Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Review Article **DOI: 10.26479/2021.0702.04**

BIOREMEDIATION OF PETROLEUM HYDROCARBON BY MICROORGANISMS: A REVIEW

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ABSTRACT: Increasing industrialisation, continuous population growth and high demand of petroleum products have led to effects economic growth and development of a country. The dependency on fossil fuels has resulted in serious ecological issues and causes adverse impact on the human health have been seen over recent decades. Therefore, a number of different physical and chemical approaches are used for reduction of hydrocarbon pollution released from various sources in the environment. But the methods are very costly and time consuming so an alternative mode for treatment of hydrocarbon pollution may be bioremediation by using of microorganisms. Bioremediation has been used to degrade hydrocarbon or oil spills contaminants through microbe such as bacteria, fungi and algae. These are playing an important role in Bioremediation process of petroleum hydrocarbons from contaminated environment by the involvement of enzymatic capabilities of the indigenous hydrocarbon-degrading microbial populations. Therefore, present review article has emphasized on hydrocarbon pollution and its remediation by microorganism. The aim of this study is to review and bring together a range of recent researches in the area of bioremediation of hydrocarbon pollution. Bioremediation is a newly accepted idea that gaining a worldwide interest.

Keywords: Petroleum, Hydrocarbon Pollution, Biodegradable, Bioremediation.

Article History: Received: February 28, 2021; Revised: March 09, 2021; Accepted: March 28, 2021.

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1.INTRODUCTION

In present time Hydrocarbon based products are the major source of energy for industries and daily life [1]. The petroleum constitutes or hydrocarbons are one of the major environmental pollutants. Several polluted environmental areas their abundance and persistence have been reported [2]. Accidental spills and leaks occur regularly during the refining, production, exploration, transport and storage of petroleum and petroleum products. The estimated amount of natural crude oil seepage was 600,000 metric tons per year [3]. The main cause of water and soil contamination is releasing of hydrocarbon into the environment whether accidently or due to anthropogenic activities [4]. Hydrocarbon contamination in soil cause extensive damage of local flora and fauna's system due to accumulation of pollutants in animal and plant tissue may cause diseases and death [5]. The most common strategies used for soil remediation includes mechanical, burying, evaporation and washing but these processes are very expensive and leads to incomplete decomposition of contaminants [6].

What is spilled oil and Hydrocarbon

The crude oil is a liquid petroleum that containing thousands of hydrocarbon components. Each of them has unique chemical behaviour that makes it either easily biodegradable, quite difficult to digest or non-degradable [7].

