

Role of microbes in bioremediation of hydrocarbon associated pollution

Función de los microbios en la bioremediación de la contaminación asociada a la hidrocarbonos

NG Rakesh*, Devki, Varsha Gupta, Deepesh Kumar Neelam, Ravi Kant Rahi
Department of Microbiology, JECRC University, Jaipur, Rajasthan, India

* Author for correspondence, e-mail: ng.rakesh3000@gmail.com

ABSTRACT

Almost two-thirds of the energy source of the world depends on petroleum and hydrocarbon production and there is an increase in demand. There are various reports about the spillage of crude oil around the world and is of major concern for our environment. The traditional methods of remediation of crude oil spilled areas are unconventional, expensive, and leave toxic intermediates that are harmful to the environment and other living creatures including human beings. Such problems can be overcome by the use of microbes for remediation of spilled petroleum hydrocarbons and drawing the attention of researchers to improve the current conditions. Biodegradation by microbes is significantly less expensive and posed little to no harmful effects on the environment. In this review paper, a clear insight of microbial bioremediation is expressed and the advantages of implementing the role of microbes in the bioremediation of crude oil and hydrocarbon related pollution. In addition, the interaction between the microbe and the environment are discussed and the necessary research and development regarding this field. The advancements in the genetic engineering field of bioremediation and introduction of GMOs in the controlled environments and the processes that hinder field trials.

Keywords: bioremediation, crude oil, biodegradation, microbial remediation, oil-spills.

RESUMEN

Casi dos tercios de la fuente de energía del mundo depende de la producción de petróleo e hidrocarburos y hay un aumento de la demanda. Hay varios informes sobre el derrame de petróleo crudo en todo el mundo y es de gran preocupación para nuestro medio ambiente. Los métodos tradicionales de remediación de áreas derramadas de petróleo crudo son poco convencionales, costosos y dejan productos intermedios tóxicos

que son dañinos para el medio ambiente y otras criaturas vivientes, incluidos los seres humanos. Estos problemas pueden superarse mediante el uso de microbios para la remediación de hidrocarburos de petróleo derramados y llamando la atención de los investigadores para mejorar las condiciones actuales. La biodegradación por microbios es significativamente menos costosa y presenta pocos o ningún efecto dañino sobre el medio ambiente. En este artículo de revisión, se expresa una idea clara de la biorremediación microbiana y las ventajas de implementar el papel de los microbios en la biorremediación de la contaminación relacionada con el petróleo crudo y los hidrocarburos. Además, se discute la interacción entre el microbio y el medio ambiente y la investigación y el desarrollo necesarios en este campo. Los avances en el campo de la ingeniería genética de biorremediación e introducción de OGM en los ambientes controlados y los procesos que dificultan los ensayos de campo.

Palabras clave: biorremediación, petróleo crudo, biodegradación, remediación microbiana, derrames de petróleo.

INTRODUCTION

Our world is facing major problems regarding environmental issues. It is not surprising that such problems can be solved by creatures that can't be seen by our naked eyes. Bioremediation is the process of degrading organic and inorganic pollutants by enzymatic action on them (Admassu & Korus 1996). The most widespread pollution in the ocean is caused by oil spills. Crude oils and their derivatives in aquatic and terrestrial environments are a huge source of environmental concerns (Oboh et al. 2006).

Polycyclic aromatic hydrocarbons (PAHs) are aromatic hydrocarbons with two or more fused benzene rings in various structural configurations (Blumer, Blumer, et al. 1977, Vichi, Pizzale, et al. 2005) and does not contain substituents of heteroatoms. Heavy PAHs that contain more than four rings are more stable and more toxic than the light PAHs that have up to four rings (Kuppusamy et al. 2016). It only consists of carbon and hydrogen combined in simple to complex ring structures, it has a wide variety of physical, chemical, and toxicological characteristics (Cerniglia 1992). Most PAHs occur as hybrids encompassing various structural components, such as naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, Benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene. The increase in hydrophobicity and electrochemical stability depends on the increase in size and angularity of the PAHs (Harvey 1991).

