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REVIEW ON ENVIRONMENTAL DEGRADATION OF PETROLEUM HYDROCARBONS IN MARINE ENVIRONMENT

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ABSTRACT

Biodegradation is a process of breakdown of organic compounds into simpler compounds with the help of enzymes. Marine environment get contaminated by Petroleum hydrocarbons and these contaminates are removed by methods like chemical method but these are harmful to marine life through its toxic components. Another method is biological method in which degradation has been done with the help of microorganisms. In this process many bacteria take part for degradation because the composition of oil is different so single bacteria cannot degrade whole oil spill. Degradation can be done by two ways; aerobic degradation and anaerobic degradation, both processes have been done by different mechanisms and pathways because various types of bacteria are used in the process and other factors such as temperature, concentration etc are also variable. Despite of all this, still scientist need to understand more on highly toxic compounds like PAHs and photodegradation which is also very essential topic for consideration.

KEY WORDS: **Biodegradation, petroleum hydrocarbons, PAHs, toxicity, bacteria.**

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INTRODUCTION

Biodegradation is a process of breakdown of organic material into simpler compounds by the help of enzymes. These petroleum hydrocarbons contaminate the environment of water bodies. This fact encouraged the interest of scientist to investigate the fate of oil distribution in the marine environment[2-3]. Petroleum is also known as crude oil obtained from decaying organic matter. It contains more than thousands of compounds but mainly consisting of carbon and hydrogen. Petroleum is the major energy source in the world[1]. Sometimes such spills are naturally cleaned by a specific group of bacteria known as hydrocarbonoclastic bacteria (HCB). The growth and concentration of these bacteria increases extensively in areas of oil spill and by adding nutrients like nitrogen and phosphorous but it also has negative impact on ecosystem[4-9-10]. Best studied envoy of this group is *Alcanivorex borkumensis.* It is also the only one to have its genome sequenced. Many other toxic compounds are also present in crude oil like pyridine and they are degraded by specific genera like *Micrococcus* and *Rhodococcus.* Oil tar balls can be degraded by *Chromobacterium, Micrococcus, Bacillus, Pseudomonads, Candida, Saccharomyces* and others[17]. In the cleanup of the Deepwater Horizon oil spill, some scientist preferred genetically modified microorganisms for cleanup, but some scientists suspect they might have caused health issues for people in the affected areas[28-31]. Oil fields are uniformly present everywhere in the world but mainly in the Persian Gulf region and half of the production of crude oil is transported by sea through tankers. All tankers takes on counterbalance water but if discharged it will contaminate the marine environment and this cause major damage to our ecosystem. Other sources of contamination are industrial and other activities at near the harbor areas. The ratio of these sources is almost 50% comes from natural seepage, less than 9% from catastrophic release and about 40% of non tanker operational discharge and urban runoff[7]. Petroleum hydrocarbons sample comprise of thousands of organic compounds so it has a very complex structure because it does not contain single representatives. Of these compounds, once released into the environment by bacterial degradation or photodegradation, and create toxicity in the environment. It is complicated or even impossible, to discriminate between petroleum hydrocarbons of anthropogenic and biogenic origin. Scientists become attentive on the marine environment, because of the largest spills, these are The Exxon Valdez (1989), the Nahodka oil spill, the Erica spill (1999) and the Prestige spill (2002)[2-3]. Hydrocarbon contaminations are hazardous to the health because of its carcinogenic mutagenic and potent immune-toxicants they effects humans, animals and of course on plants. New methodological breakthroughs in sequencing, genomics, proteomics, and bioinformatics are imaging and producing vast amounts of information[1]. There are numerous mechanisms to degrade petroleum from the environment, both biological and chemical. The most well known are evaporation and degradation by bacteria, because it has less harmful effect on the environment because it depends on light temperature and other acidic factors.

