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Pseudomonas in Biodegradation

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ABSTRACT

Due to careless waste disposal, illegal waste dumping and accidental spills large number of hazardous compound are released into the environment. The most common chemicals involved in environmental contamination are petroleum hydrocarbons, polynuclear aromatic hydrocarbons (PAHs).these products are hazardous to the surroundings as well as to life forms. The most common technique to remove pollutants from the natural environment and convert the pollutants to a less harmful product using the microbiological community of the contaminated environment is bioremediation. Pseudomonas sp. is the most frequently found bacteria in nature which is used in biodegradation process. Degradation of petroleum hazards by Pseudomonas sp. sp. is the best carrier based inoculums. Pseudomonas sp. degrades the various hydrocarbons by the different catabolic pathways. The final products can be carbon dioxide, water and simpler compounds which do not affect the environment. Pseudomonas sp. used to biodegrade of Naphthalene, Pyrine and Phenanthrene.

Keywords*: Petroleum hydrocarbons, PAHs, Pollutants, Biodegradation, Pseudomonas.*

INTRODUCTION

The development of human society all over history has lead to rising disruption of the natural equilibrium and the rate of different types of pollution. The planet depends on oil, and the use of oil as fuel has lead to intensive economic development worldwide. The great need for this power source has led to the gradual exhaustion of normal oil capital. on the other hand, mankind will witness the results of oil utilization for centuries after its termination. Ecological pollution with petroleum and petrochemical products has been recognized as a significant and serious problem[1].

Worldwide industrial and agricultural developments have released a large number of natural and synthetic hazardous compounds into the environment due to careless waste disposal, illegal waste dumping and accidental spills. There are numerous sites in the world that require cleanup of soils and sludge. In the United States, it has been estimated that contaminated site treatment costs may approach 1.7 trillion dollars over the next 30 years[2].

Oil spills in the environment cause long-term damage to aquatic flora, soil ecosystems, human health and natural resources. Petroleum oil spills tend to be associated with offshore oil rigs and tankers in marine related accidents. In contrast, land oil spills often go unnoticed by everyone except environmentalist, yet land oil spills contribute to the pollution of water supply and soil. Typical sources of land oil spill include accidents as well as oil from vehicles on the road. Characterization of spilled oil and its derivatives is very important in order to predict the behavior of oil and its long-term effects on the environment, and in order to select the proper cleaning methods. The potential danger which petroleum hydrocarbons pose to humans and the environment makes testing and characterization of the biodegradation and biotransformation processes of hydrocarbons in contaminated soil necessary in order to develop bioremediation techniques for cleaning such soils to levels that ensures its safe disposal or reuse[3].

Polycyclic Aromatic Hydrocarbons (Pahs)

The most common chemicals involved in environmental contamination are petroleum hydrocarbons, polynuclear aromatic hydrocarbons (Benzo (a) pyrene), solvents, pesticides, lead, chlorinated hydrocarbons (CFH), heavy metals (Cr, Cd Pb, MTBE, Zn and As) and Gasoline. Soil contamination with polycyclic aromatic hydrocarbons (PAHs) is a vital problem worldwide. Soil organic carbon (SOC) is one of the important factors that can influence the concentrations of PAHs in soils. Polycyclic aromatic hydrocarbons (PAHs) are widely distributed and relocated in the environment because of the incomplete combustion of organic matter.

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds containing carbon and hydrogen and are composed of two or more fused aromatic rings in linear, angular and cluster arrangements. PAHs are the pollutants present in air, soil and sediments. These compounds enter into the environment from many ways. PAHs and their derivatives are results of incomplete combustion of organic materials which arise from natural combustion like; forest fires and volcanic eruptions. PAHs are widely found in high concentrations at many industrial sites, particularly those associated with petroleum, gas production and wood preserving industries[4]. PAHs are a group of several hundred individual organic compounds, which contain two or more aromatics rings and generally occur as complex mixtures rather than single compounds. PAHs are classified by their melting and boiling point, vapor pressure, and water solubility, depending on their structure[5].

Bioremediation

Bioremediation is a complex process, with biological degradation taking place in the cells of microorganisms which absorb pollutants, where if they have specific enzymes, the degradation of pollutants and their corresponding metabolites will take place. Hydrocarbons from oil are used as a source of nutrients and energy for microorganism growth, and at the same time microorganisms decompose them to naphthenic acids, alcohols, phenols, hydroperoxides, carbonylcompounds, esters, and eventually to carbon dioxide and water[6].

