New Way to Implement Hydrocarbon degradation in Soil Through Bioremediation

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ABSTRACT

Because of industrialization, crude oil is widely used in manufacturing factories, refineries, and power plants, causing severe oil contamination in the environment. Enormous oil spills leak into wetlands and the ocean, leading to biodiversity reduction, ecosystem fluctuations, and public health concerns. This research aims to explore a tactic to improve the efficiency of oil degradation in wetland areas. Based on a recent study in the country of Georgia, bio-surfactants are proven to be the critical component of a bio-remediator. By breaking the structure of long-bond hydrocarbon, biosurfactant shows a strong ability in degrading heavy diesel. My project authenticates the performance of bio-surfactants by comparing them with traditional bacteria-based oil degraders. Combining the data from the field and experimental studies, the research concludes that in the same period of bioremediation on wetland soil, bio-remediator with rich biosurfactant has a better effect on the wetland soil recovery. The final hydrocarbon degradation is twenty percent higher than that done by the traditional bio-remediator compared to the soil sample under traditional bioremediation treatment. Also, the results are consistent among different types of oil spills. The conclusion of the research provides an improvement direction for the bioremediation in the San Francisco Bay wetland land area, which is playing a role as a clean water filtration system, wildlife habitats, and resources for local economic development. With a more advanced hydrocarbon degradation technology, bioremediation projects in San Francisco Bay wetland can deal with more environmental and social problems.

KEYWORDS

bioremediation, hydrocarbon, biosurfactant, wetland destruction, oil remediation.

INTRODUCTION

Fossil fuel use has increased across the world as globalization and economic development have advanced. Specifically, the global commercial system keeps consuming diesel as the primary fuel for goods production and increases the necessity for international transportation and coastal heavy industry. Such a trend significantly enlarges the scale and amount of marine transport and offshore oil refinery. However, with increases in the use of oil and gas has come an increase in oil spills through urban runoff, industrial discharge, and vessel accidents. (Cakir. 2021) Notably, industrial operations emit vast diesel pollution into coastal ecosystems. The crude oil pollution flowing into the soil slows the transport of oxygen into the root zone, and such delay in oxygen availability caused by oil hydrocarbon permeation can increase stress on wetland plants, which are unable to supply enough oxygen to their root system. (Fisk, 2017) In 2012, an estimated 4.9 million barrels of crude oil spills from coastal refineries and oil extraction machines were leaked into the Gulf of Mexico and had a severe impact on the wetland system team of Louisiana's Mississippi River Delta ecosystem. (Mendelssohn et al. 2012) Similar oil spills happen all over the world. Therefore, considering the importance of wetlands in drinking water management, animal survival, and the local economy, oil degradation has become critical for the sustainable development of the world.

Nevertheless, the traditional bioremediation methods for cleaning up oil spills are often ineffective and potentially harmful. For oil clean-up projects in the wetland areas, most environmental management institutions would implement in situ burning on marshes, but residues left behind burning would harm the vegetation's roots and rhizomes, slowing the rate of ecosystem recovery. (Hoff. 1995) Other oil-degradation methods involve chemical dispersants in wetlands which would suppress the bioremediation process driven by native oil degraders, weakening the soil's natural oil-degradation ability. (Kleindienst et al. 2015) In recent years, bioremediation using local microbes living on hydrocarbons has emerged as a cost-effective and environmentally friendly technology to clean up the oil-polluted site (Onwurah et al., 2007). More importantly, harvesting the oil degraders locally could exclude the bacteria's adaptation failure and increase the bio-remediation efficiency because micro-oil-degraders' growth is closely linked to the chemical features of the surrounding environment. (Belotte et al.,2003) But, one problem with oil-degrading bacteria is that they tend to be locally adapted to soil conditions and cultivation systems. Thus, different types of bacteria tend to have various physical and biological characteristics. This makes it difficult to use the same type of bacteria to clean up oil spills in different places and inevitable to revise the bacterium harvesting plan, increasing financial and time costs. For example, a commonly used oil-degrading bacteria in China, *Pseudomonas putida*, can only be harvested by centrifuge at 6000 rpm and stored by the bacterial inoculation pot with an initial concentration of approximately 1.0 × 107 CFU g−1 dry sediment in a 4 °C refrigerator. (He et al. 2019) Incompatibility of unchanged bacteria harvesting methods might even disturb the process of bio-stimulation and bioaugmentation used to cultivate the population of oil-degrading bacteria.

