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Bioremediation of Hydrocarbons via Geobacillus

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Abstract

Hydrocarbon remediation is a vital aspect of environmental cleanup, especially in soil and groundwater to ensure the health and safety of the public's drinking water. A possible solution to this contamination is bioremediation, the use of microorganisms to degrade pollutants. This research analyzes the effectiveness of *Geobacillus* as a hydrocarbon degrader for the use of bioremediation. Twelve strains were identified from oil-contaminated sources and grown in minimal media with crude oil as the carbon source. Growth was observed in the crude oil samples, demonstrating the possibility of bioremediation of hydrocarbons via *Geobacillus*.

Introduction

Environmental remediation has long been centered on the cleanup of soil and groundwater to ensure the health and safety of the public. A main priority of this remediation is hydrocarbon-contaminated areas, as this pollution is a serious threat to the safety of drinking water. A possible solution to this contamination is bioremediation, the use of microbes to degrade hydrocarbons.[1] This research analyzes the effectiveness of *Geobacillus* as a hydrocarbon degrader for the use of bioremediation. The objectives of the research are as follows:

1. Determine specific strains of *Geobacillus* for hydrocarbon degradation,
2. Determine effectiveness of *Geobacillus* for bioremediation of hydrocarbons, and
3. Determine effectiveness of degradation of specific hydrocarbons.

Background

Crude oil is a complex mixture of hydrocarbons of varying sizes and structures, such as alkanes, aromatics, and cycloalkanes. As shown below in Figure 1, oil production occurs all over the world. The highest production of oil occurs in Russia, Saudi Arabia, and the United States, with over 10 million barrels a day each.[1]

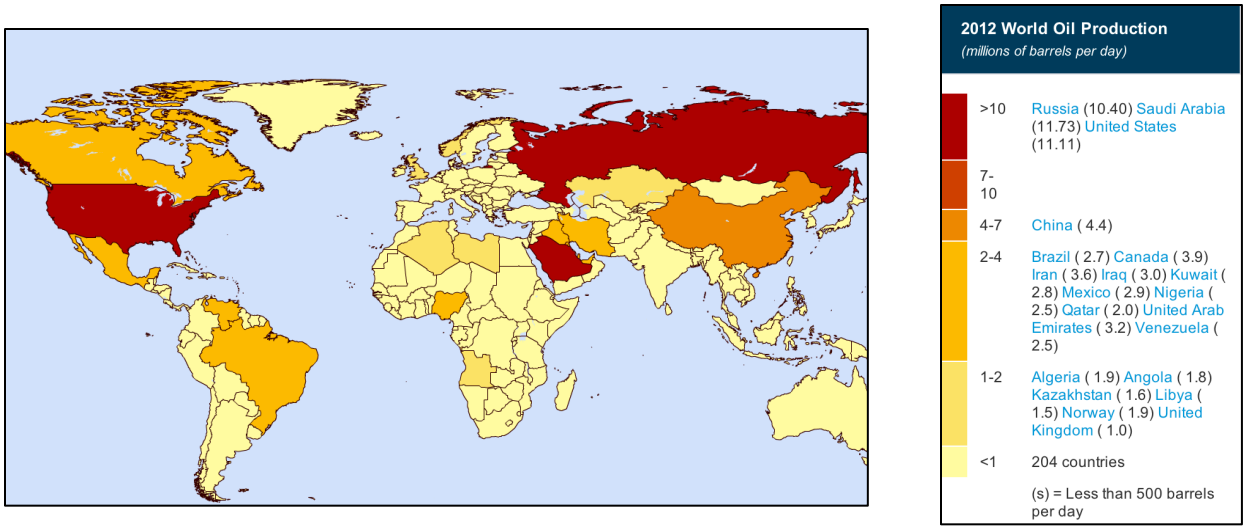

Figure 1. *World oil production in 2012.*

The 2012 consumption of oil is shown below in Figure 2. With the largest consumptions occurring in China and the United States, each country with over 10 million barrels consumed daily.[1]