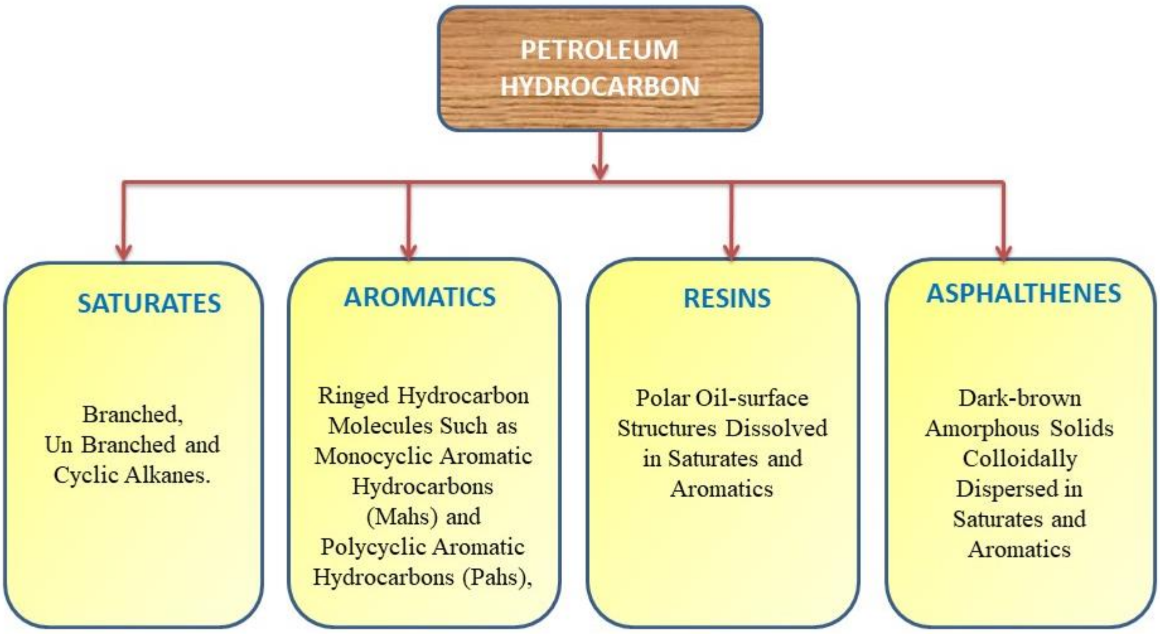

Fig 1: Showed that petroleum hydrocarbon grouped into four categories

When substances originate from aquatic algae are laid down during millions of years it creates crude oil [8]. Hydrocarbons are mostly composed of compound with different solubility, volatility and susceptibility for biodegradation. In the spilled oil there are aliphatic and aromatics compound that are difficult to degrade and an aliphatic fraction with double covalent bond and aromatics with rings those are more condensed and resistance to biodegradation [9]. PAHs (Petroleum Aromatic Hydrocarbons) are very common contamination of petroleum in the environment that are considered to be potentially mutagenic and carcinogenic [10]. The BCF (Breast Cancer fund, 2013) reported that DNA of living organisms damaged by the heavy PAHs. (i.e., they are genotoxic) and are implicated in human breast cancer. So, because of this a large number of studies on the biodegradation of PAHs degradation have done in order to safeguard the environment and biodiversity from severe long term ecological and medical damage by oil spills [7].

Effect of Petroleum Hydrocarbon on Environment

Ecosystem of our Marine and land both suffer from the impact of oil spills in similar ways. The impact of living organisms could either be direct, indirect or delicate and chronic [11]. In the direct effect some common problems like suffocation, anoxia (when surface of ocean cover with thick and sticky oil and inhibits oxygen from dissolving in water), and due the viscous nature of crude oil inhibition of movement of animals within the soil, river or ocean can be occur. On other hand the direct effect includes undersized growth (in both plant and animal) reproductive and morphological disorder etc [12]. Acute narcosis mortality (it is a state of unconscious that leads to death) cause by the Short- term effect of hydrocarbon pollution, acute exposure of feather and fur causing hypothermia. And the long-term effect includes exposure of embryos to weathered oil, intake of contaminated prey or foraging in polluted sedimentary pools. Our marine Ecosystem suffers a greater environmental damage compare to terrestrial ecosystem mainly as a result of the greater difficulty of controlling the spills [13]. Considering the nature and degree of hydrocarbon contamination in soils and so as to foresee how fruitful oil remediation approaches will be, understanding the destiny and conduct of such contaminants in the earth is crucial. During an oil slick, enduring happens and the oil is exposed to an assortment of physicochemical procedures [14]. These procedures can adjust the organization and properties of the oil influencing the level of hydrocarbon corruption, sequestration and communication with soil microorganisms. The destiny and spread of these mixes on the subsurface depend on the consistency and amount of the oil. In the earthbound condition, the destiny of oil hydrocarbons is affected by (a) the organization and physical properties of the dirt, for example, molecule size, porosity, natural issue substance, porousness and surface zone and (b) the physical and compound properties and creation of oil-based commodities including air dissemination coefficient, dissolvability in water and breaking point [15]. The biodegradability of oil hydrocarbons can likewise be influenced by the focus and bioavailability of the contaminants. Hydrocarbon bioavailability alludes to the division of contaminants that can be used or changed by the dirt microbial network. Sorption is likewise a significant factor affecting the total corruption of natural poisons in the dirt. Decreased sorption of the hydrocarbon division builds protection from desorption bringing about expanded industriousness inside the dirt natural framework. In defiled soil, two hydrocarbon portions ought to be viewed as while picking bioremediation treatment: right off the bat the irreversibly adsorbed hydrocarbons; this division isn't bio available and viewed as non-biodegradable. The subsequent bit is the bio available division which can desorbs and diffuse in the strong particles as a water solvent portion [16]. Oil hydrocarbons can be fractionated and sequestered inside the dirt through sorption to natural issue or diffuse into the three-dimensional structure of the natural issue. Following the underlying oil slick, the physical connections become increasingly convoluted; this known as matured defilement. The biodegradable portion of natural poisons in soils is the division that is effortlessly desorbed to or from the dirt particles and exist in the watery stage. It is entrenched that as the collaboration between soil particles and toxins increment, there will be a corresponding decrease in contaminant extraction and biodegradation [17]. Hydrocarbon portions that are all the more firmly sorbed onto soil natural issue are increasingly obstinate and impervious to debasement contrasted with unstable or solvent hydrocarbons. This is a significant thought when structuring or applying a procedure for the corruption of debased soils as petrogenic hydrocarbons will in general unequivocally adsorb to the dirt [18].