Bioremediation is best technique to completely remove PAHs from the environment or convert them to less harmful compounds. Bioremediation of suspended naphthalene or 2-methylnaphthalene as a single substrate and their mixture was studied using the bacterium *Pseudomonas sp. putida*. Polycyclic aromatic hydrocarbons (PAHs) are one of the major groups of these contaminants[7]. PAHs constitute a diverse class of organic compounds consisting of two or more aromatic rings with various structural configurations[8]. Being a derivative of benzene, PAHs are thermodynamically stable. In addition, these chemicals tend to adhere to particle surfaces, such as soils, because of their low water solubility and strong hydrophobicity, and this result in greater persistency under natural conditions. This persistency combined with their potential carcinogenicity makes PAHs problematic environmental contaminants[9,10].

Bioremediation is the use of living organisms to degrade or detoxify hazardous wastes into harmless substances such as carbon dioxide, water and cell biomass. It uses relatively low-cost, low-technology techniques, which generally have a high public acceptance. Bioremediation is considered a non-destructive, cost- and treatment effective and sometimes logistically favorable cleanup technology, which attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low technology techniques, which generally have a high public acceptance and can often be carried out on site1. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate[11,12].

Bioremediation can be divided into two types: first is natural attenuation, which can be applied when the natural conditions are suitable for the performance of bioremediation without human intervention, and second is engineered bioremediation, which is used when is necessary to add substances that stimulate microorganisms. The first one is more attractive because of its low cost, minimum of maintenance and

minimal environmental impact. Still, this technology is applicable only in cases when the natural level of biodegradation is higher than the degree of pollution migration. Nevertheless, this technology is more often used as a supplement to the other technologies, or after finished engineered bioremediation in order to prevent migration of pollution from the treated area. Engineered bioremediation is faster than natural attenuation because it includes microbial degradation stimulation, by controlling the concentrations of pollution, oxygen, nutrients, moisture, pH, temperature, etc[13,14]. Engineered bioremediation is applied when it is essential to carry out cleaning in a short time or when the pollution is very rapidly expanding. Its application reduces the costs due to the shorter treatment of land and lower number of sampling and analysis, and it is important for political and psychological needs when the community is exposed to pollution.

***Pseudomonas sp.* Involved in Biodegradation:**

Some microorganisms found are capable of the transforming and degrading the pollutants, which can also contaminate the environment. Some species of microorganisms: bacteria, yeasts, and fungi obtain both energy and tissue-building material from petroleum. The fuel eating bacteria known as *Pseudomonas sp.* have evolved a taste for hydrocarbons are the major component of fossil fuels. Degradation of oils by *Pseudomonas sp.* is the best carrier based inoculums. These bacteria are found in different environments such as soil, water, and plant and animal tissue. Various different species of this bacterium are opportunistic pathogens that affect humans, animals, and plants.

Biodegradation is a natural process by which microbes break down oil into other substances. The final products can be carbon dioxide, water, and simpler compounds which do not affect the environment. *Pseudomonas sp.* sp. ubiquitous in soil and water are considered as scientific and technological importance. They comprise a taxon of metabolically versatile organisms which is capable of utilizing a wide range of simple and complex organic compounds. They are known to be involved in biodegradation of natural or man-made toxic chemical compounds[15]. *Pseudomonas sp.* is a prolific producer of a number of extra cellular enzymes (like lipase). Bioremediation is the optimization of biodegradation. Two forms of technology can accomplish this acceleration: One is fertilizing (adding nutrients) and the other is Seeding (adding microbes). These additions are necessary to overcome certain environmental factors that may prevent biodegradation. Petroleum is a complex mixture of thousands of compounds.

These can be divided into four major groups: the alkanes, the aromatics, the resins, and the asphaltenes. In general, the alkane fraction is the most biodegradable rather than the polar fraction (i.e., the resins and asphaltenes) is resistant to biological degradation. The aromatic compounds, especially the polycyclic aromatic hydrocarbons (PAHs) are of intermediate biodegradability, but these are of most concern owing to their toxicity and tendency to bioaccumulat[16]. The bioavailability of weekly soluble hydrophobic compounds for microbial conversion is usually low and thus limits their degradation rate in aqueous medium. The use of surfactants has been found to degradation of crude oil[17,18].