Due to unsustainable oil explorations, marine habitats are becoming unfit for inhabitants of the environment. The presence of heavy compounds is the major concern

during petroleum hydrocarbon pollution. Carbon chains that are branched and consist of more than twenty or more carbons are relatively resistant to biodegradation. The major component of crude oil consists of the low and medium molecular weight PAHs while the rest of it is made up of the high molecular weight. It is of great environmental concern that the contamination of these known carcinogenic and toxic components in the soil and aquatic bodies may cause harmful effects on humans and other life forms (Gupta et al. 2016). PAH is made up of two or more benzene rings and is a potent carcinogen in nature (Hamdi et al. 2007). They become less capable of future microbial degradation as they tend to interact with organic-phase and non-aqueous matter (Johnsen et al. 2005).

Such compounds move to a higher level of the food chain and can harm higher life forms through biomagnification (Vinothini et al. 2015). The problem of oil spills not only arises from accidents of supertankers or oil well blowouts, but natural disasters also initiate oil spills (Olowomofe et al. 2019).

The main possible routes for human exposure of PAHs are from breathing ambient indoor air, eating food containing PAHs, smoking cigarettes, or breathing smoke from open fireplaces, from the fossil fuels that we use to drive our cars, cook our food (Zhang, Cui, et al. 2015).

The microbes degrade the hydrocarbons in single or multiple ways using their enzymes. Most of the microbes are hydrocarbon specific hence are impotent of degrading hydrocarbons of all forms. But some of them are versatile enough to degrade a wide range of hydrocarbons (Sathishkumar et al. 2008). There is no doubt that micro-organisms degrade petroleum hydrocarbons, but the problem that arises is that the degradation of the crude oil by natural process is a very time-consuming process and hence does not meet the environmental demands (García et al. 2015). The cost of the current remediation process is very high and is not as reliable as they generate toxic intermediates. More than 70 genera of microbes have the ability to degrade and obtain energy from more than one type of hydrocarbons (Feng et al. 2007, Liang et al. 2009, Haderlein et al. 2006). The advancement of technologies towards genetic engineering can be potent for the bioremediation of the crude oil-contaminated areas in a more efficient and cost-effective way than the existing traditional methods.

DEGRADATION OF PAHs (POLY-AROMATIC HYDROCARBONS)

The process of biodegradation of PAH present in the environment is mediated by micro-organisms having different and most often interconnected metabolic pathways (vila et al. 2015).

In recent studies, sphingomonads and actinobacteria are being focused on their activity on high molecular weight compounds. Sphingomonads are able to oxidize a wide range of polyaromatic substrates even though their main preference is low molecular

weight (Stolz and Andreas 2009). *Sphingobium sp.* PNB dihydroxylated phenanthrene on carbons 1,2, 3,4, or 9,10 (Khara et al. 2014) initiating oxidation in different positions. Whereas *Sphingobium sp.* KK22 grew on phenanthrene and transformed the HMW PAHs fluoranthene, benzo(a)anthracene, and benzo(k)fluoranthene (Kunihiro et al. 2013, Maeda et al. 2014). Mobile genetic elements or plasmids were found to have similar genetically similar arrangements in *Sphingobium yanoikuyae B1*, *Sphingobium sp.* P2, *Novosphingobium aromaticivorans F199* or *Sphingomonas sp.* LH128 showing widespread distribution and horizontal transfers in sphingomonads (Stolz and Andreas 2009). It is found out in the recently studied that PAHs and monochromatic compounds separately induce two estradiol degradation pathways mediated by genes located in the large plasmid pLA1 and the chromosome of the marine bacterium *Novosphingobium pentaromaticivorans US6-1* (Yun et al. 2014). Such a kind of plasticity in the catabolic activity and short time for duplication helps in mitigation of a wide range of aromatic compounds leading to the degradation of PAHs in a very short period of time. Fig.1 shows the process of degradation by microbes.

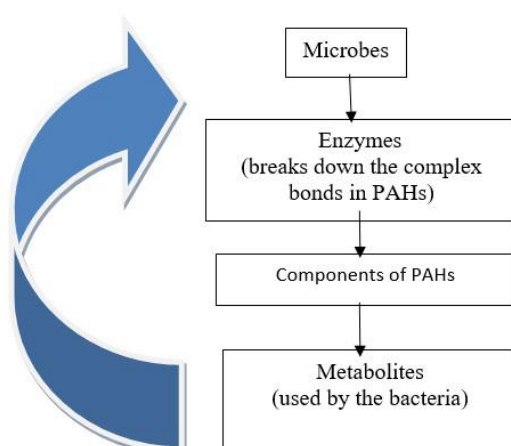


Fig.1., The action of degradation of PAHs by microbes.

