The Development of Microcosms to Achieve Anaerobic and Aerobic 1,4-Dioxane Biodegradation

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ABSTRACT: 1,4-Dioxane, a potential carcinogen, is frequently found at sites contaminated with chlorinated solvents. A major challenge in addressing 1,4-dioxane contamination concerns chemical characteristics that result in migration and persistence. This study aims to develop enrichment cultures capable of 1,4-dioxane biodegradation for the final goal of using these cultures in bioaugmentation. Microcosms using agricultural soils, river sediments (Michigan) and sediments from a 1,4-dioxane contaminated site (California) were established under a range of redox conditions (aerobic, nitrate amended, iron amended, sulfate amended and methanogenic). The experimental setup included triplicates of live microcosms and abiotic controls amended with 5 to 10 mg/L 1,4-dioxane. Concentrations of 1,4-dioxane over time were determined using a GC/MS combined with solid phase microextraction. A significant difference ($p<0.05$) in 1,4-dioxane concentrations between abiotic controls and the live microcosms was observed for two of the agricultural soils and the river sediment soil under methanogenic conditions. The aerobic samples from all three agricultural sites also illustrated significant decreases in 1,4-dioxane concentrations in the samples compared to the controls.

INTRODUCTION

1,4-Dioxane, also known as diethylene dioxide, is a polar cyclic ether and was commonly used as a stabilizer in 1,1,1-trichloroethane (TCA) formulations (Adamson et al., 2015; Derosa et al.,1996). It is now frequently detected at sites contaminated with TCA and other chlorinated solvents. 1,4-Dioxane has been classified as a probable human carcinogen (Group 2B) by the USEPA based on evidence from animal studies (Zenker et al., 2003). It is now well recognized that there is a critical need to develop management strategies for this emerging contaminant, due to its widespread occurrence.

1,4-Dioxane shows high persistence and migration potential in the environment due to its chemical characteristics. It has low vapor pressure, low octanol-water partition coefficient and high solubility (U.S. EPA, 2017). Thus, contamination in surface water and groundwater are challenging to treat. As a result, 1,4-dioxane has been detected in over 50 superfund sites in the U.S (Mohr et al., 2010). The traditional remediation methods, such as air stripping or activated carbon, are largely ineffective. Treatments using hydrogen peroxide, ozone, UV light or sonication are possible (Adams et al., 1994; Coleman et al., 2007; Son et al., 2009; Stefan and Bolton., 1998). However, the ex-situ methods can be costly at high concentrations. Natural attenuation can be an option, but it may not be feasible as a treatment methodology at most sites due to the slow in situ removal of this chemical (Adamson et al., 2015).

Given the limitations associated with traditional remediation methods, interest has turned to the use of microorganisms to degrade 1,4-dioxane. Towards this goal, the current project is aimed at developing enrichment cultures capable of aerobic and anaerobic 1,4-dioxane biodegradation for their ultimate use in bioaugmentation.

MATERIALS AND METHODS

Chemicals and Materials. 1,4-Dioxane was purchased from Thermo Fischer Scientific Chemicals Inc. (MA, USA). Ethylenediaminetetraacetic acid (EDTA) iron (III) sodium salt, sodium sulfate, sodium nitrate and humic acid were purchased from Sigma-Aldrich (MO, USA). All stock solutions and dilutions were prepared using DI water. The three agricultural samples were collected from farmland at Michigan State University (MSU). Contaminated sediment cores were obtained from two sampling locations of a site in California. The site was contaminated with trichloroethylene (TCE), 1,1-dichloroethene (1,1-DCE) and 1,4-dioxane. The river sediment samples were collected from Red Cedar River in Okemos, Michigan. The samples were stored in the dark at 6 ˚C until use.