Recently a research group invented a more advanced technology named BioCure and announced that such a tactic could be universally used to harvest oil-degrading bacteria in polluted wetlands. According to the reports, "The BioCure approach is radically different and involves analyzing the soil sample, identifying the native bacteria populations and rapidly – within days! – isolating a strain of oil-eating bacteria that will thrive and clean up best." (Gassmann. 2019). The method employed by BioCure, not only harvests the oil degraders but also isolates the microbes producing the most bio-surfactant from the soil. In this way, the bio-surfactant, with a strong emulsification ability, could extract the oil degraders outside of the soil and destroy the structure of long-chain hydrocarbons so that the bacteria can more easily consume the oil pollution. (Karlapudi et al. 2018)

My project intends to introduce a way to improve the bio-remediation process in the oil-polluted wetland in Bay Area. By analyzing the data from the field station in the country of Georgia and lab experiments, the central research question of this research was designed to illustrate the functionality and feasibility of BioCure technology as an improvement direction for bioremediation project on oil contamination in San Francisco Bay. To demonstrate the flexibility of BioCure's strategy, I tested the advantage of BioCure's approach by comparison with commercial bacteria-based oil cleaner, using the soil samples from San Francisco Bay. My research also analyzed the dataset about the performance of BioCure technology on different types of oil spills (from the field station in the country of Georgia).

METHODS

Soil sample preparation

To simulate the soil condition of the San Francisco Bay wetland, I took soil from a few inches below the surface in the root zone and collected 500ml of soil in a 1L plastic bottle at Berkeley Campus near Strawberry Creek for soil analysis. I then removed debris such as rocks, roots, and clumps to eliminate impurities that might be influential to the experimental result. I placed the remaining soil into a glass jar and added a tablespoon of powdered dishwasher soap to help keep the particles separate. After filling the jar with water and capping it tightly, I shook it well to moisten all soil particles. After 24 hours, once it was not turbid, I was able to clearly organize the component of the soil sample. Then, I collected peat moss and unfertilized agricultural sand from Oxford Field Station (UC Berkeley Greenhouse Lab) to create 1 gallon of uncontaminated soil sample (10% peat moss and 90% unfertilized agricultural sand). In the end, I granulated the 3406.5ml of dry peat moss chunks with 100 ml of hot water and mix them evenly with 378.5 ml of agricultural sand in a 5-gallon bucket. I then set the soil sample for 72 hours to evaporate the water.

Crude oil preparation and soil contamination

To replicate the oil pollution in San Francisco Bay, I used the same oil that was spilled in the Deepwater Horizon event in the Gulf of Mexico—Louisiana Sweet Crude oil. This crude oil sample was provided to our lab by the EPA. I planned to use 567.75 ml of raw crude oil to reach the level of 15% of crude oil contamination. I then took the crude oil with serological pipettes and heated it to 260 Celsius with an experimental hot plate, so that crude oil could be turned into petroleum with higher fluidity and less viscosity. I then poured 757.5 ml of petroleum into the bucket (rinse with 75 % ethanol) filled with the undefiled soil sample and stirred it up for 15 minutes.

Control group and experimental groups establishment

After setting the oil-polluted soil sample for 3 hours at room temperature, I split the soil sample into three groups with three 400 ml replicates each. All the samples in the control and experimental groups were contained in 500ml beakers. I used three groups to test the difference between oil-degradation by natural decomposition, oil-degrader with a high concentration of bio-surfactant, and commercial bacteria-based cleaner. To simplify the procedure of the experiment, I chose Vermitea as the bio-surfactant, which is proven to have a significant abundance of biosurfactant-producing colonies in the long-term oil-polluted soil as the bio-remediator of BioCure Technology. (Chiang. 2013) I used a product from EPA's top-rated bioremediation company as the method for groups of normal bacteria-based oil degradation. I added no extra variables into the natural decomposition group. I slowly stirred up the samples in each beaker with a glass rod and placed them on a clean bench.

