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Biodegradation and Bioremediation of Petroleum Hydrocarbons in Marine Ecosystems by Microorganisms: A Review

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ABSTRACT

Concern about increasing incidents of petroleum hydrocarbon spills and spillage into different marine environments is rising day by day due to enhanced human activities in marine water. The toxic compounds of spilled petroleum hydrocarbon in marine water lead to the immediate death of numerous marine organisms as well as initiate various vicious biogeochemical cycles in the marine environment resulting in prolonged toxic impacts on the marine environment. Recently, many sophisticated techniques, including physical methods, chemical methods, and biological methods, have been developed and adopted for the treatment of marine environments polluted with petroleum compounds. However, biological treatment is one of the most promising methods in this field by which microorganisms such as bacteria, fungi, and algae are used for biodegradation of pollutants such as the spilled petroleum hydrocarbon into neutralized or eco-friendly compounds. This review has been focused on different aspects of the pollution of the marine ecosystem by oil, mainly Petroleum hydrocarbons, the fate of spilled oil in marine environments and the role of microbial communities in it, as well as various techniques, especially the bioremediation and biodegradation of spilled oil including the factors affecting the capacity of techniques. Moreover, some future aspects of research in the field of biodegradation and bioremediation of spilled oil have been proposed.

INTRODUCTION

Mineral oil is an important resource to produce the necessary energy for daily life. It is considered one of the most important materials to produce petroleum fuels such as kerosene and gasoline, and many other chemical compounds such as plastics, chemical reagents, solvents, and pharmaceuticals products. Moreover, more and more oil-related economic activities such as oil extraction, refining, transport, and marketing are being carried out because of the increasing global demand for oil for leading modern life. Importantly, half of the activities related to the transportation of the world's petroleum are achieved by seas and oceans (Varjani & Upasani 2016). As a result of this increased transportation of petroleum compounds by seas and oceans, there has been a significant risk of pollution of marine water by the incidence of oil spills from drilling oil wells, transmission pipelines, and transfer oil tankers around the world. Hence, marine environments become one of the largest and most important reservoirs contaminated with petroleum hydrocarbons resulting in a severe form of pollution (Mahjoubi et al. 2018, Ławniczak et al. 2020). The pollution of marine environments with hydrocarbons is a global concern because of the potential consequences of this pollution on different environments, humans, and marine organisms (Ahmed & Fakhruddin 2018). Hence, rapid action is essential for the treatment of spilled hydrocarbons in a polluted environment. However, treatment of a polluted environment by chemical or physical methods is very expensive as well as difficult to use as compared to the biological methods in which biological agents, mainly microorganisms are used to clean and remove various chemicals from contaminated aquatic environments (Zheng et al. 2013, Ruhi 2017, Saha et al. 2017, Nasrin 2019, Rahman et al. 2019). However, biological methods such as the biodegradation of hydrocarbon pollutants take a longer period and have more complex mechanisms, depending on the amount and nature of hydrocarbon contaminants and the availability of microorganisms (Xu 2018). Hence, microorganisms that degrade hydrocarbons play the main function in the biodegradation of contaminated environments, where these microorganisms flourish and adapt to these polluted environments (Adeleye 2018).