Experimental Setup. The anaerobic microcosms were established in 30 mL amber serum bottles with 5 g of each agricultural soil or sediment sample. Each of the three agricultural soils (1, 2 and 3) or sediment sample were used for each of the four redox conditions (methanogenic, nitrate reducing, sulfate reducing and iron reducing). Triplicates of samples and the abiotic controls were established, resulting in 72 microcosms. The abiotic controls were autoclaved thrice at an interval of 24 hrs before introducing them to anaerobic conditions. A 9 ml solution was added for each treatment, as follows: a) sodium nitrate (10 mM), b) sodium sulfate (10 mM), c) ethylenediaminetetraacetic acid (EDTA) iron(III) sodium salt (10 mM) with humic acid (0.25 g/L) and d) water for methanogenic condition (Table 1). All solutions were purged under a stream of nitrogen gas (oxygen free) for 60 minutes before adding each to the microcosms. Two weeks after the initial set up, 1 mL of 1,4-dioxane was added to each microcosm for a final concentration of 5 mg/L.

The samples, closed with septa, were incubated in the anaerobic chamber at 20 ˚C. The anaerobic chamber was maintained with gaseous mix of approximately 5% H_2, 90% N_2 and 5% CO_2. The vials were sealed using BiMetal vial crimp with PTFE/silicone septa to maintain the microcosms under anaerobic conditions.

The aerobic microcosms were established using two soil cores (Core 7A and 10A) from the contaminated site (15 g) and the three agricultural soils (10 g) in 30 ml amber serum vials (Table 1). Basal salts medium (BSM) containing K_2HPO_4 (32.4 g/L), KH_2PO_4 (10 g/L), NH_4Cl (20 g/L) and trace metal solution with disodium salt (1.23 g/L), $MgSO_4.7H_2O$ (2 g/L), $FeSO_4.7H_2O$ (0.12 g/L), $MnSO_4.H_2O$ (0.03 g/L), $ZnSO_4.7H_2O$ (0.03 g/L) and $CoCl_2.6H_2O$ (0.01 g/L) were added to the microcosms (Parales, 1994). 1,4-Dioxane was added to the microcosms after adding 25 ml of the BSM media. The microcosms were established to have 10 mg/L 1,4-dioxane. For the aerobic microcosms, the experimental design included duplicates of samples and abiotic controls for each soil/ sediment.

All microcosms were placed on a shaker at 200 rpm and maintained at 20 ˚C. The aerobic microcosms were opened periodically to replenish oxygen. The nitrate amended microcosms were tested for methane after 200 days of incubation using a GC (Hewlett Packard 5890).

TABLE 1. Sample types, redox conditions and amendments used for this study

Redox Condition	Sample Type	Amendments	
Nitrate Reducing	Agricultural Soil 1	$NaNO_3$ (10 mM) + DI water + 1,4-dioxane (5 mg/L)	5 g soil/ sediment + 10 ml solution
	Agricultural Soil 2		
	Agricultural Soil 3		
	River Sediment		
Sulfate Reducing	Agricultural Soil 1	$NaSO_4$ (10 mM) + DI water + 1,4-dioxane (5 mg/L)	5 g soil/ sediment + 10 ml solution
	Agricultural Soil 2		
	Agricultural Soil 3		
	River Sediment		
Iron Reducing	Agricultural Soil 1	EDTA Iron(III) sodium salt (10 mM) + humic acid (0.25 g/L) + DI water+ 1,4-dioxane (5 mg/L)	5 g soil/ sediment + 10 ml solution
	Agricultural Soil 2		
	Agricultural Soil 3		
	River Sediment		
Methanogenic	Agricultural Soil 1	DI water + 1,4-dioxane (5 mg/L)	5 g soil/ sediment + 10 ml solution
	Agricultural Soil 2		
	Agricultural Soil 3		
	River Sediment		
Aerobic	Agricultural Soil 1	BSM media + 1,4-dioxane (10 mg/L)	10 g soil + 25 ml solution
	Agricultural Soil 2		
	Agricultural Soil 3		
	Contaminated core 10A	BSM media + 1,4-dioxane (10 mg/L)	15 g sediment + 25 ml solution
	Contaminated core 7A		

