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### Phycoremediation of crude oil-contaminated water: Current microbial remediation protocol and effect on the ecosystem: A review

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#### ABSTRACT

Owing to the extensive use of petroleum hydrocarbons in the modern world, petrochemical derivatives have become one of the major environmental pollutants with ensuing environmental devastation. Pollution of aquatic environments by hydrocarbon via numerous anthropogenic and natural sources has become even more worrisome. The detrimental effects of this pollution on the aquatic micro and macroorganisms, including fishes, birds, and mammals, have been thoroughly documented. The associated health impacts of crude oil pollution on humans and the environment have resulted in the current microbial remediation protocol to alleviate the effect on the ecosystem. The physicochemical protocols of cleaning the aquatic environments of hydrocarbon pollutants have been found deficient because of the high cost and the need for high-tech equipment and expertise. The ability of diverse microorganisms to degrade hydrocarbon pollutants as sources of carbon has been well studied for over three decades. These studies have focused mostly on bacteria. The bacterial-based crude oil remediation protocol is an effective means of remediating crude oil-polluted environments. It is environmentally friendly and cost-effective. It is even more effective when in collaboration with different species of bacteria (consortium) or when in association with plants (rhizodegradation). However, bacterial-based metabolism of organic pollutants, like crude oil, comes with severe reduction in dissolved oxygen, giving rise to longer time for microbial adaptation to the pollutants and the actual degradation, thus raising questions on the application of bacterial-based remediation protocols. Studies on the hydrocarbon metabolism by algae are already available with some details on the oxygenic metabolic pathways. The present review, therefore, highlights the potentials/advantages of algae in crude oil remediation. In addition to algae's direct involvement in the breakdown of hydrocarbon pollutants, it provides an enabling environment, like the copious supply of oxygen, for indigenous aerobic microbes equally involved in the remediation.

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#### 1. Introduction

The value of life on planet Earth depends generally on how healthy Earth is. As humans toil for their livelihood, the quality of Earth has deteriorated due to man-made

toxic pollutants discharged at every corner of Earth (Raghunandan et al., 2018). Synthetic and organic fertilizers, industrial and pharmaceutical wastes, electronic wastes, pesticides, herbicides, and heavy metals have all been incriminated as environmental

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contaminants. They have been found to have not only polluted the terrestrial, but also underground and surface waters, rendering them unfit for man's consumption and for other domestic uses (David, 2005; Dhananjaya et al., 2019; Idris et al., 2013; Jingyu et al., 2014; Kaplan, 2013; Soma, 2014).

Another very important environmental contaminant is petroleum (the crude and refined products). The increased global demand and almost complete dependence on crude oil and its numerous derivatives as source of energy and wealth have made hydrocarbon one of the most important environmental contaminants, and consequently, a huge threat to human health, especially in the third world countries (Antwi-Akomeah et al., 2018a; Bayat et al., 2015; Ikhimiukor and Nneji, 2013; Łukasz et al., 2020; Yaqoob et al., 2019).

## 2. Crude Oil

Crude oil is a naturally occurring flammable oily liquid composed of aliphatic and aromatic hydrocarbons and other heterocyclics in varied concentrations (Wiley et al., 2008). It is usually found in very large quantities as deposits in rocks far beneath the Earth's lithosphere. Crude oil is a complex mixture of predominantly hydrogen and carbon in varying molecular weights, which earned the name "hydrocarbon." However, it also contains some organic nonmetals, like oxygen, sulfur, and nitrogen (Tissot and Welte, 1984), collectively called heteroatoms and heavy metal atoms that occur in very minute proportions, occasionally referred to as impurities (Venetz et al., 2003). It consists of three major distinctive categories: paraffins, naphthenes, and aromatics (Varjani et al., 2018).

This grouping was necessary as it aids in complete understanding of the physical properties of the petroleum in general as each type of hydrocarbon exhibits unique properties. In addition to the hydrocarbon molecules which form the pillar of the petroleum, the heteroatoms blend with the different variety of hydrocarbons in a distinctive manner and also depict the numerous physical properties such as color, viscosity, and fluidity. The physical natures of the hydrocarbon directly influence the properties of crude oil. These physical natures include the number and arrangement of carbon atoms in the molecule, the number and position of carbon-carbon bonds, and presence/concentration of heteroatoms (Wiley et al., 2008; Yasin et al., 2013).

### 2.1. Significance of crude oil in modern times

Crude oil provides fuel for various means of transportation: on land, at sea, and in the air, as well as gas for cooking and for electricity generation. It is a feedstock in the chemical industries, in the manufacturing of medicines, plastics, detergents, cosmetics, insecticides, building and road construction materials, paints and fertilizers (Das and Chandran, 2011; James, 2002). Crude oil has remained the primary source of foreign earnings (Latifa et al., 2018), a major determinant of foreign relations and an important ingredient in international relations, which has always determined the strength of foreign policy, vis-à-vis the way a nation is regarded and/or treated by others (Ebel, 2005; Eze, 2015; Pitkin, 2013). These numerous useful applications turned crude oil into the lifeblood flowing through the arteries and veins of the world's economic greatness and about the most sought after natural resource (Eze, 2015; Osondu, 2017).

### 2.2. Crude oil pollution of aquatic environments

Petroleum inputs into the waters follow through two major means. The first means involves the various human activities which are connected to petroleum extraction and processing, petroleum transportation, and petroleum storage and usage. Activities including onshore and offshore drilling of crude oil, building and operating illegal coastal oil refineries, equipment failure, and pipeline corrosion/rupture (NRC, 2003), crude oil tanker accidents on the road, careless and random discharge of crude oil-rich wastes such as petrochemical effluents, expended motor oil, and reckless discharge of remnant crude oil into the soil by operatives while washing oil tankers all contribute to crude oil spillage on the terrestrial environment and eventual pollution of the aquatic environments (Abioye et al., 2012; Antwi-Akomeah et al., 2018b; Genovese et al., 2014; UNEP, 2011; Zielinski et al., 2006).

