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Oil Spill Cleanup: Role of Obligate Hydrocarbonoclastic Bacteria

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Introduction

Over 70% of earth's surface is made up of oceans, which are essential for sustaining the life and controlling the planet's temperature. The abundance of marine life supports ecosystems by supplying essential services like carbon sequestration, oxygen generation, and climate regulation. Beyond their importance to the ecosystem, seas are essential for sustaining economy, providing food, assisting with industry, and promoting leisure and cultural pursuits. Oceans are used as way of transport between the countries for their trade majorly oil trade by the shipping industries. The global crude oil market is estimated to generate trillions of dollars in revenue annually. In 2022, the total value of global crude oil production was approximately $2.8 trillion, with OPEC countries accounting for a significant portion of this revenue. Trade activities can pose a risk of accidents, potentially resulting in oil spills. During the trade there may be possible of causing the accidents which leads to oil spill. This not only causes the pollution but also inflict harm upon marine life and huge economic loss. To mitigate the adverse impacts of oil spills, prompt cleanup is imperative. While conventional methods involving physical and chemical processes are employed globally, they often prove time-consuming and expensive. An economically viable and eco-friendly alternative involves use biological organisms, such as oil-eating bacteria, to efficiently remove oil from the ocean surface. Hydrocarbonoclastic bacteria (HCB) represent a diverse group of prokaryotes with the unique capability to degrade and utilize hydrocarbon compounds as a source of carbon and energy.

Impact of Oil Spill on Aquatic Organisms

Oil spill disaster have severe economic consequences, affecting various sectors and leading to short-term and long-term financial losses. Approximately 1.3 million tonnes of petroleum are believed to enter the marine environment annually. Oil spills can kill a lot of sea animals. For example, more than 66.7% of the different kinds of sea animals (including worms, shellfish, crabs, and insects) were killed on the beaches that were the worst affected by the Prestige oil spill 2002 when compared to previous data of 1995 and 1996 (De *et al*., 2005). Hydrocarbons contaminate marine birds and mammals' feathers and fur, causing the loss of water-repellent properties, which can lead to death due to hypothermia**.** Some of hydrocarbons will be remain for long time (such as PAHs) affecting physiological, genetic, growth and fecundity of marine organisms such as, after the Braer oil spill in the Shetland Islands in 1993, there was a fishing ban for more than 6 years because the fish and shellfish were contaminated with oil. Figure 1 depicts the fate of oil in marine ecosystems, revealing that when spilled in the marine environment, oil follows an intricate pathway and undergoes a prolonged natural degradation process. There is an urgent need to develop a cost-effective and efficient process for quickly removing oil from the sea. Utilizing microbial organisms through biological methods is a promising approach for achieving this goal.

