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REVIEW ARTICLE

Bacterial Degradation of Polycyclic Aromatic Hydrocarbons for sustainable environment: An overview

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ABSTRACT
Nowadays, activities associated with petrochemical industries are among the crutial environmental concerns in terms of releases of hydrocarbon contaminates in ecosystem. These are the organic toxic pollutants associated with neurotoxic and carcinogenic activities in humans. Conventional methods for disposal of these pollutants have their limits in terms of effectiveness and high cost. Application of nonpathogenic microbial stains for solving this problem is a promising tool for treatment of contaminated sites. These biological agents i.e. "Microbes", utilize complex toxic hydrocarbon contaminates into their metabolic cycles and transform them into simple nontoxic forms with complete mineralization. Various physical and chemical factors are responsible for influencing the phenomena. Numerous residential micro flora naturally do have inbuilt properties for degrading these toxic pollutants from soil and water ecosystems. The present review deals with the successful bioremediation techniques and approaches practiced using microbes for petroleum hydrocarbon degradation. The road ahead for implementation of these organisms for the welfare of the environment and finally, public health is indeed long and worthwhile.
Keywords: *Petrochemical industry, Hydrocarbon contamination, Bioremediation, Residential microflora.*

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INTRODUCTION

In daily life and industrial applications, petroleum based products are the key sources for energy, but accidental exposes of these hydrocarbons by anthropogenic activities into the ecosystems (water and soil) causes major problems in terms of pollution [1]. Random discharge arises usually from refining, expedition and transport of fossil gasoline & fossil fuel compounds storage. Contamination of soil by using natural compounds causing harms to the neighborhood system as an accumulation of harmful elements causes lethal effect or mutation in plant tissue and animals [2].

The time in general utilized for the dirt amendment comprises of mechanical, covering, vanishing, scattering and clothing. Nonetheless, the advances square degree sumptuous and can reason fragmented disintegration of impurity. The method of bioremediation, portray just as used of a bacterium to expel poisons inferable from their mixed metabolic abilities is a developing strategy for the evacuation and corruption of numerous natural contaminations together with the consequences of oil industry. Also, bioremediation innovation is frequent to be noninvasive. Biodegradation through characteristics populaces of bacterium speaks about the vital gadget through which oil and other organic impurities can be expelled from the earth.

The potential of oil smooth bioremediation is based aloft one's accommodation to installation and collect up altitude that abutment larger oil biodegradation raise inside the attenuated condition.

The oil concrete and admixture attributes and oil apparent place are moreover analytical enticements of bioremediation prosperity. Two altered solutions are there to deal with bioremediation of oil discharge: (a) Bio-stimulation, where the development integrated oil degraders are activated with the aid of the gain of dietary supplements or added improvement co-substrates, & (b) Bio-augmentation, where oil generic aspersing bacilli are supplemented to the typical microbial population.

A lot of absolute investigations accept focused on assessing the elements influencing oil bioremediation or testing advantaged items and gratifies across analysis ability thinks about [3].

MECHANISM OF PETROLEUM HYDROCARBON DEGRADATION

Aerobic conditions bring about the speedy and whole degradation of many organic pollutants [4]. Oxidative process includes start of intracellular attack of organic pollutants and oxygenases and peroxidases are responsible for the enzymatic key reaction of activation and oxygen incorporation. The stepwise organic pollutants transformation to common of the central intermediary metabolism occurs by degradation of secondary pathways, example is the tricarboxylic acid cycle. Acetyl-CoA, central precursors, pyruvate, succinate metabolites results into the biosynthesis of cell biomass. Gluconeogenesis helps in the synthesis of sugars that are important for growth and other biosynthesis process. Petroleum hydrocarbons degradation is started by specific system of enzymes. Initial charge of oxygenases on xenobiotics is shown in figure 3 [4].Some other mechanisms include (1) Microbial cell attachment with substrate (2) Biosurfactants production [5]. The pathway of biosurfactant production is well known and studied, however the mechanism of uptake that is associated to the bond of oil droplet with cell is still unspecified.

DEGRADATION OF ORGANIC COMPOUNDS BY MICROBES

Oil biodegradation is a framework which depends upon the attributes & upon ad measurement of organic compounds. Hydrocarbons of oil can be an alone into some instructions: the aromatics, the pitches (carbazoles, pyridines, amides, quinolones, and sulfoxides), the soaks, and the asphaltenes (porphyrins, ketones, phenols, esters and unsaturated fatty acids) [6].

