



Using Bacteria to Take Carcinogens Out of Water



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Abstract

1,4-Dioxane was commonly used as a stabilizer of chlorinated solvents and as a solvent in several commercial and industrial processes, leading to widespread groundwater contaminations. 1,4-Dioxane is a toxic chemical compound with potential carcinogenic effects. The unique physical and chemical properties of 1,4-dioxane have created challenges in efficiently removing it from water. Bioremediation is a promising method for treating 1,4-dioxane contaminated water as it is potentially cost-effective and eco-friendly. This study explores the use of bacterial cultures to degrade 1,4-dioxane and assesses the effectiveness of different cultures. Two 1,4-dioxane-degrading cultures, CL1 and WCD1, were enriched by periodically spiking 1,4-dioxane at 100 mg/L. The results demonstrate that the two bacterial cultures were able to effectively degrade 1,4-dioxane under aerobic conditions. Six pure cultures CL1A, CL1B, CL1C, WCD1A, WCD1B, and WCD1C were isolated by using agar plating. The isolated cultures will be monitored for their ability to degrade 1,4-dioxane. The cultures will be identified and characterized by using partial 16S rRNA gene sequencing. The findings from this study are expected to contribute in developing efficient means of treatment for this persistent environmental pollutant.

Introduction

1,4-dioxane is a synthetic organic compound. It has been used in various industrial and commercial applications. However, due to its persistence in the environment and potential health effects, 1,4-dioxane has become a significant concern for water contamination.

1,4-dioxane has been detected in groundwater and surface water sources across the United States, posing a risk to human health and the environment. Its unique physical and chemical properties make it challenging to remove from water using conventional treatment methods.

Bioremediation, the use of microorganisms to degrade contaminants, has emerged as a promising method for treating 1,4-dioxane. Bacterial cultures have been found to efficiently break down 1,4-dioxane, converting it into harmless byproducts.

This study explores the use of bacterial cultures to degrade 1,4-dioxane and presents the results of laboratory experiments conducted to investigate the effectiveness of bioremediation for removing 1,4-dioxane from contaminated water. The findings of this research can provide valuable insights into the potential of bioremediation as a sustainable and cost-effective solution for 1,4-dioxane contamination in water sources.

Materials

- Micropipettes
- Gas Chromatograph & Mass Spectrometer
- Autoclave
- 1,4-dioxane
- Biosafety Cabinet
- Various glassware and plastic containers
- Personal protective equipment
- Various chemicals and solutions

Methodology

Culture enrichment

Two 1,4-dioxane-degrading cultures, CL1 (column reactor culture) and WCD1 (wastewater culture), were enriched by periodically spiking 1,4-dioxane at 100mg/L.

Pure culture isolation

1. The enriched culture was serially diluted to achieve a couple ranges of dilutions. Each dilution was used on a separate petri dish using the spread plate technique.
2. A 100uL bacterial sample was placed into a 1,4-dioxane rich agar growth medium. The bacteria feeds off of the 1,4-dioxane within the agar. Extra 1,4-dioxane was added on the opposite side of the petri dish to prevent drying out during the incubation period(s). The petri dish was sealed with Parafilm.
3. The plates were placed into an incubator at a 25°C in order to promote microorganism growth.
4. After a 2 week incubation period, the petri plates were removed from the incubator and analyzed for areas of clear single colony growth. The single colonies were identified and transferred to plasticware with synthetic groundwater media and 100mg/L of 1,4 dioxane. The containers were placed on a shaker.
5. The cultures will be monitored for ability to biodegrade 1,4-dioxane. The concentration of 1,4-dioxane will be monitored overtime using a GC-MS machine.

Pure culture identification.

16S rRNA sequencing will be used to analyze the DNA that make up the microorganism.