The second means, which is not man-made, involves natural crude oil seepage into the aquatic environment (Kvenvolden and Cooper, 2003; NRC, 2003). The natural seeps are completely natural occurrences where crude oil leaks from the geologic layers underneath the floor of the sea to the water surface. While it is usually an indicator to the geologists that the area has large reserve of crude oil (Osondu, 2017), these seepages discharge large amounts of crude oil into water bodies, causing serious aquatic environmental catastrophes (Leifer and Wilson, 2017; NRC, 2003).

### 2.3. Crude oil pollution of international water ways

The major causes of heavy crude oil spills and consequent pollution of the international waters are large vessel accidents/attacks (Aditi et al., 2015; Gambrell, 2019; Pérez-Peña et al., 2019), blowout of crude oil pipelines/wells (Sardrood et al., 2013), and natural seepages (NRC, 2003).

#### 2.3.1. Instances of oil tanker accidents

In 1989, the *Exxon Valdez* spilled 40,000–50,000 tons of oil into the Prince William Sound. In 1999, *MV Erika's* sinking released more than 37,000 tons of heavy fuel. In 2002, the sinking of *MV Prestige* resulted in the spillage of approximately 63,000 tons of crude oil into the Mediterranean (Yaima, 2011). In 2018, an Iranian-owned oil tanker, *Sanchi*, had a head-on collision with a Hong Kong-flagged cargo ship, *CF Crystal*, discharging 136,000 tons (960,000 barrels) of condensate natural gas into the sea (Madrigal, 2018). In July 2020, a Japanese oil tanker, *MV Wakashio*, discharged her entire content into the water at Pointe d'Esny as it ran aground a coral reef (Mikhail, 2020). In August 2020, a Chinese oil tanker, *Long Qing 1*, carrying 3,000 tons of gasoline was involved in a head-on collision with another cargo, discharging and polluting the water body with her content (Islamuddin, 2020).

#### 2.3.2. Oil well blowout

Oil well blowouts usually occur naturally when there are noticeable earth tremors causing fissures in the seabed or when there is serious reservoir pressure and the pressure control procedures fail. Blowouts are sure signs of oil deposits; they are also very dangerous, wasteful, and capable of covering the entire environment, including the aquatic habitats with barrels of crude oil (Robert et al., 2003). Selected instances include the 1910 blowout at the Midway-Sunset Oil Field in Kern County, California, which discharged more than 100,000 barrels per day (Walsh, 2010); the 1956 oil well blowout in Qom, Iran, gushing out 120,000 barrels of crude oil per day (Robert et al., 2003); the 1979 blowout of the offshore oil well (Pemex well) spilling 0.5 Megatons of crude oil into the Gulf of Mexico (Patton et al., 1981); and the Deepwater Horizon drilling rig explosion in April 2010, which resulted in the crude oil spillage that discharged into the Gulf of Mexico (Harris, 2010).

#### 2.3.3. Marine piracy and ship attacks

Deliberate attacks against ships on international waterways, either for robbery, intentional destruction

of enemy's property to get even, for ransom, as a show of strength, or intentional targeting of offshore production platforms as a calculated war strategy, have been thriving for a very long time. While reasons for attacks may vary, the impacts of attacks usually are closely similar: loss of properties, loss of lives, fatal injuries, and, of course, environmental pollution (International Maritime Organization, 2012). Few instances include the sinking of 42 oil tankers by the German submarines during WWII, discharging about 417,000 tons of petroleum products (Koons and Jahns, 1992). The Iran and Iraq War of 1981–1987 recorded 314 attacks on oil cargos causing oil spillage of over 260,000 tons into the Persian Gulf (Holloway and Horgan, 1991). In 2019, two oil cargos, *Kokuka Courageous* and *Front Altair*, carrying methanol and naphtha, respectively, were attacked near the Strait of Hormuz causing heavy spillage into the aquatic environments (Gambrell, 2019; Pérez-Peña et al., 2019).

#### 2.3.4. Natural seepages

Natural crude oil seepages from the geologic strata underneath the floor of the sea to the water surface also do occur with resultant increase in the load of crude oil in the international waters, causing serious environmental pollution (Leifer, 2019; NRC, 2003). Assessments of crude oil seepage on a global scale are completely based on very minimal information obtained from a limited number of measurements. For instance, crude oil seepages have been recorded in the Gulf of Mexico (Leifer and MacDonald, 2003), offshore Norway (Muyakshin and Sauter, 2010), the North Sea (Leifer, 2015), offshore Svalbard in the Norwegian Arctic (Veloso et al., 2015), and the arctic Laptev Sea (Leifer et al., 2017).

Through these various ways of contamination, the aquatic environment is turned into a sort of dumpsite for petroleum, causing considerable and almost irreparable damage to aquatic environments (Yilei et al., 2020). The quality of the aquatic environment, therefore, deteriorates on a daily basis in both developing and developed worlds with ensuing health, social, and economic consequences (Aditi et al., 2015; Ikenna et al., 2016; Ikhimiukor and Nneji, 2013; Ovuakporaye et al., 2012).

### 2.4. General effects of crude oil pollution of aquatic environments

Irrespective of the sources of contamination, the truth remains that the impact is usually devastating (O'Reilly et al., 2001). The most important adverse impact of crude oil contamination of water sources



is that it renders the water unfit for human uses; the attractiveness will be lost due to the oily sheen that is conspicuous on the water surface. The obnoxious smell emanating from such water makes it completely unfit for any domestic use, especially drinking. Such polluted water is also unfit for bathing or recreation as the skin may absorb the hydrocarbon or other elemental constituents (Davies and Abolude, 2016; Lindén and Pålsson, 2013; Nganje et al., 2015; UNEP, 2011).

#### 2.4.1. Effects on fishes

People living in the riverine areas are usually fish farmers (Esclamado, 2011; Rhoan, 2011; UNEP, 2011). Depending on the severity of the contamination (Silliman et al., 2012; Teal and Howarth, 1984), mature and immature fishes and their eggs, as well as their larvae, suffer exceedingly when exposed to crude oil, causing damage to the gill structures, weakening of the heart, alteration in respiratory rates, compromised reproduction, damages to vital organs, and finally death (Albert et al., 2012; Kuehn, 1995; Moles and Norcross, 1998; Willette, 1996).

