Bioremediation And The Application Of Bacteria In Combating Anthropogenic Oil Spill Pollution

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CMNS2300: Critical thinking and writing for sciences and technology

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April 06, 2023

Abstract

Oil spills have caused major environmental disasters over the past decades, impacting marine environments and shorelines. The methods currently used by scientists to remediate these sites are inefficient and have resulted in major impacts on the ecosystem at low rates of success. Bioremediation is an innovative and environmentally friendly technique, consisting of the use of added or pre-existing bacteria for the degradation of hydrocarbons that make up the oil. Bioremediation has proven to have a major impact on affected sites and represents a promising mechanism for the degradation of organic waste. Scientists have developed three different methods for the application of bioremediation to the affected sites, bioaugmentation, biostimulation, and biosurfactants, with bioremediation showing good rates of hydrocarbon degradation while also being a safe alternative for the environment. Current bioremediation rate studies are performed under controlled lab settings producing inaccurate results. Future studies should be focused on determining the rates of biodegradation in the field due to environmental factors that could affect these rates. Studies on the use of fungi degradation should also be studied further.

Introduction

Oil spills are a major issue and have been the cause of some of the largest environmental disasters in history. In the past decade alone, the BP Deepwater Horizon Spill in the Gulf of Mexico and the Exxon Valdez spill along the Alaskan shoreline released an estimated 779 million and 41.6 million litres of oil respectively. Both spills had major impacts on the environment, ecosystem, and industry, and some areas have still not recovered completely.

Crude oil can be extremely toxic and difficult to remove due to hard-to-degrade components called hydrocarbons. Thousands of different hydrocarbons make up oil, they can be light, heavy, and aromatic (cyclic). During both spills, different strategies for oil spill cleanup were implemented: the use of chemicals, mechanical extraction, and burning. However, these methods were not entirely effective, were expensive, and impacted the environment long after the spill itself.

Exxon Valdez 41.6 million liters	BP Deepwater Horizon 779 million liters estimated by the National Incident Command's Flow Rate Technical Group (FRTG)
North Slope Heavy Oil (API 29) tanker spill with known volume discharged as surface spill on Bligh (near island shorelines) impacted cobble/rocky shorelines, major storm in area with 50 mph winds 2 days after spill bioremediation used extensively fate of oil remnants still studied more than 21 years after spill much scientific and operating experience gained is applicable to other spills	Light Louisiana Oil (API 35.2) well leak with uncertain flow rates, large amounts of methane also released discharged at well head in 1500 m of seawater 77 km offshore impacted deep-sea cloud of fine droplets of low concentration oil, marshes and sandy beach shorelines, 84 days to stop leak aerial and subsurface dispersants used extensively fate of oil remnants yet to be determined largest remediation and emergency response to an oil spill ever, worldwide

Table 1. Comparison of Exxon Valdez spill and BP Deepwater Horizon spills and impact. Table and data retrieved from "Oil Biodegradation and Bioremediation: A Tale of the Two Worst Spills in U.S. History" article (Atlas & Hazen, 2011).

As bioremediation is relatively new, research is still being conducted in trying to identify oil-degrading bacteria as well as determine accurate biodegradation rates. This is difficult as there are many environmental factors to consider when implementing bacteria. It is also unclear how bioremediation compares to current methods of oil spill cleanup.

This literature review aims to determine if the use of bacteria and bioremediation represents the final solution to anthropogenic marine pollution.

To answer this question, this paper examines the dangers of current common methods, the current known oil-degrading bacteria, the methods of bioremediation, and the current most accurate study on biodegradation rates. It will also examine the advantages and disadvantages of bioremediation.

Discussion

Due to the difficulties of physical removal, bioremediation, the process of organic waste degradation using microorganisms, has become a promising alternative. Bioremediation proved effective in the Exxon Valdez and Deep Water Horizon spills prompting extensive laboratory tests. Trials were performed to determine whether the addition of fertilizer would enhance the rates of oil biodegradation (Atlas & Hazen, 2011).

Crude oil is composed mostly of organic hydrocarbons, which can occur naturally in aquatic biomes, and act as an essential part of the ecosystem. Most hydrocarbons present in crude oil can occur safely in marine ecosystems in low concentrations. Indigenous bacteria have adapted to degrade these molecules naturally under ideal conditions, (Atlas & Hazen, 2011) converting them to carbon dioxide and water.