Contamination of groundwater is also a potential problem. The other impact noticed was on surface water, mostly nearby the streams, which receive a lot of untreated effluent containing oil and grease as well as non-biodegradable detergents. Emtiazi *et al.,* studied the biodegradation of petroleum oil by a *Pseudomonas sp.* isolated from a petroleum-contaminated soil was instable. They were showed that the isolates were immobilized on Perlite, they were more stable for oil degradation. The generation time was for degradation of petroleum oil, dodecane and octadecane was 20, 22, and 25 h. [19].

Catabolic Pathways of PAHs degradation:-(Naphthalene and Pyrine)

Jones *et al.,* recognized the biodegraded petroleum-derived aromatic hydrocarbons in marine sediments. They investigated the immense biodegradation of alkyl aromatics in marine sediments which eventuate prior to detectable biodegradation of n-alkane profile of the crude oil and the microorganisms namely: *Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas sp. , Sphingomonas, and Rhodococcus* were observed to be involved for alkyl aromatic degradation[20].

Zhang *et al.,* investigated a bacterial isolate, designated as DQ8, was capable of degrading diesel, crude oil, n-alkanes and polycyclic aromatic hydrocarbons (PAHs) in petroleum. Strain DQ8 was assigned to

the genus *Pseudomonas sp. aeruginosa* based on biochemical and genetic data. The metabolites identified from n-docosane as substrate suggested that P. aeruginosa DQ8 could oxidize n-alkanes via a terminal oxidation pathway[21].

Malik *et.al.,* investigated the metabolic capability of 15 bacterial isolates isolated from oil contaminated site by using enrichment culture technique which were able to degrade aromatic and polyaromatic fractions. The results showed that the aromatics compounds (benzene, toluene and xylene) were vaporized in the 4th day of incubation, while the efficiency on polyaromatic fractions (anthracene, phenanthrene and pyrene) was 46.17 to 55.3% after 24 days of incubation. The ability of degrading long chain n-alkanes and crude oil at high concentrations makes the consortium potentially useful for bioremediation and microbial enhanced oil recovery[22]. Ashok *et.al.,* isolated four bacterial strains which were able to degrade naphthalene, anthracene or mixture of both from the soil of oil refinery. Out of four isolates two of them were identified as belonging to the genus *Micrococcus*, and other two were identified as *Pseudomonas sp.* and *Alcaligenes* respectively. These species degrade at the rate of 89%, 67.5% and 92.1°/o of high molecular weight plasmid DNA[23].