Table1: Summary of experimental groups

Control Groups	**Type of remediator**	**Added before aeration**
Control bin	None	400ml polluted soil
Vermitea bin	10g Vermitea	400ml polluted soil
Oil-degrading Bacteria bin	10g Act Cleaner	400ml polluted soil

Hydrocarbon concentration testing

After 48 hours, I took 20 grams of each sample with glass jars and place them in an incubator. Then, I sent them to an analytical lab (McCampbell Analytical, Inc.), which is working with our lab, to operate a chromatographic-based Total Petroleum Hydrocarbon (TPH) test. Firstly, to measure the oil biodegradation rate, McCampbell lab researchers performed extraction and analysis of hydrocarbon compounds according to a modified version of EPA Method 3510C. In the beginning, the lab researchers extracted and quantified total petroleum hydrocarbons (TPHs). Then, with TurboVap reconstituted in hexane (100%) they operated the chromatographic analysis to monitor and determine the oil biodegradation rate. Furthermore, the researchers obtained the data on bacteria growth by measuring the cellular protein concertation. Total cellular protein was extracted for each sample using 1 ml of 2% SDS lysis buffer (50 mM Tris-HCl buffer with 2% [wt/vol] SDS) followed by room temperature incubation for 20 min by the lab researchers (Overholt et al. 2016) The lab sent me the collected data about the hydrocarbon-degrading and bacteria growth quantification procedure in 24 hours.

Data analysis

Based on the data from the field station in the country of Georgia and my experiments, I utilized ANOVA to test the significance of my hypothesis that the treatment group (Vermitea) could degrade more hydrocarbon from the soil than the control groups (Natural remediation and commercial act cleaner). Specifically, I employed one-way ANOVA to authenticate my argument. I performed statistical tests in Python (Girden. 1992)

RESULTS

The functionality of BioCure in oil-degradation

In the control-group experiment, evaluating the hydrocarbon concentration in soil samples before and after the bioremediation with the Total Hydrocarbon Petroleum test, I concluded the hydrocarbon degradation of the different bio-remediation methods (Figure 1).

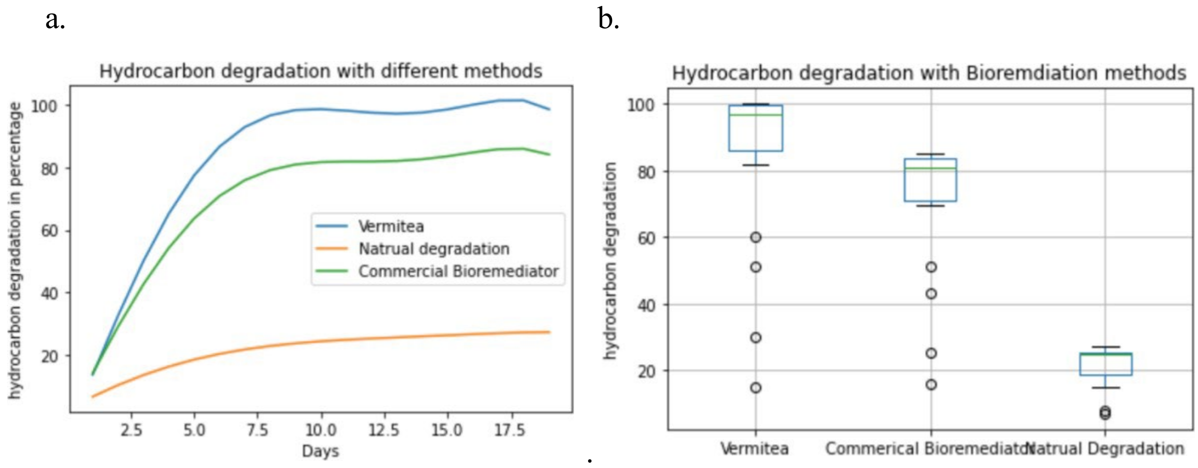

Figure 1. Comparison of hydrocarbon-degradation abilities of the different bioremediation tactics (Blue: BioCure (Vermitea), Natural degradation (Orange), Commercial Bacteria-based degrader (Act Cleaner)) in the soil polluted by Gulf of Mexico—Louisiana Sweet Crude oil. The BioCure demonstrates stronger oil-degrading ability than Natural degradation and Commercial Bacteria-based degrade ($p = 0.0134$).

Between the three groups, I found a significant difference in oil-degradation capabilities (one-way ANOVA, $p=0.0134$). BioCure method degrades more than 20 percent of oil than the other two.

