Harnessing M. *luteus* in an In-situ Bioremediation Technique to Reduce Environmental Contamination by Total Petroleum Hydrocarbons

Rithvik Akella

Abstract

Many parts of the world are subject to increasing petroleum pollution which affects some places more than others. Bioremediation is one of the methods that can be used to combat the incurred negative effects, but not all countries have the money to spend on such endeavors. Furthermore, it is known that countries with developing economies rely heavily on agriculture as their main industry. For example, many parts of southern India are agriculturally-based economies. Petroleum pollution has also been an issue in such places. Exposing agricultural lands to such a pollutant can harm the local economies, not to mention it is a health hazard for locals. This study calls for an evaluation of the effectiveness of M. *luteus* in an in-situ bioremediation approach as opposed to ex-situ. In-situ bioremediation is completed in the environment as opposed to ex-situ which is done in a lab setting. In recent years, many ex-situ techniques have been proposed and implemented, but the issue with these is that the process is overbearing in terms of time. An in-situ technique can be implemented directly into the environment which is more efficient than the constant removal and addition of environmental content. The methods will involve M. *luteus* cultured in plates uncontaminated and contaminated by petroleum. Observing the difference in growth between different conditions will provide insight into the relative efficacy of M. *luteus* in a petroleum-polluted environment.

Introduction

The agricultural lands of South Asian countries are major contributors to the world's agricultural economies, producing major crops such as Rice, Wheat, and Sorghum(*South Asia - Crop Production Maps*, n.d.). Crops such as these are exported and used across the globe. However, there is a growing issue of pollution, especially in major exporters of these groups. One of the biggest polluters of South Asian lands are Hydrocarbons that take residence in the soil. Like many pollutants, Hydrocarbons(HCs) are harmful to both the humans that work the land as well as the environment itself. HCs can originate from many different points including industrial activities, energy production, and transportation(*Sources of Soil Pollution in Asia and the Pacific*, n.d.). Petroleum HCs can be structurally varied. Two of their main constituents are important groups of hydrocarbons called alkanes and aromatic hydrocarbons. There are different classifications of alkanes but the groups of interest are the acyclic alkanes: normal and branched. Normal alkanes are usually C_5 up to several hundred, but generally, they lay between C_5 and C_{40}. Branched alkanes are generally C_9 to C_{25}. The aromatic hydrocarbons are composed of one or more benzene rings(Clayton, 2005). Aromatic Hydrocarbons are often associated with the main organic coil contaminants, usually diffusing into the soil after some form of a combustion reaction. The stability of the aromatic rings is what makes them such stubborn polluters. South Asia happens to be third on the list of highest Polyaromatic Hydrocarbon emissions despite efforts to reduce its presence(*Global Status and Trends of Soil Pollution by Organic Contaminants*, n.d.).

Though Petroleum Hydrocarbons negatively impact humans and many ecosystems, they can still be largely reduced by a few species of bacteria. Many microorganisms cannot survive long when their habitat is compromised by petroleum, but there are a few exceptions to this rule(Truskewycz et al., 2019).

There have been sightings of different species of bacteria working under different conditions to reduce oil contamination through the process of biodegradation, a technique used in bioremediation. A rather popular group of marine bacteria that has been observed to take up crude oil is the Pseudomonas family. Though it takes some time for these marine species to multiply enough to consume oil spills in the ocean, they can be effective over a longer period of time. An example that can be used to study this is the Deepwater Horizon Oil Spill in 2010 that spanned up to a couple of months(*Who Thinks Crude Oil Is Delicious? These Ocean Microbes Do*, 2015). Another example of this phenomenon in a soil environment is Alcanivorax *borkumensis*. The study analyzed the effectiveness of the crude enzymes contained within A. *borkumensis*, proving its inherent efficacy against petroleum hydrocarbon degradation(Kadri et al., 2018). Though this study only proved the effectiveness of the crude enzyme, it alludes to the potential of the microbial ability to degrade petroleum hydrocarbons in aquatic and terrestrial environments.