#### 2.4.2. Effects on aquatic birds

Significant exposure to crude oil disrupts the structure of the feathers, thereby destroying the insulation properties of feathers (IPIECA-IOGP, 2015; O'Hara and Morandin, 2010). This causes the birds to lose their ability to cover themselves from cold, resulting in hypothermia. The birds also lose the ability to fly, which may result in drowning or become prey to other aquatic animals (Giri and Anuprakash, 2016), irritation of the eye, and the skin may also result (Jenssen, 1996; Tseng, 1993). Ingestion of the pollutant may cause severe damage to the vital organs like the liver, kidney, and blood cells (Jiang et al., 2010).

#### 2.5. Human exposure to crude oil and associated health effects

Direct and consistence body contact with sufficiently high concentrations of crude oil can result in irritation of the skin, redness of the skin, skin edema, blisters, dermatitis, and rashes (Aguilera et al., 2010; ATSDR, 2009). Also, hydrocarbons contain volatile organic compounds (VOCs), such as ethylene, propylene, formaldehyde, and benzene (de Gouw et al., 2017). These VOCs can enhance the penetration of crude oil into the sub-skin, resulting in skin cancer (Jia et al., 2019; Major and Wang, 2012; Ramirez et al., 2017).

Exposure to sufficient concentrations of vaporized hydrocarbons can lead to reddish eyes, watery/itchy eyes, and even poor vision. It may also result in

dizziness, shortness of breath and other respiratory disorders, throat irritation unproductive cough, headache, confusion, and the possibility of mental health impairment (Aguilera et al., 2010; ATSDR, 2009; Carrasco et al., 2007).

Drinking of crude oil-contaminated water and/or consumption of potentially contaminated seafood are the major ways through which crude oil gets into the human digestive system (Barron, 2012; Gohlke et al., 2011). The health effects of ingestion of petroleum hydrocarbon begin almost immediately after intake. Illness usually begins with uneasy and emotional distress, general body weakness, and back pain (D'Andrea, 2013). This is immediately followed by nausea, vomiting, and moderate to severe abdominal pain (Bosch, 2003; Gema et al., 2007). Ingestion of a large quantity of crude oil can result in the erosion of the abdominal and intestinal walls, resulting in frequent and excessive bowel movements, producing watery feces, loosing of bodily fluid and electrolyte, shock, and, if not quickly attended to, death may follow (Albert et al., 2012).

#### 2.6. Remediation

Remediation is a conscious process of removing any materials: synthetic and organic fertilizers, industrial and pharmaceutical wastes, electronic wastes, pesticides, herbicides, heavy metals, and, of course, hydrocarbon (David, 2005; Dhananjaya et al., 2019; Idris et al., 2013; Jingyu et al., 2014; Kaplan, 2013; Soma, 2014), otherwise referred to as pollutants, or conditions which may be injurious to humans, animal, or microbes, alter the physical, chemical, or biological characteristics of the receiving environment (soil, air, or water body), and/or produce a general offending esthetic sensibility, thereby reducing the importance and usage of such environment. The sole aim of remediation is to return the environment to its original state prior to contamination and avert any associated consequences (Zabbey et al., 2017).

Different remediating protocols have been employed in cleaning up of an environment polluted with crude oil. These protocols, which may be physical, chemical, or biological (Yao et al., 2012), depending on the type of environment, terrestrial or aquatic, have their advantages that made them stand out among other protocols and also have certain drawbacks that present them as bad tools for the job (Uchechukwu et al., 2007; USEPA, 2016).

#### 2.7. Effective cleanup of crude oil-contaminated aquatic environments

Oil spills in the aquatic environment have been a big threat to mankind since the discovery and usage of oil

because of the associated pollution (Wu et al., 2017). This is credited to the unavailability of a reliable protocol for complete removal of this pollutant without altering the environment or leaving behind evolved compounds that may even be more dangerous to man and the environment than the original pollutant (Abioye, 2011; UNEP, 2011). Another very important challenge is that crude oil spillage on the surface of fast-moving water bodies contaminants is usually very quickly transported to very far away from the point where the spillage occurred. While removing the contaminant presents a serious challenge, the contaminant is quickly moved to a new environment, creating more problems as it goes (Frank and Boisa, 2018). Therefore, many of the traditional remediation protocols have become obsolete and not able to meet up with the present challenge, leaving the environment to its own fate (Minai-Tehrani et al., 2009).

### 2.7.1. Traditional mechanical/chemical protocols of remediating the aquatic environment

The complete removal of crude oil-contaminated water and discharging the same to a safer location, known as siphoning, has been used for small nonflowing water bodies. The use of booms and skimmers as an immediate action taken to temporarily control and contain current oil spillage also proved helpful as the equipment prevent the contaminant from extending to the shorelines and stop the oil from being carried farther away from the point of occurrence to ease recovery (Fang and Wong, 2006; Obi et al., 2014). Organic materials have also been used as sorbents. Spongy material, like sawdust, straw, and talc, and most recently human hairs have been used as sorbents (Gavin, 2020). These materials are poured on the oil sheen and allowed for weeks or even months for them to soak up the oil. On-the-spot controlled burning of spilled crude oil is another physical method of environmental remediation that can be employed on the open surface waters where there are no nearby floating oil tankers, ships, speed boats and fishermen, using the oil floating on the water surface as fuel (Obi et al., 2014).

The chemical method of remediation is basically the application of known chemicals called dispersants on the water surface to weaken the oil molecule, hasten its disintegration, and expunge the oil from the water surface to avoid further pollution of the water (Graham et al., 2016; Lindstrom and Braddock, 2002; Sogbanmu et al., 2017). Generally, dispersants are produced specifically to suit a particular contaminated aquatic environment. For instance, during the remediation of Deepwater Horizon oil spill, two types of

dispersants, Corexit 9500A and Corexit 9527A, were specifically produced and registered under the federal guideline list of dispersants that can be used during a spill (United States Coast Guard, 2011; USEPA, 2014).