BIOSURFACTANT

The surface tension between air-water and water-oil interfaces is reduced by surfactants and because of their property to increase aqueous solubility to non-aqueous phase liquids they are used to separate oily materials from a particular media (Yin et al. 2009, Martins et al. 2014). Biosurfactants are amphipathic compounds excreted mostly by microbes for growth on water-immiscible substrates that have the ability to reduce surface and interfacial tension at the phase boundary making the substrate available for nutrients uptake and metabolism. They function similarly to the chemical ones (Cunha et al. 2004, Singh et al. 2007).

In the recent studies of strains isolated from Creosote contaminated soil consortium which has an efficient amount of PAH and biosurfactant producing bacterial species like *Bacillus stratosphericus*, *Bacillus subtilis*, and *B. megateriumi*, *Ochrobactrum sp.* and *P. aeruginosa*, are the only few bacteria that are efficient in producing biosurfactant (Bezza et al. 2016). The decrease in degradation of PAHs with increasing ring number was also confirmed earlier by Tikilili and Chirwa (2011). A finding by (Tripathi et al. 2019) aiming to develop an effective bacterial consortium for enhanced biodegradation. The biosurfactant was identified as rhamnolipid from *O. anthropi* IITR07, *S. maltophilia* IITR87, *P. mendocina* IITR46, and *P. aeruginosa* IITR48 and glycolipid from *M. esteraromaticum* IITR47. The Biosurfactants produced exhibited stability under extreme pH, temperature, and salt concentration and are able to remove crude oil. The strains of *B. subtilis* and *P. aeruginosa* exhibited antimicrobial properties that could be utilized in cleanup in oil spillage and the PAHs present in the contaminated soil.

With the advancement of technology, more attention is drawn to the ability of bacteria to produce biosurfactants because of its ecofriendly environmental process (Lotfabad et al. 2009). Biosurfactants have many advantages over their counterparts which are synthesized chemically. The degradation period is less and the toxicity is found to express the higher value of effective concentration than the synthetic dispersants (Satyanarayana et al. 2012). Many biosurfactants are least affected by physical factors such as temperature, pH, and ionic tolerances (Muthusamy et al. 2008).

FACTORS AFFECTING PRODUCTION OF BIOSURFACTANTS

Nutrient availability and Environmental conditions: The nature of the carbon substrate affects the quality and quantity of the production of biosurfactant production (Rahman et al. 2008). In *Arthrobacter paraffineus* ammonium salts and urea are preferred but in *P. aeruginosa* nitrate is used for the production of biosurfactant (Adamczak & Bednarski., 2000).

For production in large amount, optimum temperature, pH, aeration and its salinity is required. The best results are reported in pH 8.0 (natural pH of the sea) and temperature about 25-30°C (Desai and Banat 1997; Zinjarde & Pant, 2002). Increased trehalose synthesis is induced by temperature stress in *Rhodococcus spp.* (Matvyeyeva & Aliieva, 2014).

For the optimization of production of biosurfactants four main factors are responsible (i) a C/N ratio higher than 10, with a maximum production with C/N ratio = 13/1, (ii) the presence of phosphorus in the culture medium, with a maximum production with a C/N/P ratio = 13/1/2, (iii) a constant presence of dissolved oxygen in the medium, and (iv) a pH superior to 7. Purification of the produced biosurfactant

allowed its characterization, with a CMC = 42 mg/L, and the determination of the Molar Solubility Ratio for 4 PAHs with this biosurfactant (Cazals et al. 2020)

FACTORS RESPONSIBLE FOR EFFECTIVE DEGRADATION OF POLLUTANTS

The natural attenuation of crude oil is a very slow process due to the imbalance of nutrients in the environment (Joy et al. 2017). For the microbes to degrade the pollutants effectively, we need to optimize the condition of the environment to clear the way for increased microbial action.

Bioavailability: Substrate availability is of major importance for microbes to bind and carry out bioremediation. It is also assumed that bound substrates are not available for the microbes until it undergoes desorption (Miller and Alexander, 1991). If the substrate bioavailability is increased and the degradation reactions have not inhibited the mobilization of the contaminant might be beneficial. It is also important to consider the impact of the environment on the survival and activity of the microbe (Crawford et al. 2015). Several studies have shown that extracellular enzymes of microbes facilitate the disintegration of complex polymers in EPS to less complex molecules (Jones and Lock, 1993; Romaní and Sabater, 2000; Espeland et al. 2001).