Hydrocarbon spills removal Techniques

A complex of mechanical, synthetic, and natural methodologies can be applied for the remediation of oil hydrocarbon contamination. The usually utilized mechanical systems incorporate assortment and skimming, cleaning, water flushing, working, just as cutting vegetation and consuming [19]. Mechanical expulsion of oil slicks is generally used as an underlying system for tidying up in sea-going and earthly situations. In any case, they can be costly and need particular hardware [20]. Accordingly, different strategies can be considered. In situ consuming of oil is an elective treatment, which can be utilized for snappy evacuation of thick film of oil spilled on a water body or land. Be that as it may, its application is restricted by the state of the earth. For instance, some plant networks like needle grasses are delicate and might be harmful or disposed of by fire [21]. Likewise, in situ consuming could compromise human wellbeing and ecological assets because of the smoke and the likelihood of blaze back and optional flames. Warm desorption is an *ex-situ* consuming strategy that is developing in notoriety and use. It utilizes warmth to consume, break down, or demolish the contaminants in soil leaving the mineral substance of the dirt after treatment [22].

Bioremediation

In 1960 George M. was the person who invented the technology of bioremediation [23]. Bioremediation is a technique for removal, degradation and transformation of pollutants or contaminants to less harmful substance through biological resources. This technology has been accepted as mostly bioremediation process occurs at aerobic condition but microbial degradation of recalcitrant compound can also occur in anaerobic environment [24]. Bio remediators are microbial agents which help in performing the function of bioremediation [25]. Biodegradation is a mechanism which is done by natural population of microorganisms to remove petroleum and other hydrocarbon pollutants from the environment and it is cheaper than other remediation technologies [26]. The process of bioremediation become easy when the microorganisms which are degrading the contaminants occurs naturally in environment [27].