FUEL OIL POLLUTION

Fuel oil is a natural combination of hydrocarbons that may be in a liquid, solid or gaseous state depending on both pressure and temperature. The extracted oil is not pure containing some impurities such as carbon dioxide, hydrogen sulfide, and some other complex components such as sulfur, nitrogen, oxygen, and other impurities (Hamidian et al. 2020). At the beginning of the twentieth century, great global economic changes were introduced. Consequently, these changes increased the encouragement of the industrial and technological revolution and relied on it as a pattern of prosperity. These changes led to a rise in the demand for the extraction, purification, processing, and transportation of petroleum products, which in turn caused the discharge of large quantities of petroleum waste, around 67 million tons waste per year (Nath & Cholakov 2009). Thus, pollution caused by releasing of fuel oil and their waste into the ecosystem become one of the main types of global pollution, and thus this problem has become the focus of attention to both developing and industrialized countries because of its deleterious effects on both living organisms as well as different environments (Luna et al. 2013). The pollution of the seas and oceans by fuel oil mainly results from the routine operations of oil extraction, refining, and washing operations as well as accidents during oil exploration, extraction, and transport operations. Moreover, natural drift from the seabed, sediment erosion, atmospheric sedimentation from incomplete oil combustion, runoff of rivers and oil-contaminated lands and waste, shipping and clearance, and illegal discharges also contribute to the pollution of marine water (Souza et al. 2014). The spilled fuel oil spreads into the water very quickly with a thickness of 1 mm. Fuel oil pollutants spread in the form of a thin coat on the marine water, which in turn interrupts the mechanisms of gas exchange between atmospheric oxygen and water surface, and thus the reduction of the amount of dissolved oxygen in the water resulting in a higher rate of mortality and morbidity of marine organisms. In addition, fuel oils imprinted into aquatic environments have toxic effects on humans and other terrestrial organisms depending on marine environments directly or indirectly (Hranova 2006). Petroleum hydrocarbons are non-homogenous compounds, so scrubbing these compounds from the marine environment is a difficult process. However, the natural biological process is one of the main ways to clean the environments that are contaminated with hydrocarbons. This microbial degradation occurs naturally in different environments, where the potential microbial communities are available for decomposition and removal of oil pollutants naturally from the environment (Varjani 2017).

THE FATE OF FUEL OIL IN THE MARINE ENVIRONMENT

Pollution of the marine environment by several hundred million gallons of crude oil products annually has been reported and its fate depends on many factors, whether physical, chemical, or biological (Saadoun 2015). These include biological processes, evaporation, hydrolysis, water emulsification, dispersion, sinking, sedimentation, and photolytic oxidation. Biological processes include microbial degradation, ingestion, and the assimilation of oil into the environment (Neff 2012). During these processes in the environment, the changes occur in the structure as well as in the physical and chemical characteristics of the original pollutants. However, the most vital weathering process is initiated in the water environment within 48 hours of oil spills, resulting in evaporation of the components of medium and light oil with a low boiling point into the atmosphere. The evaporation process may be liable for the loss of 33% to 66% of the amount of oil leaked into the aquatic environment (Yakimov 2004). For instance, one-third of the fuel oil spilled from the supertanker Amoco Cadiz evaporated in just three days. Many factors control the process of evaporation such as the common structure of the oil, wind velocity, surface area, air and water temperature, intensity of solar radiation, and abandoned metals (Fig. 1).

RESPONSE OF MARINE MICROBIAL COMMUNITY TO FUEL OIL POLLUTION

Microbes that decompose hydrocarbons vigorously are usually found in the oceanic environment, where spilled hydrocarbons are used by these microbes as an energy source resulting in their enhanced growth and multiplication. Moreover, the number of hydrocarbons degrading microbes is also increased because of changes in some of the catalysts and other physiological factors in polluted marine ecosystems (Labud et al. 2007). Thus, the normal structure of the microbial community is affected by the overgrowth of hydrocarbon-degrading microbes in the marine ecosystem polluted with petroleum hydrocarbons (Xu 2018, Truskewycz 2019). For example, significant changes in the structure of the microbial community in the oil-polluted water of the Caspian Sea and the Persian Gulf were reported by Hassanshahian et al. (2010). Hence, the determination of the main microbial communities that are working on the degradation