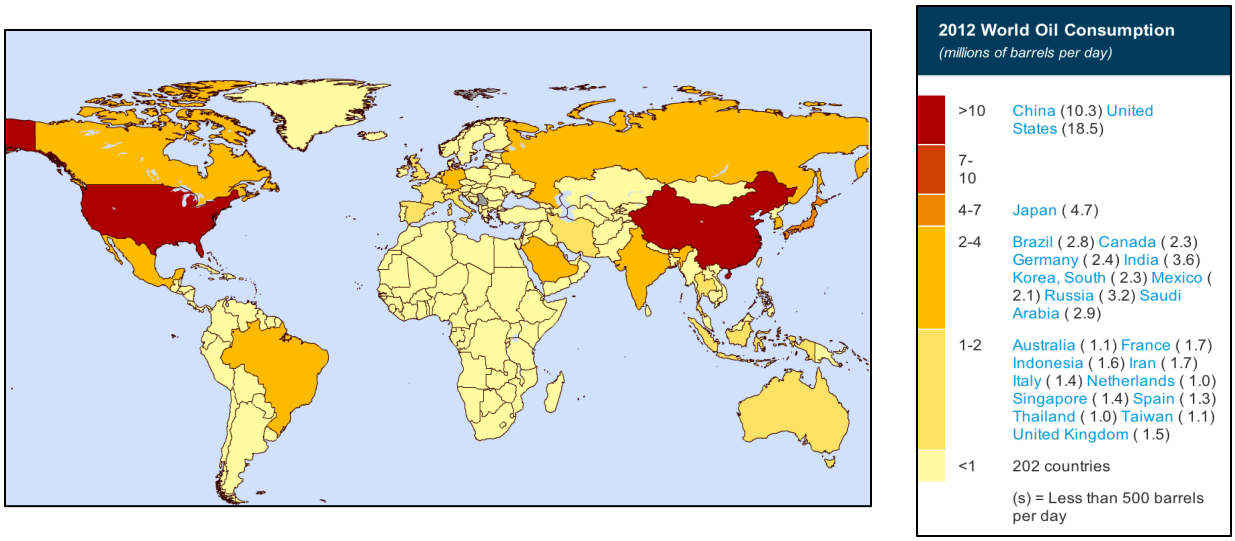

Figure 2. *World oil consumption in 2012.*

From Figures 1 and 2, it can be observed that oil production and consumption occur all over the world, which can lead to oil pollution. Oil leakage is possible in all aspects of oil production, from mining, to storage, to transportation, to the end use of the final product. Groundwater and soil contamination is a large aspect of oil leakage.

Oil pollution has long been a priority for environmental restoration. Groundwater and soil contamination threatens the health and safety of the public, as this pollution can lead to drinking water contamination. Therefore, it is necessary to develop environmentally friendly technologies to combat this pollution, one solution being bioremediation.

The technique of bioremediation utilizes biological organisms to cleanup contaminants or pollutants from a designated area. The two most prevalent bioremediation methods are biostimulation and bioaugmentation. Biostimulation involves the addition of oxygen and mineral nutrients, such as phosphorus, nitrogen, and trace metals, to enhance the already present bacteria in the environment. Bioaugmentation is the direct addition of microorganisms to the desired site.[2]

Geobacillus has been identified as bacteria capable of bioremediation. An image of *Geobacillus* grown on a TBAB agar plate is shown below in Figure 3. This bacterium is

gram-positive and rod-shaped. It is spore forming and has an optimum growth temperature of 50-60°C, classifying it as a thermophile.[3] The bacterium is naturally occurring in various soils and sediments, such as volcanic environments, hot springs, and ocean sediments.[4] The use of *Geobacillus* for bioremediation of hydrocarbons has an increasing interest due to their low toxicity and growth in high temperature, which has been found to increase degradation.[5]