GC/MS Analysis. GC/MS with Agilent 5975 GC/single quadrupole MS (Agilent Technologies, CA, USA) equipped with a CTC Combi Pal autosampler was used for analysis of 1,4-dioxane concentrations. A sterile 1 mL syringe with 22 Ga 1.5 in. (3.81 cm) needle was used to collect 0.7 ml sample from each microcosm in 40 ml amber glass vials for GC. A method was developed to analyze 1,4-dioxane using solid phase micro extraction (SPME). The SPME fiber was inserted in the headspace of the vial and exposed to the analyte for 1 minute before being injected into the GC for thermal desorption. The fiber coating can adsorb the analytes in the vapor phase. Splitless injection was executed and the vials were maintained at 40 ˚C. In this experiment, SPME fiber assembly with 50/30µm Divinylbenzene/ Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) and 24 Ga needle was utilized. The initial oven temperature was 35 ˚C and was programmed to increase at a rate of 20 ˚C/min to 120 ˚C. Once it reached 120 ˚C, it increased at a rate of 40˚C/min to 250˚C, which was maintained for 3 min. VF5MS column was used with Helium as the carrier gas in constant flow mode at a flow rate of 1 ml/min. The conditioning of the SPME fiber was at 270 ˚C for 60 min at the beginning of each sequence. The data analysis was achieved using the Waters MassLynx software.

RESULTS AND DISCUSSION

1,4-Dioxane concentrations were monitored periodically for over 300 days for the microcosms established under anaerobic conditions. The aerobic microcosms, in which agricultural soils were used as inocula were analyzed for over 100 days. The aerobic cultures with contaminated sediment inocula were analyzed for 78 days. cultures were

maintained on a shaker at 200 rpm to facilitate better mixing and contact with the soil microbes.

The results indicate a significant difference ($p<0.05$) in 1,4-dioxane concentrations between the controls and the live microcosms for two soils (1 and 2) under methanogenic conditions, suggesting biological degradation of 1,4-dioxane is occurring in these microcosms over time (Figure 1). The agricultural soil microcosms that were initially amended with nitrate produced methane after more than 250 days (indicating a change to methanogenic conditions). Under these conditions, by the end of the incubation period, all three soils illustrated a significant difference in 1,4-dioxane concentrations between the live microcosms and abiotic controls (Figure 2). In the iron amended samples, decreases in 1,4-dioxane concentrations were observed over time and yet there was no significant difference from the abiotic controls (Figure 3). One agricultural soil (soil 1) initially amended with sulfate also showed a significant decrease in 1,4-dioxane concentrations in the samples compared to the controls (Figure 4). In addition, the aerobic samples indicated a significant decrease in 1,4-dioxane concentrations in all three agricultural soils (Figure 5). The results from the contaminated sediment from California are uncertain because a decrease was noted between the samples and controls at day 1 (Figure 6). Decreases in 1,4-dioxane was also observed in the river sediment sample after approximately 120 days of incubation under methanogenic conditions (data not shown).