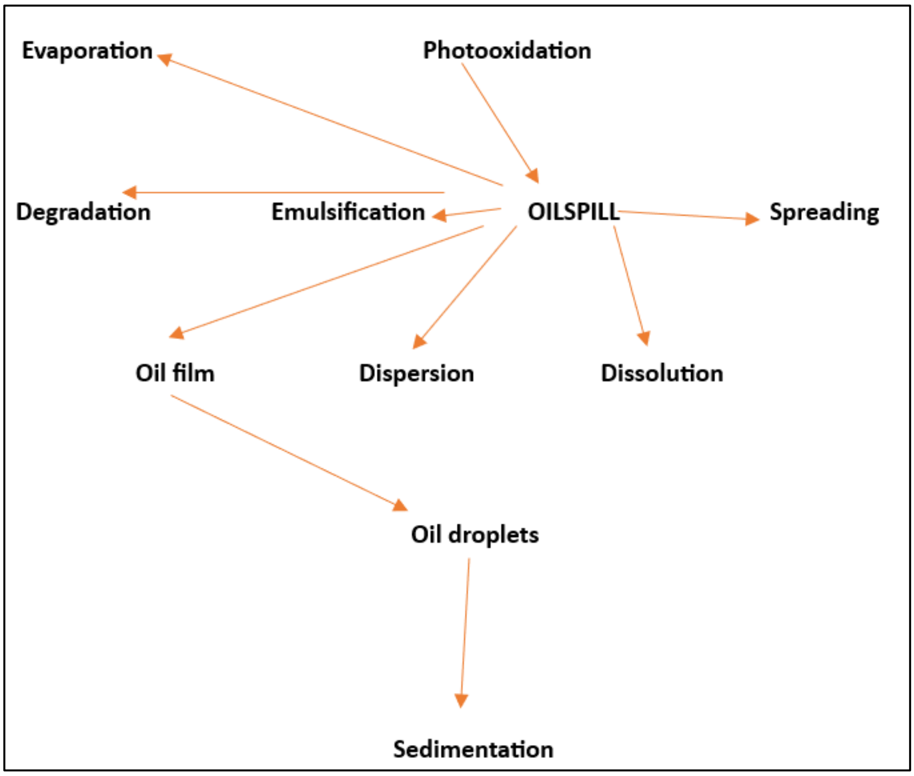

Figure 1: Fate of oil spill in marine environment

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Obligate Hydrocarbonoclastic Bacteria (OHCB)

Hydrocarbonoclastic bacteria utilizes hydrocarbons as a substrate. Some of these bacteria use exclusively hydrocarbons as growth substrates known as obligate hydrocarbonoclastic bacteria (OHCB) which plays a significant role in biological removal of petroleum hydrocarbons. Hydrocarbon is the principal carbon source for more than 175 bacterial species across seven phyla of bacteria and archaea, and for a similar number of fungal genera. Only in the past 20 years have the most significant oil-degrading bacteria been discovered (e.g., *Cycloclasticus, Alcanivorax, Fundibacter, Oleis-pira16*) (Hazen *et al.*, 2016). OHCB are marine bacteria that belong primarily to the Proteobacteria subclass and have restricted growth capabilities. They were highlighted as "highly specialized obligate hydrocarbon utilizers" with a potential role in oil spill cleanup. According to taxonomy, they comprise the several species such as *Thallassolituus, Oleiphilus, Oleispira, Cycloclasticus*, and *Alcanivorax. Cycloclasticus* species flourish later with complex hydrocarbons, whereas *Alcanivorax* species colonize first. OHCB enrichment was reported in marine environments contaminated with oil, indicating their role in oil removal. After spilling the most prominent phenomenon was seen as the significant rise in the population of *Alcanivorax* species, which break down branched and straight-chain alkanes, and *Cycloclasticus* species, which break down polycyclic aromatic hydrocarbons. A variety of alkane hydroxylase systems allow *Acinetobacter* spp., which are frequently isolated from oil-contaminated marine environments, to metabolize both short- and long-chain alkanes. While *Thalassolituus* spp. occasionally outcompete *Alcanivorax* spp. in temperate environments, *Oleispira*, the obligate alkane-degrading psychrophile, is more frequently linked to oil spills in cold marine environments. The oil's composition and level of saturation, in addition to the surrounding environmental factors—temperature and nutrient concentrations in particular—all influence the microbial response to an oil spill at sea.

Distribution

The distribution of OHCB is worldwide. The species *A. borkumensis* that is linked to marine invertebrates appears to represent a unique ecological niche that has easily accessible hydrocarbons that the animal partners produce. Though they are also widely distributed, *T. oleivorans* and *Cycloclasticus* spp. have only been found in the Northern Hemisphere. Currently, 16S rRNA gene sequences of 59 *Thalassolituus*-like bacteria from microbial communities living in both terrestrial (subsurface caves and groundwaters) and marine (Baltic, Barents, Mediterranean, North, Okhotsk, and South China seas, as well as the Atlantic, Pacific,

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and Polar oceans) environments are available in the GenBank and RDP (Ribosomal Database Project) databases. The distribution of the psychrophilic OHCB *Oleispira antarctica*, in contrast to the cosmopolitan OHCBs covered above, is currently restricted to the colder waters found at high latitudes. According to 16S rRNA gene analysis, *Alcanivorax* strains were most prevalent after an oil spill.