### 2.7.2. Drawbacks of mechanical/chemical remediation protocols

Siphoning is only applicable for small localized/point source-contaminated sites (Khan et al., 2004). It is laborious and expensive, and the dumpsite could become a source of contamination to another environment (Jain et al., 2005). The use of booms and skimmers is highly expensive (Obi et al., 2014), while heating the oil may transform it into a new daughter compound, turning the oil into its gaseous form and releasing greenhouse gases into the atmosphere, resulting in air pollution. Moreover, the heat created by the burning of the oil can also be deleterious to soil or aquatic microbes (Zabbey et al., 2017). The use of dispersant needs an aircraft for spraying on the affected site and application of chemical on a contaminated water source implies addition of more contaminants to the already contaminated water and as such makes the water unsuitable for domestic uses. Also, the dispersants may become too toxic and may have deleterious or long-term effects on relevant aquatic lives (Nduka, 2011).

## 2.8. The concept of bioremediation

Biological-based environmental remediation/recovery from environmental pollution is as old as the environment itself. Prior to the advent of scientific methods of environmental remediation and recovery, nature already has her own spontaneous ways of self-purification and correction of abnormality resulting from any sort of pollution; organic and inorganic compounds, heavy metals, electronic wastes, chemicals, and, of course, crude oil (Raja et al., 2014).

By definition, bioremediation is the use of living agents; bacteria (Bender and Phillips, 2004; Mishra et al., 2001), fungi (Singh, 2006), and plants (Mackova et al., 2006) with potentials to degrade, alter, immobilize, detoxify, remove, and transform organic or inorganic compounds or elements, otherwise hazardous contaminants, in soil or in water to nonhazardous waste products in order to cleanup the polluted environment (Sardrood et al., 2013). Bioremediation technique, unlike the chemical and mechanical techniques, is effective; it does not need specialized machines and expertise; it is inexpensive and an environmentally friendly means of cleaning up contaminated environments, particularly crude oil-polluted environments (Obi et al., 2014).

### 2.8.1. Microbial-mediated remediation protocol

Microbial-mediated remediation protocol is a biological-based remediation technique that employs the potentials of microbes to achieve environmental cleaning (Gadd, 2010). Microbes are the smallest forms of life, otherwise known as microorganisms that inhabit our environment. Microbes are so small that they cannot be seen with unaided eyes. They live in the soil, in water in the air and even on human and animal bodies as ecto- and endoparasites. Typical examples and, of course, the most commonly encountered are the bacterial, viral, parasitic, and fungal species (Genetic Science Learning Centre, 2014).

Microbial remediation is a spontaneous and active reaction of microbes, usually under very unfavorable conditions, to a certain environmental pollutants that have devastated their immediate habitat and threatened their very existence (David, 2018). Because of the nutritional versatility of microbes (Abatenh et al., 2017) and in their attempt to escape the ruinous effects of the pollutants, microbes create a unique means of turning the harmful pollutants into a useful delicacy (Abatenh et al., 2017; David, 2018). Hence, microbes are able to survive, grow, and adapt in severely contaminated environment, irrespective of the nature and concentration of the contaminants, and consequently, transform toxic pollutants to less toxic/nontoxic compound or completely break them down to their elemental forms (Shweta and Pratyosh, 2020).

### 2.8.2. Conventional microbial-based remediation protocols

Bioaugmentation and biostimulation have been the commonly used microbial-based remediation protocols. The protocols are based on the microbial ability to spontaneously degrade pollutants as they search for food (Abatenh et al., 2017; David, 2018). Bioaugmentation is a deliberate introduction of microbes that have been grown in isolation under prescribed conditions, screened, and confirmed to possess some degrees of pollutant-degrading or removal abilities for preferentially particular contaminants into the polluted environment. This is purposely to reinforce the autochthonous microbes and boost their remediation capacity for a speedy and efficient performance, or to out-compete the incapable native microorganisms, inhabit the vacated metabolic niche within the contaminated environment in order to perform a specific remediation task (Alexis et al., 2016; Da Silva and Alvarez, 2010). Studies have revealed some inherent drawbacks associated with bioaugmentation. Notably,

most exogenous microbial cells die almost immediately as they are delivered into a polluted site. This is mostly a result of competition between the introduced organisms and indigenous ones for dissolved  $O_2$ , substrates, and food. Environmental temperature and pH changes, trauma from pollutant load, and hunting by protozoa and other hostile agents, including phages, could also lead to the death of the introduced organisms (Alexis et al., 2016; Bouchez et al., 2000; Songzhe et al., 2009; Stroo et al., 2013). On the other hand, biostimulation involves the adjustment of certain identified physical and chemical factors within a polluted environment; dissolved  $O_2$ , nutrient and water availability, environmental temperature and pH; which may completely hinder or slow down the rate of proliferation of the autochthonous organisms and impede biodegradation in that particular environment. It may also involve the importation and injection of the identified limiting factors; dissolved  $O_2$ , nutrient, and/or water, from the outside into the polluted environment, enhance the rate of microbial growth, stimulate microbial activities, and facilitate biodegradation of the contaminants by the indigenous microorganisms (Biello, 2010). Organic and/or inorganic stimulants have been used. Palm kernel husk ash (Adenipekun and Lawal, 2011), groundnut/rice husk, coconut shell (Nyankanga et al., 2012), plantain peels, cocoa pod husk (Agbor et al., 2012), *Moringa oleifera*, soya beans (Danjuma et al., 2012), and chicken droppings (Ofoegbu et al., 2015) are among the organic stimulant in use. On the other hand, nitrogen phosphorus potassium, one of the inorganic fertilizers widely used for soil enrichment, has proven to be a good inorganic stimulant which enhances microbial activities and facilitates biodegradation of crude oil pollutants (Christopher et al., 2017). The possible scarcity of native microbes capable of degrading the target pollutant (hydrocarbons) (Hosokawa et al., 2009), the possibility of the added nutrients in the water to rapidly scatter in and probably taken away from the reach of the autochthonous microbes or to locations where the pollutants are not visibly pronounced (Christopher et al., 2017) form the major impediments to the application of biostimulation, especially on the aquatic environment.

### 2.9. Current microbial-based remediation protocols of crude oil-contaminated aquatic environments

The traditional microbial-based remediation protocols (bioaugmentation and biostimulation) have shown some level of inadequacy, especially when applied as sole remediation protocol of crude oil-contaminated aquatic environments particularly against high



concentrations of the pollutants like massive marine oil spillage (Mapelli et al., 2017). However, considering the abilities of microbes in breaking down pollutants and turning them into delicacies, scientists are continually engaged in formulating means of harnessing and deploying these microbial abilities in microbial remediation systems for effective and optimum results (Mouna et al., 2018; Nur et al., 2020).