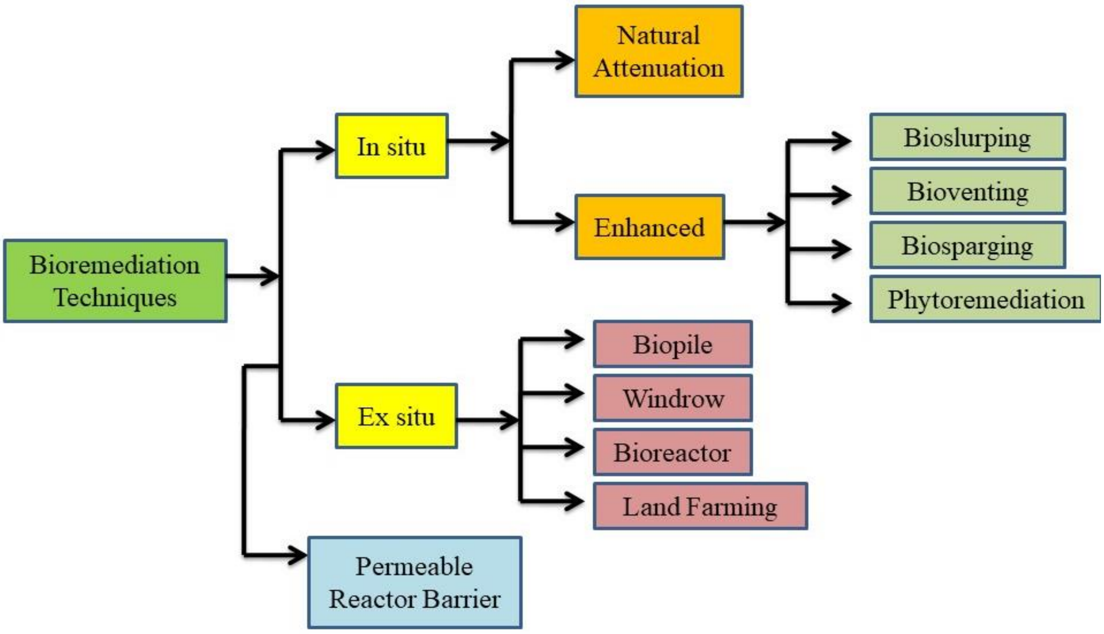

Fig 2: Types on Bioremediation Technique

Strategies for bioremediation

There are two main strategies for bioremediation that are bioaugmentation and bio stimulation. Bioaugmentation is a process in which high concentration and single strains or consortia population are added to the contaminated site which contain recalcitrant toxic compounds [28]. This process is mainly performed at the site where the microorganisms are present but does not contain the metabolic route for the contamination present in the site or there are sufficient number of microorganisms present at that particular contaminated site. Bio stimulation is a process in which rate of biodegradation are enhanced by the help of microorganisms by determining and adjusting the factors such as nutrients of the contaminated site [29].

Bioremediation of Crude oil or Hydrocarbon

The use of bioremediation process for degrade contaminating substance was initially developed to treat contamination of hydrocarbon like petrol and oil spills [30]. Now a days there are more than seventy known genera of oil degrading microorganism like bacteria, fungi, algae etc. which are capable of breaking down a specific group of hydrocarbons. In soil there are lots of microorganisms like bacteria such as *Achromobacter* sp, *Acinetobacter*sp., *Bacillus* sp. and fungus like *Allescheria* sp., *Aspergillus* sp., *Candida* sp. which are capable of degrading hydrocarbons like oil and petrol [31]. These microorganisms are naturally found in oil contaminated soil mostly due to hydrocarbon contamination [32]. The bioremediation is a process in which we use microorganism to detoxify or remove pollutants and it is help in degradation of many environmental pollutants including the product of petroleum industry [33]. In addition, this technology is believed to be cost effective and non-invasive [6]. Bioremediation of oil spills depends on ability to establish and maintain conditions that favour enhanced oil biodegradation rates in the contaminated environment. Lots of review articles have covered various factors that influence the rate of oil biodegradation [34]. The important requirement of this mechanism is the presence of microorganisms with the appropriate metabolic capabilities. When microorganism is present then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that sufficient concentration of nutrients and oxygen are present. The other important thing is the physical characteristic of oil and oil surface area because it determines the success of bioremediation [5].