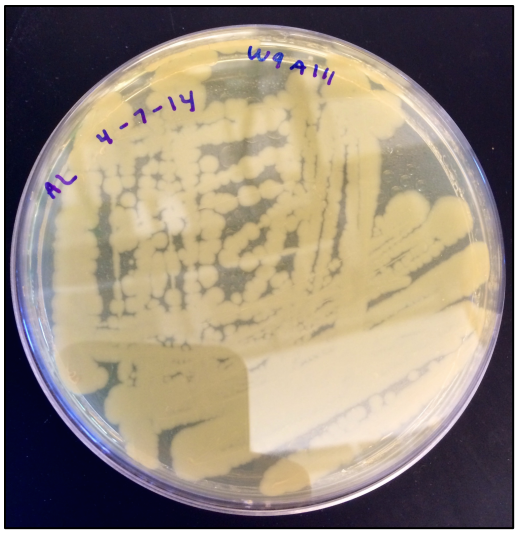

Figure 3. *Geobacillus strain W9A111 on TBAB agar plate.*

Some of the factors that affect hydrocarbon degradation are soil characteristics, such as the nutrients and pH, quality and quantity of the contaminants, source of the pollutants, and temperature. Increasing the temperature of the environment can have many positive effects on the degradation of hydrocarbons, such as decreased viscosity, higher solubility, and faster diffusion of hydrophobic contaminants.[2] Due to the benefits of a high temperature environment, the interest in thermophiles, such as *Geobacillus*, as hydrocarbon degraders is increasing.

Thermophiles grow most effectively in hot climates, such as deserts and areas with hot summers. They have faster growth rates than mesophiles, which grow at moderate temperatures, because of the higher growth temperature. Thermophiles are also more resistant to physical and chemical denaturation. Along with the benefits of thermophiles, *Geobacillus* is a good option for bioremediation of hydrocarbons because it is naturally occurring, making it both cost effective and environmentally safe.

The metabolic pathway utilized for hydrocarbon degradation by thermophiles is through the OCT plasmid that contains the *alk*B gene. This gene encodes the proteins necessary to degrade alkanes from primary alcohols, to aldehydes, to fatty acids.[6]

Hydrocarbon degradation can occur through two methods: interfacial accession or through the use of biosurfactants. Interfacial accession is the direct contact of the cell and the hydrocarbon and when a biosurfactant is used the cell contacts an emulsified hydrocarbon.[7]

Surfactants have been shown to enhance metabolic activity of hydrocarbon degraders. Some hydrocarbon-degrading microorganisms have been found to produce biosurfactants to aid this degradation. There are two types of biosurfactants, one that lowers the surface and interfacial tensions and the other, bioemulsfiers, which stabilizes oil-water emulsions without a significant reduction of surface tension.[5]

Experimental Method

The first step necessary to begin research was to identify which strains of *Geobacillus* would most likely be good hydrocarbon degraders. From a list of strains already present in the laboratory, twelve strains were identified as being isolated from oil-contaminated sources, such as oilfield formation water, an oilfield, and oil-contaminated soil. These strains are listed below in Table 1.

Table 1. *Geobacillus strains identified for possible growth in crude oil.*

Strain	Isolation Source	Location
91A1T	Oilfield Formation Water	Liaohe, China
92A2	Oilfield	Mykhpaiskoe, Western Siberia, Russia
W9A95	Oil-contaminated soil	Taiwan
W9A96	Oil-contaminated soil	Taiwan
W9A101	Oil-contaminated soil	Taiwan
W9A103	Oil-contaminated soil	Taiwan
W9A104	Oil-contaminated soil	Taiwan
W9A108	Oil-contaminated soil	Taiwan
W9A109	Oil-contaminated soil	Taiwan
W9A110	Oil-contaminated soil	Taiwan
W9A111	Oil-contaminated soil	Taiwan
W9A113	Oil-contaminated soil	Taiwan

From a freezer stock, the strains were grown in 5 mL of TGP, a liquid culture, for 24-hours in a 60°C oven. The recipe used to make 1 L of TGP solution is detailed in Table 2, below.