### 2.9.1. Construction of microbial consortium

Constructing a microbial consortium involves isolation and screening of different microbial species with very active crude oil-degrading abilities and exposing them to the pollutant (hydrocarbons) for a long time, such that they have undergone a sort of genetic modification. Hence, they become accustomed to the toxic effects of the pollutant and can even draw their source of livelihood from the pollutant (Atlas and Bartha, 1992; Patowary et al., 2016).

Crude oil is a complex mixture of numerous hydrocarbon and nonhydrocarbon compounds, including trace metals; some, especially the polycyclic aromatic hydrocarbons (PAHs), are very difficult to biologically degrade; making them to persist in the environment (Ghosal et al., 2016; Mouna et al., 2018). The idea behind microbial consortium as a remediation protocol is to engage the collaborative capabilities of many species of microbes in tackling the persistent pollutants (Patowary et al., 2016). This has become highly recommendable because of (a) microbe substrates specificity; (b) some types or components of hydrocarbon; the PAHs and long-chain hydrocarbons ( $C_{32}$  and above) are chemically nonreactive and need a specific enzyme system to become activated (Elumalai et al., 2017); and (c) long-chain hydrocarbons ( $C_9$  and above) are generally hydrophobic, thus not soluble in water (Singh et al., 2012), making them unavailable to the organisms. This may hinder the proliferation of many hydrocarbon-dependent microbial species and delay or obstructs biodegradation of the pollutant (Joutey et al., 2013).

In the microbial consortium protocol, the task of degrading the hydrocarbon pollutant is distributed to members of the microbial population that make up the consortium according to their abilities and enzymatic oxidation attributes (Elumalai et al., 2017). Many of the thermophilic bacterial genera, such as *Pyrococcus*, *Sulfolobus*, *Methanothermobacter*, *Thermomicrobium*, *Geobacillus*, and *Thermus* species are known to have and possess the ability to secrete degradative enzymes and growth factors, particularly the alcohol dehydrogenases (ADHs) (Helia and Philip, 2003; Wasoh et al.,

2019) and biosurfactants which increase the solubility and bioavailability of those hydrophobic hydrocarbons for easier degradation (Wang et al., 2011).

A robust bacterial consortium system was developed for crude oil bioremediation in Assam, India. Different bacterial species capable of biosurfactant production and ability to secrete degradative enzymes were used. The available result showed that the consortium degraded up to 84.15% of total petroleum hydrocarbon after few weeks of incubation (Patowary et al., 2016).

In another study, microbial consortium was also employed in Malaysia to degrade paraffin wax in Malaysian crude oil. Several bacterial strains (*Geobacillus*, *Parageobacillus*, and *Anoxybacillus* species) were employed, each producing different degradative enzymes such as alkane monooxygenase, ADH, lipase, and esterase needed for the degradation of paraffin wax. It was, thus, recommended the application of these strains in crude oil reservoirs, crude oil processing, and recovery units will generally improve the quality of the oil (Nur et al., 2020).

### 2.9.2. Use of bacterial cells immobilized on organic biocarriers

Immobilizing microbes on a biocarrier involves isolation of highly potential bacterial strains from crude oil-contaminated environment, screening them for crude oil utilization, and adsorbing the biofilm layer onto the carrier (Hsu et al., 2004). Biocarriers are usually made from organic materials like wheat straw, rice straw, coconut husk fiber, wood chips, palm leaf raffia, loofa sponge, and corncob (Dang et al., 2020; Zohra et al., 2018). These organic materials are accessible, non-toxic, biodegradable, and, therefore, cannot repollute the environment (Cai et al., 2013). The main reasons for immobilizing the cells on organic materials are to improve the stability and retentive base for the microbial cells, provide longer remediating operating life span, and ensure microbial cell survival (Zacheus et al., 2000). This protocol is environment-friendly, does not require expensive machines, and high-tech appliances; hence, it is cost-effective (Dang et al., 2020).

This protocol was applied in the removal of crude oil from water obtained from Nile River, Egypt, in a batch experiment. Four bacterial strains, *Pseudomonas aeruginosa*, *Enterobacter sakazakii*, *Klebsiella oxytoca*, and *Bordetella bronchiseptica*, were isolated, screened, and immobilized on finely blended corn qgualh, Egyptian loofah, palm leaf raffia, and used sponge (Samhan et al., 2017). The cells and carriers were incubated for 72 hours and observed for biofilm formation.

The result obtained from the experiment showed high proliferation of the immobilized cells, resulting in complete bacterial colonization of the organic biocarriers and consequently biofilm formation. The study, therefore, concluded that the use of crude oil-degrading bacteria as a consortium immobilized on biocarriers was efficient in the removal of crude oil from contaminated water (Samhan et al., 2017; Sihag et al., 2014).

### 2.10. Plant-microbial synergism

Plant-microbial synergism in the degradation of environmental pollutants (rhizodegradation) occurs naturally as indigenous microbes that inhabit the rhizosphere spontaneously facilitate plants' ability to produce enzymes involved in pollutant degradation (Ahmad et al., 2020; Garbisu and Alkorta, 2001). This protocol can also be constructed by screening plants with abilities such as having fibrous roots capable of providing suitable habitats for rhizospheric microbes and being able to survive severe crude oil pollution (Ahmad et al., 2020; White et al., 2006). Such plants are, thus, inoculated with plants' growth-promoting bacteria (Ahmad and Khan, 2012; Tara et al., 2014), while the rhizosphere is stimulated with screened microbes (Ahmad et al., 2020; Ali et al., 2020). While the plants are also directly involved in pollutant degradation (phytoremediation) (Fatima et al., 2018; Vaziri et al., 2013), they also secrete certain exudates that nourish the diverse microbial community in the rhizosphere (Ahmad et al., 2020; White et al., 2006) that are capable of utilizing crude oil (Nie et al., 2011; Minoui et al., 2015).

