



## Review Article

## Biodegradation of petroleum hydrocarbon polluted soil

Ehenenden Iyobosa<sup>1</sup>, Meng Xianagang<sup>1,\*</sup>, Ning Hai Jun<sup>1</sup>, Shu Fang<sup>1</sup>, Wang Zhennan<sup>1</sup><sup>1</sup>Dept. of Chemical and Biological Engineering, School of Chemical and Biological Engineering, Lanzhou Jiaotong University, Gansu Lanzhou, China

## ARTICLE INFO

## Article history:

Received 09-03-2020

Accepted 13-03-2020

Available online 20-07-2020

## Keywords:

Crude oil

Hydrocarbons

Soil pollution

Bioremediation

Bacteria

Biosurfactant

Gene

## ABSTRACT

Crude oil contributes a major percentage to the source of energy used on earth, however, hydrocarbon components have been classified to the family of carcinogens and neurotoxic organic pollutants. The inappropriate drilling, transportation, and usage lead to the increment of soil, air, and water body petroleum hydrocarbon pollution. If this menace is not put in check as a matter of urgency, it can cause an epidemic outbreak in an affected community, shortage in agriculture produces output, threatening of soil useful microbial biome and environmental disaster. Bioremediation as promising modern biotechnology is capable of mineralizing hydrocarbon pollutants into water, carbon dioxide, cell proteins, and an inorganic compound. Indigenous or genetically modified microbes are able to secrete enzymes for the synthesis of biosurfactants that break down organic pollutants into a less toxic form. Bioremediation is not only potent in degrading organic pollutants, but it is also environmentally friendly and cheap compared to other non-natural methods. Therefore, this paper combined and presents a review of empirical researches done on microbial bioremediation of petroleum hydrocarbons pollutants in different countries.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (<https://creativecommons.org/licenses/by-nc/4.0/>)

## 1. Introduction

Petroleum hydrocarbon carbon contamination with environment has been a big threat to human and his natural surroundings.<sup>1</sup> The media that is affected by these pollutants are soil, water and air.<sup>2</sup> It brings change in the environment that resulted in way of introducing harmful effects that altered the quality of human life, animals, plants and microorganisms.<sup>3</sup> To reduce the harms caused by pollution, the three media mention above need to be subjected to physical, chemical, mechanical or biological treatment.<sup>4</sup> Generally in china, according to<sup>5,6</sup> more than 8% of china's arable land is severely polluted. This was reaffirmed by<sup>7</sup> saying that one fifth area of China's arable land is polluted, and the polluted arable land equal the size of Taiwan to a degree that farming should be prohibited there in totality. The United States Environmental Protection Agency(USEPA) most targeted sites of hazardous wastes

clean-up programs are made of 600 of 1408 different kinds of hydrocarbons.<sup>8–10</sup> Soil pollution is considered to be a lifethreatening problem because all the plant consumed by human and the animal is cultivated from the soil, therefore, proper attention most paid to soil pollution to reduce it to the barest minimal if not totally eliminated. Although soil remediation is of a high capital and labour intensive project, but due to man poise for cheaper method for a polluted soil remediation, much have been done using microorganisms to bioremediate contaminated soil which proved positive. This method termed to be labour and capital cheap and environmentally friendly.<sup>11</sup> However, not all bacteria have the adaptability to degrade pollutants like petroleum hydrocarbon especially in the soil, according to<sup>12</sup> it has been proven that microorganism's capability and adaptability to degrade pollutants, depend on the individual strains ability to produce suitable biosurfactans and the formation of biodegrading association that is very strong and reliable throughout the degradation period. Also, it depend on the adaptability of the microorganism to carry

\* Corresponding author.

E-mail address: [ehenedenyobosa@gmail.com](mailto:ehenedenyobosa@gmail.com) (M. Xianagang).

out its physiological functions under natural condition that is characterized with limited water supply, low availability of nutrients and adverse pH condition.

## 2. Hydrocarbon Contaminated Soil

The present of crude oil in the soil usually by spilling altered its properties. Therefore,<sup>7,13</sup> define crude oil pollution as the contamination of the environment by crude oil. The products of crude oil (Hydrocarbons, solvents, etc.) used as a source of energy in the oil industry have polluted about 80% of lands. There exist hundreds different combination of polycyclic aromatic hydrocarbons (PAHS), however, 28 compounds was identified to be hazardous by US EPA (United States Environmental Protection Agency) in 2008.<sup>14</sup> Crude oil is made up saturated and unsaturated aliphatic hydrocarbon, monocyclic and polycyclic aromatic hydrocarbon;<sup>15</sup> these components are pollutants that contaminates the soil but aromatic hydrocarbon are the most highly hydrophobic which rendered them difficult to degrade.<sup>16</sup> Low molecular weight PAHs (LMW) and high molecular weight PAHs (HMW) are the classes of PAHs. naphthalene, fluorene, phenanthrene and anthracene with two to three (2-3) aromatic rings are LMW PAHs which are less toxic, while HMW PAHs of 4-7 aromatic rings (chrysene, Triphenylene, Pyrene, pentacene, Corannulene, and coronenes) are highly toxic, carcinogenic to human health and difficult to degrade<sup>17,18</sup> Petroleum hydrocarbon pollution in the soil reduces the level of environmental biomass, taxas and the biodiversity. The presence of hydrocarbon in the soil can alter both the physical and chemical properties of the soil, especially the C/N ratio and soluble salt content.<sup>7</sup> According to research done by,<sup>19</sup> the presence of petroleum hydrocarbons in the soil have significant effects on the soil properties, it was noticed in observable differences in data obtained when comparing crude oil-polluted soil and non-polluted soil. Hydrocarbon polluted soils were slightly acidic whereas the control soils set towards neutrality. Crude oil reduced water infiltration into the soil and also causes low permeability. Hydrocarbon pollution increase soil organic content, it reduces the concentration of nitrogen, phosphorus and calcium in the soil.<sup>19,20</sup> And an increase in hydrocarbon level in the soil caused low fertility of the soil, which negatively affect the agricultural productivity which reduces the source of livelihood of both human and animals in that geographical area.<sup>1</sup>