Some strains of bacteria, in particular, *Bacillus subtilis, Pseudomonas flurescens, Alcaligenes sp., Micrococcus roseus, Acinetobacterlwoffi, Corynebacterium* sp., Bacillus sp., Flavobacterium sp., and P. aeruginosa were apprenticed through attenuated beacon which could attenuate unrefined petroleum.

Microorganisms square measure the form gt dynamic specialists in oil corruption, and that they fill in as essential degraders of spilled in oil corruption, and that they fill in as essential degraders of spilled oil in condition [7]. A number of microscopic organisms square measure even renowned to sustain specially on hydrocarbons [8]. Passage [9] recorded twenty five genera of organic compound corrupting microbes and twenty five genera of organic compound dishonorable growths that were separated from marine condition. A comparative arrangement by Bartha and Bossert [10] enclosed twenty two genera of microscopic organisms and thirty one genera of parasites. In previous days, the degree to that microscopic organisms, yeast, associated thin parasites take an interest within the biodegradation of oil hydrocarbons was the topic of unnatural investigation, nevertheless gave off a bearing of being part of the biological community and neighborhood ecological conditions. Das and Mukherjee accounted unrefined oil from oil attenuated clay from North East Indies. Acinetobacter sp. was absolute for the adapted abusage of n-alkanes of amendment breadth C 10- C 40 as a sole babyhood of carbon [11].

Soil, marine, fresh water have different degradation rate marine 15 to 20°, soil 30 to 40°, and fresh water 20 – 30 °(figure 1).

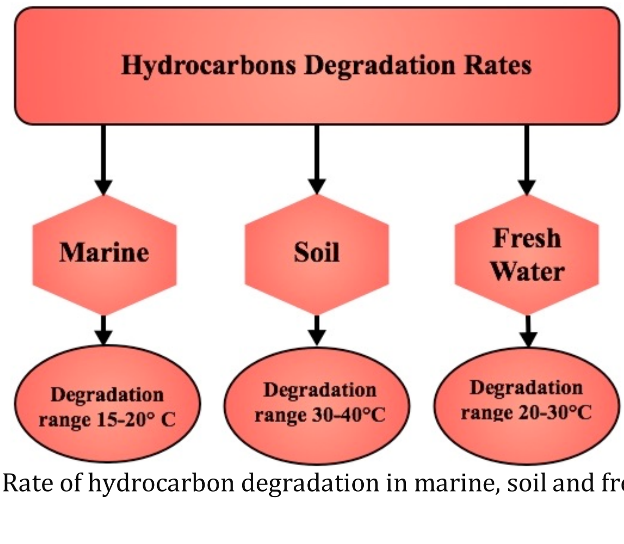

Figure 1: Rate of hydrocarbon degradation in marine, soil and fresh water.

FACTORS AFFECTING MICROBIAL DEGRADATION

- **Temperature**

Temperature plays a very significant role to degrade PAHs by the availability of the in situ microorganisms. Predominantly throughout the year temperature is not optimum at contaminated sites for the process of bioremediation.PAHs solubility increases with temperature which ultimately enhances the bioavailability of PAH molecules. Whereas, if there is rise in temperature the solubility of oxygen decreases because of which the metabolic activities of microorganisms decreases. There is wide range of temperature at which PAHs gets degraded, many studies believes and focuses on mesophilic temperature instead of transformation at very high or low temperatures. However, it is apparent that microorganisms have adapted to metabolize PAHs at extreme temperatures.

In the process of bioremediation, the vital role is played by temperature [12].

Because of temperature not only hydrocarbons get affected but also microbes consuming them also get affected [13].

It influences chemical and physical state of contaminants, soil matrix, gas solubility, microbial growth rate, as well as metabolism of microbes [14].

As temperature raises hydrophobic pollutants solubility increases, viscosity decreases, transfer and diffusion long chain n-alkanes enhanced to the water phase from the solid phase stated by [15].

In the study of Thamer [16], they have proclaimed crude oil can be biodegraded by 80% with the help of B. thuringiensis in 27 days. In their study they have determined that it can happen because of the bacterial enzymes, emulsion material production, availability of optimal temperature and environmental factors, due to which the efficiency of bacteria can be raised in the process of degrading crude oil constituent.