Plant-microbial synergism was applied in the remediation of crude oil-contaminated Yellow River Delta coastal wetlands in China (Yilei et al., 2020). The herbaceous plant used was the *Suaeda salsa* (L.), a strong salt tolerant plant commonly found in Yellow River Delta (He et al., 2016). Organic exudates, including alkanes, esters, alcohols, amines, and organic acids, released from the plant following reaction to the severe crude oil pollution attracted and significantly encouraged the growth and colonization of diverse and active microbial communities, including crude oil-degrading and biosurfactant-producing bacteria within the rhizosphere (Sara et al., 2018).

The result indicated that it was not difficult to notice crude oil removal from this collaborative effort. Many of the root exudates have similar structure to the crude oil contaminants (Singer et al., 2003). So, it was easier for the microbes to degrade the organic contaminants within rhizosphere even when the root exudates are exhausted as microbes that utilize the root

exudates are also able to degrade petroleum hydrocarbons (Yergeau et al., 2014).

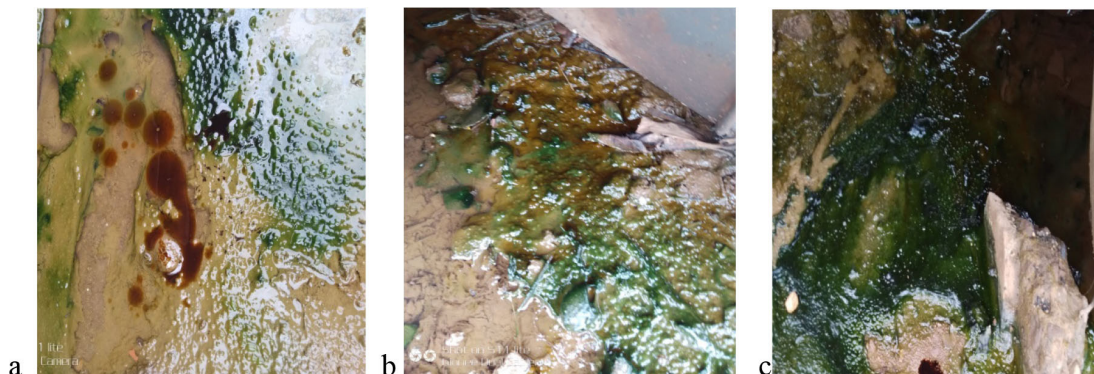
#### 2.10.1. Application of synthetic biology

The application of natural organisms, either in the single species or as a consortium in the crude oil remediation of contaminated environments, has been found wanting; firstly, because of the time frame before the actual degradation of the pollutant, and secondly, the difficulty of a particular organism degrading all the diverse components of the pollutant (Bharagava et al., 2019; Dangi et al., 2019; Kumar et al., 2019), as individual biological agents show remarkable substrate specificity (Ghosal et al., 2016; Mariana et al., 2020; Nur et al., 2020). These drawbacks spurred the scientific community into a quest to discover and construct novel microbial-based remediation systems that can target specific individual recalcitrant pollutants for optimum results (Shweta and Pratyosh, 2020).

Synthetic biology simply means the creation of artificial organisms. It involves the chemical synthesis of artificial DNA, arranging them in a catalogue of DNA sequences and assemblage into new genomes. The vision of synthetic biology is to create organisms (artificial cells) that do not exist previously in nature and possess entirely new or enhanced unique traits and capabilities (Gutmann, 2011). In the field of environmental biology, this advanced technology is targeted at designing tailor-made biological entities capable of engaging recalcitrant compounds with precision and minimal time consumption (Dangi et al., 2019; Elizabeth and Neil, 2020). Synthetic biology is different from genetic engineering. While genetic engineering involves the transfer of particular natural genes from one microbe or cell to another, synthetic biology is the artificial synthesis of entirely new organism from a set of standard genetic parts (Gutmann, 2011). This protocol is advantageous in that it potentially yields organisms that are better suited for specific intended purposes, while the disease-causing characteristics could be excluded during the assembling (Dvořák et al., 2017; Hunter, 2013).

At present, synthetic biologists are only able to design and construct organisms based on the template of already existing organisms and have them behave according to design specifications (Gibson et al., 2010). Consequently, the bacterial genome, *Caulobacter ethensis*-2.0, made completely by an engineered biological system capable of carrying out specific functions was created (ETH Zurich, 2019; Venetz et al., 2019), followed by the creation of active variants of *Escherichia coli* (Fredens et al., 2019; Zimmer, 2019).





**Plate I.** *Spirogyra* proliferating in a copious supply of spent motor oil on day 1 (a), day 7 (b), and day 14 (c). Source: Ezugwu Mini Algal garden, Field work, 2021.

Literature studies are already available on the metabolism of recalcitrant environmental pollutants using synthetic systems (Dvorak et al., 2014). However, studies are still in progress on the metabolism potentials of these synthetic systems against environmental pollutants like crude oil, pesticides, dyes, heavy metals, plastics, and other xenobiotics (Hemmat-Jou et al., 2018; Jaiswal et al., 2019; Lebrazi and Fikri-Benbrahim, 2018; Paniagua-Michel and Fathepure, 2018; Rucká et al., 2017; Wu et al., 2020; Yadav et al., 2015). Moreover, the biosafety of these synthetics are also being rigorously examined to ensure human safety (Shweta and Pratyosh, 2020).

#### 2.10.2. Application of nanobiotechnology in microbial-based crude oil remediation

Nanotechnology is a novel domain of scientific research concerned with innovative creation of materials, entities, and devices based on atomic, molecular, and supramolecular scales for industrial purposes. An important field where nanotechnology has found application is in the synthesis and usage of nanoparticles (NPs)/nanomaterials. These are extremely ultra-fine particles of matter whose sizes, at least from one dimension range between 1 and 100 nm, are practically undetectable by unaided human eyes (Benjamin et al., 2019). Commonly used NPs, which are also synthesized under microbial assistance (Xiaojiang et al., 2019), include silver, aluminum, silicon dioxide, palladium, titanium dioxide, carbon-manganese oxide, gold, selenium, sodium silicate, diamond, zirconium dioxide, aluminum oxide, tungsten, disulfide zinc oxide, boron nitride, molybdenum disulfide, and  $\gamma$ -aluminum oxide (Taylor et al., 2013; Valenti et al., 2018).