## 3. Soil bioremediation and its Mechanism

Bioremediation activities were first noticed among Romans around 600BC, where they used bioremediation to clean their wastewater, though their method was not as developed as today's biotechnological methods. Bioremediation was officially invented by George Robinson when he used

microbes to degrade an oil spill on the coast of Santa Barbara;

California in the late 1960s.<sup>21</sup> Ever since this successful attempt, human has indulged on this natural method to treat contaminated environments. Bioremediation is the process of elimination or reduction of pollutants from the environment. It is an environmental or aesthetics waste detoxification mechanism with the help of microorganisms, plants, or their enzymes. However, when the waste detoxification is done at the contaminated site, it is referred to as In-situ Bioremediation. Contrarily, when the detoxification of waste is done outside the original site of the contamination is called Ex-situ Bioremediation.<sup>22</sup> This process involves relocating the contaminants from the contamination site before treatment is administered in a controlled environment. Also, bioremediation can be in a form of bioattenuation which solely depend of natural process of degradation, biostimulation where indigenous microorganisms are stimulated by addition of nutrients, water, and electron acceptors or electron donors to the soil, and bioaugmentation where microorganisms of high degradation ability are inoculated to the soil to enhance bioremediation.<sup>23</sup> Every bioremediation technology depends on many factors which include; the types, amount, and toxicity of a pollutant chemical species present in the soil site conditions and microbial activity (indigenous or exogenous strains).<sup>24</sup> Bacteria remediation can be anaerobic; in the absence of oxygen as an electron acceptor, bacteria can use organic chemicals or inorganic anions as alternative electrons acceptors. The bacteria involves in this bioremediation do not employ the utilization of oxygen during their metabolic pathway. And bacteria can also be aerobic; the bacteria involve employing oxygen to carry out effective biodegradation of pollutants in the soil. In general, oxygen acts as an electron acceptor by accepting the electrons from oxidizing organic substances (reduction) the organic substance oxidized loses electrons to oxygen (oxidation). For this study, aerobic bioremediation is explained in detail. Mineral oil contents and halogenated products of petrol chemicals are the most important classes of organic pollutants in the soil; therefore, the enzymatic- metabolic activities of aerobic bacteria specifically pertinence for the speedily and complete biodegradation of such organic compound and their various products. The optimal degrading potential of aerobic microorganisms can only be attained if the organic pollutant is accessible to the biodegrading microorganisms.<sup>25</sup> When bacteria pick up organic pollutants as illustrated in Figure 1, the first intracellular remediation approach is enzymatic (oxygenases and peroxidases) oxidative process, cell biomass synthesis particularly from the metabolites in the center precursor, e.g., acetyl-CoA, succinate, pyruvate, and Sugars. These are required for various biosynthesis and growth. They are synthesized by gluconeogenesis

and finally, the organic pollutants are converted into intermediates of the intermediary metabolism by the surrounding degradation pathways, e.g., the tricarboxylic acid cycle.<sup>26</sup>



Fig. 1: Bacteria bioremediation procedures.<sup>27</sup>

#### 4. Influencer factors of biodegradation of Petroleum hydrocarbon

##### 4.1. Temperature

A slight increase in temperature of the soil tends to increase the bioremediation rate while decrease temperature reduces the rate of bioremediation.<sup>28</sup> There was a significant reduction in the level of total PAH and phenol with an increase in temperature of inoculated treatment in mesocosm studied. There was observable increased in desorption and bioavailability with an increase in temperature.<sup>29</sup> This could also be a result of the effect of temperature on microbial enzymatic makeup.

##### 4.2. Nutrients

Nutrients play a major role in bioremediation; microbes need a commensurate amount of nutrients to carry out their metabolic activities, cell division, and growth. Trace elements that will serve as electron donor and electron acceptor are added into the soil in a form of organic (compost) and inorganic (fertilizer) this not only stimulates but also accelerate bioremediation.<sup>30</sup> Bioremediation of petroleum hydrocarbons polluted soils can be enhanced with appropriate selection, combination and application of suitable nutrient to mesocosm.<sup>31</sup>

##### 4.3. Bioavailability of Contaminant:

Availability of contaminant has a significant effect on the metabolic component of a cell, this can be view in three contaminant's availability concentration levels, firstly, at the absent of contaminants, biodegradation will not occur because there will be insufficient energy supply to induce bioremediation. Secondly, at a low contaminant concentration level, bioremediation will occur but at a slow rate, this is a result of no spontaneous increase in the microbial population because there is no availability of energy required for cell division, therefore, the cell will maintain a resting stage. Finally, if there is enough

bioavailable contaminant, energy supply will increase thereby inducing biodegrading, at this time it will be in a growing stage, due to cell division and an increase in growth rate.<sup>32</sup>

##### 4.4. Moisture content

Optimum microbial growth can only occur in the presence of water, soil water content regulates oxygen diffusion. From a research work, it was found out that keeping the moisture content of the soil at an optimum value is critically important for a successful bioremediation process. It was also stated by an author that modification of water content of sandy clay soil to 60% of its field capacity, and moisture adjustment is a proper strategy to degrade the contaminated soil.<sup>28,33</sup>

##### 4.5. Electron donor, Oxygen content and Redox Potential:

The potential inhibitory effect of molecular oxygen is negated by excess addition of electron donor. Degradation is inversely proportional to redox potential, increase in oxidation of redox conditions, will result is decrease is bacterial perchlorate degradation. Electron acceptors such as sulfate, manganese oxides and iron oxides have significant effects on redox potential.<sup>34</sup>

##### 4.6. PH

Soil pH alteration by liming process can be of a great important in PAH degradation, as some hydrocarbon feeding bacteria attained maximum potential for degradation in certain level of pH range.<sup>35</sup> However, in the presence hydrocarbonoclastes 2% Crude, changes in pH have no significant effect on the growth of bacterial strains.<sup>36</sup>