Oil degradation on different oil spill types

Taking the crude oil sample from different clients, data from the country of Georgia shows the consistency of BioCure's method. The decrease in hydrocarbon by BioCure was performed in the field station in the country of Georgia and the total area is 179000 square meters)

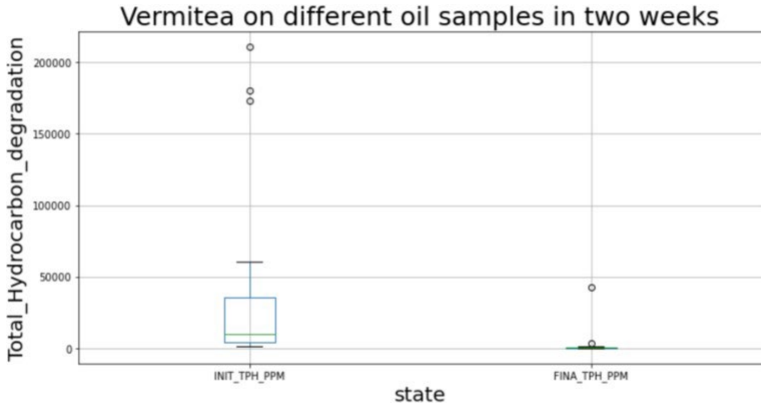

Figure 2. Hydrocarbon removal ability in different oil types. The graph shows the oil-degradation result based on nine different soil samples. After a 19-day experiment by the team in the country of Georgia, the finial hydrocarbon concentration is close to 0ppm. The maximum and minimum initial hydrocarbon concentrations are 178000ppm and 65ppm. The significant decrease in oil pollution was proved by ($p = 0.0167$).

DISCUSSION

To address the severe environmental destruction issue caused by crude oil pollution, my research explores a more effective tactic for bioremediation. BioCure technology turns the latest academic theories into practical applications. Scientists believe that due to biosurfactant's emulsification effect that reduces the tension between hydrocarbons and other bacterium's cell surface, oil-degradation methods require bio-surfactant-producing bacteria as the major bio-remediator. (Kumar et al. 2006) However, the environmental management department and institute need a solid reason to switch the bioremediation technology from bacteria-based to biosurfactant-based. My research provides a comprehensive conclusion on the advantages of biosurfactant–based bioremediation by comparison with traditional methods and analysis of different target oil types. Overall, my research found with a high concentration of bio-surfactant, bio-remediators can perform an effective hydrocarbon degradation in different locations and oil pollution types.

The biosurfactants' ability of oil-degradation

In this experiment, I measured the advantages of BioCure technology over other oil-degradation methods by testing the oil-degrading functionality of Vermitea, which is an artificial oil cleaner made of worm casting, deionized Milli-Q water, fish hydrolyzate, kelp fertilizer, and humic acid. (Chiang. 2013) Such a combination could ensure the maximum level of bio-surfactant in the bio-remediator. In my study, by comparing Vermitea's performance with the final hydrocarbon concentration after treatment with a commercial bio-remediator and natural hydrocarbon decomposition, I found that Vermitea showed a steadier and more efficient bio-remediation rate. According to the mean values of results from different groups, Vermitea decreases approximately 20% more hydrocarbon than commercial cleaner and 80% more than natural degradation. (Figure 1) This makes sense because, in bioremediation, the oil-degradation is driven by the oil-consuming ability of microbe bacteria. In the traditional bioremediation method, Researchers typically isolate and identify the most efficient hydrocarbon degraders from the environment and implement bioaugmentation. However, common bacteria harvesting methods can only find the best oil-consuming bacteria with a small variation from the surrounding environment. (He et al. 2019) Nevertheless, it is hard to find the bacteria that can degrade petroleum hydrocarbons through the whole process and will also be efficient in other environments too, so commercial bacteria-based bio-remediator might become less effective in certain soil types. In the mechanism of bacteria's oil-degrading, the self-producing bio-surfactant acts as chemically active surface compounds synthesized by specific groups of microbes that utilize different substrates like simple sugars, oils, and hydrocarbons from a contaminated environment (Parthipan et al., 2017) So, once the amount of biosurfactant falls to a certain level, researchers need to harvest new oil-degrading bacteria to ensure the bio-remediation efficiency. My experiment proves that the necessity to harvest multiple kinds of bacteria by different tactics might be caused by bacteria's limited capability to break the tension between hydrocarbon and soil. Therefore, this research provides convincible evidence that using BioCure technology can be the substitution for bacteria harvesting method revision due to its better hydrocarbon removal ability (Figure 1). Generally, to address the scarcity of biosurfactants, BioCure operates DNA isolation and extraction from the soil to identify the bio-remediator with the strongest ability to produce bio-surfactant so that the bacteria can benefit from a consistent emulsification effect by biosurfactant. However, because modern industrialization development enlarged both the concentration and range of crude oil spills, the volume of bio-surfactant generated from the local oil-degrading bacteria is often insufficient. Large-scale biosurfactant production from bacteria is challenging because such result depends on complex genetic systems such as operons, non-ribosomal peptide synthetases, and multiprotein assembly complexes. (Araújo et al., 2020) But, in the process of bioaugmentation of bacteria under field study, other soil chemical properties and toxic pollutants tend to disturb the bacteria's metabolism and confine the biosurfactant production from the bio-remediators (Nikolopoulou and Kalogerakis. 2019).