Results

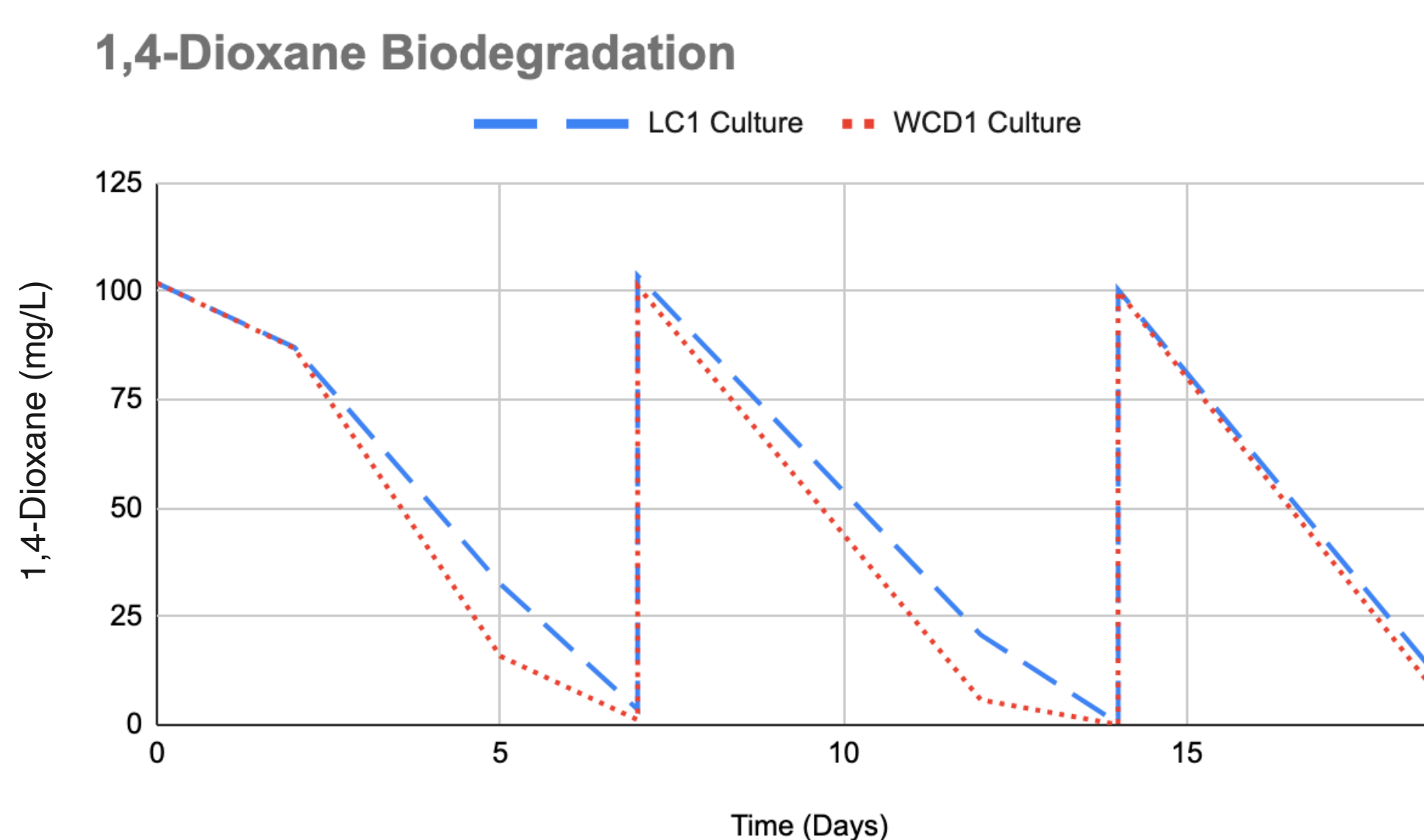


Figure 1: 1,4-Dioxane biodegradation by CL1 and WCD1

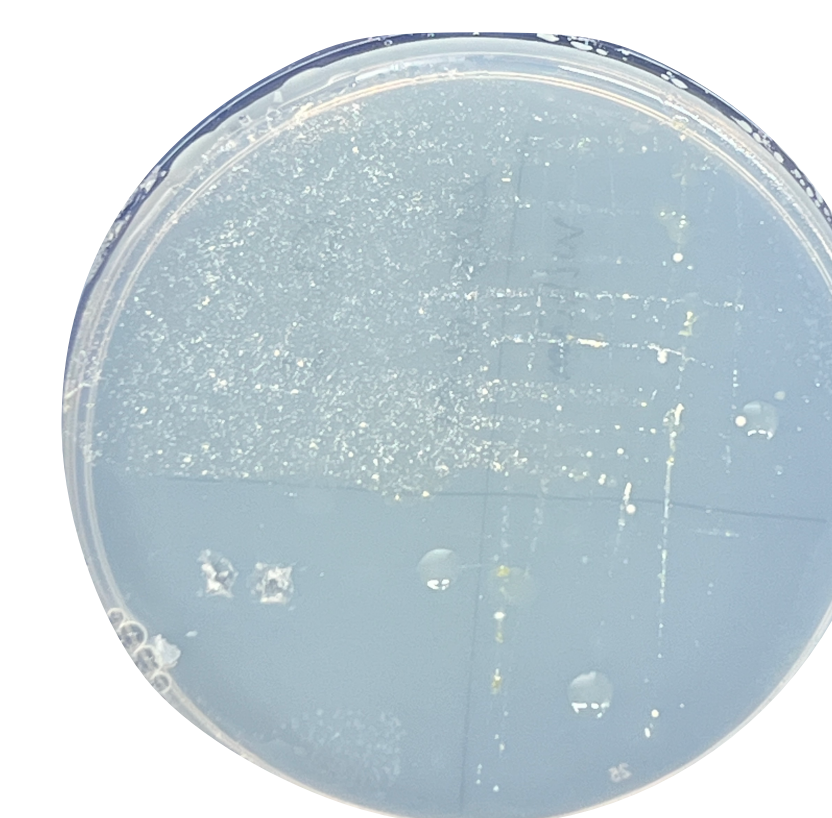


Figure 2: CL1 (undiluted) agar plate

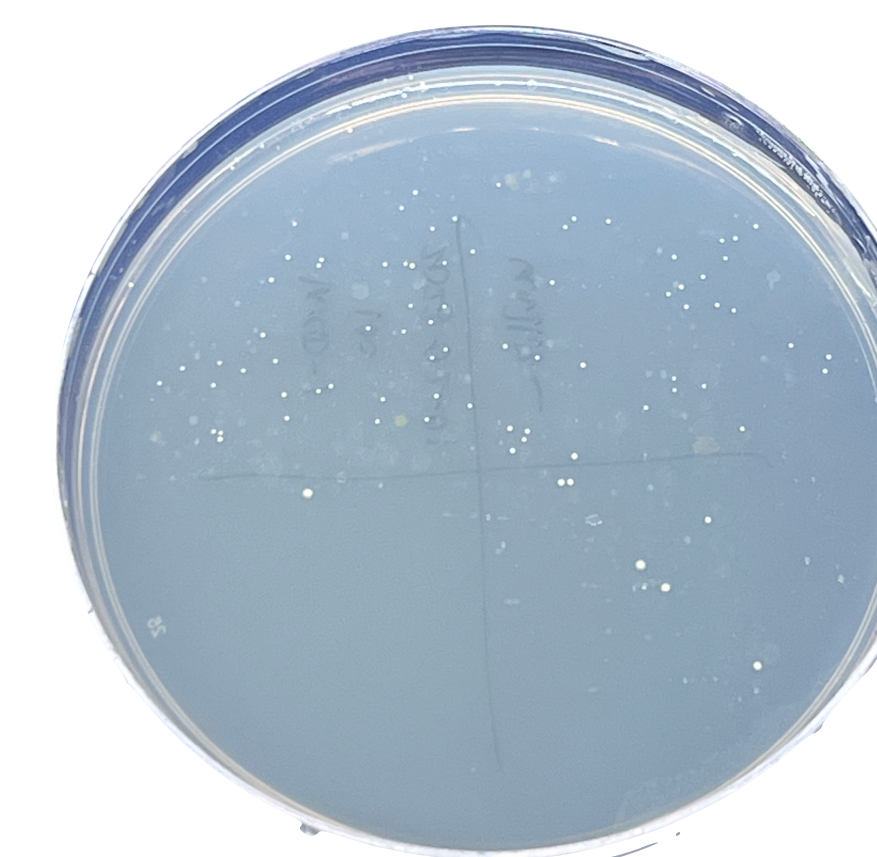
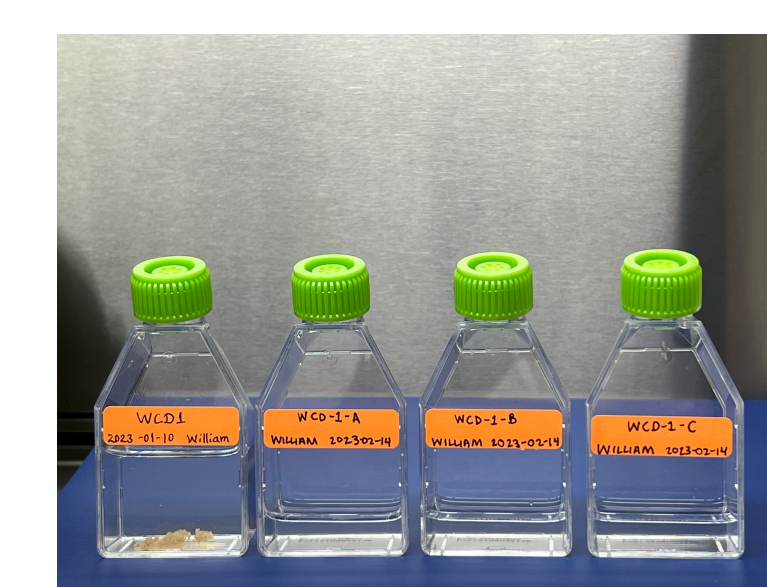
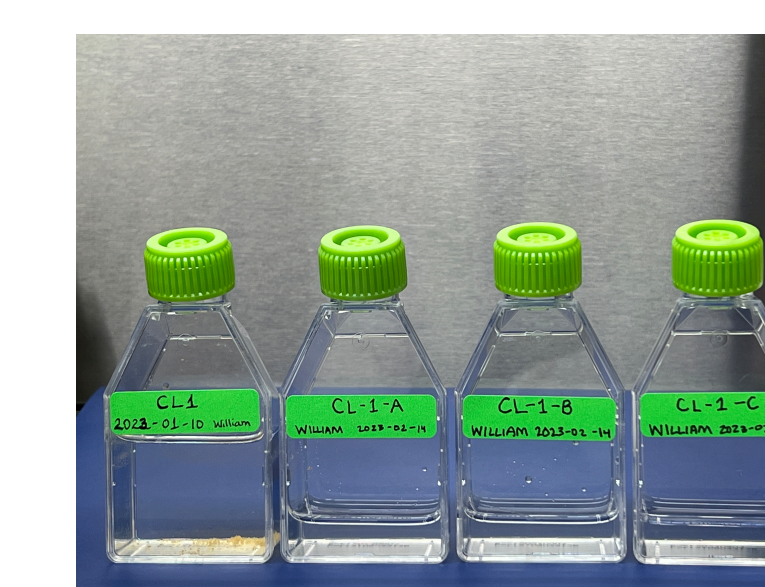


Figure 3: WCD1 (diluted 100x) agar plate



WCD1 and WCD1A, WCD1B, WCD1C pure culture isolations (Fig. 4a)



CL1 and CL1A, CL1B, CL1C pure culture isolations (Fig. 4b)

Conclusions

- ✓ Two bacterial cultures were able to effectively degrade 1,4-dioxane under aerobic conditions.
- ✓ Six pure cultures were isolated by using agar plating.
- ✓ The isolated cultures will be monitored for their ability to degrade 1,4-dioxane.
- ✓ The cultures will be identified and characterized by using partial 16S rRNA gene sequencing.

References

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