Bioremediation involves the use of these microorganisms, whether native or additive, which is enhanced by chemicals and nutrients to maximize degrading efficiency. Studies conducted on rates of bioremediation found petroleum-hydrocarbon losses as high as 1.2% per day (Atlas & Hazen, 2011).

Current Common Methods

Common oil spill recovery methods used today include; mechanical, chemical, and burning. These methods are not fully effective and are highly harmful to the environment.

During the BP spill, one of the first responses was to apply more than 1 million gallons of the oil dispersants Corexit 9527A and Corexit 9500A to the sea surface and more than 770 thousand gallons to the sub-sea (Rico-Martínez et al., 2013). However, this dispersant was highly toxic when combined with oil at a 1:30 ratio. Burning methods are also considered highly toxic because of the gas generated which contributes to greenhouse gasses.

Based on historical application, the only method that does not have a significant negative impact is mechanical removal. This consists of the use of floating booms to contain the spilled oil. However, this method is expensive and not highly effective, collecting only around 3% of the oil spilled during the BP spill (Ramseur, 2011).

Types of Bacteria

Microorganisms possess a wide variety of metabolic pathways, enabling countless microbes to be oil degraders. Yeast, fungi, bacteria, and archaea have all shown the ability to break down hydrocarbons.

Bacteria were the first microbes recruited to consume oil and therefore have been studied and implemented the most. These bacteria have been isolated from oil-rich environments where they thrive by consuming oil hydrocarbons for energy and nutrients. Once grown in the lab their DNA can be sequenced to identify enzymes and oil-degrading pathways as well as to observe the rates at which oil is degraded. As of 2016 over twenty individual microorganisms had been isolated, identified, and investigated.

Table 2 is collected from “A Review on Biotechnological approaches applied for marine hydrocarbon spill remediation” by Ramahi published in 2022. It shows all of the bacteria studied as of 2016 as well as whether the bacterium is aerobic or anaerobic, whether its genome has been sequenced, the target oil component (alkanes, PAHs, etc.), and its habitat. The sheer size and variety in this table demonstrate the potential of bioremediation. There is a wide range of bacteria that can be utilized in different habitats for specific oil targets as well as operating with or without oxygen. This means that bioremediation could be instituted in virtually any environment. With more research, this list will only grow larger making bioremediation an even more viable means of oil spill cleanup.

A/AN	Genome	Microorganism	Phylogeny	Target Substrate	Habitat	References
A	Y	*Alcanivorax borkumensis*	*γ-proteobacteria, Alcanivoracaceae*	*n*-alkanes	Seawater, sediment, beach sand, coastal salt marsh	[8,9]
A	Y	*Alcanivorax dieselolei*	*γ-proteobacteria, Alcanivoracaceae*	*n*-alkanes	Seawater, sediment	[10]
A	Y	*Marinobacter hydrocarbonoclasticus*	*γ-proteobacteria, Alteromonadaceae*	n-alkanes, PAHs	Seawater, sediment	[11]
A	Y	*Cycloclasticus pugetii*	*γ-proteobacteria, Piscirickettsiaceae*	PAHs	Sediment	[12,13]
A	Y	*Oleispira Antarctica*	*γ-proteobacteria, Oceanospirillaceae*	*n*-alkanes	Seawater	[14]
A	N	*Oleibacter marinus*	*γ-proteobacteria, Oceanospirillaceae*	*n*-alkanes	Seawater	[15]
A	N	*Oleiphilus messinensis*	*γ-proteobacteria, Oleiphilaceae*	*n*-alkanes	Seawater, sediment	[11]
A/AN	Y	*Pseudomonas pachastrellae*	*γ-proteobacteria, Pseudomonadaceae*	*n*-alkanes, PAHs	Sediment, beach sand	[16,17]
A/AN	Y	*Pseudomonas stutzeri*	*γ-proteobacteria, Pseudomonadaceae*	*n*-alkanes, PAHs, BTEX	Seawater, marine sediments, beach sand	[18]
A	N	*Halomonas halodurans; Halomonas organivorans*	*γ-proteobacteria, Halomonadaceae*	*n*-alkanes	Seawater, sediment	[19,20]
A	Y	*Thalassolituus oleivorans*	*γ-proteobacteria, Oceanospirillaceae*	*n*-alkanes	Surface seawaters, sediments, coastal and estuarine areas	[21]
A	Y	*Alteromonas naphthalenivorans*	*γ-proteobacteria, Alteromonadaceae*	PAHs	Seawater, tidal flat sediment	[22]
A	Y	*Acinetobacter venetianus*	*γ-proteobacteria, Moraxellaceae*	*n*-alkanes	Surface water, sediment.	[23]
A	Y	*Dietzia maris*	*Actinobacteria, Dietziaceae*	*n*-alkanes, PAHs	Seawater, deep sea hydrothermal field	[24]
A	N	*Rhodobacter* sp. *SS12.29; Rhodococcus* sp. *ice-oil-488 s*	*γ -proteobacteria, Rhodobacteraceae*	PAHs	Seawater	[25]