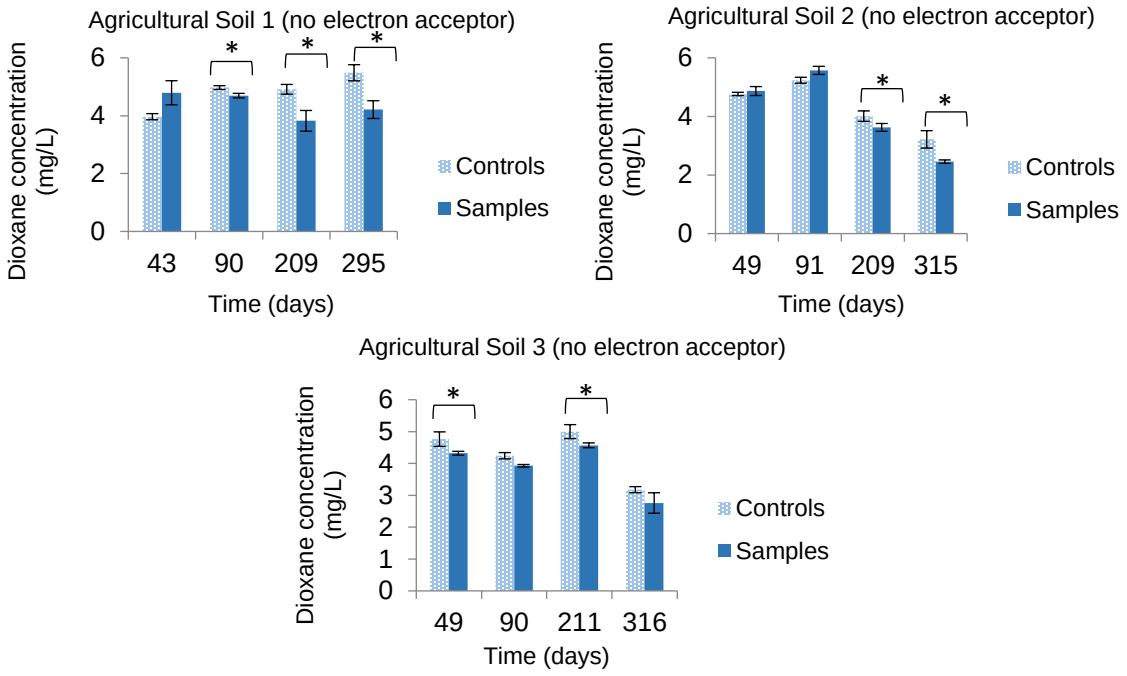

FIGURE 1. Average 1,4-dioxane concentrations in triplicate live and control microcosms with no electron acceptor added, using agricultural soil as inocula. The bars represent standard deviations from triplicate microcosms. Significant differences ($p<0.05$) between the samples and controls are shown with an asterisk and bar.

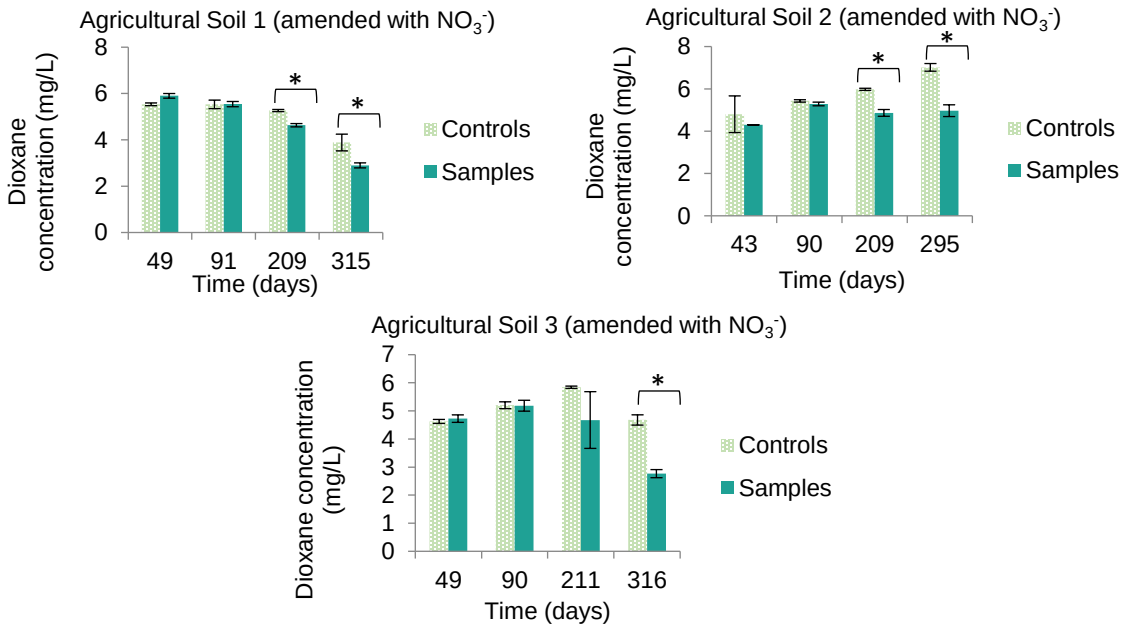

FIGURE 2. Average 1,4-dioxane concentrations in triplicate nitrate amended live and control microcosms using agricultural soil as inocula. The bars represent standard deviations from triplicate microcosms. Significant differences (*p*<0.05) between the samples and controls are shown with an asterisk and bar.