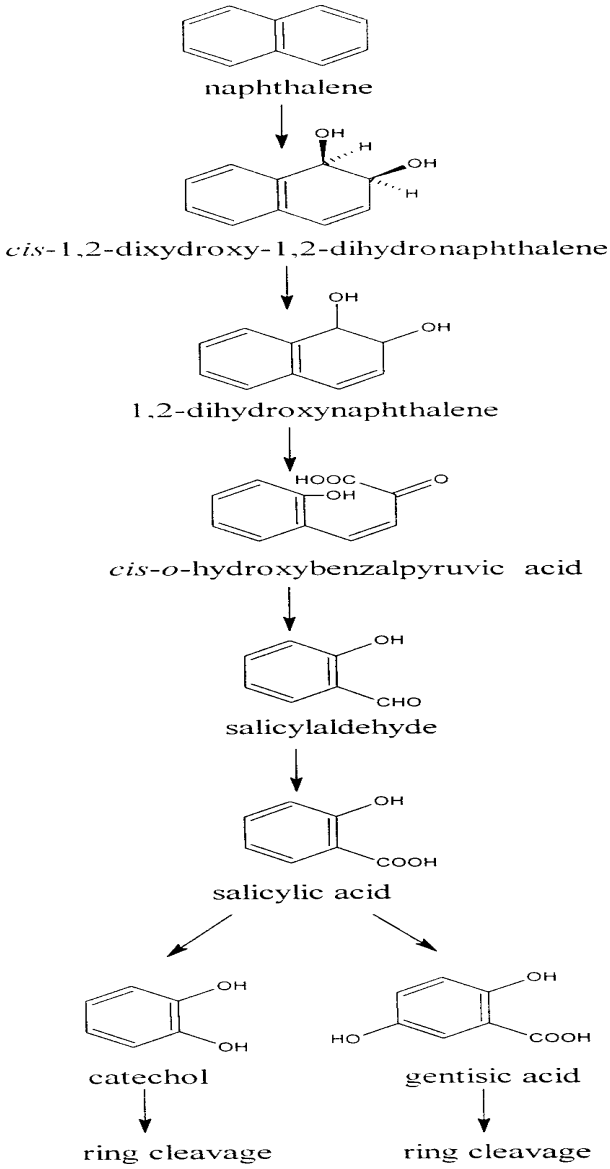
Naphthalene

Naphthalene is the primitive and most soluble PAH in nature. It is found naturally in fossil fuels like; coal and oil. Burning of fuels, tobacco or wood produces naphthalene as a product. It has a strong odour smells like coal tar or mothballs. It is used to make products like; moth balls, dyes, leather goods, and as a household fumigant. Its melting point is 80.2ºC and the molecular weight is 128.2. It degrades more rapidly as compared to other PAHs found in nature. Bacterial strains that are able to degrade aromatic hydrocarbons have been isolated mainly from the soil. These generally belong to gram-negative and are from the genus *Pseudomonas sp.*

Davies and Evans were the first ones who investigated biochemical sequence and enzymatic reactions which leads to the degradation of naphthalene. Naphthalene dioxygenase enzyme is required for studying naphthalene activity however; it is unstable in nature. Naphthalene oxygenase has also been isolated from cells of *Corynebacterium renale* which was able to use naphthalene as a main source of carbon and energy[24]. Degradation of naphthalene is best studied due to its simplicity. It starts through the multicomponent enzyme called naphthalene dioxygenase which attacks on the aromatic ring to form *cis*-(1R, 2S)-dihydroxy-1,2-dihydronaphthalene (*cis*-naphthalene dihydrodiol). The *cis*-naphthalene dihydrodiol formed by naphthalene dioxygenase and it is subsequently dehydrogenated to 1,2-dihydroxynaphthalene by an enzyme *cis*-dihydrodiol dehydrogenase. 1,2-dihydroxynaphthalene is metabolized to salicylate via 2-hydroxy-2*H*-chromene-2-carboxylic acid, *cis*-*o*-hydroxybenzalpyruvate and 2-hydroxy-benzaldehyde. However, 1,2-dihydroxynaphthalene is non enzymatically oxidized to 1,2-naphthaquinone. Salicylate convert to catechol via decarboxylated, and it is further metabolized by ring fission in *meta* and *ortho*-pathways.

Plasmid NAH7 in *Pseudomonas putida* G7 used to encode bacterial degradation of naphthalene. NAH7 has two operons which contain the structural genes for naphthalene degradation. One operon contains the gene for the upper catabolic pathway and encodes the enzymes necessary for the conversion of naphthalene to salicylate. The second operon contains the gene for the lower catabolic pathway and encodes the enzymes necessary for the metabolism of salicylate through the catechol meta-cleavage pathway to pyruvate and acetaldehyde. Parales *et al.,* reported aspartate 205 in the catalytic domain of naphthalene dioxygenase which is a necessary residue in the major pathways of electron transfer to mononuclear iron at the active site. Several naphthalene degrading bacteria were isolated from oil contaminated soil in a crude oil extraction and desalination center in Omidieh, Ahvaz, Iran. Bacterial strains were isolated and identified by biochemical and morphological tests. This investigation exhibited the most of the isolated bacteria were belong to *Staphylococcus sp, Corynebacterium sp, Pseudomonas sp. Bacillus sp, and Micrococcus sp.* These species were able to degrade naphthalene. The efficiency of naphthalene as the only source of carbon and energy was evaluated by High performance liquid chromatography (HPLC) analysis. HPLC analysis showed that the *Bacillus sp.* and *Pseudomonas sp.* are capable to degrade naphthalene 86% and 80%, respectively and *Corynebacterium sp and Staphylococcus sp,* degrade77% and 69% after one week incubation[25].

Fig.1:The proposed pathway for naphthalene biodegradation by bacteria



Survey *et al*., studied the soil near several gas stations in Karachi, Pakistan. He has isolated and identified 60 bacterial strains including *Staphylococcus* (11.5%) *Corynebacterium* (5%), *Bacillus* (10%), *Pseudomonas sp.* (8.3%), *Escherichia* (33.3%) *and Klebsiella* (10%). These bacteria were capable of degradation of the hydrocarbons. Coral *et al* successfully isolated 50 bacterial strains that all belong to *Pseudomonas sp.* and were capable of degrading naphthalene. Since native bacteria of contaminated areas are in contact with aromatic compounds, these bacteria should be able to degrade the materials surrounding them. 38

Walczak *et al* and Bestett *et al.,* have studied the degradability of naphthalene by native bacteria. In this study, the most dominant naphthalene degrading bacteria isolated from the Maroon II oil field belonged to *Pseudomonas sp.* and *Bacillus.* Therefore, the results of this study showed that these bacteria are native in this area[26,27].