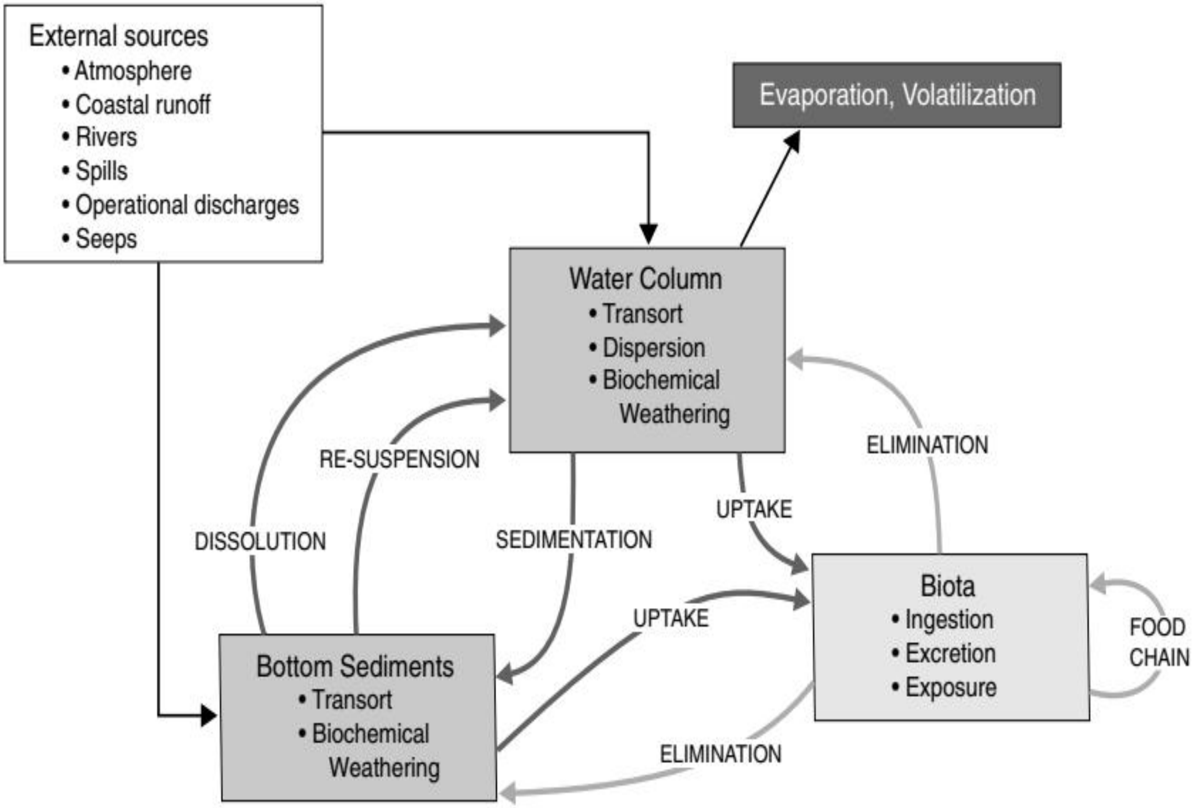

Fig. 1: A model for the fate of oil in the aquatic environments (Board et al. 2003).

of hydrocarbon pollutants is very important for understanding and developing treatment processes for contaminated sites.

The process of natural biodegradation is one of the efficient ways of removing hydrocarbon pollutants from different environments, especially non-volatile hydrocarbons from crude oil. Commonly, microbes such as bacteria, fungi, and yeast convert hydrocarbon compounds into simpler compounds, some of them are not soluble in water (Cappello 2012). It is hard to accomplish optimal biodegradation and bioremediation values for hydrocarbon pollutants in a short period, because of some non-biodegradable compounds which need more time to deteriorate, as well as many variables that impact the degree and range of biodegradation and bioremediation of contaminants (Cappello 2012, Varjani & Upasani 2017). Moreover, the quantity of some nutrients such as phosphorus and nitrogen is inadequate to support the growth of microorganisms in marine ecosystems naturally, however, supplementation of these substances to contaminated sites encourages the growth of hydrocarbons degrading microbes in marine ecosystems (Hassanshahian et al. 2010). However, some microbial communities finally can adapt to hydrocarbon contamination as they act to resist the toxic effects of these pollutants, thereby increasing the number of microbial organisms that use hydrocarbon contaminants as food sources (Mahjoubi et al. 2018).