#### 5. Bioaugmentation

In recent years, researchers have shown that aerobic microorganisms have the adaptability to degrade hydrocarbon in the soil but are limited by accessible oxygen, which will act as an electron acceptor to sustain and keep the growing population. For these, researchers developed a means to put this problem in check through bioaugmentation. Bioaugmentation, as the word implies, involves the addition of natural, cultured or genetically modified microorganisms with required metabolic adaptability into treatment which could be contaminated soil, water or sewage for degradation of targeted pollutants or pollutants. When bacteria are exposed to pollutant for a long period it evolved adaptive abilities such as increased in content of cyclopropane fatty acids, Saturation of membrane fatty acids, isomerization of cis unsaturated fatty acids to their appropriate trans isomers, Production of stress proteins, Changes in cell morphology and Toxic pollutants as substrates for the efflux system.<sup>37</sup>

In hydrocarbon contaminated soil, selected hydrocarbon-degrading microorganisms are inoculated into the soil to speed up the biodegrading capacity of the hydrocarbon in the soil. A single oil-degrading strains inoculate into hydrocarbon culture shows high degrading capacity as it was noticed by a researcher, an isolate G7 (*Brevibacillus agri*) had high ability to degrade aromatic fraction (61,14%), isolate G3 (*Pseudoxanthomonas taewanensis*) had high ability to degrade aromatic fraction (38,27%) and resin fraction (29,26%).<sup>38</sup> It was also reported by a researcher that *P. aeruginosa* BAS-Cr1 was able to degrade oil sludge with more than 80% degradation of TPH at 5% and 10% concentration within 42 days of treatment.<sup>39</sup> Findings by another researcher show an increase in the degradation rate of 4-chloronitoben-zene (4CNB) degradation in soil microcosm by inoculation of pure cultured *Pseudomonas putida* ZWL73.<sup>40</sup> However, studies have shown that the use of consortia with different kinds of aromatic-degrading bacteria has been more efficient in degrading pollutants as compared with using selected single strain.<sup>41,42</sup> Assessing the degrading mineralize anthracene, phenanthrene and pyrene abilities of a selected microbial consortium of *Mycobacterium fortuitum*, *Bacillus cereus*, *Microbacterium* sp., *Gordonia polyisoprenivorans*, *Microbacteriaceae* bacterium, and *Fusarium oxysporum*. Within 70 days, an average degradation of 96% to 99% was recorded in mineralize anthracene, phenanthrene, and pyrene present in the soil, with an initial dose of (250, 500 and 1000 mg kg<sup>-1</sup>). It was also observed that the PAH in the soil was degraded by 70% by the same consortium within the same incubation period of 70 days.<sup>43</sup> Researches have shown that when a consortium is immobilized, its soil hydrocarbon degradation ability increases. A researcher<sup>44</sup> compared the differences between biostimulation and bioaugmentation treatments on crude oil- contaminated soil; the researcher reported that treatment with bacteria immobilized peanut hull powder has the most effective treatment of hydrocarbon biodegradation in soil. According to,<sup>45</sup> immobilization of microbial cells system for bioremediation possess many advantages with few includes; resist to toxic chemical attack, solvent, heavy metals, temperature, and pH, providing suitable microbial environmental conditions, protection against shear damage, high flow rates of and volumetric productivities, elimination of cell washout problems at high dilution rates, providing cell high biomass. Biocarriers enhanced diffusion of oxygen in the soil, nutrient mass transfer to the bacteria and improved water-retention capacity that served as a limiting factor for bioremediation such contaminants as crude oil.<sup>4,43</sup>

### 5.1. Biostimulation for Hydrocarbons Degradation

Biostimulation is a method of remediation of a polluted environment through the addition of stimulants like

nutrients, oxygen, and water to the environment of the contaminated site which could be soil, water or sludge, to initiate rapid multiplication and growth of the microbial biome for rapid bioremediation. The stimulation of microorganism's activities by the addition of substrates, oxygen vitamins, and other microorganisms tolerated compounds to enhance the degradation of pollutants such as petroleum hydrocarbon is termed biostimulation.<sup>2</sup> it also involves the enhancement of cometabolism.<sup>47</sup> The depletion of

Nitrogen and phosphorus in petroleum-contaminated soil can be responsible for the low degradation of hydrocarbon in contaminated sites.<sup>48</sup> The importance of nutrients for biodegradation of a contaminated site was noticed in the positive correlations of nitrogen and phosphorus with hydrocarbon.<sup>49</sup> Abundant nitrogen and phosphorus in crude oil contaminated site goes a long way in stabilizing the even distribution of microbial community and the richness if the site which is an essential factors for biodegradation of hydrocarbon.<sup>50</sup>

## 6. The effect of Biosurfactant on Hydrocarbon Biodegradation

Biosurfactants is amphipathic configured polymers with prominent hydrophobic and hydrophilic moieties which enable them to mold micelles that gathered at the interface between liquids of different polarities such as water and wax, they are polymers that are partial or total extracellular secreted by bacteria.<sup>55,56</sup> It modified the surface properties of bacteria cells; increase the bioavailability hydrocarbon and the surface area of hydrophobic water-water insoluble substances like petroleum crude oil. Biosurfactant can function at extreme pH and salinity, as well as variable temperature conditions, less toxic, biodegradable and non-hazardous.<sup>57,58</sup>

Biosurfactants which are generally derived from the secondary metabolites of microorganisms,<sup>59</sup> are important biomolecules in environmental biotechnology because of its application in oil industries especially oil spill sites bioremediation, recovery of oil, cleaning of oil storage tank from sludge and environmentally friendly characteristics.<sup>60</sup> The physical and chemical properties of microbial biosurfactants such as biodegradability, foaming, and environmental compatibility give them the edge over their equivalent.<sup>60</sup>