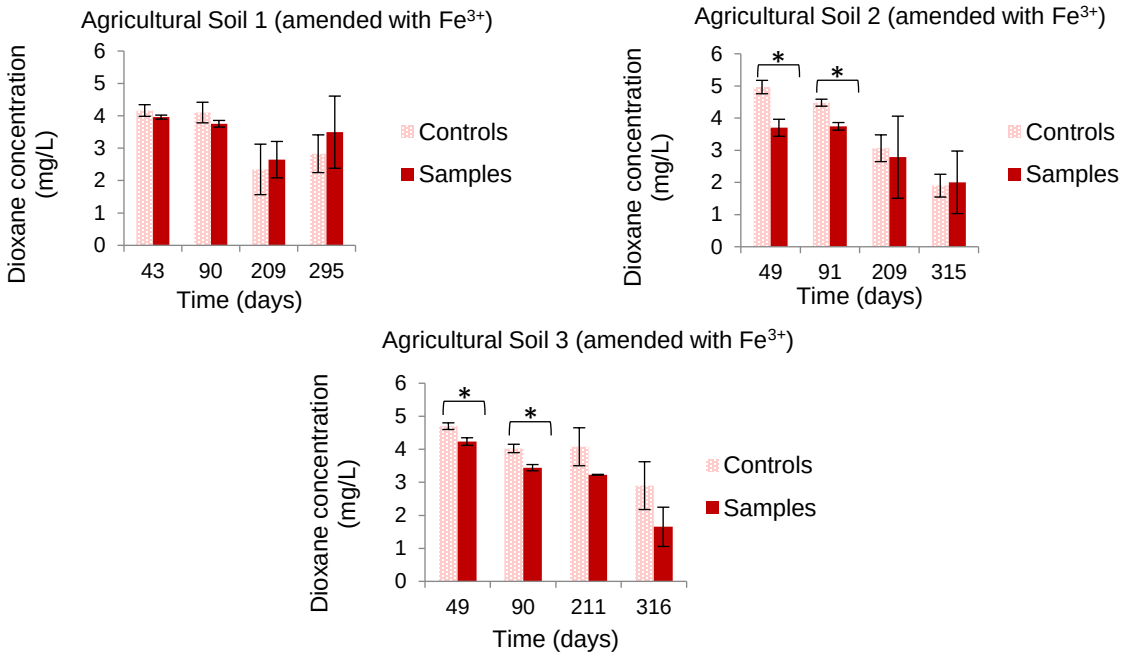

FIGURE 3. Average 1,4-dioxane concentrations in triplicate iron amended live and control microcosms using agricultural soil as inocula. The bars represent standard deviations from triplicate microcosms. Significant differences (*p*<0.05) between the samples and controls are shown with an asterisk and bar