Oxygen

For petroleum hydrocarbons (PHs) degradation in the environment the oxygen concentration has been adamant as the rate-limiting variable [17]. Type of soil and the rate of consumption of microbial oxygen determine the oxygen availability in the soil [18]. It is also denoted by some studies that anaerobic degradation of PHs by microorganisms can happen at negligence rates [18]. Substrate oxidation by oxygenases in the catabolism of all aromatic, aliphatic and cyclic compounds by microbial is a leading step in the biodegradation process [19].

Bioavailability

Bioavailability can be defined as the chemicals present in the soil which can be taken and transformed by living organisms. It also defined as the influence of the physical, chemical and microbiological factors to the extent and rate of biodegradation. By restricting the hydrocarbons bioavailability can put the significant effect on the community of microbes, pH, and the extent of deterioration of the hydrocarbon. The area which is accessible to microorganisms is the bio available part of hydrocarbons. PHs is classified as hydrophobic organic pollutants and also has low bioavailability. Chemicals have little water solubility that makes them resistant to photolytic breakdown [20].

Microbial community

PHs can be degraded by some algae, yeast, bacteria and fungi. Availability of microorganisms which can catabolise pollutant is the main aspects which influence petroleum hydrocarbon degradation. Furthermore, contribution to the degradation of hydrocarbons in the soil has been made by fungi and bacteria. PHs has been utilized as a source of food by the microorganisms which can be effortlessly found in huge amount near oil contaminated places such as gas stations, shipping lanes, oil fields, oil seeps, ports and other places which have similar facilities.

- **Pressure and salinity**

Microbial growth and their products gets hinder due to high temperature and salinity [13]. It is described that salinity and pressure are two important attribute of ecosystem like deep seas and saline lake are polluted due to petroleum hydrocarbons. It has been noted by [21] that when there was no addition of NaCl than there was 41% of the degradation of crude oil (incubated for 4 months) where as it was 12% of the crude oil degraded when 50g/L NaCL was added. It was suggested by Qin [22] that the salinity majorly influences biodegradation and bioremediation process, as well as it also impacts growth of microorganisms and their diversity. Salinity adversely influences key enzyme's activity in the process of degradation of hydrocarbon [23].

Toxicity

Principle of biological treatment is to destroy pollutants and toxins from the inadequate environment by the usage of microorganisms. Lately, the usage of bioreactor for the treatment of contaminated PAH gas work soil evaluated both removal and buildup of oxy-PAHs, such as quinonens, PAH- ketones, as well as coumarins.

pH

There are many sites which are contaminated with polyaromatic hydrocarbons (PAHs) which are not at the optimal pH for bioremediation. Leaching of the materials present at the retired gasworks sites are responsible for the increase of pH of the naïve soil which results in the unfavorable conditions for microbial metabolism. Initial micro organisms present at the contaminated site will not have the capacity to transmute PAHs under acidic or alkaline conditions and the reason for this behavior of microorganisms is the pH of the contaminated sites

Therefore, it is necessary to regulate the pH of such sites.

Water activity

The cause of the restriction of hydrocarbon's biodegradation in terrestrial ecosystems is the availability of water for metabolism and microbial growth. With 30-90% water saturation in oil sludge biodegradation was optimal. There is a direct impact of availability of water on the growth and movement of microorganisms.