Pyrene

Pyrene is a polycyclic aromatic hydrocarbon, composed of four fused benzene rings and the chemical formula is $C_{16}H_{10}$. It forms during incomplete combustion of organic compounds. It is a byproduct of gasification processes and other incomplete combustion processes. This colourless solid is the smallest

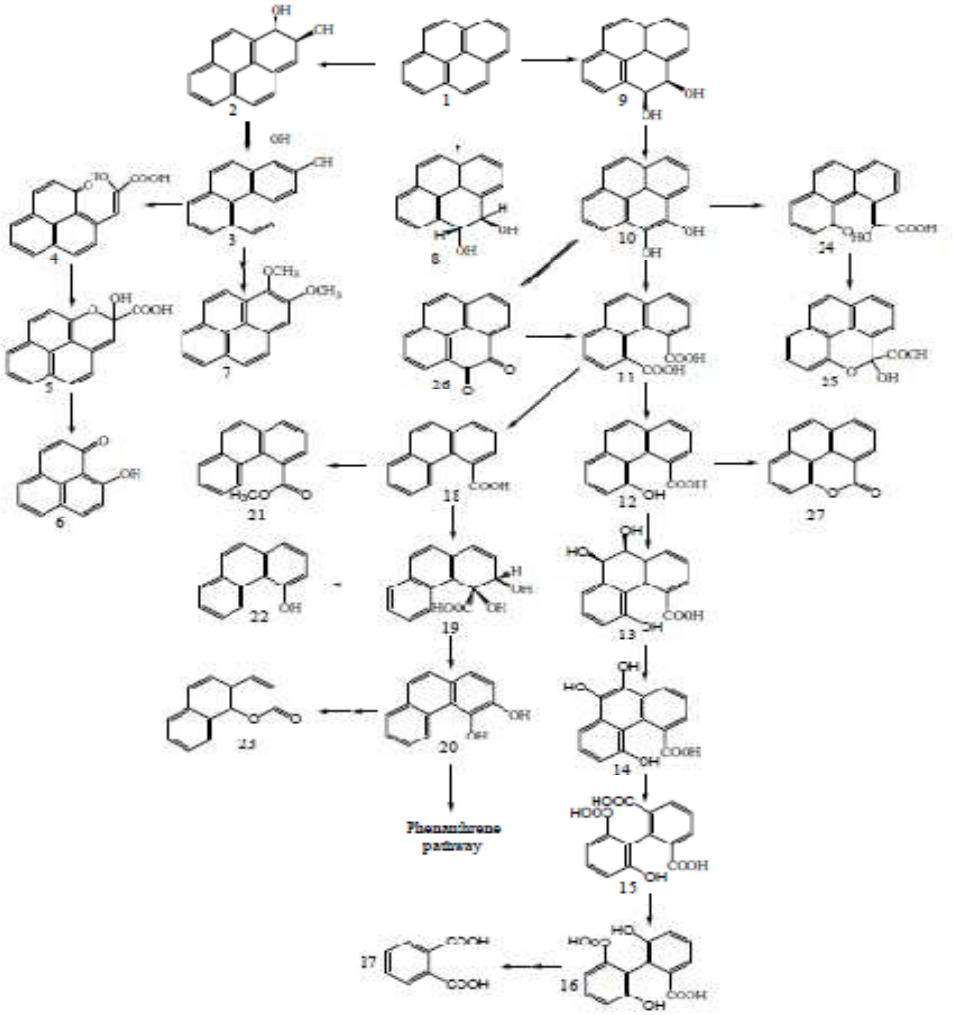
peri-fused PAH (one where the rings are fused through more than one face). Its melting point is 145-148ºC. Many bacteria have been isolated which are capable of degrading pyrene. *Mycobacterium* (Gram-positive species) use it as a sole carbon and energy source to perform their metabolic activity. *Mycobacterium* spp. is known to have high cell surface hydrophobicity and adhere to the emulsified solvent droplets. Other pyrene degrading strains isolated include *Rhodococcus* sp., *Bacillus cereus*, *Burkholderia cepacia*, *Cycloclasticus sp.* P1, *Pseudomonas sp. fluorescens*, *Pseudomonas sp. stutzeri*, *Sphingomonas sp.* VKM B-2434, *Sphingomonas paucimobilis*, and *Stenotrophomonas maltophilia*. Heitkamp *et al.,* found the three products of ring oxidation, pyrene-*cis*-4,5-dihydrodiol, pyrene-*trans*-4,5-dihydrodiol, and pyrenol, and four products of ring fission, 4-hydroxyperinaphthenone, 4-phenanthroic acid, phthalic acid, and cinnamic acid by multiple analyses, including UV, infrared, mass spectrometry, NMR, and GC. The formation of pyrene-*cis*-4,5-dihydrodiol by dioxygenase and pyrene-*trans*-4,5-dihydrodiol by monooxygenase suggested multiple initial oxidative attacks on pyrene. Pyrene-1,2-diol derived from the dioxygenation at pyrene 1,2-C positions is metabolized to 4-hydroxyperinaphthenone *via cis*-2-hydroxy-3-(perinaphthenone-9-yl)-propenic acid and 2-hydroxy-2*H*-1-oxa-pyrene-2-carboxylic acid[28].