Biosurfactant is an effective biostimulant in crude oil hydrocarbon degradation. This was found out in a study of effect of biosurfactant and fertilizer on biodegradation of crude oil by marine isolates of *Bacillus megaterium*, *Corynebacterium kutscheri* and *Pseudomonas aeruginosa* by researchers, according to the research findings, Biosurfactants alone are capable of promoting biodegradation process, if the polluted site have the required nutrients. However, little significant increase in

**Table 1:** Different countries bioaugmentation with the microbial system and nutrient used

<b>Countries</b>	<b>Microbial system used</b>	<b>Nutrients used</b>
USA	Pure or mixed cultures of Bacillus, Clostridium, Pseudomonas, and Gram-negative rods; mixed cultures of hydrocarbon degrading bacteria; mixed cultures of marine source bacteria; spore suspension of Clostridium; indigenous stratal microflora; slime- forming bacteria; ultramicrobacteria	Molasses 2–4%, Molasses and ammonium nitrate addition, addition of Free corn syrup and mineral salts, Maltodextrine and organic phosphate esters (OPE), Salt solution, Sucrose 10% +Peptone 1% + NaCl 0.5–30%, Brine supplemented with nitrogen and phosphorous sources and nitrate, Biodegradable paraffinic fractions + mineral salts, Naturally contain inorganic and organic materials + N, P sources.
Russia	Pure cultures of <i>C. tyrobutiricum</i> ; bacteria mixed cultures; indigenous microflora of water injection and water formation; activated sludge bacteria; naturally occurring microbiota of industrial (food) wastes.	Molasses 2–6% with nitrogen and phosphorous salt addition ; Water injection with nitrogen and phosphorous salt and air addition ; Waste waters with addition of biostimulators and chemical additives ; Industrial wastes with salts addition Dry milk 0.04%.
China	Mixed enriched bacterial cultures of Bacillus, Pseudomonas, Eurobacterium, Fusobacterium, Bacteroides; Slime-forming bacteria: Xanthomonas campestris, Brevibacterium viscogenes, Corynebacterium gumiform; Microbial products as biopolymers.	Molasses 4–6%; Molasses 5% + ; Residue sugar 4% +; Crude oil 5% ; Xanthan 3% in waterflooding.
Australia	Ultra microbacteria with surface active Properties.	Formulate suitable base media.
Bulgaria	Indigenous oil-oxidizing bacteria from water injection and water formation	Water containing air C ammonium and phosphate ions ; Molasses 2%
Canada	Pure culture of <i>Leuconostoc mesenteroides</i> .	Dry sucrose C sugar beet molasses dissolved in water.
Former Czechoslovakia	Hydrocarbon oxidizing bacteria(predominant <i>Pseudomonas</i> sp.);sulfate-reducing bacteria	Molasse.
England	Naturally occurring anaerobic strain, high generator of acids; special starved bacteria, good producers of exopolymers.	Soluble carbohydrate sources ; Suitable growth media (type E and G).
Former East Germany	Mixed cultures of thermophilic Bacillus and Clostridium from indigenous brine microflora	Molasses 2–4% with addition of nitrogen and phosphorous source.
Hungary	Mixed sewage-sludge bacteria cultures (predominant: Clostridium, Desulfovibrio, and Pseudomonas).	Molasses 2–4% with addition of sugar and nitrogen and phosphorous source.
Norway	Nitrate-reducing bacteria naturally occurring in North Sea water.	Nitrate and 1% carbohydrates addition to injected Sea water.
Poland	Mixed bacteria cultures ( <i>Arthrobacter</i> , Clostridium, Mycobacterium, Peptococcus, and Pseudomonas).	Molasses.
Romania	Adapted mixed enrichment cultures (predominant: Bacillus, Clostridium, Pseudomonas, and other Gram-negativerods).	Molasses 2–4%.
Saudi Arabia	Adequate bacterial inoculum according to requirements of each technology.	Adequate nutrients for each technology.
The Netherlands	Slime-forming bacteria ( <i>Betacoccus dextranicus</i> ).	Sucrose-molasses 10%.
Trinidad-Tobago	Facultative anaerobic bacteria high producers of gases.	Molasses 2-4%.
Venezuela	Adapted mixed enrichment cultures.	Molasses.

Source<sup>46</sup>

**Table 2:** Biostimulation using difference organic wastes

Biostimulation contents	Bioremediated Contaminant	TPH % degradation	Bioremediation Duration	Conclusions	References
Cow dungs and Sewage slug	Used engine oil	94% and 82%	98 days	Cow dung and sewage sludge can be an effective organic amendment for the biodegradation of used lubricant contaminated soil	4
Tea leaf, soy cake and potato skin	Petroleum Hydrocarbon diesel fuel	40-89%	126 days	The study therefore proves the viability of using soy cake amendment in remediating hydrocarbon contaminated soil.	2
Peanut hull powder (15% w/w, no immobilized cells)	Contaminated soil samples from an oil storage site	38%	12 weeks	Oil degradation was enhanced using peanut hull powder as biocarrier. As it enhanced the transferred of biomass, water, oxygen, nutrients and hydrocarbons	43
Brewery, Spent grains, Banana skin and Spent mushroom compost	Petroleum Hydrocarbons used engine oil	79% and 92% for 5% oil contamination 36% to 55% for 15% oil contamination	84 days	There was significant removal Of TPH using the organic nutrient sources.	51
Non-Sterile poultry wastes	Polluted Mangrove Swamp Soil	70%	42 days	Non-sterile poultry waste can effectively and efficiently enhance removal of petroleum from polluted site.	52
Domestic wastewater sludge	Crude oil-contaminated soil	98.3%	30 days	The addition of nutrient and inoculum would be the best option for hydrocarbon biodegradation.	53
Sugar cane bagasse, empty fruit bunch of Oil palm tree	Petroleum hydrocarbon crude oil	100%, 97%	20 days	There was significant increase in the rate of biodegradation of petroleum hydrocarbon using the above supplements which stimulate bacteria growth and metabolism.	54

the rate of biodegradation was observed when compared to the treatments where biosurfactant and fertilizer were combined.<sup>61</sup>