Rate of degradation: In order to increase the rate of degradation and contaminant availability supplementation with exogenous surfactants may also be considered (Churchill et al. 1995). A study by Doshe (1994) has shown that the transport of phenanthrene is enhanced by the extracellular bacterial polymers in the sand column (Dohse and Lion, 1994). Several types of extracellular enzymes are secreted by heterotrophic microbes, assisting the degradation of EPS components (Decho, 1990). EPS production and extracellular enzyme activity are enhanced by the addition of oil and dispersant Corexit (Kamalanathan et al. 2018). During the deepwater horizon oil spill, the oil and the Corexit can act as carbon sources to the microbes in addition to the EPS. It can also lead to a significant change in the hydrocarbon degraders community of microbes (Bacosa et al. 2015, Kleindienst et al. 2015, Doyle et al. 2018). By gravimetric analysis, *P. aeruginosa* and *B. subtilis* express maximum degradability of crude oil by 88.75% and 87.41% (Veerapagu et al. 2019).

BIOAUGMENTATION

Bioremediation of hydrocarbon polluted areas is accomplished mainly by the indigenous microorganisms present in the particular with the help of other diverse groups of microbes (Sebiomo et al. 2010). Bioaugmentation is the process of adding microbial community which is endogenous or exogenous metabolically active in the site of petroleum hydrocarbon contaminated site when the metabolic activity is low. When

considering bioaugmentation, consulting the local regulation is a crucial step to decide which biota could serve the best according to the needs (Ueno et al. 2007). Choosing a suitable strain with the effective catabolic potential to carry out degradation of hydrocarbons and knowledge of types and level of the contaminant is required for a more effective bioaugmentation process (Forsyth et al. 1995, Vogel 1996). Vidali stated that in the remediation of contaminated soil, the application of microbial cultures is limited as the non-indigenous microbial consortium does not perform well as compared to the indigenous microbial population for the introduction and the survival of the new microbial population, the soils that are exposed to petroleum hydrocarbons for a longer period of time have indigenous microbial communities with more efficient biodegradation capabilities if the contaminated soil is managed well. It is reported that mixed culture of bacteria has the potential of maximum degradation due to the reason that there is no single strain of bacteria that has the capability to degrade all the components of the crude oil (Bento et al. 2005, Adebuseye, 2007).

BIOTECHNOLOGY ROLE IN BIOREMEDIATION

Genetic engineering changes the properties of an organism by manipulating the genes for a specific purpose with the help of technologies (Vert et al. 2012). It can be used to alter the genes of bacteria to produce microbes that have multiple characteristics and capable to perform different activities like degrading heavy oils with the ability to thrive in extreme environments (Sen 2008; Patel et al. 2015). For monitoring of bioremediation, (Sayler and Ripp 2000) developed a genetically modified strain of *P. fluorescens* strain HK44 designed to detect an environmental contaminant and indicating with the help of bioluminescence. Field release of genetically engineered microorganisms (GEMs) is often done in an environmentally monitored area because of the difficulty and lengthy process required to obtain the permission of the government. The present strategies to eliminate GEMs from the environment after introduction can be a risk because of the gene transfer potential than the modifications done for bioremediation (Ensley & DeFlaun, 1995).

CONCLUSION, SUGGESTIONS, AND FUTURE DEVELOPMENTS

Microbes play an important major role in the bioremediation of crude oil and PAHs contaminated areas. Such microbes modify the outer membranes of their cell producing surfactant and exert out harmful toxic materials to survive the harsh condition of the environment. The enzymes produced cleave the complex hydrocarbons chains and convert them into simpler molecules so that the microbes can utilize them as a carbon source. In recent findings, many strains of microbes possess genes that are capable of degrading more than one form of hydrocarbons (e.g. aromatic, aliphatic). With the help

of genetic engineering and more in deep research, it is possible that such potent strains of microbes can be exploited to get the best possible results in bioremediation of sites contaminated by the spillage of crude oil or PAHs.

ACKNOWLEDGEMENTS

All listed author(s) are thankful to JECRC University for providing the related support to compile this work.

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Received: 12th January 2021; Accepted: 18th August 2021; First distribution: 05th September 2021.