OIL SPILL CLEANING BY BIODEGRADATION AND BIOREMEDIATION

Many methods namely physical, chemical and biological techniques are used to clean the areas contaminated with oil spills to protect the floras and faunas from further destruction and extinction. However, cleaning up the contaminated environments is often very difficult because of the influence of several factors. The physical or chemical method could be the first option that can be taken immediately after oil spills, even though both techniques have some drawbacks such as the requirement of many auxiliary materials, failure to complete elimination of the leaky oil, and further damage to the environment by the chemicals used for treatments (Kukkar 2020). Because of such disadvantages of physical and chemical methods, biodegradation and bioremediation have appeared as alternative approaches for removing oil from polluted environments.

Biodegradation

Biodegradation of hydrocarbons is an important process in which microorganisms are applied to improve the natural cleaning procedures of hydrocarbon contaminants. However, the process of biodegradation of hydrocarbons is subject to many factors and a series of vital and often different interactions between microorganisms and other factors which mostly occurs on the surface of the contaminated water, and in the beaches, the sediments, and water column of the marine ecosystem. Ultimately, this method relies on the consumption of hydrocarbons by microorganisms to produce food, energy, and carbon dioxide. Biodegradation initiates instantly after the oil spill into the aquatic environment. Microbes naturally degrade oil in the water, and thus they grow and thrive in the contaminated environment due to the availability of food. This process continues with the presence of biodegradable hydrocarbons in the contaminated environment, culminating in the first month of the oil spill and decreasing over time to the depletion of nutrients from the environment. Nutrients (N, P, and K) play an important role in the thriving of potent microbial communities resulting in a faster rate of degradation of hydrocarbons along the polluted shoreline (Shewfelt et al. 2005). However, effective biodegradation of petroleum hydrocarbons by microorganisms is dependent on some physicochemical factors namely temperature, pH, dissolved oxygen in water, and nutrients that regulate the growth of the microorganisms as well as some biological factors such as an enzymatic activity that control the potency of biodegradation (Leahy & Colwell 1990, Varjani & Upasani 2017, Ahmed & Swargiary 2021). In addition to these, many other factors influence the biodegradation rates, for example, the chemical composition and the physical condition of the oil, the type, and quantity of spilled oil, the characteristics of the polluted ecosystem, etc. (Haritash & Kaushik 2009).

Bioremediation

Bioremediation is one of the alternative techniques for the treatment of pollutants which is an inventive technique that relied on microorganisms to reduce toxic pollutants to harmless compounds such as H2O, CO2, CH4, and biomass without disturbing the ecosystem in the polluted environment (Ron & Rosenberg 2014). There are many types of microorganisms that have the potential for bioremediation of spilled hydrocarbon pollutants. These include yeast, fungi, algae, and bacteria (Naeem & Qazi 2019). The rate of bioremediation can be enhanced by the supplementation of some elements such as N to the oil-contaminated environments (Wang et al. 2011). Even if the marine environments polluted with spilled oil are cleaned, some compounds may last for decades on these beaches and environments, threatening the organisms living in these ecosystems (Owens et al. 2008).

Depending on the application process, the bioremediation process can be classified into two types, in situ bioremediation, and ex-situ bioremediation. In situ bioremediation techniques are the most desirable options, because of their low cost and avoiding drilling and transport of contaminants where treatment is done on-site. On contrary, ex-situ bioremediation which is the removal of the contaminant's physical material for treating contaminated environments is very costly as well as problematic due to the tough extraction of contaminants from underwater or soil (Farhadian et al. 2008). This technique is achieved by drilling and removal of contaminated soil and water, including composting, land farming, bioreactors (Vidali 2001), and biopiles (von Fahnestock & Wickramanayake 1998). Bioremediation processes can also be classified into two main approaches, i) Biostimulation and ii) Bioaugmentation (Varjani et al. 2013).