Biosurfactant secreting bacteria are very efficient in crude oil hydrocarbon degradation, investigating the effect of the Addition of Biosurfactant Produced by *Pseudomonas* sp. On Biodegradation of Crude Oil by researchers, they find out that growth of the bacterial isolate on crude oil has been associated with the production of biosurfactants, they conclude that the crude oil metabolizing bacterium is able to secrete surfactants which further enhance the hydrocarbon degradation.<sup>62</sup> Some examples of biosurfactants of commercial and detoxification importance are; rhamnolipid, a glycolipid type biosurfactant produced by *Pseudomonas aeruginosa*, lipopeptide biosurfactant commonly known as surfactin produced by *Bacillus subtilis*, arthrofactin from *Pseudomonas* species, iturin and

lichenysin produced by *Bacillus* species, mannosylerythritol lipids (MEL) from *Candida*, emulsan from *Acinetobacter* species, alasan from *Acinetobacter radioresistens*, serrawet-tin from *Serratia* species, viscosin, amphisin, putisolvin, hydrophobin, lokisin and tensin etc.<sup>63</sup>

## 7. Genome Sequence Basis for Crude Oil Degradation

Perusal proteomic analyses of strains have been potential tools to examine the relationship between various pathways encoded in the genome.<sup>64</sup> This has revealed some potentially crude oil degradation endowed bacteria. These bacteria have a genome with some genes that saves as a genetic base for the production of secondary metabolites from which biosurfactants are derived. Crude oil degradation genes regulates glycolipid, thioesterases and peptide synthetases synthesis,<sup>65–67</sup> it is well known that lipopeptides are biosynthesized through the

**Table 3:** Genes associated with crude oil degrading biosurfactant synthesis

Microbes	Genes	Biosurfactant	References
Bacillus subtilis	ituD, ituA, ituB, and ituC.	Iturin	73,74
Bacillus licheniformis	licA, licB and licC	Lichenysin	75,76
Pseudomonas sp. MIS38	arfA, arfB, and arfC	arthrofactin	77
Pseudomonas sp	rhlA, B, R and I.	rhamnolipid	70
Pseudomonas fluorescens	ViscAR and ViscBCR	viscosin	78
Pseudomonas syringae	GacA/GacS and amsY	Amphisin	79
Pseudomonas putida	dnaK, dnaJ and grpE. psoA, psoB and psoC	Putisolvins	80
Acinetobacter radioresistens	AlnA, AlnB and AlnC SrfAA,	Alasan Surfactin	81,82
Bacillus sp	SrfAB, SrfAC and SrfAD or SrfA-TE, sfp.		

ribosome-independent pathway with non-ribosomal peptide synthetases (NRPSs) enzymes moderated by genes.<sup>68</sup> A genus *Polymorphum* SL003B-26A1<sup>T</sup> endowed with a gene that coded for some vital enzymes such as ketoreductase (RhlG), 3-oxoacyl-(acyl- carrier protein) reductase and acyltransferase, phosphomannomutase (AlgC) that contribute majorly to the synthesis of glycolipids, which equipped this strain for the degradation of both saturated and unsaturated aliphatic hydrocarbon, monocyclic and polycyclic aromatic hydrocarbon, as well enhanced their perpetual adaptation in the crude oil polluted environment.<sup>69</sup> The expression and regulation of these enzymes are coordinated at the transcriptional level of at least two quorum sensing system.<sup>70,71</sup> The genetic regulated biosurfactant synthetic pathways have not been fully studied, however, according to,<sup>72</sup> there are three major processes involved in biosurfactants biosynthesis which are; biosynthesis of 3-hydroxy-heneicosanoic acid from specific carbon sources through fatty acid, synthesis of hexapeptide by a series of enzymatic condensations from the N-terminal of Leu to the C-terminal of Gly; and the 3- hydroxy-heneicosanoic acid may undergo an enzymatic condensation process, being incorporated at the C- and N- terminals of the hexapeptide to produce a cyclic lipopeptide. Some of the genes that played vital roles in crude oil degradation are illustrated in table3 with their biosurfactants.

## 8. Conclusion

This work illustrates some methods of crude oil polluted soil bioremediation, effects of PAH on both physical and chemical properties of the soil and some factors that can affect the rate of PAH biodegradation. Environmental PAH pollution has been a threat to man and his immediate surroundings; cleansing of petroleum hydrocarbon pollution has caused many countries the huge amount of capital and financial resources. Understanding the mechanism of some petroleum hydrocarbons degrading bacteria can be very useful when applied to petroleum hydrocarbons contaminated sites like soil, sewage, and water body. Some microorganisms feed on crude oil as a source of carbon and

generate energy for their metabolic activities; this is possible because some microbes like some species of bacteria secrete enzymes that can degrade petroleum hydrocarbons. However, most of the tested degradation was carried out in a controlled environment, therefore the probability of recording failure might be high if the degradation is carried out in a non-control environment, because unfavourable climatic factors can alter biodegradation. Moreover, there have been none 100% recorded degradation in the experimental crude oil degradation test. Therefore, further studies need to be carried out on crude oil degradation to understand the degradation mechanisms of some bacteria. A critical study of the genetic characteristics of some already known crude oil degrading bacteria will help researchers to discover new strains with higher degradation capability. More research still needs to be carried out to determine other mechanisms that bacteria make use of to degrade crude oil in their environment.

## 9. Source of Funding

None.

## 10. Conflict of Interest

None.