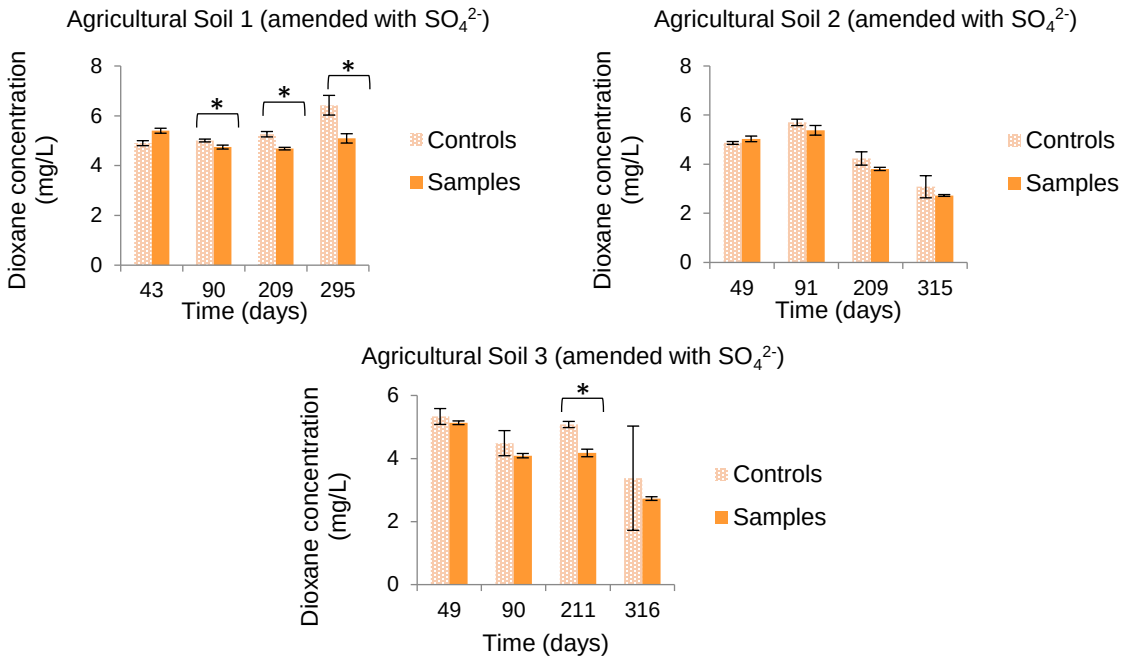

FIGURE 4. Average 1,4-dioxane concentrations in triplicate sulfate amended live and control microcosms using agricultural soil as inocula. The bars represent standard deviations from triplicate microcosms. Significant differences (p<0.05) between the samples and controls are shown with an asterisk and bar.

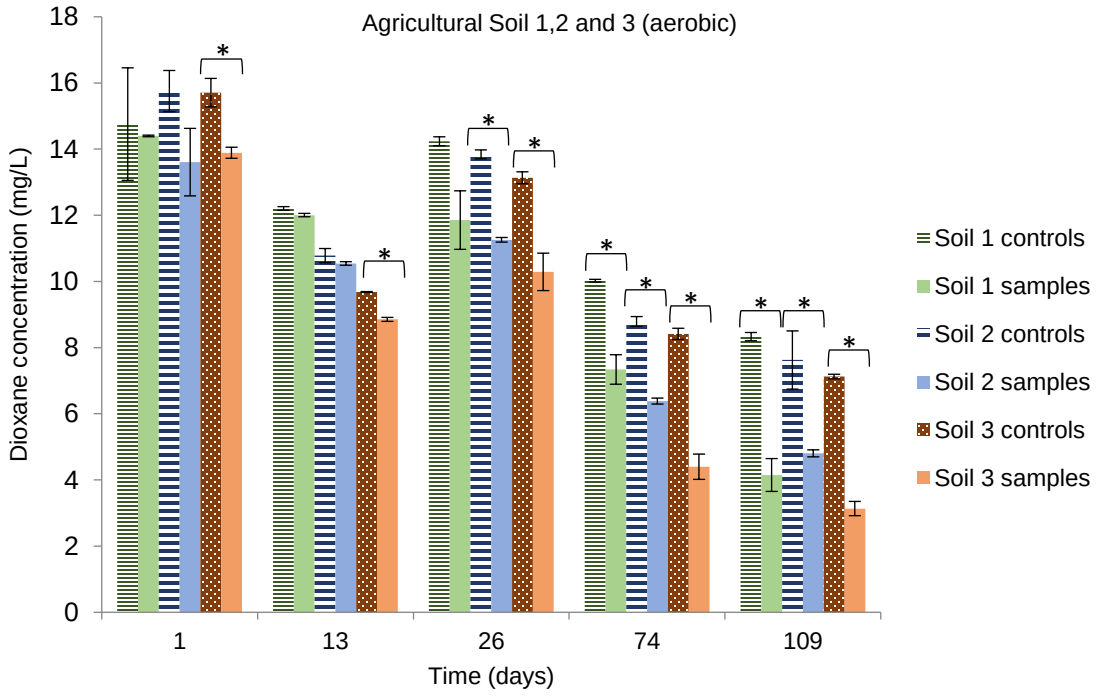

FIGURE 5. Average 1,4-dioxane concentrations in aerobic duplicate live and control microcosms using agricultural soil as inocula. The bars represent standard deviations from duplicate microcosms. Significant differences (p<0.05) between the samples and controls are shown with an asterisk and bar.