A/AN	Genome	Microorganism	Phylogeny	Target Substrate	Habitat	References
A	N	*Sphingopixis* sp.	*γ -proteobacteria, Sphingomonadaceae*	PAHs	Seawater	[25]
AN	Y	*Desulfatibacillum alkenivorans*	*γ -proteobacteria, Desulfobacteraceae*	*n*-alkanes	Sediment	[26]
AN	N	*Desulfosarcina-Desulfococcus cluster strains*	*γ -proteobacteria, Desulfobacteraceae*	Short chain *n*-alkanes	Sediments of marine HC seeps	[2,27]
AN	N	*Desulfococcus oleovorans*	*γ -proteobacteria, Desulfobacteraceae*	*n*-alkanes, aromatic HCs	Sediment	[28]
A	Y	*Bacillus pumilus*	*Bacilli, Bacillaceae*	*n*-alkanes, PAHs	Sediment	[18,29]
A	N	*Bacillus stratosphericus*	*Bacilli, Bacillaceae*	PAHs, BTEX	Seawater	[6]
AN	Y	*Archaeoglobus fulgidus*	*Euryarchaeota, Archaeoglobaceae*	*n*-alkanes	Shallow marine hydrothermal system	[30]
AN	Y	*Thermococcus sibiricus*	*Euryarchaeota, Thermococcaceae*	*n*-alkanes	Oil reservoir	[31]
AN	Y	*Ferroglobus placidus*	*Euryarchaeota, Archaeoglobaceae*	Aromatic HCs	Shallow marine hydrothermal system	[32,33]
AN	N	*Dothideomycetes-related taxa*	*Fungi*	PAHs	Beach sediment, tarballs, salt marshes	[34,35]

Table 2. Shows current bacteria researched for oil-degrading properties. Retrieved from "A review on biotechnological approaches applied for marine hydrocarbon spills remediation", (Rahmati 2022)

Types of Bioremediation

The application of bioremediation in the field can vary, however, it can generally be categorized into three primary methods; bioaugmentation, biostimulation, and the use of biosurfactants. The core idea of these methods is to manipulate the microbiota or environment in some way to maximize the oil-degrading efficiency of the indigenous bacteria community.

Although several species of bacteria have been studied for their oil-degrading properties as of now it is not as simple as injecting them into a pollution site and waiting (Smith & Kostka 2014). When adding a foreign bacterium to a pollution site there are environmental factors affecting the survivability and degradation rates of the bacteria. There are also risks to the ecosystem as any additive bacteria can become invasive. To overcome these issues, scientists use bioaugmentation, biostimulation, and biosurfactants.

Bioaugmentation

Bioaugmentation is the addition of bacteria to an indigenous consortium to aid in oil consumption. These bacteria can be oil degrading themselves or release enzymes/chemical energy that can be used by the indigenous bacteria. Studies have shown that bioaugmented communities can degrade 60-75 % of diesel oil in only 40 days (Ramahti 2022). An example of a bacteria utilized in this way is Alcanivorax borkumensis which releases alkane hydroxylases and lipases that cleave hydrocarbons into easily consumable fragments.