## References

- Oyem I, Rank OL, Lawrence I. Effects of Crude Oil Spillage on Soil Physico- Chemical Properties inUgborodo Community. *Int J Modern Engg Res.* 2013;3(6):3336.
- Agamuthu P, Dadrasnia A. Potential biowastes to remediate diesel contaminated soils. *Glob NEST J.* 2013;15(4):474–84.
- Abosede EE. Effect of Crude Oil Pollution on some Soil Physical Properties. *IOSR J Agriculture Vet Sci.* 2013;6(3):14–7.
- Agamuthu P, Tan YS, Fauziah SH. Bioremediation of Hydrocarbon Contaminated Soil Using Selected Organic Wastes. *Procedia Environ Sci.* 2013;18:694–702.
- He GG. The Soil pollution crisis in China: a cleanup presents daunting challenge; 2014.
- Jayanta S, Saha K. Soil Pollution - An Emerging Threat to Agriculture. In: Status of Soil Pollution in Indian; 2017. p. 271–315.
- Marinescu M, Toti M, Tanase V, Carabulea V, Georgiana P, Calciu I. An assessment of the effects of crude oil pollution on soil properties.

- Ann Food Sci Technol.* 2010;11(1):94–9.
8. Duan L, Naidu R, Thavamani P, Meaklim J, Megharaj M. Managing long-term polycyclic aromatic hydrocarbon contaminated soils: a risk-based approach. *Environ Sci Pollut Res.* 2015;22(12):8927–41.
  9. Rengarajan T, Rajendran P, Nandakumar N, Lokeshkumar B, Rajendran P, Nishigaki I. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pac J Trop Biomed.* 2015;5(3):182–9.
  10. Okere UV. Biodegradation of PAHs in 'Pristine' soils from different climatic regions. *J Bioremed Biodegrad.* 2011;s1. doi:10.4172/2155-6199.S1-006.
  11. Obi LU, Atagana HI, Adeleke RA. Isolation and characterization of crude oil sludge degrading bacteria. *Springer Plus.* 1946;5. doi:10.1186/s40064-016-3617-z.
  12. Aldisi Z, Jaoua S, Al-Thani D. Isolation, Screening and Activity of Hydrocarbon- Degrading Bacteria from Harsh Soils. Isolation, Screening and Activity of Hydrocarbon- Degrading Bacteria from Harsh Soils. 2016. Proceedings of the World Congress on Civil, Structural, and Environmental Engineering (CSEE'16) ; 2016.
  13. Minai-Tehrani D, Minoui S, Herfatmanesh A. Effect of Salinity on Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) of Heavy Crude Oil in Soil. *Bull Environ Contam Toxicol.* 2009;82(2):179–84.
  14. Gan S, Lau EV, Ng HK. Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). *J Hazardous Mater.* 2009;172(2-3):532–49.
  15. Moubasher HA, Hegazy AK, Mohamed NH, Moustafa YM, Kabiell HF, Hamad AA. Phytoremediation of soils polluted with crude petroleum oil using *Bassia scoparia* and its associated rhizosphere microorganisms. *Int Biodeterioration Biodegradation.* 2015;98:113–20.
  16. Budavari S. The Merck index: an encyclopedia of chemicals, drugs and biological, 12th edn. NJ Merck, White house Station; 1996.
  17. Kuppusamy S, Thavamani P, Megharaj M, Naidu R. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by novel bacterial consortia tolerant to diverse physical settings – Assessments in liquid- and slurry-phase systems. *Int Biodeterioration Biodegradation.* 2016;108:149–57.
  18. Gereslassie T, Workineh A, Liu X, Yan X, Wang J. Occurrence and Ecological and Human Health Risk Assessment of Polycyclic Aromatic Hydrocarbons in Soils from Wuhan, Central China. *Int J Environ Res Public Health.* 2018;15(12):2751.
  19. Moses E, Uwah EI. The effect of crude oil pollution on some soil fertilities parameters in Ikot Oboreyin, Ikot Abasi Akwa Ibom State Nigeria. *Merit Res J Environ Environ Sci Toxicol.* 2015;3(2):17–024.
  20. Benka-Coker MO, Ekundayo JA. Effects of an oil spill on soil physico-chemical properties of a spill site in the Niger Delta Area of Nigeria. *Environ Monitoring Assess.* 1995;36(2):93–104.
  21. US Microbics. Annual Report FY-2003. Available from: <http://www.bugsatwork.com/USMX/BUGS%20Report%20PRIN>.
  22. Lin TC, Pan PT, Cheng SS. Ex situ bioremediation of oil-contaminated soil. *J Hazardous Mater.* 2010;176(1-3):27–34.
  23. Owsianiak M, Dechesne A, Binning PJ, Chambon JC, Sørensen SR, Smets BF. Evaluation of Bioaugmentation with Entrapped Degrading Cells as a Soil Remediation Technology. *Environ Sci Technol.* 2010;44(19):7622–7.
  24. Guang-Guoying. Integrated Analytical Approaches for Pesticide Management. Academic Press Inc; 2018.
  25. Johnson D. Pollutants — Persistent Organic (POPs). Reference Module in Earth Systems and Environmental Sciences; 2005.