Table 2. *TGP recipe for 1 L solution.*

Material	Amount
Sodium Chloride	5 g
Tryptone	17 g
Soy Peptone	3 g
Dipotassium Phosphate	2.5 g
Sterile Glycerol	4 mL
40 g/L Sodium Pyruvate	100 mL

A minimal media was then prepared with 2% carbon source, either glucose or crude oil. The minimal media recipe is detailed below in Table 3.

Table 3. *Minimal media recipe for 125 mL of culture with glucose or crude oil as carbon source.*

Material	Concentration	Amount
Biotin	100 μg/mL	1.25 mL
Thiamine·HCl	500 μg/mL	0.25 mL
Nicotinic Acid	500 μg/mL	0.25 mL
$CaCl_2$ Anhydrous	5%	12.5 μL
$FeCl_3 \cdot 6H_2O$	0.5%	12.5 μL
$ZnSO_4 \cdot 7H_2O$	5%	12.5 μL
$MnCl_2$	10 mM	12.5 μL
Mineral Salts	-	12.5 mL
KPO_4 Buffer	-	6.25 mL
Yeast Extract	2%	6.25 mL
Carbon Source	-	2.5 mL

From the TGP cultures, a ratio of 1:100 (0.05 mL TGP culture into 5 mL minimal media) was used to grow the cells in the minimal media. The cultures were grown in a 60°C oven for a 24-hour growth period. Large test tubes with small volumes were used to promote mixing within the tubes. Crude oil was added directly to each test tube to ensure proper nutrients to each culture and evenly distributed oil. The crude oil used was Texas Raw

Crude: Real Crude Oil Samples. After a 24-hour growth period in a 60°C oven, a 1 mL sample of each culture was transferred to a cuvette in order to perform optical density readings, being careful not to transfer remaining crude oil.

Cell growth was measured via optical density readings. Optical density, a measure of absorbance, is a measure of the light that is scattered by the number of particles present in a sample. The higher the optical density reading, the more turbid the sample is, indicating a higher number of cells. The more cells present in the cultures indicate a higher growth rate of the sample. [8]

To properly determine positive growth, three controls were utilized. To ensure proper growth and no oven contamination, samples were tested with no inoculum, one for each carbon source. Minimal media with glucose as a carbon source was used as a positive control, as all the strains tested have been shown to have positive growth with glucose. Strain 95A1 was chosen as a negative control, as it is not isolated from an oil-contaminated source, it should not be able to utilize crude oil as a carbon source for growth.

Results and Discussion

After a 24-hour growth period, growth was observed in all samples, with minimal growth in 95A1 strain with crude oil. The results obtained are shown below in Figure 4.

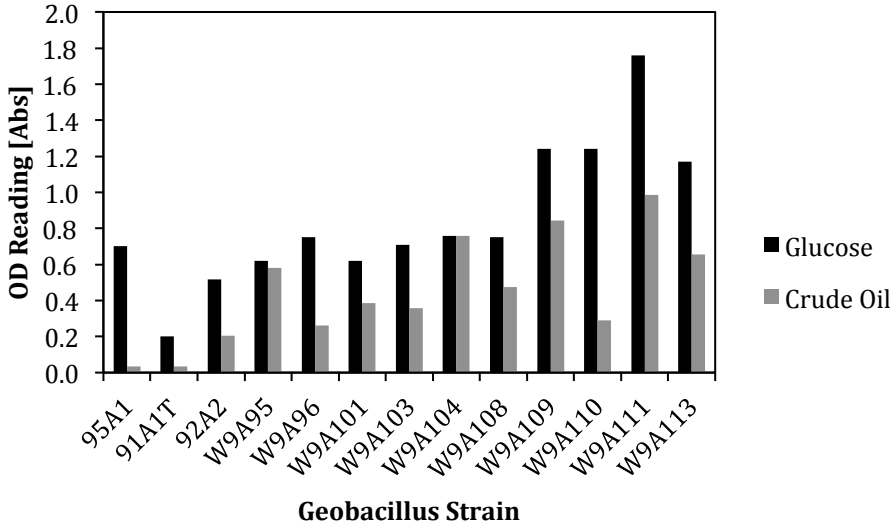

Figure 4. *Cell densities measured for various Geobacillus strains with glucose and crude oil as carbon sources.*

Varied growth was observed throughout all strains because the minimal media used was developed for thermophiles and is not optimized for all strains. Therefore, it is necessary to relate relative cell growth for each strain.