Biostimulation

Biostimulation is defined as an environmental modification process to encourage the growth of existing microbes as well as to stimulate the biodegradation of pollutants to be treated in the modified environment. This process is applied in practice by adding nutrients or substances, for example, carbon, nitrogen, and phosphorus to the environment to be treated, as well as by ensuring appropriate environmental conditions such as moisture and oxygen content to promote microbial growth and natural biodiversity of microorganisms. Nitrogen, Phosphate, and molecular oxygen concentrations in seawater are important examples of abiotic factors. Therefore, Nitrogen and Phosphate are used to reduce the limit of these nutrients, which spurs the prosperity of degrading bacteria for petroleum hydrocarbons. Nitrogen and Phosphate based fertilizers such as nitrates, ammonium phosphate, urea, and phosphate, can be used in such cases because they have high solubility in water (Nikolopoulou & Kalogerakis 2009). Moreover, uric acid which is used as a source of nitrogen by several bacterial species, including *Alcanivorax* strains, can be a potent biological catalyst for the bioremediation of spilled oil (Ron & Rosenberg 2014).

Bioaugmentation

Bioaugmentation is defined as the use of particular strains of microbial organisms, which could be either isolated or genetically modified to increase their capability for controlling pollution, for example cleaning spilled oil in contaminated marine ecosystems (Nzila et al. 2016). This process relies on the capability of microbes to metabolize pollutants and turn them into less toxic and less dangerous compounds. Biodegradation occurs naturally in contaminated environments by indigenous microorganisms which usually have limited capacity to degrade these compounds because of the complex structure of compounds and unfavorable environmental conditions. Therefore, bioaugmentation is required in such conditions so that those complex compounds can be degraded completely by the specialized microbial organisms (Nzila et al. 2016).

Bioaugmentation also called the sowing of microorganisms in the contaminated sites, is used in marine ecosystems polluted with oil spills to promote the decomposition of spilled oil. This leads to the increase of microorganisms, where these microbes use hydrocarbons as the sole source of energy and carbon. These microbial organisms are called hydrocarbonoclastic microorganisms, mainly bacteria including many strains such as *Cycloclasticus*, *Thalassolituus*, *Alcanivorax*, *Oleispira*, and *Oleiphilus* (Nzila et al. 2018). *Cycloclasticus* strains prefer growth on aromatic hydrocarbons, for example, anthracene, phenanthrene, and naphthalene, while *Oleispira* sp. and *Oleiphilus* grow on aliphatic hydrocarbons, alkanoates, and alkanols (Nzila et al. 2018). Similarly, the strains of *Alcanivorax* sp. can grow on branched alkanes and n-alkanes only (Head et al. 2006).

Aerobic and Anaerobic Bioremediation

The rate of bioremediation of hydrocarbon pollutants is less in the absence of oxygen (anaerobic) as compared with that in the presence of oxygen (aerobic). The bioremediation of hydrocarbons in the presence of oxygen is faster and easier because most species of bacteria, algae, and fungi have the potential to degrade in aerobic conditions (Haritash & Kaushik 2009). The addition of oxygen to contaminated environments increases the rate of biodegradation several times than the rates that occur naturally in these environments. In aerobic environments, microbes usually break down the hydrocarbon contaminants by forming alcohol by adding an unsaturated ring of polycyclic aromatic hydrocarbons or a hydroxyl group to the end of the alkanes. Hence, these compounds are easily soluble in water with oxygen. Moreover, aerobic microbes have a greater potential to degrade a broad range of hydrocarbons than anaerobic microbes resulting in effective bioremediation of hydrocarbon pollutants in aerobic conditions (Saxena et al. 2013). Despite the slow rates of anaerobic bioremediation, this is also important for the complete degradation of many hydrocarbons in the absence of oxygen, for example, deterioration of polycyclic aromatic hydrocarbons was reported within 90 days, while benzene degradation occurred in 120 weeks (Meckenstock 2016).