Classification

Depending on substrate (hydrocarbon -aromatic or aliphatic) OHCB can be classified into two groups (figure 2). Whereas *Neptunomonas* and *Cycloclasticus* species have evolved to use a variety of polycyclic aromatic hydrocarbons, *Alcanivorax*, *Marinobacter*, *Oleiphilus*, *Oleispira*, *Oceanobacter*, and *Thalassolituus* species use a variety of branched- and/or straight-chain saturated hydrocarbons (Wentzel et al., 2007). But numerous other "non-obligate" hydrocarbonoclastic bacteria have been identified as marine bacteria that can break down naphthalene, phenanthrene, and chrysene. These bacteria belong to the genera *Vibrio*, *Pseudoalteromonas*, *Marinomonas*, and *Halomonas (*Melcher *et al., 2002).*

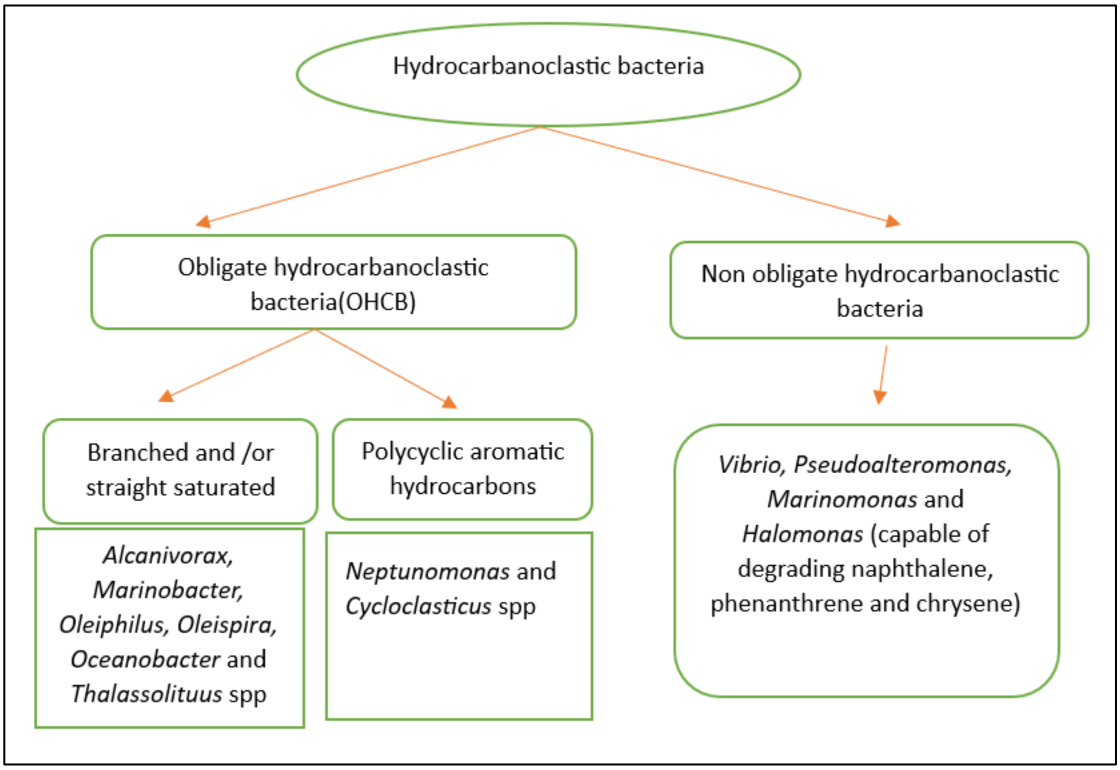

Figure 2: Classification of Hydrocarbonoclastic bacteria

Factors of Hydrocarbon Degradation process

Numerous hydrocarbons can be broken down by bacteria in both aerobic and anaerobic environments. Thus, while anaerobic hydrocarbon degraders are mainly found in anoxic sediments and within hydrocarbon seeps, aerobic hydrocarbon-degrading bacteria are

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widespread throughout the marine water column and in oxic sediments even in deep waters. At a depth of 2000–5000 meters, oil biodegradation occurs easily, as seen in the Arctic, Antarctic, and even polar ice (Bazylinski *et al.*, 1989). Aliphatic paraffins, which are straight-chain hydrocarbons, generally decompose more readily than aromatics, which are ring-shaped hydrocarbons. Hydrocarbons without double bonds, or unsaturated hydrocarbons, decompose more readily than those with double bonds. Compared to hydrocarbons without branches, hydrocarbons with branches break down more slowly. However, marine microbes also readily degrade the tiny, ring-shaped hydrocarbons, often known as low molecular weight aromatics, present in petroleum. The ability of microorganisms to utilize hydrocarbons as a source of carbon and energy has been recognized for nearly six decades. ZoBell (1946), highlighted three key factors regarding this process:

1. **Abundance of hydrocarbon-utilizing microorganisms:** A vast array of microorganisms possesses the capacity to metabolize hydrocarbons as their sole carbon and energy source.
2. **Ubiquitous distribution:** These microorganisms are widely distributed in various natural environments, indicating their adaptability and ecological relevance.
3. **Influence of oil composition and environmental factors on hydrocarbon utilization:** The efficiency of hydrocarbon utilization by microorganisms is significantly influenced by the chemical composition of the petroleum mixture and the prevailing environmental conditions.