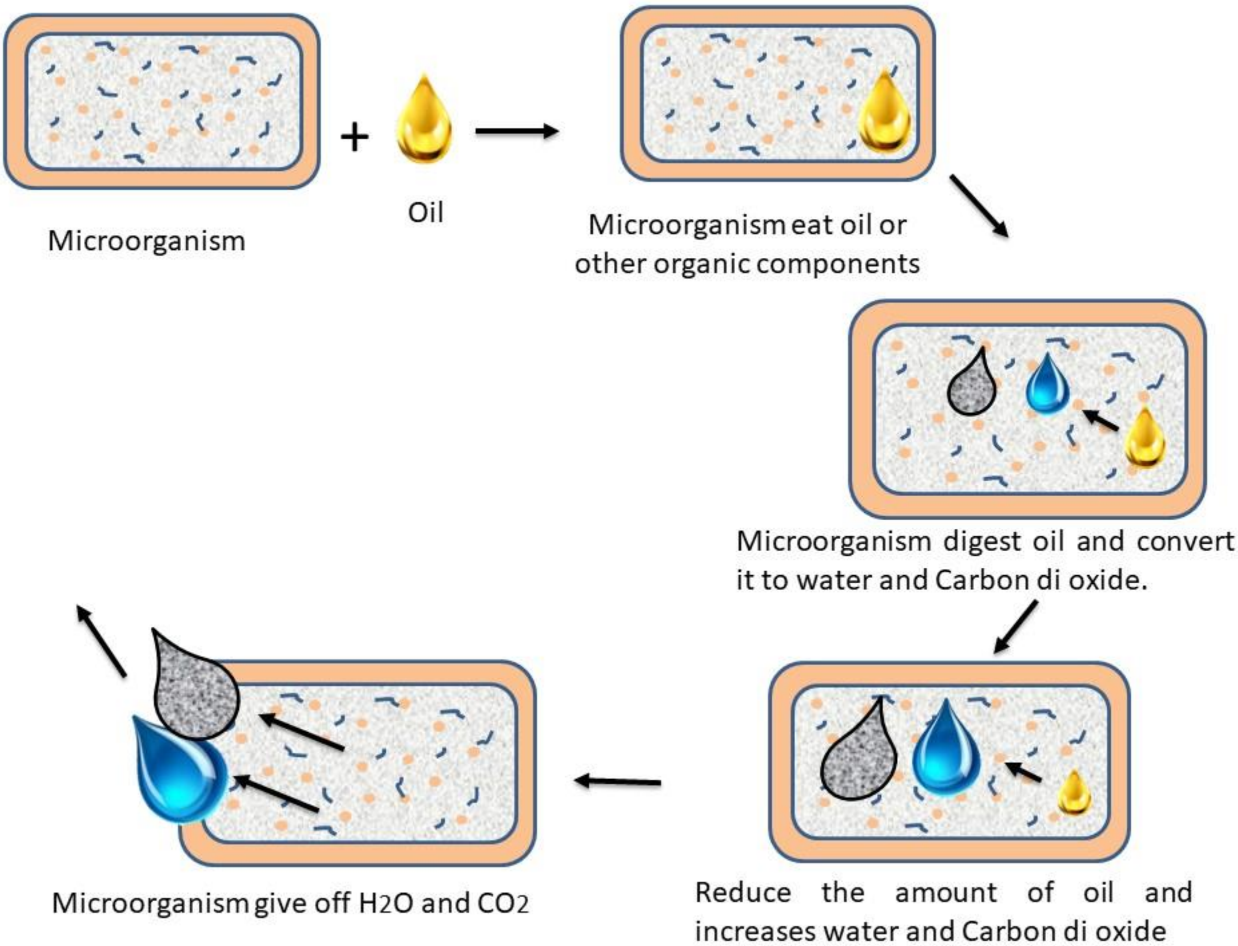

Fig 3: The process of aerobic biodegradation

Bacteria which have the ability to degrade hydrocarbon or oil spills called Hydrocarbon degrading Bacteria. There are lots of bacteria reports on the degradation of oil spills these types of bacteria use oil as a food material [35]. In oil degradation Bacteria are the most active agent and in the process of hydrocarbon degradation they work as a primary degrader [36]. Biodegradation of oil can occur under both aerobic and anaerobic condition. Although lots of bacteria are metabolize hydrocarbon pollutants, a single bacterium does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil. Mixed bacterial colonies have the very strong hydrocarbon degradation potential because the genetic information of more than one organism is necessary to degrade the complex mixtures of hydrocarbon compounds present in oil contaminated areas [37]. Some bacteria namely *Pseudomonas sp.* *Brevibacillus sp. Bacillus*sp, *Corynebacterium* sp, *Staphylococcus* sp., *Streptococcus* sp., *Shigella* sp, *Alcaligenes* sp, *Acinetobacter* sp., *Escherichia* sp. are helpful to degrades hydrocarbon or oil spills [38].