BIODEGRADATION AND BIOREMEDIATION OF FUEL OIL BY MICROORGANISMS

There are many types of microorganisms such as bacteria, algae, and fungi that decompose hydrocarbons in the environment, but bacteria and fungi are common and efficient members of them (Table 1) (Treu & Falandysz 2017, Kaur 2018). There are a few species of algae and protozoa with the ability to degrade hydrocarbons (Kachieng'a & Momba 2017). A particular group of hydrocarbons can be metabolized by an individual type of microorganisms while a consortium of microbes with wide enzymatic abilities can degrade down-mixed and compound hydrocarbons, for example, crude oil compounds in soil, freshwater, and marine waters (Varjani 2017).

Biodegradation by Bacteria

Oil degradation passes through a series of different and sequential stages. Bacteria is the first group of microorganisms that are responsible for the first attack on oil spills to generate medium compounds that are later used by diverse types of other microorganisms (Hallbeck 2010). In marine environments, more than 200 species and 100 genera of microbes are known to degrade petroleum hydrocarbons; most of them are bacteria (Brakstad 2014). There are several potential strains of oil-degrading bacteria, such as *Oleiphilus*, *Cycloclasticus*, *Alcanivorax*, *Neptunomonas*, *Marinobacter*, etc. These bacteria act on the degradation of alkanes and aromatic hydrocarbons (Nzila et al. 2018). The other potential bacterial groups that degrade hydrocarbons in seawater and soil are *Acinetobacter*, *Achromobacter*, *Actinomycetes*, *Arthrobacter*, *Alcaligenes*, *Nocardia*, *Bacillus*, *Flavobacterium*, *Corynebacterium* sp, and *Pseudomonas* sp. (Villela et al. 2019). Similarly, there are some genera of bacteria such as *Rhodococcus*, *Corynebacterium*, *Mycobacterium*, *Pseudomonas* sp., *Brevibacterium, Arthrobacter* and *Nocardia* that degrade gaseous hydrocarbons, especially propane and/or butane (Giebel et al. 2011).

Biodegradation by Algae

Phytoremediation is an important process in the treatment of petroleum hydrocarbon, which is a type of biological treatment by using micro-algae or macro-algae to clean the solid, liquid, or gaseous pollutants from contaminated soil, wastewater, and air (Naeem & Qazi 2020). However, the use of micro-algae in the bioremediation of petroleum hydrocarbons is still a major area of research. Some studies have shown that some algae including green algae, red algae, and brown algae have the potential to degrade some hydrocarbons into less harmful compounds to the environment, indicating their ability to handle crude oil pollution (Naeem & Qazi 2020). For example, the algae *Prototheca zopfi* and *Chlorella vulgaris* have the capacity for biodegradation of hydrocarbons resulting in a decrease in alkanes, iso-alkanes, and aromatic hydrocarbons in the contaminated environment (Walker et al. 1975, Kalhor et al. 2017). In addition, the dry weight of *Chlorella vulgaris* increased with increased concentration of pollutants, indicating that crude oil has a positive effect on the Growth of types of algae (Kalhor et al. 2017).

Biodegradation by Fungi

Fungi play a very significant role in the biodegradation of petroleum hydrocarbons in polluted aquatic environments (Table 1). In addition, the sediment contaminated with

Table 1: A list of microorganisms that degrade petroleum hydrocarbons in marine environment.