The role played by NPs in enhancing microbial remediation is still sketchy, and hence only very few works are available in this regard. However, the following

views about NPs are worth noting. Firstly, the surface area per unit mass of a material is increased when such material is reduced to nanoscale and this increases the overall rate of reaction of such material. Secondly, because of the increased rate of reactivity, the smallest quantity of activation energy is thus needed to bring catalytic reaction of NMs into actions (Ragaa et al., 2019; Rizwan et al., 2014). Hence, it has been established that NPs, when sufficiently in contact with the microbial cells and through their catalytic activities increases the microbial proliferation and colonization of the contaminated site, resulting in enhanced and accelerated pollutant degradation (Mayasar et al., 2020; Rizwan et al., 2014; Shan et al., 2005).

In a laboratory experiment, to access the rate of microbial degradation of hydrocarbon in the presence of a known NP, two fungal species, *Aspergillus flavus* strain AF15 and *Trichoderma harzianum* strain TH07, were incubated with and then without known concentrations of biological-assisted synthesized silver nanoparticle (AgNPs) in Saudi Arabia (Mayasar et al., 2020). The results obtained from the treatments indicated that an effective degradation of hydrocarbon (26.4% after 3 days and 49.3% after 7 days) was by the collaborative effort (consortium) of the fungal isolates (Al-Hawash et al., 2018). However, incubation of the fungal consortium with the AgNPs seemed to yield an optimal microbial degradation as the percentage degradation rose to 31.6% after 3 days and 57.8% after 7 days (Mayasar et al., 2020).

In another laboratory trial, bacterial cells coated with nickel (II) oxide NPs synthesized under green approach were used to remediate water contaminated with petroleum hydrocarbons from Suez Canal, Egypt. The bacterial cells involved were *Halomonas xianhensis*, *Halomonas zincidurans*, *Pseudomonas stutzeri*, and *Halomonas salifodinae*. The result obtained

from this trial indicated that there was complete degradation of two-membered rings of polyaromatic hydrocarbons and higher percentage of degradation of four-membered rings of polyaromatic hydrocarbons. The complete degradation was credited to the increased microbial activities as catalyzed by the introduced NPs (El-Sheshtawy et al., 2017; Ragaa et al., 2019; Shan et al., 2005).

### 2.11. Phycoremediation of crude oil-contaminated water

Algae form an important component of the microbial community that inhabits the aquatic and terrestrial environments. Different species have been observed growing spontaneously on the surfaces of water, at the shorelines or at the bottom of freshwater bodies where they find nutrients (Cruces et al., 2010; Frances, 2016). Many of them have also been found growing and thriving unaided in sewages and even in severe crude oil-polluted terrestrial and aquatic environments; a clear indication that they can utilize crude oil as nourishment (Aditi et al., 2015).

Macroalgae have been used for a long time in the removal of pollutants in the environments, mostly heavy metals (Abioye et al., 2020) and inorganic industrial pollutants (Wojtyła and Baran, 2018). These treatments have mostly been applied on soil environments or at wastewater discharge sites (Nithiya et al., 2018). Only very few accounts of their involvement in the biodegradation of hydrocarbon are available; with *Chlorella*, *Scenedesmus*, and *Raphidocelis* species tested on PAHs degradation (Aditi et al., 2015; Diana et al., 2020; Nithiya et al., 2018; Pankaj et al., 2019). This is partly because more studies have not been conducted on the hydrocarbon-degradation potentials of algae and their metabolic pathways on the various crude oil components. Hence, information about algal species that strictly utilize hydrocarbon as sole carbon source is very scanty (Pei et al., 2019). However, with the expansion of interest on how to manage hydrocarbon contaminants, there is a strong belief that the application of algae in the cleaning of petroleum-polluted environments may just be the missing piece of the puzzle.

### 2.12. Advantages of phycoremediation

Generally, the importance of algae on planet Earth can never be overemphasized. Algae use the greenhouse gas, CO<sub>2</sub>, as they grow photosynthetically; reducing the gas emissions, global warming, and battling climate change. In the process, algae generate about half of the oxygen needed for the sustenance of planet Earth.

In addition to being cheap and eco-friendly, phycoremediation requires less labor and does not need high-tech expertise (Bhola et al., 2014; El-Gendy and Nassar, 2021).

Algae have a unique characteristic of a -providing oxygenic cycle during growth (Bhaskar, and Suresh, 2019). By metabolizing the hydrocarbon pollutants during the photosynthetic growth in the presence of sunlight and CO<sub>2</sub>, algae release O<sub>2</sub> into the environment, a key electron acceptor which is used by heterotrophic aerobic organisms also participating in the degradation of the pollutants. The aerobic oxidation of hydrocarbon by the native heterotrophic bacteria yields CO<sub>2</sub> which is used by the algae, in the presence of light for photosynthesis, thereby making the clean-up a lot faster (Ghasemi et al., 2011; Pankaj et al., 2019).

Another important advantage of phycoremediation is the ability of the algae to grow very fast even in severely polluted sites; with few of them capable of doubling in large mass and colonizing the entire polluted sites within few hours. They make use of any available organic/inorganic carbon substrate in the presence of environmental CO<sub>2</sub> and sunlight to earn a living, and by populating polluted sites within a shorter time, the rate and efficiency of bioremediation is enhanced (Diana et al., 2020). This solves the problem of long adaptation time needed in bacterial-based remediation protocol (Plate I).

Algae provide the ingredients needed for microbial hydrocarbon degradation in a hypersaline environment. The level of salt in an environment greatly affects the rate of hydrocarbon bioremediation in the said environment. When the concentration of salts in a solution becomes high, it makes hydrophobic organic compounds insoluble. This increases the tendency of the organic molecules to get adsorbed to any available particle. This is termed “salting out” and the consequence is that the organic compound becomes biologically unavailable and the carbon source becomes out of reach to the microbes (Means, 1995; Turner and Rawling, 2001). In addition to the bio-unavailability of organic compounds to bacterial cells due to the salting out effect, the availability of dissolved oxygen also decreases with increase in salt concentration (McGenity, 2010). These generally affect the rate of bacterial growth and seriously reduce the population of microbes capable of utilizing hydrocarbons in hypersaline environments.