Bacteria Super Bug

Prof. Chakrabarty in 1971 developed a method in which genes are transferred through genetic cross-linking using plasmid transfer technique as a result a new stable bacterial species known as *Pseudomonas putida* was produced and this bacterial species was able to degrade oil [39]. For the construction of *Pseudomonas putida,* he used four strains of *Pseudomonas* sp., containing plasmid and then he isolated the plasmids from each strain and found that octane, camphor, xylene, and naphthalene, respectively can be degraded. Then all the four plasmids were inserted into one strain of *Pseudomonas putida* which has capability to degrade octane, camphor, xylene, and naphthalene and then this species of bacteria was called as superbug and for this research Prof. Chakrabarty was awarded with U.S patent [40]. *Pseudomonas putida* (*P. putida*) is a gram negative, straight or curved rods shape bacteria with dimensions of range size between 0.5 and 1.0 µm × 1.5 - 4.0 µm. range of temperature for their growth is 0-42ºC and the optimum temperature is 35ºC. *Pseudomonas putida* are chemoorganotrophic, aerobe obligate and aerobically respiratory metabolisms and can live saprophytically in soil and water. *Pseudomonas putida* can degrade aromatic hydrocarbon by the formation of diol followed by cleavage of aromatic ring and formation of diacid such as cis-muconic acid takes place. Recombinant DNA Advisory Committee has certified a model microorganism known as *Pseudomonas putida* KT2440 as a biosafety strain for recombinant DNA manipulation. *P. putida* KT2440 is a bacterial strain which is capable of degrading aromatic compounds [41].

Fungi

Fungal bioremediation process also called mycoremediation. In the process of the degradation of any harmful compound leads by fungi, and this is happened because of extracellular and intracellular enzymes which catalyse various reactions [42]. Fungi have extracellular multienzyme complexes and it uses in the breaking down the natural polymeric compounds. Mycelia of the fungi also helps to colonize and penetrate substrate rapidly. These qualities lead to fungi as an important hydrocarbon degrader [43]. When we compare fungi with other microorganisms, fungi has an advantage that it contain the ability to secrete classes of enzymes and due to the secretion of enzymes fungi can interact with several types of polyaromatic hydrocarbons with high degree of nonspecific activity [44]. Fungi can grow in extreme conditions and thus fungi can tolerate high concentration of recalcitrant compounds [45]. Some fungal species namely *Amorphoteca* sp., *Neosartorya* sp, *Talaromyces* sp., *Graphium* sp., *Rhodotorula mucilaginosa, Geotrichum* sp, *Trichosporon* sp., *A.niger, Mucor* sp. are found to degrades oil spills or Hydrocarbon pollution [46].

