Microbial Degradation for Bioremediation

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The Ala Wai Canal was built as a conduit for runoff from major watersheds and functions as a drainage canal and a sediment basin. Due to sediment accumulation, periodic maintenance dredging is required. Contaminants such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, and pesticides, as well as microbial pathogens, may be present in the sediment. Microbes utilize organic compounds for energy and/or growth, either producing new organic compounds as side products or carbon dioxide and water. The objective of this study was to determine if indigenous microbes could utilize potentially carcinogenic PAHs, specifically phenanthrene, as a carbon source.

In previous studies, microbes present in the dredged sediment were isolated and identified. Since these microbes have been thriving in contaminated sediment for years, we hypothesize that these microbes may have potential for bioremediation. To determine the phenanthrene degradation capabilities of the four selected indigenous microbes, each strain was inoculated into three different media: 25ppm phenanthrene in basal Bushnell-Haas medium, basal Bushnell-Haas medium (negative control), and Marine Broth (positive control). The growth of these microbes was monitored through a series of turbidity tests and cell counts using a hemocytometer. The degradation activity of phenanthrene was studied using gas chromatography and calorimetry.

The results of this study showed that the four microbial strains are able to grow in the presence of phenanthrene and cause a decrease of phenanthrene. Therefore, these microbes are promising candidates for bioremediation of the contaminated sediments.

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Introduction

This project deals with the sediment from a man-made estuary called the Ala Wai Canal located on the south side of Oahu, Hawaii and on the border of Waikiki. The Ala Wai Canal was built as a drainage canal for major streams and road runoff. The Ala Wai Canal also served as a sediment basin, preventing sediment from entering coastal waters. It now is serving as a recreational area for kayaking, canoeing, and some fishing. In order to maintain optimum flood control, the canal needs to be dredged periodically.

The dredged sediment from the canal is contaminated with pesticides, heavy metals, pathogens, and polycyclic aromatic hydrocarbons (PAHs) from petroleum products. This contamination raises a health issue and disposal problem. In this project, we focused on the contaminant, PAHs, which are anthropogenic carcinogens.

In previous studies, microbes present in the dredged sediment were isolated and identified by 16S rDNA extraction and sequencing. After numerous literary searches, AW 4 (Ferrimonas), AW 12 (Erythrobacter sp.), AW 16 (Rhodobacter sp.), and AW 22b (Psedoalteromonas haloplanktis), were found to be among the most promising for degrading PAHs and were therefore selected for investigation of bioremediation potential. We believed that the four selected indigenous bacteria could degrade PAHs, specifically one called phenanthrene, because the bacteria have acclimated in the contaminated sediment and adapted to high levels of toxicity. If bacteria can degrade phenanthrene, they can then be used for bioremediation of PAH-contaminated sediment.

For this project, we focused on aerobic respiration of microorganisms. In aerobic respiration, microorganisms utilize organic compounds and oxygen for energy, either producing new organic compounds as side products, or carbon dioxide and water. The objective of this study was to first determine if the bacteria can grow at all in the presence of phenanthrene, which is potentially toxic to the bacteria. The second objective was to find out if the bacteria can utilize phenanthrene as a carbon source and thus degrade phenanthrene.

Methods

We inoculated each of the bacterial strains into three different media—basal Bushnell-Haas medium (referred to as B-H here on), 25ppm phenanthrene in basal B-H medium, and marine broth. The B-H medium served as a negative control, and marine broth served as the positive control. The bacteria should not grow in the B-H medium, which lacks a carbon source, and should grow the most in the marine broth, which is heterotrophic rich.

There are three factors we investigated to find out if the bacterial strains degraded phenanthrene: bacterial growth; disappearance of phenanthrene: and energy given off from degradation of phenanthrene. To measure bacterial growth, we used turbidity tests with a spectrophotometer and cell counts with a hemocytometer. To measure disappearance of phenanthrene, the phenanthrene from media that had been inoculated for at least two weeks was extracted. The extractions were injected into a gas chromatograph. To measure energy given off from degradation of phenanthrene, we used calorimetry.

RESULTS

The turbidity tests indicated an increase in growth when there was an increase in absorbance. As expected, the marine broth yielded the greatest increase in absorbance, and therefore the most growth with both Ferrimonas and Erythrobacter. With Ferrimonas, there was very little, or no growth in the B-H with phenanthrene and B-H without carbon because the change in absorbance was small. From these results, we cannot determine if more growth occurred in the B-H with phenanthrene than in the B-H without carbon, although the turbidity for the B-H with phenanthrene is slightly greater than that of the B-H without carbon. With Erythrobacter, we observed a greater bacterial growth in B-H with phenanthrene than in B-H without carbon. Rhodobacter and Pseudoalteromonas followed the same trends as Ferrimons. The cell counts in various media indicated growth when there are large cell counts. With all four strains, marine broth showed the greatest concentration of cells, followed by B-H with phenanthrene and B-H without carbon. Based on the higher numbers in B-H with phenanthrene than in the B-H without carbon, bacteria are probably utilizing phenanthrene as a carbon source.

For the concentration of phenanthrene in extractions indicated that with Ferrimonas, Erythrobacter, and Rhodobacter, there was about a 25% decrease in phenanthrene compared to the control. With Pseudoalteromonas, there was about a 10% decrease in phenanthrene compared to the control. As we expected, the concentration for the inoculated B-H without carbon was zero.

Based on the observed decreases in phenanthrene and increasing growth of bacteria, we deduced that these strains have the ability to degrade phenanthrene. In summary, high cell counts correspond with high percentages of degraded phenanthrene. Ferrimonas, Erythrobacter, and Rhodobacter all had high cell counts and therefore high phenanthrene degradation. On the other hand, Pseudoalteromonas had a lower cell count, and therefore lower phenanthrene degradation.

CONCLUSION

Our study has generated substantial evidence supporting our hypothesis that bacterial strains isolated from the Ala Wai Canal can grow in the presence of and degrade phenanthrene. These results are beneficial for the maintenance of public health as well as the environment. We conclude that the bacterial strains investigated are promising candidates for bioremediation—a solution that will keep our home in the islands of Hawaii clean and safe for locals and visitors alike.

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