Algae are able to endure hypersalinity and can even desalinate salty environment (Gan et al., 2016; Petronia et al., 2020; Scheres and van der Putten, 2017). The adaptation and self-protection to changes in salinity is

via a series of molecular and biochemical approaches to withstand the effect of hypersalinity. Firstly, algae produce and accumulate osmolytes, such as glucose, fructans, sucrose, trehalose, and raffinose, which are highly soluble low-molecular-weight organic compounds that influence the characteristics of cytoplasmic fluid and allow an efficient pumping of sodium and/or potassium ions (Erdmann and Hagemann, 2001; Slama et al., 2015). Secondly, when algae are exposed to excessive salt, they usually develop new vacuoles for the sole purpose of storing salt ions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) (Singh et al., 2018). Thirdly, the algae like any other photosynthetic lives produce and express abscisic acid, a hormone which elicits expression and manifestation of a series of genes that respond to the detrimental effects of salt (Cutler and Krochko, 1999; Jose et al., 2017; Shinozaki and Yamaguchi-Shinozaki, 2007).

Hence, algae can grow optimally in both fresh and salt water environments where they generate oxygen via photosynthesis; a key factor needed to increase the population of other members of the microbial community and facilitate their growth and hydrocarbon uptake (Petronia et al., 2020). Obviously, the application of phycoremediation, therefore, becomes more advantageous not only because of the direct involvement in the hydrocarbon degradation but also for creating the enabling environmental conditions needed for other members of the microbial community to optimally grow and contribute their quotas in the cleansing of the environment.

### 2.13. Hydrocarbon phycoremediation pathways and the significance of dissolved oxygen

Since only few works on algal degradation of petrochemical contaminants are available, information on the catabolic pathways of biodegradation of hydrocarbon compounds in algae is also equally scanty (Jacques and McMartin, 2009). However, there are detailed studies on phycoremediation pathways for direct involvement of algal degradation of some components of hydrocarbon (Lika and Papadakis, 2009; Lovell et al., 2002; Martins et al., 2015; Subashchandrabose et al., 2013; Thies et al., 1996). The studies established that these pathways follow two major routes: the ortho- and meta-cleavage pathways, as activated by enzymatic systems (El-Gendy and Nassar, 2021; Pankaj et al., 2019).

For instance, the breakdown of phenol by macro- and microalgae, as reported by El-Gendy and Nassar (2021), was reportedly via the ortho-cleavage pathway as they successfully split the catechol aromatic

ring between the two carbon atoms bearing hydroxyl groups as activated by catechol 1,2-dioxygenase which employs  $\text{Fe}^{3+}$  cofactor. The resultant intermediate product is the *cis, cis*-muconic acid which is further degraded to  $\beta$ -keto adipate before entering into the citric acid cycle (Das et al., 2015; Surkatti and Al-Zuha, 2018). On the other hand, a species of golden algae, *Ochromonas danica*, was able to breakdown phenol via the meta-cleavage pathway as it was able to cleave the catechol aromatic ring between a hydroxylated carbon and an adjacent nonhydroxylated carbon atom using the catechol 2,3-dioxygenase which employs  $\text{Fe}^{2+}$  cofactor. The resultant intermediate, which is produced via *cis*-2-hydroxypenta-2,4-dienoate, is the 2-hydroxy muconic semialdehyde with further degradation to acetaldehyde and/or pyruvic acid (Semple and Cain, 1996).

In another study, the autotrophic microalgae biodegradation pathway of toluene was determined as they were employed in the degradation of the petroleum hydrocarbon from polluted sites (Pankaj et al., 2019). The mechanism of degradation involves photosynthetic capturing of photons and subsequent generation of electrons (Kruse et al., 2005). The generated electrons are transferred to the inorganic electron acceptor via electron carriers such as nicotinamide adenine dinucleotide. The harvested energy through this process is stored as adenosine triphosphate. Phycodegradation of toluene equally follows both the ortho- and meta-cleavage pathways which are activated by an enzyme system, toluene oxygenase, and dissolved oxygen. The intermediate product is a 3-methylcatechol which is further degraded into pyruvate/acetyl-CoA with consequent involvement in the Journal - Typeset and glyoxylate cycle (Hammed et al., 2016).

The report shows that the initial reaction for oxygenic degradation of hydrocarbon species begins with the addition of molecular oxygen (El-Gendy and Nassar, 2021). In other words, initiation of hydrocarbon degradation becomes almost impossible in the anoxygenic condition. Even in algae-bacterial synergism, the oxygenic hydrocarbon degradation pathway, even by the bacteria, still needs the introduction of molecular oxygen (Naas et al., 2014) and the depletion of dissolved oxygen retards the entire process. Since the depletion of oxygen saturation level is a major setback in the bacterial-based remediation protocol (Das and Deka, 2019), application of phycoremediation directly as the sole remediating system or in synergism with screened bacteria may just be what is needed to cleanse our environments of petrochemical pollutants.



### 3. Conclusion

The traditional physicochemical remediation protocols are helpful as they provide a fast means of confronting the crude oil pollution as soon as it occurs. However, these protocols have become obsolete because of the expensive nature and need for high-tech equipment and expertise. Microbes, fungi, and bacteria have the spontaneous abilities to break down hydrocarbon pollutants and turn them into delicacies (microbial-based remediation protocol). This protocol has also shown to be ineffective especially when applied as sole remediation system of crude oil-contaminated aquatic environments, particularly against high concentrations of pollutants like massive marine oil spillage. The use of these agents in hydrocarbon degradation is environmentally friendly, cost-effective, and thorough. However, the time frame needed for microbial adaptation and colonization is usually a huge drawback. Algae use the greenhouse gas, CO<sub>2</sub>, as they grow photosynthetically: a tool against global warming and climate change. Algae are able to grow very fast, doubling in large mass and colonizing the entire polluted sites within the shortest time. In the process, algae generate about half of the oxygen needed for the sustenance of planet Earth. Algae have the ability to proliferate in extremely salty habitats and also generate the essential oxygen needed for microbial hydrocarbon degradation in the said environment. Obviously, the application of phycoremediation, therefore, becomes more advantageous not only because of the direct involvement in the hydrocarbon degradation but also for creating the enabling environmental conditions needed for other members of the microbial community involved in the environmental remediation.

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