Micrococcus *luteus* possesses comparable capabilities. Existing in all parts of the human integumentary system, this species of bacteria is seldom harmful to human existence. The potential of M. *luteus* to reduce petroleum hydrocarbons safely combined with its versatility makes it a highly applicable species of bacteria in bioremediation.

This study examines the growth capabilities of M. *luteus* in a petroleum environment to examine its feasibility in application towards petroleum biodegradation.

Materials and Methods

The materials used were: Micrococcus luteus, petroleum jelly(petroleum), a thermometer, a styrofoam incubator, sterile absorbent paper, and nutrient agar plates. M. *luteus* was obtained from Carolina Biological Supply. All nine cultures were prepared using the streak plate method.

A trial was run with two control groups, to test current claims that M. luteus has an evident response to petroleum. One experimental group was also present.

I. Positive control

The positive control group consisted of the three agar plates with only M. *luteus* cultured on the nutrient agar plates.

II. Experimental Group

The experimental group was run with agar plates of M. luteus and three different diameters of absorbent paper inoculated with petroleum. The small diameter was 0.5 cm, the medium diameter was 1 cm, and the large diameter was 1.5 cm.

III. Methods

M. luteus was thawed at 40 degrees C. During this time, petroleum jelly was also melted at 40 degrees C.

For the positive control group, a swab was inoculated with bacteria and its growth medium and then rubbed across nutrient agar plates.

The same was repeated for the experimental group with the addition of two disks of equal diameter into each of the three agar plates in the experimental group.

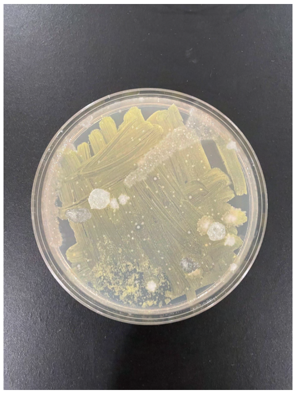
All the plates were then incubated in the styrofoam incubator at 25 degrees C.

Plates were incubated for one week and then photographed at the end of this timeframe.

Results and Discussion

As expected, the positive control group exhibited normal growth, as observed in Figure 1 below, confirming the feasibility of M. *luteus*.

In Figure 2(below), the plates with disks of large diameters, 1.5 cm, displayed higher growth towards the disks.



In Figure 3(below), the specimens with medium diameters, 1 cm, displayed less growth towards the disk.

In Figure 4, the plates with the disks of the smallest diameter, 0.5 cm, displayed the least growth around the disks.

Overall, there was still ample growth in all cultures, except there appeared to be fewer colonies around the disk as the diameter of the disk decreased. There appears to be a correlation between the presence of petroleum and the growing preference for M. *luteus*. M. *luteus* appears to have an evident affinity for petroleum presence.

This knowledge can be applied in a bioremediation project to remediate lands contaminated with petroleum. Furthermore, M. *luteus* does not have environmental specifications in terrestrial environments, making it manipulatable across many environments.

Many current bioremediation projects involving bacteria thus far have studied only *ex-situ*, or off-site, bioremediation techniques. The applicability of M. *luteus* across many environments gives rise to a potential *in-situ*, or on-site study.

In addition, *in-situ* bioremediation is arguably preferable to *ex-situ* for the reason of efficiency. *Ex-situ* techniques, though requiring less time, will be more costly and excavation-heavy. *In-situ* trumps this approach in the long run because of its capability to treat a large area at once with a lower possibility of contamination than *ex-situ techniques.*

In addition, *in-situ* techniques remain more versatile than *ex-situ* techniques. Studies have been conducted showcasing the potential of *in-situ* bioremediation in harsh hydrocarbon-rich environments such as Arctic soils(Rike et al., 2003).

Applying the results on a larger scale may not be as difficult as imagined. With the flexibility of M. *luteus*, it has the potential to grow across many conditions and it is already proved that M. *luteus* has favored environments with increased petroleum. Scalability is a crucial quality of M. *luteus* that furthers its appeal in an *in-situ* bioremediation approach.