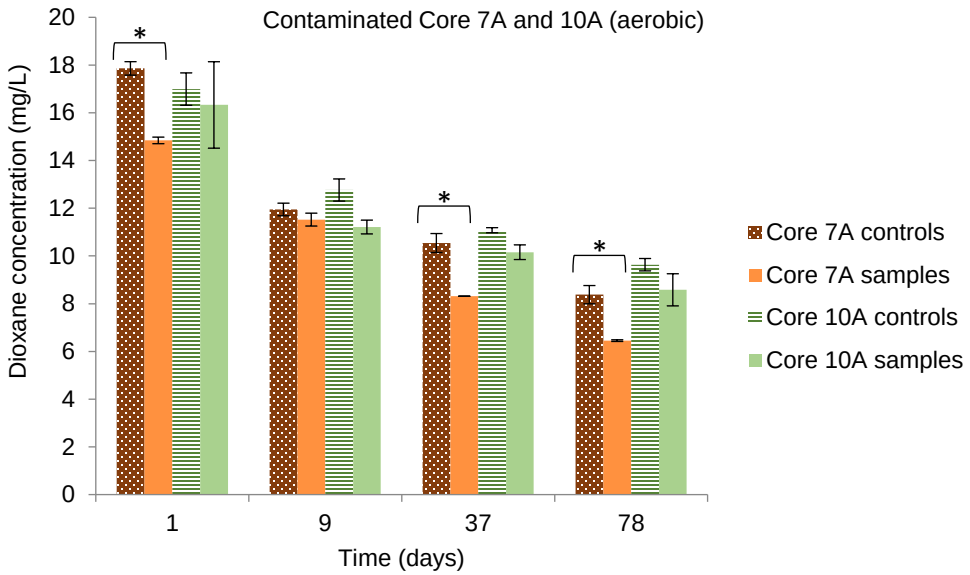

FIGURE 6. Average 1,4-dioxane concentrations in aerobic duplicate live and control microcosms using contaminated sediment as inocula. The bars represent standard deviations from duplicate microcosms. Significant differences (p<0.05) between the samples and controls are shown with an asterisk and bar.

The results suggest 1,4-dioxane biodegradation occurred in the aerobic samples. Further experiments will be conducted to determine if the contaminated sediments are also capable of 1,4-dioxane biodegradation.

To date, the majority of research has focused on the aerobic degradation of 1,4-dioxane. To our knowledge, no anaerobic 1,4-dioxane degrading isolates have been identified and only two studies have examined 1,4-dioxane biodegradation under anaerobic conditions.

One project (SERDP ER-1422) investigated 1,4-dioxane degradation over a range of redox conditions (aerobic, nitrate reducing, iron reducing, sulfate reducing and methanogenic) (Steffan., 2007). The work involved microcosm experiments with soil and groundwater from a site heavily contaminated with the chlorinated solvents and 1,4-dioxane. In these tests, nitrate, nitrite, sulfate and ferric iron were added as electron acceptors. In another set of experiments, samples from across a vegetable oil biobarrier were investigated, without the addition of electron acceptors, as it was expected that the biobarrier had resulted in a range of redox conditions. Notably, 1,4-dioxane was not degraded in any of the anaerobic microcosms during >400 days.

Another study produced more promising results, documenting 1,4-dioxane biodegradation under iron reducing conditions using an enrichment originating from wastewater treatment plant sludge (Shen et al 2008).

The current study add to this limited knowledge on the susceptibility of 1,4-dixoane to biodegradation under anaerobic conditions. A key finding of the current work is that 1,4-dioxane biodegradation can occur under methanogenic conditions. These results are important because 1,4-dioxane is a co-contaminant of the chlorinated ethenes, which are frequently remediated under methanogenic conditions.