BioCure's hydrocarbon removing ability in different oil spill types

My second part of the experiment also concludes that the BioCure technology shows intense oil-degradation ability in an oil spill sent from different companies. By demonstrating BioCure technology's feasibility on different target objects, I authenticated that BioCure technology can perform as expected in real-world circumstances. This is important because most oil spills include mixed crude oil types and the ability to clean up different lengths of a hydrocarbon chain with one technology is valuable. (Gassmann. 2019) According to my project, the BioCure technology could resolve various oil spill types with nearly 90% percent degradation. (Figure 2) My results indicate that BioCure technology employs bacteria with sufficient concentration of bio-surfactant which could break the chemical bound in the long-chain hydrocarbon, and bio-remediator could consume a large sum of heavy and light diesel regardless of the surrounding environment

All in all, for the central research question of my study, my experimental results show that BioCure technology could be a direction for improving the bioremediation project in San Francisco Bay. Additionally, for the first sub-questions of my project, the hydrocarbon degradation results of the control groups indicate that high biosurfactant concentration bio-remediators (BioCure technology) display a better degrading ability than the traditional bioremediation tactics. (Figure1) Also, for the second sub-question, the BioCure technology demonstrates a consistent hydrocarbon-degradation ability in different contexts of oil contamination (Figure 2)

Limitations and future directions

Therefore, future studies should focus on the cultivation of bacteria with genetics that allows it to have a stronger ability to generate biosurfactants. Another direction is to create a more efficient artificial bio-remediator like Vermitea. Depending on the relationship between plant biomass and hydrocarbon concentration, BioCure might trigger ecosystem recovery. Lettuce is proven to be a plant that is very sensitive to soil specifically, decreased hydrocarbon concentration not only reduces the soil toxicity but also enhances the chlorophyll contained in the soil. Since chlorophyll, the primary resource for photosynthesis, plays a vital role in plants' physiology and productivity, the whole ecosystem could benefit from the improved bioremediation technology. (Baruah et al.,2014) So, evaluating the impact of the anaerobic property of crude oil on the vegetation before and after the bioremediation by BioCure can indicate its biological value in environmental protection. Moreover, for my project, the lack of result comprehensiveness might be caused by the design of my experiment. The comparison groups should have also contained objects with different soil samples from other field stations in San Francisco Bay so that I could account for the error and bias led by the properties of soils in the hydrocarbon-degrading ability of bio- remediators. Also, the number of replicates needs to be more than 6, but I only created 3 due to the time and financial cost. With more data, I could construct a model to predicate the oil- degrading performance of BioCure technology under different conditions.

Broader Implications and conclusions

With my project, I have fully demonstrated the advantages and functionalities of BioCure technology in the context of oil-polluted wetland soils. Considering the bio-remediator with a high bio-surfactant concentration as the target object of the oil-degrading bacteria harvest process, researchers can save the time and funding cost of the harvesting strategy revision.

The commercial application of BioCure technology is proven to be reliable by my study on the oil samples from multiple clients who required bioremediation to fix the environmental issues led by their industrial crude oil emissions. Furthermore, as I used Vermitea to simplify the bioremediation process of BioCure technology, my experimental result can broaden the application of artificial oil-cleaners in environmental restoration projects.

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