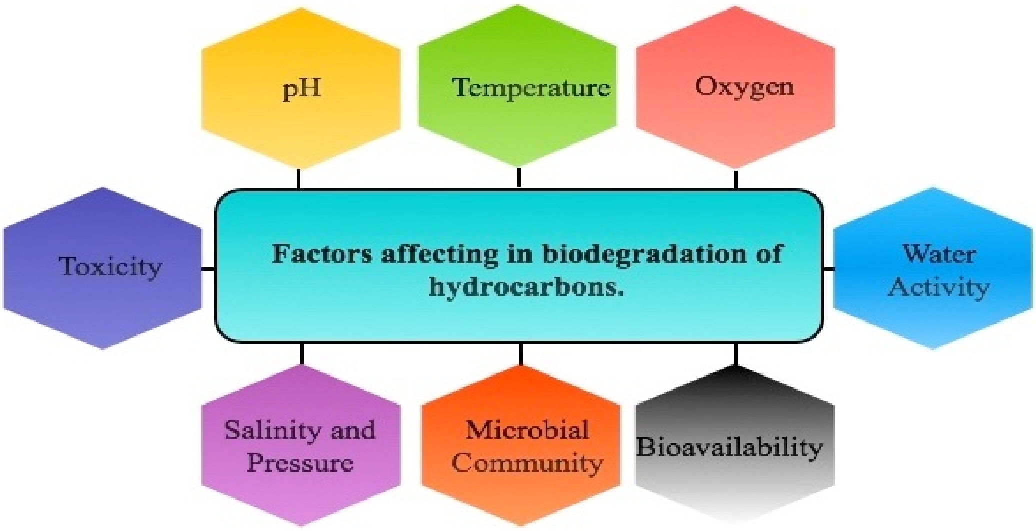

Figure 2: factors affecting biodegradation rate of hydrocarbons

COMMERCIALLY AVAILABLE BIOREMEDIATION AGENTS

Bioremediation agents defied by U.S.EPA that increase biodegradation rate to diminish the effect of discharge include enzyme additives, microbiological cultures, or nutrient additives [24]. Bioremediation constitutes of biostimulation agents and bioaugmentation agents that depend upon two important approaches to bioremediation of oil spills. Various products of bioremediation in early 1990s have been suggested and promoted by vendors, as "ultimate solution" to oil spills [25]. 15 bioremediation agents list has been compiled by U.S EPA which is a part of Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule and National Oil. The National Contingency Plan (NCP) and Clean Water Act. However, modification of record was done, and no. was deduced to 9 of bioremediation agents. The study demonstrated efficacy of bioremediation products in more in laboratory as compared in the field [26]. Reason behind this is complicated conditions of real world cannot be simulated in laboratory such as biological interactions, spatial heterogeneity, nutrient mass transport limitations, and climatic effects. Thus, studies on field and their applications are the only tests and more persuasively demonstrated for the efficacy of bioremediation products. There are limited nutrient additives which are revolutionized and are marketed specifically a soil spill cleanup which are commercial bioremediation agents. Reason lies as common fertilizers are inexpensive, readily available if properly used. But their limitations because of wave and tide action, various products of organic nutrient are that they are rapidly washed out. Various products of organic nutrients like product of oleophilic nutrient are marked as bioremediation agents by certain analysis. Four agents that have been put in this category by NCP Product Schedule are Inipol EAP22, Oil spill Eater II (OSE II), BIOREN 1 and BIOREN 2. Use of Inipol EAP22 (Societe, CECA S.A., France) as a nutrient add-on and popular bioremediation agent for oil spill cleanup. Oil Spill Eater II (Oil Spill Eater International, Corp) is other product. It is enzyme/ nutrient additive consisting of "readily available carbon, phosphorus, vitamins and nitrogen for rapid colonization of naturally occurring bacteria". European EUREKA BIOREN researchers did a field trial in an estuary environment to increase the potency of (BIOREN 1 and 2). The two nutrient products were procured from fish meals in a form of granules with urea and super phosphate as phosphorus and nitrogen sources and proteinaceous material as the carbon source. Main distinction between two formulations was biosurfactant present in BIOREN 1 and it showed that its presence was responsible for the evaluated oil degradation rate as compared to BIOREN 2 which is without any surfactant is less effective.

Phytoremediation

This technology use plants so as to supervise diversified environmental pollution problems that includes soil cleanup and underground contaminated with hydrocarbons and other life-threatening substances. Usage of Phyto-transformation, phyto-volatilization, rhizo-remediation, and hydraulic control, are various mechanisms to be used for the remediation of diverse contaminants. This is a cost effective phytoremediation process useful for the sites which have larger areas as well as shallow levels of residue with nutrient, metal or organic pollutants or where contamination is not a danger. The treatment required is small or where vegetation is used as a final cap and closure of the site [27]. In the in-situ treatment steps the usage of phytoremediation helps in decreasing the land disturbance and also helps in eliminating liability and transportation costs which are related with offsite disposal and treatment. Remediation system was effectively used and was further improved and phytoremediation novelty has helped in the treatment of petroleum hydrocarbons contamination over the past fifteen years have bestowed much applicable enlighten. The usage of this technology is for the contaminated sites remediation. Contaminant fate and pathways of transformation which also includes the metabolites identity, there is not much information. Barely a data exist which supports the removal rate of contaminants and efficiencies directly attributable to plants under field conditions. The interrogation at a site which is contaminated by hydrocarbons was done. Removal of total petroleum (TPH) was interrogated at several field site that were contaminated with crude oil, or petroleum refinery wastes and diesel fuel [28]. Primary remediation method, with trees grasses were often planted at sites with organic contaminants.