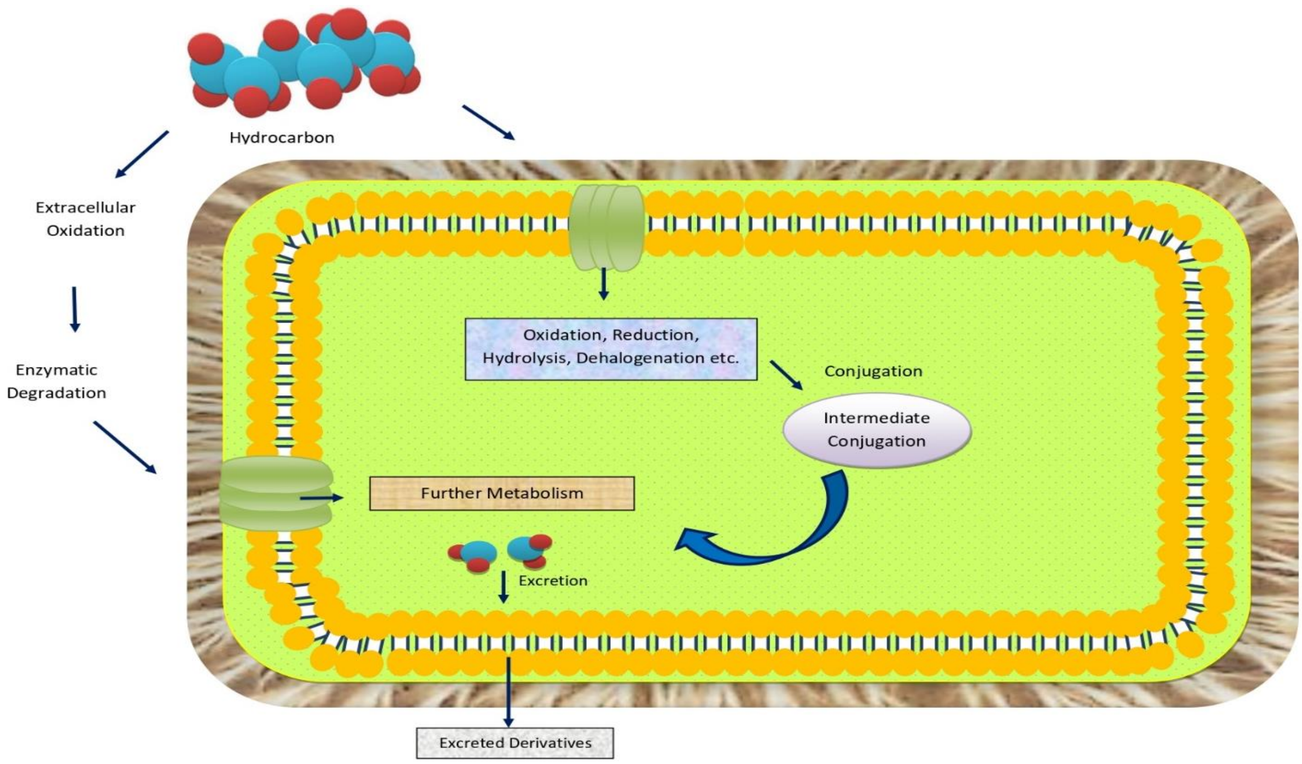

Fig 4: Simplified diagram of fungal interactions with petroleum hydrocarbons

Algae

Algae are very important member of both marine aquatic ecosystem and terrestrial eco system and plays a vital role in degradation of hydrocarbon [5]. Research on the degradation of hydrocarbon through green algae is a great importance from environmental point of view because the pollution of oil spills has become one of the major problems in aquatic ecosystem [47]. According to Tahri *et al* algae *Prototheca zopfii* is capable of degrading n alkanes and iso-alkanes as well as hydrocarbon [48]. Tang *et al* reported that microalgae *Scenedesmus obliquus* is used for to build an artificial microalgal bacterial consortium for degradation of crude oil [49]. Some Algal species namely *Chlorella vulgaris, Scenedesmus platydiscus, S. quadricauda, S. capricornutum, P. rotothecazopfi, Monoraphidium braunii, Agmenellum quadruplicatum, Selenastrum capricornutum, Scenedesmus obliquus, Phytoplankton* are helps to degrades hydrocarbon and oil spills [48,49,50,51].

Enzymes Involved in degradation

Various microorganism like bacteria, cyanobacteria, algae, fungi are capable of degrading different molecules of petroleum hydrocarbon under different conditions of environment, such asaerobic and anaerobic conditions at varied salinities and pH. The genes which are responsible for degrading petroleum enzymes production may be located on chromosomal or plasmid DNA [52].