Figures

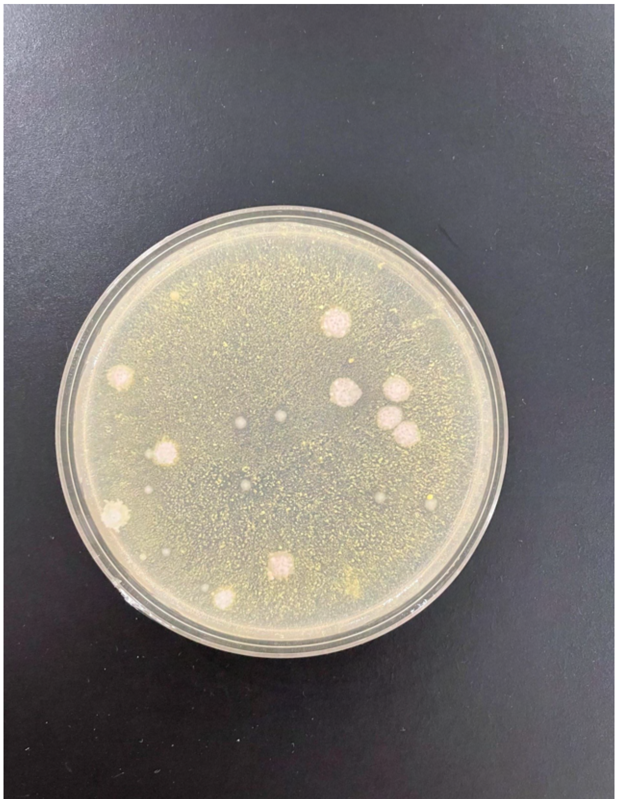


Figure 1* with only M. luteus

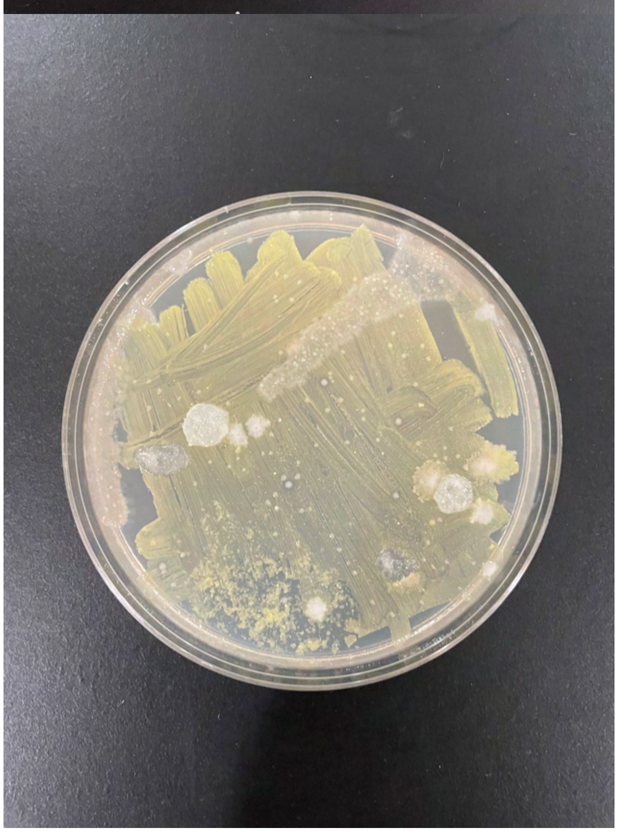


Figure 2* with M. *luteus* and small(0.5 cm) petroleum-inoculated disks

Figure 3* with M. *luteus* and medium (1.0 cm) petroleum-inoculated disks

Figure 4* with M. *luteus* and large (1.5 cm) petroleum-inoculated disks

Notes: Yellow growth corresponds to M. *luteus* and the two clear circles are the disks inoculated with petroleum.

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