1.1. Composition of oil

Hydrocarbons differ in their solubility, from polar to non polar compounds. Petroleum contain organo-metallic compounds other than hydrocarbons. Petroleum consists of the following constituents: saturates aromatics, resins and asphaltenes[3]. A Saturate hydrocarbon means no double bonds in their structure. They are categorized into alkanes and cycloalkanes. Saturates represents the most crude oil ingredients. Aromatic hydrocarbons with one or numerous aromatic rings are usually substituted with dissimilar alkyl groups. Resin and Asphaltenes contain non-hydrocarbon polar compounds with the addition of nitrogen, sulfur, and oxygen atoms. Petroleum recovered from different reservoirs varies extensively and according to its composition it may ranges from the very low molecular weight hydrocarbons to very high[3-7]. A hydrocarbons chemical structure affects its biodegradation in two ways. First, the molecule may contain substituents that cannot react with inducible enzymes. Next, the structure might decide the compound to be in a physical state where as microbial degradation does not occur easily. Often, the larger and more compound the structure of a hydrocarbon, the more little by little it is oxidized. Adding aliphatic side-chains increases the susceptibility of cyclic hydrocarbons to microbial attack[14-21]. The other factors also present on microorganism like microbes excrete emulsifiers that increase the surface area of the substrate. Some of these microorganisms are *Pseudomonas putida, Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus laterospore*[7]. Hydrocarbons can be very fluid component or very viscous and volatile non volatile component. Hydrocarbons are viscous component and viscosity of polluting oils is very important property and it determines the dispersion of the hydrocarbon mixture and also the surface area available for microbial attack. In the environment, the concentration of organic compounds also affects the level of tolerance as well as will affect the number of organism present. At low concentrations, all fractions are possibly to be attacked. Though, at elevated concentrations, only those fractions most susceptible to degradation will be broken down. It has been shown that the higher concentrations of gasoline in contaminated water were related to elevated counts of microorganisms[15]. Degradation of substituted cycloalkanes appears more readily than unsubstitued cycloalkanes because substituted cycloalkanes get easily oxygenated and can precede ring cleavage and then normally occurs as substituted forms[3]. Asphaltic components have complex structure which is difficult to analyze with current methodology so degradation and the metabolic pathway are less well understood. The elucidation of the biochemical fate of asphaltic petroleum compounds is a major challenge for future research on petroleum biodegradation[7]. The soluble hydrocarbons are more toxic and most harmful fraction for the environment than non soluble hydrocarbons. Water soluble fractions of aromatics and poly aromatic hydrocarbons (PAHs) are most harmful and consider as carcinogenic and mutagenic and come under toxicological studies and have low solubility only 3%. Most dangerous PAHs are benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene[21-25-30].

1.2. Fate of petroleum in the environment.

When petroleum comes in water it forms a layer and gets separate and forms a layer between water air and sediment. The insoluble part forms a layer of 0.01 to 3.0 mm thickness on the water layer. When the hydrocarbons have become very high concentration, then non-aqueous phase liquid (NAPLs) can be formed[19]. The remaining hydrocarbons are present in aqueous layer and form a film on the water surface. The lighter fractions are removed within twenty-four hours by evaporation. The evaporation range of hydrocarbonsdepends on its number of carbon atoms present, alkanes have possible until an 18 carbon chain. The mass loss of heavier oil is 0.1% and for lighter oil is 17.3% due to evaporation[25].