CONCLUSION

Significant decreases in 1,4-dioxane concentrations ($p<0.05$) were observed in both aerobic and anaerobic microcosms. Removal was noted under aerobic conditions, with shorter lag times. The aerobic cultures demonstrate 45 to 56% removal in the agricultural soils in approximately 3 months. The most consistent biological removal was noted in the microcosms with no added electron acceptor (methanogenic), however, long lag times were observed. These microcosms illustrated 20 to 25% decrease in approximately 300 days. Also, a decrease in the 1,4-dioxane concentration was noticed in the iron amended cultures from agricultural soils (5 g) in both the samples and the abiotic controls. Some samples showed up to 70% decrease in 1,4-dioxane concentrations compared to earlier time points, suggesting a non-biological removal mechanism could be important.

Further anaerobic microcosms have been established under methanogenic conditions with media and sodium lactate to stimulate microbial growth and confirm the biological removal reported here. Also, additional microcosms have been established for DNA extraction to identify the dominant microorganisms in the 1,4-dioxane degrading enrichments. Further plans include combining the developed enrichment cultures with the bioaugmentation culture SDC-9 to determine if the chlorinated solvents can be reduced during 1,4-dioxane biodegradation. Future work will include an internal control to improve 1,4-dioxane concentration measurements.

Based on these data sets, additional microcosms have been established (no electron acceptor amendment) to confirm these results and provide samples for DNA extraction, high throughput sequencing and microbial community analysis

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REFERENCES

Adams, C. D., P.A. Scanlan and N.D. Secrist. 1994. "Oxidation and Biodegradability Enhancement of 1,4-Dioxane Using Hydrogen Peroxide and Ozone." *Environmental Science and Technology*, *28*(11): 1812–1818.

Adamson, D. T., R.H. Anderson, S. Mahendra and C.J. Newell. 2015. "Evidence of 1,4-dioxane attenuation at groundwater sites contaminated with chlorinated solvents and 1,4-dioxane." *Environmental Science and Technology*, *49*(11): 6510–6518.

Coleman, H. M., V. Vimonses, G. Leslie and R. Amal. 2007. "Degradation of 1,4-dioxane in water using TiO2 based photocatalytic and H2O2/UV processes." *Journal of Hazardous Materials*, *146*(3): 496–501.

Derosa, C. T., S. Wilbur, J. Holler, P. Richter and Y. Stevens. 1996. "Health evaluation of 1, 4-dioxane." *Toxicology and Industrial Health*, *12*(1): 1–43.

Mohr, T. K. G., J.A. Stickney and W.H. DiGuiseppi (Eds.). 2010. Environmental Investigation and Remediation: 1,4-Dioxane and other Solvent Stabilizers. CRC Press.

Parales, R. E., J.E. Adamus, N. White and H.D. May. 1994. "Degradation of 1 , 4-Dioxane by an Actinomycete in Pure Culture."*Applied and Environmental Microbiology*, *60*(12): 4527–4530.

Son, H. S., J.K. Im and K.D. Zoh. 2009. "A Fenton-like degradation mechanism for 1,4-dioxane using zero-valent iron (Fe0) and UV light." *Water Research*, *43*(5): 1457–1463.

Shen W, H. Chen, S. Pan. 2008. "Anaerobic biodegradation of 1,4-dioxane by sludge enriched with iron-reducing microorganisms." *Bioresource Technology*, 99(7): 2483-2487.

Stefan, M. I., and J.R. Bolton. 1998. "Mechanism of the degradation of 1,4-dioxane in dilute aqueous solution using the UV/hydrogen peroxide process." *Environmental Science and Technology*, *32*(11): 1588–1595.

Steffan, R. J. 2007. ER-1422: *Biodegradation of 1,4-dioxane*. SERDP Final Report.

U.S. EPA. (2017). *Technical Fact Sheet – 1,4-Dioxane*.

Zenker, M. J., R.C. Borden and M. Barlaz. 2003. "Occurrence and Treatment of 1,4-Dioxane in Aqueous Environments." *Environmental Engineering Science*, *20*(5): 423–432.