advisory action levels (e.g. California, Florida, Michigan and North Carolina have levels <5 ppb). A major challenge to 1,4-dioxane remediation concerns chemical characteristics that result in migration and persistence (Adamson et al., 2015; Mohr et al., 2010). A low organic carbon partition coefficient (log K_{OC} = 1.23) and Henry's Law Constant (5 X 10^{-6} atm. m^3mol^{-1}) make traditional remediation methods such as air stripping or activated carbon largely ineffective (Mahendra and Alvarez-Cohen, 2006; Steffan et al., 2007; Zenker et al., 2003). *Ex situ* oxidation methods including ozone and hydrogen peroxide (Adams et al., 1994) or hydrogen peroxide and ultraviolet light (Stefan and Bolton, 1998) have been commercially applied, however these can be expensive at high concentrations (Steffan et al., 2007). Given the limitations associated with traditional remediation methods, interest has turned to bioremediation to address 1,4-dioxane contamination. Numerous bacteria have been associated with aerobic metabolic and co-metabolic degradation of this chemical. The initiation of aerobic 1,4-dioxane biodegradation has been associated with various groups of soluble di-iron monooxygenases (SDIMOs). In contrast, little is known about the potential for 1,4-dioxane biodegradation under anaerobic conditions. The lack of information on the susceptibility of 1,4-dioxane to biodegradation under such conditions is a significant knowledge gap. Additional research gaps concern the relative abundance of SDIMOs and genera associated with aerobic 1,4-dioxane biodegradation across mixed microbial communities.

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2.0 OBJECTIVES

The overall objective of the current study was to determine the susceptibility of 1,4-dioxane to biodegradation over a range of redox conditions using inocula from contaminated and uncontaminated sites. The first project focused on the susceptibility of 1,4-dioxane to degradation under anaerobic conditions and the second study focused on aerobic conditions.

The specific objectives of the first study were 1) to determine the susceptibility of 1,4-dioxane to degradation under anaerobic conditions (nitrate, iron, sulfate amended, or no amendments), and 2) identify which phylotypes are enriched during 1,4-dioxane biodegradation.

The specific objectives of the second study were 1) to identify which aerobic 1,4-dioxane degrading functional genes (SDIMOs) are present across different microbial communities, and 2) to determine which genera may be using 1,4-dioxane and/or metabolites under aerobic conditions to support growth.

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3.0 TECHNOLOGY APPROACH

In the first study, the potential for 1,4-dioxane biodegradation was examined using multiple inocula sources and electron acceptor amendments (nitrate, iron/EDTA/humic acid, sulfate, no amendment). The inocula included uncontaminated agricultural soils and river sediments, as well as sediments from two 1,4-dioxane contaminated sites. Five separate experiments included the examination of triplicate live microcosms and abiotic controls for approximately one year. Compound specific isotope analysis (CSIA) was used to investigate biodegradation in a subset of the microcosms. Also, DNA was extracted from microcosms exhibiting 1,4-dioxane biodegradation for microbial community analysis using 16S rRNA gene amplicon high throughput sequencing. In the second study, laboratory microcosms and abiotic control microcosms were inoculated with four uncontaminated soils and sediments from two contaminated sites. The sample microcosms were amended with 1,4-dioxane three times over approximately 250 days. Live control microcosms were treated in the same manner, except 1,4-dioxane was not added. The microbial communities present and the relative abundance of 1,4-dioxane degrading functional genes (SDIMOs) were examined using shotgun sequencing.

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4.0 RESULTS AND DISCUSSION

In the first study, given the long incubation periods, it is likely that electron acceptor depletion occurred and methanogenic conditions eventually dominated (confirmed in one case). The iron/EDTA/humic acid or sulfate amendments did not result in 1,4-dioxane biodegradation in the majority of cases. In contrast, 1,4-dioxane biodegradation was noted in a subset of the nitrate amended treatments and no electron acceptor treatments. Notably, both contaminated site sediments illustrated significant removal in the samples compared to the abiotic controls in the no electron acceptor treatment. However, it is important to note that slow degradation rates were observed, with statistically significant concentration reductions occurring over approximately one year. In two of the three cases examined, CSIA provided additional evidence for 1,4-dioxane biodegradation. In one case, the reduction in 1,4-dioxane in the samples compared to the controls was likely too low for the method to detect a significant $^{13}C/^{12}C$ enrichment. Further research is required to determine the value of measuring $^{2}H/^{1}H$ for generating evidence for the biodegradation of this chemical. The analysis of the microbial communities indicated the phylotypes unclassified *Comamonadaceae* and *3 genus incertae sedis* were obtaining a growth benefit during 1,4-dioxane degradation in microcosms inoculated with contaminated and uncontaminated site sediment, respectively. The soil and sediment microcosms contained genera previously associated with 1,4-dioxane degradation. The soil microcosms were dominated primarily by *Rhodanobacter* with lower relative abundance values for *Pseudomonas, Mycobacterium* and *Acinetobacter*. The sediment communities were dominated by *Pseudomonas* and *Rhodanobacter*. Additional research is needed to confirm the role of these phylotypes in 1,4-dioxane biodegradation.

In the second study, 1,4-dioxane decreased in live microcosms with all six inocula, but not in the abiotic controls, suggesting biodegradation occurred. A comparison of live microcosms and live controls (no 1,4-dioxane) indicated seventeen genera were enriched following exposure to 1,4-dioxane, suggesting a growth benefit for 1,4-dioxane degradation. The three most enriched were *Mycobacterium*, *Nocardioides*, *Kribbella* (classifying as *Actinomycetales*). There was also a higher level of enrichment for *Arthrobacter, Nocardia* and *Gordonia* (also *Actinomycetales*) in one soil and *Hyphomicrobium* (*Rhizobiales*) in two soils. Although *Arthrobacter, Mycobacterium* and *Nocardia* have previously been linked to 1,4-dioxane degradation, *Nocardioides, Gordonia* and *Kribbella* are potentially novel degraders. Eleven of the twelve functional genes (all except *Mycobacterium dioxanotrophicus* PH-06 *prmA*) associated with 1,4-dioxane were found in the soil and sediment metagenomes. Reads aligning to *Rhodococcus jostii* RHA1 *prmA* and *Rhodococcus* sp. RR1 *prmA* illustrated the highest relative abundance values and were present in all eighteen metagenomes. The third most abundant was *Burkholderia cepacia* G4 *tomA3* and this was also present in all eighteen metagenomes. The contaminated site sample generated the same trend for the three most abundant genes and the values were greater compared to those in the soil metagenomes.

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5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

Overall, the current study indicates 1,4-dioxane biodegradation under anaerobic (likely methanogenic) conditions is feasible. Therefore, natural attenuation of 1,4-dioxane may occur over wider range of conditions, notably methanogenic areas where highly reducing conditions occur either naturally or as a result of enhanced reductive dechlorination. The work is novel as it is the first to document the frequency of 1,4-dioxane biodegradation over a range of redox conditions and inocula types and provides critical evidence for the feasibility of 1,4-dioxane remediation through natural attenuation. Under aerobic conditions, 1,4-dioxane degradation was observed with all inocula types, indicating 1,4-dioxane degraders are widespread. Further, a large number of genera were enriched following 1,4-dioxane degradation. The second study is innovative as it combines taxonomic and functional data to generate a more complete picture of the multiple microorganisms and genes linked to 1,4-dioxane degradation in mixed communities.

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