Figure 5 below shows no inoculum, 91A1T, and W9A11 samples in cuvettes before the optical density measurement was taken. The samples on the left show cultures in glucose minimal media and the samples on the right show cultures in crude oil minimal media. As seen in the photos, the growth is approximately the same for these three samples.

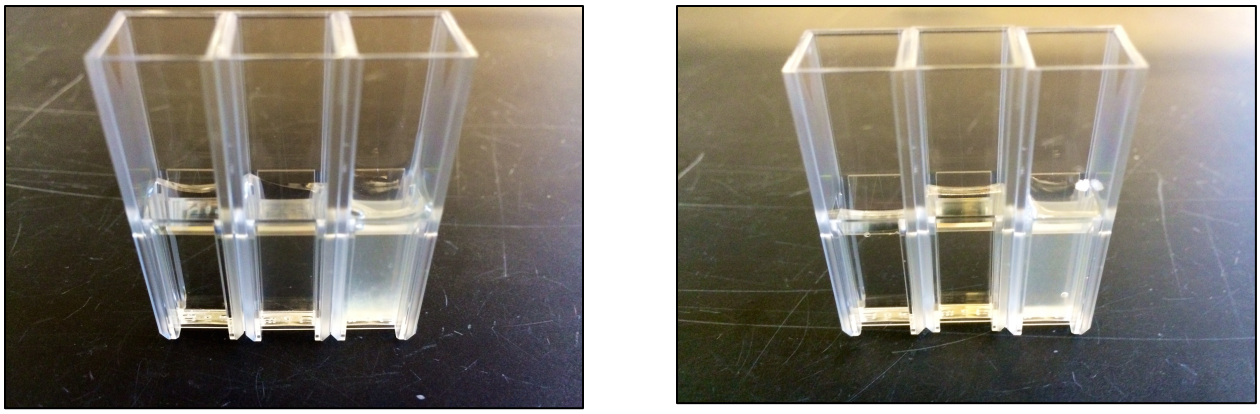

Figure 5. *No inoculum, 91A1T, W9A111 samples after 24-hour growth period in minimal media with glucose (left) and crude oil (right).*

Further Research

There are various experiments and studies that could be conducted in order to further this initial research. From this study, it has been determined that it is possible to use *Geobacillus* as hydrocarbon degraders, as growth was observed in all strains. However, in order to further the research, specific strains could be optimized by testing different media and conditions, such as temperature and amount of carbon source. Another aspect of this project that could be developed further is the toxicity of hydrocarbons to *Geobacillus*, determining if there is a point that oil will have harmful effects on the bacteria instead of utilization as a carbon source.

Another area of research for the continuation of this project is the effect of surfactants on the growth in crude oil. As previously stated, surfactants enhance hydrocarbon degradation and could be used to promote the growth of hydrocarbon degrading

microorganisms. In order to quantify the amount of hydrocarbons being degraded, gas chromatography could be used. Gas chromatography could also allow for a study on which hydrocarbons are being degraded within the crude oil. Synthetic oils could also be studied to determine the effect of specific hydrocarbons, as synthetic oils are less complex than crude oil.

Conclusions

Oil pollution is a crucial aspect of environmental restoration as oil contamination in groundwater and soil environments can affect a population's drinking water. From this research, *Geobacillus* has been shown as a viable option for hydrocarbon bioremediation, as various strains have been shown to utilize crude oil as a carbon source. Different growths were observed for each strain, but this may be attributed to the minimal media used, which is for thermophiles and no optimized for each strain. Strain W9A111 was shown to have the best growth in crude oil, and could be further optimized to show more degradation. Much research could be done to expand this project and more trials and experiments should be conducted to optimize hydrocarbon bioremediation via *Geobacillus*.

Acknowledgements

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