Genera	Species	References
Bacteria	*Aeromonas hydrophila*	(Sarwade & Gawai 2014)
	Ochrobactrum anthropi	(Bao 2012)
	Acinetobacter lwoffii	(Alkhatib et al. 2011)
	Stenotrophomonas maltophilia	(Juhasz et al. 2000)
	Erythrobacter citreus	(Udotong et al. 2008)
	Pseudomonas aeruginosa	(Thavasi et al. 2007)
	Pseudomonas xanthomarina	(Esmaeil & Obuekwe 2014)
	Rhodococcus corynebacterioides	(Gentili et al. 2006)
	Neisseria elongata	(Adoki & Orugbani 2007)
	Vibrio fischeri	(Bao 2012)
	Bacillus megaterium	(Das & Chandran 2011)
	Enterobacter cloacae, Brevibacillus parabrevis B-1	(Al-Jumaily & Al-Wahab 2012)
	Bacillus pumilus	(Maliji et al. 2013)
	Chromobacterium violaceum	(Yusoff 2008)
	Bacillus cereus	(Latha & Kalaivani 2012)
	Klebsiella pneumoniae	(Hii et al. 2009)
	Achromobacter, Acinetobacter, Alcaligenes, Actinomycetes, Achromobacter, Acinetobacter, Alcaligenes, Actinomycetes, Archrobacter, Cycloclasticus, Coryneforms, Chromobacterium, Flavobacterium, Micrococcus, Microbacterium, Mycobacterium, Nocardia, Pseudomonas, Sarcina, Serratia, Streptomyces, Vibrio, Xanthomonas	(Giebel et al. 2002)
Fungus	*Aureobasidium, Candida* *Rhodotorula*	(Singer & Finnerty 1984)
	Acremonium, Aspergillus, Cladosporium, Mortierella, Saccharomyces, Trichoderma, Verticillium	(Bogusławska-Wąs & Dąbrowski 2001)
	Fusarium solani	(AI-Jawhari 2014)
	Aspergillus fumigatus	(Chandran & Das 2012)
	Aspergillus niger	(Okoro 2008)
	Aspergillus versicolor	(Garapati & Mishra 2012)
	Cochliobolus lutanus	(Al-Nasrawi 2012)
	Aspergillus saprophyticus	(Ekundayo & Osunla 2013)
Yeast	*Aureobasidium, Pichia, Candida maltosa, Candida tropicalis, Candida apicola, Candida, Debaryomyces, Monilia, Rhodotorula, Toruplopsis*	(Bogusławska-Wąs & Dąbrowski 2001)
	Exophiala xenobiotics	(De Hoog et al. 2006)
	Candida tropicalis	(Beier et al. 2014)
	Candida lipolytica	(Das & Chandran 2011)
	Candida maltose	(Chrzanowski et al. 2006)
	Candida tropicalis RETL-Cr1	(Tuah et al. 2009)
	Pichia ohmen YH-41	(Shumin et al. 2012)
	Trichosporon asahii	(Chandran & Das 2010)
Algae	*Agmenellum, Amphora, Anabaena, Aphanocapsa, Chlorella, Chlamydomonas, Coccochlorise, Cylindrotheea, Dunaliella, Microcoleus, Nostoc, Oscillatoria, Petalonema, Porphyridium.*	(Brakstad 2014)
	Prototheca zopfii	(Vigna et al. 2002)

spilled hydrocarbons is one of the preferred places where these fungi live to use carbon derived from the hydrocarbons (Hamad et al. 2021). However, research on the biodegradation of hydrocarbons by fungi has been focused mainly on the activity of enzymes produced by fungi in biodegradation processes. Several studies have shown that fungi have the potential to degrade total petroleum hydrocarbons with a wider range of other microbial organisms such as bacteria (Hamad et al. 2021).

CONCLUSION

Bioremediation is a cost-effective and environment-friendly technology and is unique to the most promising ways to treat oil pollution in different ecosystems. It has been observed through several studies that the coasts contaminated with hydrocarbons can be recovered by biodegradation technology in 2-5 years. This paper presents the different aspects of biodegradation and bioremediation of petroleum hydrocarbon in the marine ecosystem. As oil spills increase in marine environments, there are many issues related to the process of biodegradation in marine ecosystems that need to be studied, such as the assessment of the risks involved after the bioremediation process and finding solutions. The risks of secondary products of metabolites of hydrocarbons should be determined in the environment and their treatment process should be explored. In addition, the effects of growing and developing microbes that degrade oil on local environments should be evaluated.

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