1.2.1. Hydrocarbons dissolved in water

In water, oil emulsion is formed due to the viscosity of the oil after evaporation of volatile compounds and the molecules that are absorbed in the sediment will settle down in and cannot be easily degradable. In fact bacteria are only able to degrade these hydrocarbons (e.g. degradation of PAHs) [3-19]. Only some fractions are dissolved in water after petroleum spill in the environment, and this can be as low as only 2%. Other parts are absorbed in the sediment. Lighter aromatic molecules are soluble in water, while the higher aromatic molecules containing 5 or more rings cannot be easily degradable and become persistent such as PAHs associated in sediment[23]. Recent research also revealed thatthe presence of humic acids can be very important for solvability of PAHs, because it is big molecules and easy to bind with PAHs. An important part of oil degradation in the aquatic environment is emulsification, because this leads to hydrated particles with properties differing from the molecular oil layer. It is mainly the polyaromatic or asphaltic fraction which is associated with emulsion formation[11-29]. There are several of the mechanisms known for petroleum degradation. The most studied ones are with the bacterial pathways. Light is also able to degrade a lot of petroleum hydrocarbons.

BIODEGRADATION OF PETROLEUM BY BACTERIA.

Bacteria are able to degrade most oil spills. The degradation speed of bacteria depends on the compounds, bacteria present and on the environment condition[26]. Different steps performed by different bacteria in the degradation pathway. These bacteria includes *Pseudomonads putida, Rhodococcus, Acinetobacter sp., Alcanivorax sp., Bijerinckia sp., Pseudomonads aeruginosa, Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Bacillus laterospore, Chromobactrium sp., Hallomonas cupida, Pseudomonas stutzeri, Bacillus pumilus, Bacillus sphericus, Micrococcus roseus and Micrococcus varians.*

Mechanism for petroleum degradation

There are mainly two metabolic machinery for the degradation of petroleum hydrocarbons. Aerobic degradation is most proficiently and hurriedly processed. Scientists discovered that anaerobic microorganism has the ability to oxidize and utilize crude as a complex organic substrate under aerobic conditions[10]. These microorganisms decompose most organic compounds into water, carbon dioxide and mineral matter, like nitrate, sulfate and various other inorganic compounds. They do not generate methane or hydrogen sulfide as reaction products like aerobic microorganisms. Reactions which are aerobic need lesser free energy for initiation and yield more energy per reaction[16-31]. The hydrocarbons are broken down by an enzyme-mediated reaction. In this reaction, oxygen acts as an external electron acceptor, whereas an organic component in the contaminating matter functions as the electron donor or energy source[22-31]. The general degradation pathway for alkanes involves chronological formation of an alcohol, an aldehyde and a fatty acid. This fatty acid which is formed by the previous reaction is cleaved, releasing carbon dioxide and forming a new fatty acid that is two carbon units shorter than the parent molecule in a process known as beta-oxidation. The initial enzymatic attack involves a group of monooxygenases[6-29-31]. The general pathway for aromatic hydrocarbons involves cis-hydroxylation of the ring structure forming a diol (e.g. catechol) using dioxygenase and then the ring is cleaved forming an acid. (e.g. muconic acid) and β oxidation process starts. The degradation pathway for a highly branched compound, such as pristane or phytane, may ensue by omega oxidation forming a dicarboxylic acid. In PAHs and phenol-degrading microorganisms, *Pseudomonads puitda* is mostly used strain in biodegradation. Other strains like *Bijerinckia* genus are also very active in aerobic hydrocarbon degradation[17-18]. The microorganisms which are extensively used in these process and the roles they are played in the process under anaerobic conditions during biodegradation is not fully understood[1]. In past few decades researches studying or investigate the following fact that oxygen is not available in all environments where hydrocarbons occur (e.g. in deep sediments, stagnant fresh and ocean waters and oil reservoirs) but still degradation occurs and finally they came to a decision with the discovery of new group of microorganisms in the late 1980s to degrade strictly under anoxic conditions[16-17-20]. Studies have confirmed that these microorganisms activate organic compounds by special biochemical mechanisms that differ completely from those employed in aerobic hydrocarbon metabolism.