Biodegradation of oil spills or Hydrocarbon may occur both under anaerobic or aerobic conditions. In aerobic conditions, oxygenase enzymes introduce oxygen atoms into hydrocarbons. The anaerobic degradation is catalysed by anaerobic bacteria, such as sulphate-reducing bacteria, using different terminal electron acceptors [53]. Under aerobic condition petroleum hydrocarbon degradation occur faster than anaerobic condition. But anaerobic condition is also important for bioremediation process, because in several cases the environmental conditions can include limitations of the oxygen availability, such as in mangroves, aquifers, and sludge digesters. Degradation of petroleum hydrocarbon can be achieved by the use of laccases enzymes, laccases were expressed from *Myceliophthora thermophila* (MtL) in *Saccharomyces cerevisiae*, using directed evolution, and extensively improved laccase expression. Whitely and lee reported that until 2004, there were over 1000 described enzymes involved in the biodegradation of aromatic systems [54]. Enzymes like oxygenase, dehydrogenase and lignolytic are responsible for degradation of hydrocarbons or oil spills. lignin peroxidase, laccase, and manganese peroxidase are fungal lignolytic enzymes. They are extracellular and catalyse radical formation by oxidation to destabilize bonds in a molecule [55]. In Spent Mushroom Compost (SMC), Laccase and Mn-dependent peroxidase are present abundantly, whereas the production of ligninase is reported to be low and addition of SMC enhances the rate of PAH-degradation [56]. The fungal enzymes have low specificity, which secrete several types of compounds, like *Phanerochaete chrysosporium* is a crust fungus, which degrades many harmful chemicals including benzene, ethyl benzene, xylene, toluene, organochlorines, N-heterocyclic explosives, petroleum hydrocarbons, synthetic dyes etc. Enzymes like extracellular oxidoreductases, cell-bound enzymes, and other transferase are the major group of enzymes involved in the degradation of wastes materials and pollutants [57]. There are some fungi which are producing enzymes namely laccases, tyrosinases, lignin peroxidases, manganese peroxidases, versatile peroxidases, coprinopsis cinerea peroxidase, dye-decolorizing peroxidases, caldariomyces fumago haeme thiolate chloroperoxidase, haemethiolate peroxygenases, cytochrome P450 monooxygenases, nitroreductases, reductive dehalogenases which helps to remove pollution from the environment [58].

2. CONCLUSION

Now a days we could not imagine our life without Hydrocarbon products and oil of our day-to-day life. Because these are very necessary requirements. But if we see the other aspects of hydrocarbon material these are not eco-friendly for our environment. Therefore, hydrocarbon contamination effects all forms of life and have an ability to cause several diseases in human beings, animals and plants. Hydrocarbon and oil spills on the ocean create toxic environment for the aquatic animals. Hence to solve the problem of hydrocarbon contamination it is necessary to understand the role of microorganism in hydrocarbon degradation. Because microorganisms help in converting the harmful pollutants in into less harmful product. As apart from physical and chemical treatment of hydrocarbon degradation the biological approaches that involved the microorganisms are the most effective treatment in case of hydrocarbon degradation. Bioremediation of petroleum hydrocarbon by microorganisms has potential to remediate polluted areas. There are certain environmental factors which affects the microbial bioremediation of hydrocarbons but when the condition is favorable for the growth of microorganisms the rate of hydrocarbons degradation also enhances. Hydrocarbon degrading microbes were studied extensively. But there is still a lack of knowledge on the microbial degradation of hydrocarbons. In biotechnology we can use applications of genetic engineering for the improvement degradation pathways in microorganisms. Bioremediation can be effective only where environmental conditions permit microbial growth and activity. Bioremediation has been used in different sites globally within varying degrees of success. Mainly, the advantages is greater than that of disadvantages which is evident by the number of sites that choose to use this technology and its increasing popularity through time. Generally, different species are explored from different sites and they are effective in control mechanism.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

FUNDING

Funded by Bio- Design Innovation Centre, Rani Durgavati University, Jabalpur (Sponsored by MHRD, New Delhi, India).

ACKNOWLEDGEMENT

Authors are grateful to Bio Design Innovation Centre, Rani Durgavati University, Jabalpur sponsored by MHRD, New Delhi for providing excellent facilities and for their encouragement in carrying out this works.

CONFLICT OF INTEREST

There is no conflict of interest exists.

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