  26. Fritsche W, Hofrichter M. Aerobic Degradation by Microorganisms; 2008.
  27. Samajdar A. Bioremediation using Fungi – Mycoremediation; 2018. Available from: <https://envibrary.com/bioremediation-using-fungi-mycoremediation/>.
  28. Delille D, Coulon F, Pelletier E. Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydrocarbon-contaminated sub-Antarctic soils. *Cold Regions Sci Technol.* 2004;40(1-2):61–70.
  29. Iqbal J, Metosh-Dickey C, Portier RJ. Temperature effects on bioremediation of PAHs and PCP contaminated south Louisiana soils: A laboratory mesocosm study. *J Soils Sediments.* 2007;7(3):153–8.
  30. Ruberto L, Dias R, Balbo AL, Vazquez SC, Hernandez EA, Cormack WPM. Influence of nutrients addition and bioaugmentation on the hydrocarbon biodegradation of a chronically contaminated Antarctic soil. *J Appl Microbiol.* 2009;106(4):1101–10.
  31. Kalantary RR, Mohseni-Bandpi A, Esrafilia A, Nasserli S, Ashmogh FR, Jorfi S, et al. Effectiveness of biostimulation through nutrient content on the bioremediation of phenanthrene contaminated soil. *J Environ Health Sci Engg.* 2014;12(1):143.
  32. Maier RM. Bioavailability and Its Importance to Bioremediation; 2000.
  33. Bahmani F, Ataei SA, Mikaili MA. The Effect of Moisture Content Variation on the Bioremediation of Hydrocarbon Contaminated Soils: Modeling and Experimental Investigation. *J Environ Anal Chem.* 2018;5(2):236.
  34. Shroud JD, Parkin GF. Influence of electron donor, oxygen, and redox potential on bacterial perchlorate degradation. *Water Res.* 2006;40(6):1191–9.
  35. Pawar RM. The Effect of Soil pH on Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs). *J Bioremed Biodegr.* 2015;06(03):3.
  36. Boudherhem A, Khelil AOEH. Isolation and characterization of crude oil degrading bacteria from soil of Ouargla (Algeria). *Int J Biosci.* 2017;10(6):13–9.
  37. Katarína D, Slavomíra M, Hana D. The Adaptation Mechanisms of Bacteria Applied in Bioremediation of Hydrophobic Toxic Environmental Pollutants: How Indigenous and Introduced Bacteria Can Respond to Persistent Organic Pollutants-Induced Stress; 2018.
  38. Purwasena IA, Astuti DI, Fatmawati R, Afinanisa Q. Isolation and Characterization of Oil- Degrading Bacteria from One of South Sumatera's Oilfield. In: The 2nd Annual Applied Science and Engineering Conference (AASEC 2017); 2017.
  39. Piakong MT, Zaida ZN. Effectiveness of Single and Microbial Consortium of Locally Isolated Beneficial Microorganisms (LIBEM) in Bioaugmentation of Oil Sludge Contaminated Soil at Different Concentration Levels: A Laboratory Scale. *J Bioremed Biodegr.* 2018;09(02). doi:10.4172/2155-6199.1000430.
  40. Niu GL, Zhang JJ, Zhao S, Liu H, Boon N, Zhou NY. Bioaugmentation of a 4-chloronitrobenzene contaminated soil with *Pseudomonas putida*ZWL73. *Environ Pollut.* 2009;157:763–71.
  41. Heinaru E, Merimaa M, Viggor S, Lehiste M, Leito I, Truu J, et al. Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. *FEMS Microbiol Ecol.* 2005;51(3):363–73.
  42. Ghazali FM, Rahman RNZA, Salleh AB, Basri M. Biodegradation of hydrocarbons in soil by microbial consortium. *Int Biodeterioration Biodegradation.* 2004;54(1):61–7.
  43. Jacques RJS, Okeke BC, Bento FM, Teixeira AS, Peralba MCR, Camargo FAO. Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. *Bioresour Technol.* 2008;99(7):2637–43.
  44. Xu Y, Lu M. Bioremediation of crude oil-contaminated soil: Comparison of different biostimulation and bioaugmentation treatments. *J Hazardous Mater.* 2010;183(1-3):395–401.
  45. Bayat Z, Hassanshahian M, Cappello S. Immobilization of Microbes for Bioremediation of Crude Oil Polluted Environments: A Mini Review. *Open Microbiol J.* 2015;9. doi:10.2174/1874285801509010048.
  46. Lazar I, Petrisor IG, Yen TF. Microbial Enhanced Oil Recovery (MEOR). *Petroleum Sci Technol.* 2007;25(11):1353–66.
  47. Lorenzo D, V. Systems biology approaches to bioremediation. *Curr Opin Biotechnol;*19(6):579–589.
  48. Kanissery RG, Sims GK. Biostimulation for the Enhanced Degradation of Herbicides in Soil. *Appl Environ Soil Sci.* 2011;2011. doi:10.1155/2011/843450.
  49. International Centre for Soil and Contaminated Sites. Manual for biological remediation techniques; 2006. Available from: [www.umweltbundesamt.de/sites/default/files/medien/publikation/](http://www.umweltbundesamt.de/sites/default/files/medien/publikation/)