At tie and altering hydrophobic contaminants like as PAH, TPH, as well as BTEX, huge number of fine roots was found to be potent in the surface soil. Herbs and grasses planted in between the trees rows so as to stabilize the soil and to provide protection against wind-blown dust that could move contamination offsite. Peas (*Pisum* sp.), afalfa (*Medicago sativa*), as well as alsike clover (*Trifolium hybridum*) could be utilized to regenerate poor soil. Rye (Elymus sp.), reed canary grass (Phalarisarundinacea), fescue (Vulpiamyuros), and clover (Trifolium sp.) are grown especially at contaminated sites contaminated due to petrochemical wastes. The grasses could be disposed of as compost or burned after harvesting. For removal of diesel range organics in vegetated contaminated soils, Degradation of microbes in the rhizosphere is the best mechanism [29]. This is because of PAHs being highly hydrophobic and sorption to soil, their bioavailability decreases for the phytotransformation and uptake of plant

Petroleum Hydrocarbons Biodegradation by Immobilized cells

For the bioremediation of diverse toxic chemicals study and use of immobilized cells is done. The immobilized cells simplification for separation and recovery is done by immobilization and helps in making application reusable thus reducing the overall cost. Their study demonstrated increases in the connection linking cells & hydrocarbon's droplets & increased the rhamnolipid fabrication as a result of immobilization. In a study done by [30], the rate of crude oil biodegradation is increased by bacterial cell immobilizationas compared in a vast scale of culture salinity in terms of living cells. [31] Explained as a microorganism indigenous to the site and entrapment matrix polyvinyl alcohol (PAV) cryogelation was used. Laboratory biopiles were raised to differentiate microorganisms indigenous to the site and immobilized system that were in designate of diesel removal after 32 days percentage was found much better. The experiment was performed by Rahmanin 2004 which purposes to investigate the hydrocarbon degradation in which he used immobilized bacteria in alginate beads. Results of which manifested no decrease in activity of microbial consortium biodegradation though it was repeatedly used, hence from this it was concluded, the great application for the site contaminated by hydrocarbon bioremediation is immobilization of cells.

Genetically Modified Bacteria

Attention is given to the use of genetically engineered microorganisms (GEMs) in bioremediation for the improvement of degradation of life-threatening wastes under laboratory conditions. Various bacteria help in the degradation of pollutants present in our environment. Consist of the summary on the usage of genetic engineering technology for the improvement improves bioremediation of hydrocarbon contaminants using bacteria. Higher derivative capacity was shown by genetically engineered bacteria. However, Environmental and ecological concerns and regulatory constraints are some barrier for testing of GEM in the field; hence their resolving is very important for GEM to be effective in cleaning process at lower cost. Usage of GE bacteria was used for strain monitoring, stress response, toxicity assessment, endpoint analysis, and bioremediation process monitoring. The range of analyzed contaminants comprises of aromatic hydrocarbons, nonpolar toxicants, and chlorinated compounds. For successful in-situ bioremediation using genetically modified bacteria, essential elements are the combination of ecological and microbiological knowledge, biochemical mechanisms, and field engineering designs.

CONCLUSION

In the current situation some conventional method are there but microbial degradation is the method that is less expensive and cost effective. Microbial degradation is ecofriendly or harmless process. Cleanup of organic compounds in the sub layers action is a certifiable issue. This is believable on the area that microorganisms accepted mixture frame works to a base and use assorted organic compounds as a babyhood of carbon and vitality.

The utilization of hereditarily adjusted (GM) microscopic organisms speaks to an examination boondocks with wide ramifications. The potential advantages of utilizing hereditarily changed mircobes are huge. In any case, the requirement for GM microscopic organisms might be faulty for some instances which consider that indigenous species regularly execute enough however we don't tap the maximum capacity of wild species because of our constrained comprehension of different phytoremediation components, including the control of compound frameworks that debase poisons. Consequently, in a blaze of the current audit, its ability to be articular that microbial bribery can be advised as a key articulation in the cleanup arrangement for oil hydrocarbon remediation.

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