7. Breakdown of Hydrocarbons

7.1. Enzymatic breakdown

OHCB contain dioxygenases enzyme which is membrane-bound and specific to compound classes. Because oxygenases are group specific, it follows that only a combination of different microbes can effectively degrade crude oil and petroleum fractions. Some focus on cyclic or aromatic hydrocarbons, while others break down specific alkane fractions.

7.2. Use of Biosurfactants

Biosurfactants increase the surface area of insoluble compounds by building up at the interface of immiscible fluids, reducing surface tension. This increases the bioavailability of hydrocarbons, which in turn causes them to biodegrade. It is known that the majority of microorganisms that break down hydrocarbons produce biosurfactants, which help emulsify hydrocarbons and biosurfactants, which considerably decreased the oil-water interfacial surface tension, caused a notable rise (14–32%) in the relative abundance of 16S rRNA genes

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linked to obligate hydrocarbonoclastic bacteria (OHCB). As microbial biosurfactants are amphiphilic extracellular compounds with hydrophilic and hydrophobic moiety, they can lower surface tension and assist in uptake of hydrocarbon, emulsification and dispersion of hydrocarbons.

8. Facilitating the growth of OHCB

Bacteria need utilizable, sufficient sources of nitrogen, phosphorus, sulfur, iron, and oxygen in order to grow and multiply on hydrocarbons spilled seawater. For effective hydrocarbon biodegradation, nitrogen and phosphorus must be added to seawater because it is a desert in these elements. In actuality, the supply of nitrogen and phosphorus is the rate-limiting step in the bioremediation of petroleum pollution in the sea. Fertilizers like urea, ammonium phosphate, nitrates, and phosphates can be used to meet the requirements for phosphorus and nitrogen. The primary nitrogen waste product of many insects, birds, bats, and terrestrial reptiles is uric acid. It sticks to hydrocarbons and is poorly soluble in water. These characteristics, along with the fact that uric acid makes up the majority of the widely accessible, low-cost guano fertilizer, led us to believe that it could be a valuable source of nitrogen for the bioremediation of petroleum pollutants in open systems. Uric acid is a naturally occurring product that is widely used by a variety of bacterial species, including hydrocarbonoclastic *Alcanivorax* strains, as a source of nitrogen. It was shown that commercial uric acid was a useful source of nitrogen for the growth of marine bacteria on crude oil using a simulated open system.

9. Cometabolism

The process by which a contaminant is unintentionally broken down by an enzyme or cofactor during the microbial metabolism of another compound is known as cometabolism. Cometabolic bioremediation has been used to treat a wide range of petroleum compounds in various settings, both aerobically and anaerobically. Oxygenases, such as methane monooxygenase, toluene dioxygenase, toluene monooxygenase, and ammonia monooxygenase, are necessary for the activity of numerous aerobic cometabolic biodegraders. These enzymes are incredibly potent oxidizers; methane monooxygenase, for example, is capable of breaking down more than 300 different substances. Furthermore, pulsing an electron donor or acceptor may be necessary for cometabolic biostimulation in order to lessen competitive inhibition between the contaminant and the substrate that the microbe can use. Methane pulsing has been shown to greatly enhance the rates at which methanotrophs biodegrade trichloroethylene (TCE), creosote, and oil.

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10. Conclusion

Hydrocarbonoclastic bacteria play a crucial and beneficial role in the process of oil spill cleanup. These specialized bacteria have the remarkable ability to metabolize and break down hydrocarbons present in crude oil, contributing to the natural degradation of oil pollutants in the environment. Through bioremediation, hydrocarbonoclastic bacteria offer a sustainable and environmentally friendly solution to mitigate the adverse effects of oil spills. This process harnesses the power of microbial communities to restore ecosystems impacted by oil contamination, highlighting the potential for bio-based approaches in addressing environmental challenges.

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