Anaerobic biodegradation degrades PAHs, N-alkanes, branched alkanes, cycloalkanes, and some alkenes. Generally m, p-xylenes and toluene degrade under anaerobic conditions. Benzene degradation is one typical example of it. Aerobically Benzene is cleaved. On the further hand, it is typically recalcitrant under influence of nitrate-reducing conditions[17-18]. Toluene is one of the most important and most studied hydrocarbons with respect to enzymatic and genetic characterization in the denitrifying bacteria *Azoarcus sp*. In the proposed pathway of toluene, the addition of fumarate is mediated by benzylsuccinate synthase to form benzylsuccinate. Now the reaction proceeds from benzylsuccinate to acetyl CoA and benzoyl-CoA which is a central intermediate in the anaerobic degradation of aromatic compounds. Reductive dearomatization of Benzoyl-CoA occurs and ring cleavage followed by reactions that again resemble those in the β-oxidation reactions of fatty acids[17]. The metabolic pathway for sulfate-reducing bacteria is similar to that of toluene metabolism. In this case (1-phenylethyl) succinate was detected in *Azoarcus sp*. enrichment cultures signifying addition of ethylbenzene to fumarate. There is evidence that anaerobic degradation of initially *m*-xylene, which is in analogy to toluene, through *m*-methylbenzylsuccinate to *m*-methylbenzoyl-CoA[25-1].

FACTORS INFLUENCING BIODEGRADATION

There are various factors that influence the biodegradation of petroleum hydrocarbons like temperature, oxygen concentration, humic acid and salinity.

1.3. Temperature

Temperature is the first factor which is responsible for biodegradation because the biological enzymes have an optimum temperature of around 30 to 40 degrees Celsius for every degradation pathway. When the temperature becomes higher the hydrocarbons become toxic to the bacterial membrane and at lower temperature, they cause lower metabolic rates[12].

1.4. Oxygen concentration

Oxygen is an important factor in biodegradation process. The concentration of oxygen determines whether the reaction pathway is anaerobic or aerobic. Aerobic degradation is a lot faster than anaerobic ones. The bacterias in the aerobic degradation were able to degrade 20-25% of the organic material and 90-95% of the alkanes[12-27].

1.5. Humic acid

Humic acid is a big biomolecules and is able to bind a lot of organic compounds and due to this they are able to degrade PAHs (highly carcinogenic compounds). They are naturally occurring in the aquatic environment[12-25-27].

1.6. Salinity

Salinity refers to the salt concentration present in water. When there is a higher salt concentration than lower the degradation rates. Variation in salt concentration results in lower degradation rates compared with stable concentration[8-12-25].

2. CONCLUSIONS

There has been research on petroleum degradation by microorganism for a long time. The research has been increased during 1960s and 1970s due to some large oil spills and hence there is a need for further understanding of the risks of the environment as well as humans for degradation of oil spills. Aerobically degrading bacteria are known to be popular far more than a century but vast range of novel bacteria was discovered after the Second World War. Biodegradation by light is also an important factor in degradation but its influence is very small for a long time. The toxicology of hydrocarbons is an important factor in appraisal the risk on humans and ecosystem. Researchers had developed the interest in toxicology because of PAHs are highly carcinogenic in nature. Most of Aliphatic hydrocarbons are non-carcinogenic in nature. Biodegradation is almost degrading all hydrocarbons in the environment but bigger aromatic hydrocarbons are the only compounds which are poorly degrade by bacteria. Fungi in association with bacteria are able to degrade these types. Photodegradation does not completely degrade hydrocarbons but it initiate the biodegradation by microorganisms and temperature and light intensity are the important factors. These polar groups lead to the chocolate mousse type formation and increase in solubility.

2.1. ***Further research on petroleum degradation***

Most of the studies have been done on hydrocarbons but majorly fate of petroleum in the environment which is quite complex has to be determined and one more parameter is photodegradation, it is an important process but poorly understood. The affect of each parameter on the environment is known, but the influence of these parameters is measured in relation to the influence of other parameters is rarely done. This complexity should be the central thought of developing further research. This is important because these experiments are being used to calculate the behavior of hydrocarbons in the environment, and this is always a multiparameter process.

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