Kafilzadeh *et. al.,* isolated bacterial strain from soil of the landfills in Shiraz and evaluation of their growth kinetic and investigated the pyrene degradation. The isolated bacterial strains were *Mycobacterium sp., Corynebacterium sp., Nocardia sp., Pseudomonas sp. Rhodococcus sp.* and *Micrococcus sp.* which were potentially capable to degrade pyrene hydrocarbon. After 10 days of incubation, the pyrene biodegradation value evaluated by high performance liquid chromatography (HPLC) was 89.1%, 79.4%, 75.3%, 68.2%, 62.3% and 56.8% for each strain respectively. Therefore these bacteria could be used to clean the soils which are polluted with pyrene[29]. Moody *et.al.,* reported that the degradation of anthracene and phenanthrene by *Mycobacterium* sp. strain PYR-1. After the incubation of 14 days 92% and 90 % of degradation was obtained of anthracene and phenanthrene, respectively. Metabolites of anthracene and phenanthrene by the UV-visible light absorption, high-pressure liquid chromatography (HPLC) retention times and mass spectrometry analysis were also identified[30]. Schneider *et.al.,* studied the degradation of three polycyclic aromatic hydrocarbons (PAH), pyrene (PYR), benz [*a*] anthracene (BAA), and benzo [*a*] pyrene (BaP), by isolated the *Mycobacterium* sp. strain RJGII-135 from abandoned coal gasification site soil by analog enrichment techniques. By high-resolution mass spectral and fluorescence metabolites of these PAHs were identified. The ability of this bacterium to degrade these PAH is well supported and used in remediation of sites containing mixtures of these PAH[31].

Bisht *et.al.,* isolated naphthalene and anthracene degrading bacteria from non-contaminated soil of rhizosphere of *Populus deltoids*. Four isolates were determined by HPLC analysis i.e. *Kurthia* sp., *Micrococcus varians, Deinococcus radiodurans* and *Bacillus circulans* utilizing the chrysene, benzene, toluene and xylene. Among these isolates *Kurthia* sp and *B. circulans* showed positive chemotactic response for naphthalene and anthracene. After 6 days of incubation it was found that *B. circulans* SBA12 and *Kurthia* SBA4 degraded 87.5% and 86.6% of anthracene while, *Kurthia* sp. SBA4, *B. circulans* SBA12, and *M. varians* SBA8 degraded 85.3 %, 95.8 % and 86.8 % of naphthalene respectively[32]. Pathak *et al.* investigated 4T engine oil biodegradation potential of *Pseudomonas sp. serratia* strains, were isolated from contaminated soil from Sitapura industrial area Jaipur. MSM broth was used in enrichment technique supplemented with 1% v/v hydrocarbon substrate (4T engine oil)[33]. Juhasz *et.al.,* analyzed that the degradation of low molecular weight PAHs by isolating numerous genera of bacteria, fungi and algae have been done while the degradation by high molecular weight PAHs compounds are generally recalcitrant to microbial attack, although some fungal and bacterial isolates were identified which were able to degrade four ring PAHs as sole carbon and energy source. This review concern about the presence of benzo [a] pyrene in the environment and the ability of bacteria, fungi and algae to degrade the toxic, carcinogenic and mutagenic four fused benzene ring compound, benzo [a] pyrene[34].