- long/3065.pdf.
50. Wu M, Wu J, Zhang X, Ye X. Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum-contaminated loessal soil. *Chemosphere*. 2019;237:124456.
  51. Abioye OP, Agamuthu P, Aziz ARA. Biodegradation of Used Motor Oil in Soil Using Organic Waste Amendments. *Biotechnol Res Int*. 2012;2012:1–8.
  52. Ezekoye CC, Amakoromo ER. Abiye Anthony Ibiene. Laboratory - Based Bioremediation of Hydrocarbon Polluted Mangrove Swamp Soil in the Niger Delta Using Poultry Wastes. *Microbiol Res J Int*. 2017;19(2):1–14.
  53. Alkhatib MF. An isolated bacterial consortium for crude oil biodegradation. *Afr J Biotechnol*. 2011;10(81):18763–7.
  54. Hamzah A, Phan CW, Yong PH, Ridzuan NHM. Oil Palm Empty Fruit Bunch and Sugarcane Bagasse Enhance the Bioremediation of Soil Artificially Polluted by Crude Oil. *Soil Sediment Contamination: Int J*. 2014;23(7):751–62.
  55. Karanth NGK, Deo P, Veenanadig N. Microbial production of biosurfactants and their importance. *Curr Sci*. 1999;77:116–26.
  56. Santos D, Rufino R, Luna J, Santos V, Sarubbo L. Biosurfactants: Multifunctional Biomolecules of the 21st Century. *Int J Mol Sci*. 2016;17(3):401.
  57. Nie Y, Tang YQ, Li Y, Chi CQ, Cai M, Wu XL. The Genome Sequence of *Polymorphum gilvum* SL003B-26A1T Reveals Its Genetic Basis for Crude Oil Degradation and Adaptation to the Saline Soil. *PLoS ONE*. 2012;7(2):e31261.
  58. Maier RM. Biosurfactants: Evolution and Diversity in Bacteria. *Adv Appl Microbiol*. 2003;52:101–21.
  59. Pacwa-Płociniczak M, zyna A, Plaza G, Piotrowska-Seget Z, Cameotra SS. Environmental Applications of Biosurfactants: Recent Advances. *Int J Mol Sci*. 2011;12(1):633–54.
  60. Adamczak M, Bednarski W. Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida Antarctica*. *Biotechnol Lett*. 2000;22:313–6.
  61. Thavasi R, Jayalakshmi S, Banat IM. Effect of biosurfactant and fertilizer on biodegradation of crude oil by marine isolates of *Bacillus megaterium*, *Corynebacterium kutscheri* and *Pseudomonas aeruginosa*. *Bioresource Technol*. 2011;102(2):772–8.
  62. Aparna A, Srinikethan G, Hegde S. Effect of Addition of Biosurfactant Produced by *Pseudomonas* spp. In: 2nd International Conference on Environmental Science and Technology. vol. 6. IACSIT Press; 2011.
  63. Das P, Mukherjee S, Sen R. Genetic Regulations of the Biosynthesis of Microbial Surfactants: An Overview. *Biotechnol Genet Engg Rev*. 2008;25(1):165–86.
  64. Hong YH, Ye CC, Zhou QZ, Wu XY, Yuan JP, Peng J, et al. Genome Sequencing Reveals the Potential of *Achromobacter* sp. HZ01 for Bioremediation. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01507.
  65. Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM, Chopade BA. Methods for investigating biosurfactants and bioemulsifiers: a review. *Crit Rev Biotechnol*. 2010;30(2):127–44.
  66. Yao S, Gao X, Fuchsbaue N, Hillen W, Vater J, Wang J. Cloning, Sequencing, and Characterization of the Genetic Region Relevant to Biosynthesis of the Lipopeptides Iturin A and Surfactin in *Bacillus subtilis*. *Curr Microbiol*. 2003;47(4):272–7.
  67. Moyne AL, Cleveland TE, Tuzun S. Molecular characterization and analysis of the operon encoding the antifungal lipopeptide bacillomycin D. *FEMS Microbiol Lett*. 2004;234(1):43–9.
  68. Roongsawang N, Washio K, Morikawa M. Diversity of Nonribosomal Peptide Synthetases Involved in the Biosynthesis of Lipopeptide Biosurfactants. *Int J Mol Sci*. 2010;12(1):141–72.
  69. Nie Y, Tang YQ, Li Y. The Genome Sequence of *Polymorphum gilvum* SL003B-26A1T Reveals Its Genetic Basis for Crude Oil Degradation and Adaptation to the Saline Soil. *PLoS ONE*. 2012;7(2):31261.
  70. Waack S, Keller O, Asper R, Brodag T, Damm C. Score-based prediction of genomic islands in prokaryotic genomes using hidden Markov models. *BMC Bioinformatics*. 2006;7:142.
  71. Maier RM, Soberón-Chávez G. *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. *Appl Microbiol Biotechnol*. 2000;54(5):625–33. Available from: <https://dx.doi.org/10.1007/s002530000443>.
  72. Hong YH, Ye CC, Zhou QZ, Wu XY, Yuan JP, Peng J. Genome Sequencing Reveals the Potential of *Achromobacter* sp. HZ01 for Bioremediation. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01507.
  73. Tsuge K, Akiyama T, Shoda M. Cloning, Sequencing, and Characterization of the Iturin A Operon. *J Bacteriol*. 2001;183(21):6265–73.
  74. Rahman MS, Ano T, Shoda M. Second stage production of iturin A by induced germination of *Bacillus subtilis* RB14. *J Biotechnol*. 2006;125(4):513–5.
  75. Konz D, Doekel S, Marahiel MA. Molecular and Biochemical Characterization of the Protein Template Controlling Biosynthesis of the Lipopeptide Lichenysin. *J Bacteriol*. 1999;181(1):133–40.
  76. Anuradha SN. Structural and Molecular Characteristics of Lichenysin and Its Relationship with Surface Activity. *Biosurfactants*. 2010;p. 304–15.
  77. Roongsawang N, ichi Hase K, Haruki M, Imanaka T, Morikawa M, Kanaya S. Cloning and Characterization of the Gene Cluster Encoding Arthrofactin Synthetase from *Pseudomonas* sp. MIS38. *Chem Biol*. 2003;10(9):869–80.
  78. de Bruijn I, Raaijmakers JM. Diversity and Functional Analysis of LuxR-Type Transcriptional Regulators of Cyclic Lipopeptide Biosynthesis in *Pseudomonas fluorescens*. *Appl Environ Microbiol*. 2009;75(14):4753–61.
  79. Andersen JB. Surface motility in *Pseudomonas* sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi *Rhizoctonia solani* and *Pythium ultimum*. *Microbiol*. 2003;149(1):37–46.
  80. Dubern JF, Coppoolse ER, Stiekema WJ, Bloembergen GV. Genetic and functional characterization of the gene cluster directing the biosynthesis of putisolvin I and II in *Pseudomonas putida* strain PCL1445. *Microbiol*. 2008;154(7):2070–83.
  81. Ahmed MMM, Hafez EE, Saadani MAE. Biodegradation of polyaromatic hydrocarbons by recombinant bacteria containing *Alasan* gene. *Int J Environ Pollut*. 2009;38(4):415.
  82. Zhi Y, Wu Q, Xu Y. Genome and transcriptome analysis of surfactin biosynthesis in *Bacillus amyloliquefaciens* MT45. *Sci Rep*. 2017;7(1).

## Author biography

**Ehenenden Iyobosa** Masters Research Student

**Meng Xianagang** Professor

**Ning Hai Jun** Masters Research Student

**Shu Fang** Masters Research Student

**Wang Zhennan** Masters Research Student

**Cite this article:** Iyobosa E, Xianagang M, Jun NH, Fang S, Zhennan W. Biodegradation of petroleum hydrocarbon polluted soil. *Indian J Microbiol Res* 2020;7(2):104–112.