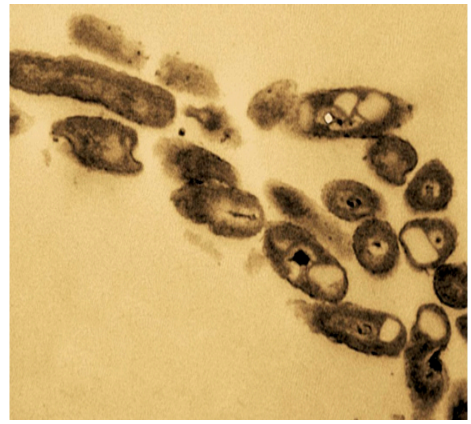

Figure 1.Alcanivorax borkumensis, an oil degrading bacteria found at the Deepwater horizon leak site, viewed under a microscope. (Lab Roots)

Biostimulation

Biostimulation is the optimization of environmental factors to maximize oil degradation. This involves the addition of chemicals that enhance bacterial growth and boost metabolic activity. The most common additives are phosphate salts and nitrate (Ramahti 2022) which are natural biodegradable nutrients. This method poses no environmental or ecosystem risk making it a very viable method while also being relatively efficient as some studies have shown rates of up to 70% of diesel degradation in only 30 days (Ramahti 2022).

Biosurfactants

Biosurfactants can disperse oil as droplets in water making it easier to degrade. Bacteria are able to produce amphipathic molecules that are able to decrease the surface tension of oil and form micelles in water. Studies have shown these molecules act similarly to man-made chemical dispersants while not leaving toxic chemical byproducts at pollution sites after clean-up has concluded.

Experimental Rates

The rates at which bacteria can degrade oil is an under-researched and complicated subject. However, there are a number of studies on bacterial oil degradation rates like the ones performed by Jerin in 2020 and Wu in 2016. These were performed under lab conditions and did not reflect the actual rates that are observed in the field (Antoniou 2022). Antoniou and his team developed the first high-pressure collection and test apparatus and performed the first experiments on oil plume degradation while keeping bacteria in environmental conditions.

With bacteria observed in conditions resembling their environment, this study was a proof of concept for in situ bioremediation and provided accurate biodegradation rates of alkanes by indigenous bacteria in the eastern mediterranean sea. This proves that bioremediation is an efficient oil spill clean-up strategy.

The results showed these bacteria possess rapid degradation rates of light and heavy alkanes as well as polycyclic aromatic hydrocarbons (PAHs). Light alkanes displayed half-lives between 0.6-9.5 days, heavier alkanes were 18-40 days and polycyclic aromatic hydrocarbons were 10-64 days. These rates decreased even more with the use of dispersants to 0.6-3.2 days of light, 2.1-7.7 days for heavy, and 265 times faster for PAHs. Figure 2 is a graph retrieved from this study showing the rapid degradation with and without dispersants.