Pyrene is a tetracyclic aromatic hydrocarbon with a symmetrical structure which is one of the top 129 pollutants as ranked by the U.S Environmental Protection Agency. Scientists collected sample from three different stations in landfills areas in the city of Shiraz, Iran. The bacterial strains were isolated and then identified by standard bacteriological tests. Isolated bacteria including *Mycobacterium sp.*, *Pseudomonas sp. Rhodococcus sp.* and *Micrococcus sp.* were potentially capable of degrading pyrene hydrocarbon. They showed high growth rate during increasing the optical density (OD600). Its biodegradation value evaluated by high performance liquid chromatography (HPLC) was 89.1%, 79.4%, 75.3%, 68.2%, 62.3% and 56.8% for each strain respectively 10 days after incubation. The highest pyrene degradation rate was found in *Mycobacterium* sp. and *Corynebacterium* sp. with 89.1% and 79.4% values; therefore these bacteria could be used to clean the soils which are polluted with pyrene degradation occurred in *Mycobacterium* and *Corynebacterium*. This indicates the high potency of these bacteria in contact with most PAHs. Thus, these two bacteria were introduced as the indicator degrader strain in the landfills' area[35].

Fig. 2:Proposed catabolic pathways of pyrene by bacteria



Compound designations: 1, pyrene; 2, pyrene-cis-1,2-dihydrodiol; 3, pyrene-1,2-diol; 4, 2-hydroxy-3-(perinaphthenone-9-yl)-propenic acid; 5, 2-hydroxy-2H-1-oxa-pyrene-2-carboxylic acid; 6, 4-hydroxyperinaphthenone; 7, 1,2-dimethoxypyrene; 8, pyrene-trans-4,5-dihydrodiol; 9, pyrene-cis-4,5-dihydrodiol; 10, pyrene-4,5-diol; 11, phenanthrene-4,5-dicarboxylic acid; 12, 4-carboxyphenanthrene-5-ol; 13, 4-carboxy-5-hydroxy-phenanthrene-9,10-dihydrodiol; 14, 4-carboxyphenanthrene-5,9,10-triol; 15, 2,6,6'-tricarboxy-2'-hydroxybiphenyl; 16, 2,2'-dicarboxy-6,6'-dihydroxybiphenyl; 17, phthalic acid; 18, 4-phenantroic acid; 19, 3,4-dihydroxy-3,4-dihydro-phenanthrene-4-carboxylic acid; 20, phenanthrene-3,4-diol; 21, 4-phenanthroic acid methyl ester, 22, 4-hydroxyphenanthrene; 23, 7,8-benzocoumarin; 24, 2-hydroxy-2-(phenanthrene-5-one-4-enyl)-acetic acid; 25, 5-hydroxy-5H-4-oxa-pyrene-5-carboxylic acid; 26, pyrene-4,5-dione; 27, 4-oxa-pyrene-5-one.

Bishnoi *et al.,* studied pyrene degradation by *Pseudomonas Putida* and *Pseudomonas paucimobilis* were selected for further study. After 42 days, *Pseudomonas Putida* degraded 59.8% of pyrene and *Pseudomonas Paucimobilis* degraded 52% of pyrene. In the current study, this bacterium also was isolated as the pyrene-using strain and its degradation value was 68.2%[36]. In another study in normal conditions by Shafiee *et al.,* after 10 days 60% of pyrene was consumed by soil bacteria. Studies on compounds with low molecular weight, e.g., the average of phenanthrene and anthracene degradation by soil bacteria, was reported as 48.44% and 30.19%, respectively, after 24 h [37].

CONCLUSION

Bioremediation offers an alternative method for detoxification of contaminant. It is a natural process which relies on bacteria, fungi and plants to alter contaminants. These organisms carry out their normal life processes using these contaminants as their source of nutrients. Metabolic processes of these organisms are capable of using chemical contaminants as energy source, rendering the contaminants harmless or less toxic in most cases. This study shall provide a better solution for bioremediation of spilled petroleum hydrocarbons in soil and water ecosystems. It will provide an idea on distribution of microorganisms in the environment which have the ability to degrade the hydrocarbons and investigation of the response of microorganisms towards different petroleum oils.

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