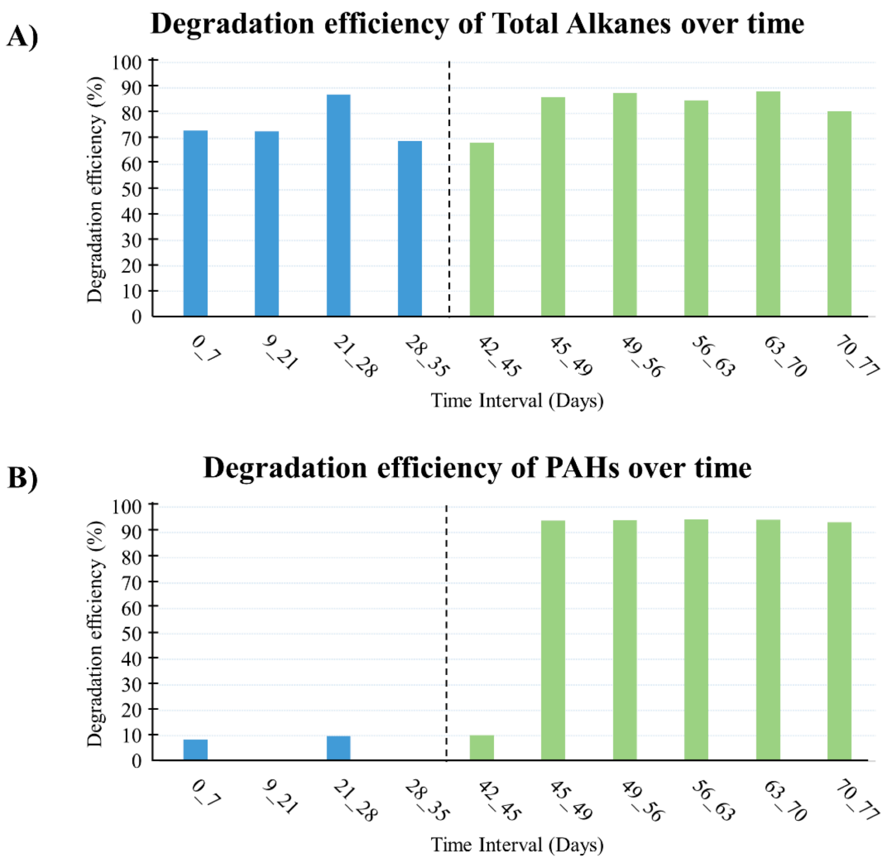

Figure 2. Total alkanes (A) and PAHs (B) degradation over a time interval where the blue bars are just oil and the green bars are oil plus dispersant, retrieved from "Emulating Deep-sea bioremediation: Oil plume degradation by undisturbed deep-sea microbial communities using a high-pressure sampling experimentation system" article (Antoniou 2022).

Advantages and Disadvantages

The main advantage of bioremediation compared to other cleaning methods is its flexibility to be applied under different environmental conditions. Rates can vary due to the pH and nutrient availability in the soil. The optimal pH is 5.5-7.8, depending on the bacteria, and the optimal temperature in marine ecosystems is 15-20°C (Ramahti 2022).

However, if environmental conditions are not ideal, this can be solved by applying fertilizers. The availability of molecules that can donate and accept electrons in energy-producing redox reactions is directly proportional to biodegradation efficiency. The addition of a molecule like oxygen through oil snorkels (Ramahti 2022) in aerobic systems is a great example of this as it can act as an electron acceptor to make energy and also become incorporated hydrocarbons to form water.

Performing ex-situ bioremediation is also an alternative in harsh environments, this consists of the creation of a closed system degradation away from the original site of the spill and can be performed independently of environmental conditions.

As stated before, bioremediation is a natural method with no toxic byproducts while playing an essential role in marine environments. It is considered to be a clean and better alternative to traditional methods.

However, the main disadvantage of bioremediation is that it is a slow process and it can take decades to completely degrade the oil hydrocarbons from the soil when not enhanced.

Conclusion

Current oil spill treatment strategies like using chemical dispersants, mechanical extraction, and burning come with significant drawbacks. They can damage the environment, are expensive, and are relatively inefficient. Bioremediation is an innovative technique involving the use of oil-degrading bacteria to clean up spills. It has been applied in the past with great success but is yet to be implemented on a large scale as governments tend to administer traditional methods.

However, bacteria are abundant in nature possessing a great variety of metabolic pathways. Because of this bacteria could be used for virtually any spill region with various specific conditions while not having negative impacts on the local ecosystem and environment. Researchers have developed three methods of bioremediation which all show promise in lab study; bioaugmentation, biostimulation, and biosurfactants. Based on recent studies and analyzed data on biodegradation rates it is apparent that this bioremediation is also a very efficient cleanup alternative.

Bacteria are easily accessible, efficient, and a natural part of the marine environment. As more research is conducted identifying more oil-degrading bacteria and developing more efficient methods in which bacteria can be utilized, it is evident that bioremediation is the final answer to combatting oceanic oil spill pollution.

Recommendations

In our research, we found several studies of biodegradation rates however all of these except the study conducted by Antiniou were done in a lab setting. We deemed these results inaccurate as they didn't represent a natural environment. We would recommend more studies of rates under environmental conditions i.e high pressure, low or high temperatures, and specific levels of pH and salinity.

Our review primarily focused on bacteria, however, we found that fungi could actually pose an even more efficient means of bioremediation. They possess the same diversity as bacteria and inhabit the same marine ecosystems but could be more efficient through the formation of extended mycelial networks which show increased overall efficiency and resistance to oil toxicity (Velez 2019). However, there is very little research or application on fungi. We would recommend an investigation comparing bacteria to fungi to conclude which organism would be more effective.

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