International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 12 Issue 4, April-2023, Impact Factor: 7.957

Invitro Biodegradation of Petroleum Hydrocarbon using Microorganism from Contaminated Soil

J. Ishwarya[1], S. Fathima[2]

[1]Assistant Professor, PG and Research Department, Department of Microbiology, Kamban College of Arts and Science for Women, Tiruvannamalai - 606603
[2]Research Scholar, PG and Research Department, Department of Microbiology, Kamban College of Arts and Science for Women, Tiruvannamalai - 606603

ABSTRACT

The use of petroleum raises the possibility of soil contamination with diesel and used engine oil, which becomes one of the main environmental issues. Even some of the components the makeup hydrocarbons are extremely toxic, mutagenic or cancer causing in nature. The present investigation was made to prove that Invitro biodegradation of petroleum hydrocarbon using microorganism from contaminated soil. The study was carried out in Kamban College of arts and science for women, Tiruvannamalai in period of January 2023 to March 2023.The physiochemical composition result gives an average value of 6.194. The soil samples rich in minerals it has the ability to give nutrients to the bacterial. Soil sample were collected from petrol bunk of Tiruvannamalai district. Using nutrient agar as a bacterial were obtained from soil samples that had been polluted with crude oil. The total bacterial count rages 1.52107 CFU/ml to 7.24107 CFU/ml.The three isolates character shows that two positive and one negative bacterium. It may note as Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cerus. The maximum level of degradation for the three isolates was up to 20%. Based on the biochemical characters and colony morphology the organism identified as isolate A as bacillus substilis, B as pseudomonas aeruginosa, C as bacillus cereus. The isolates were checked for the extent of crude oil degradation in solid and liquid medium. All the isolates were found to be the highest degradation ratio. Visual degradation of the sample shows that it was higher in case of basal broth. According to isolates A bacillus subtilis (85.6%), B pseudomonas aeruginosa (94%), and C bacillus cereus (70.6%), the ability to degrade petrol exhibits a greater proportion. From this investigation the organism pseudomonas aeruginosa have the highest efficient to degrade the hydrocarbon and eco-friendly for the degradation of hydrocarbon. Further studies are carried out to justify the potential of pseudomonas aeruginosa for the degradation of petroleum hydrocarbon.

INTRODUCTION

Petroleum is the primary energy source for daily life and business around the world. However, crude oil, a dark-brown, viscous liquid mixture comprised of petroleum and other natural hydrocarbons with different molecular weights that serve as the primary fuel source used in the production of several petroleum products. Crude Poly-aromatic hydrocarbons (PAHs) make up more than 30% of oil's composition. Additional hundreds of different hydrocarbons, including paraffins, naphthene, sulphur, organic nitrogen compounds, and aromatic compounds hydrocarbons that contain oxygen, and compounds (phenols).

The molecular weight range of crude oil, which runs from 16 to 1800, is a complicated mixture of solid or gaseous hydrocarbons that are dispersed in liquid hydrocarbons and appear liquid. Petroleum (crude) oil is a complex mixture of thousands of compounds. Petroleum hydrocarbons account for 50-98% of crude oil and are considered a major component depending on the source of the oil. Petroleum could be treated with a variety of microorganisms. Biodegradation of hydrocarbons. However, bacteria considered important biodegradable microorganisms that play a critical role in hydrocarbon degradation **(Udgire *et al.*, 2015).**

Four Main Groups of Petroleum Hydrocarbons

Hydrocarbons with aromatic rings that are saturated (branched and unbranched as well as cyclic structures), like polyaromatic hydrocarbons (PAHs), Mono aromatic hydrocarbons (MAHs), polar oil surface structures, and in aromatics

and saturates, resins evaporated. A biodegradation is a method of treatment used to get rid of many different pollutants, the contamination of soil with petroleum hydrocarbons.

With the constant rise in demand for oil and other oil-related products, petroleum hydrocarbon pollution (PHP) is getting worse. This type of contamination has drawn more attention due to its severe negative effects on both marine and terrestrial ecosystems PHP is caused by a variety of things, like using Includes lubricants and cooling agents, compressor condensates, Transporting and using oil, as well as processing metals. Oil exploitation and processing produce enormous volumes of wastes and industrial effluents, and oil transportation is typically connected with recurring crude oil spills. Oil-contaminated effluents typically originate from a variety of sources and are full of toxins such phenols, polyaromatic hydrocarbons (PAHs), and petroleum hydrocarbons. Which are dangerous and challenging to break down?

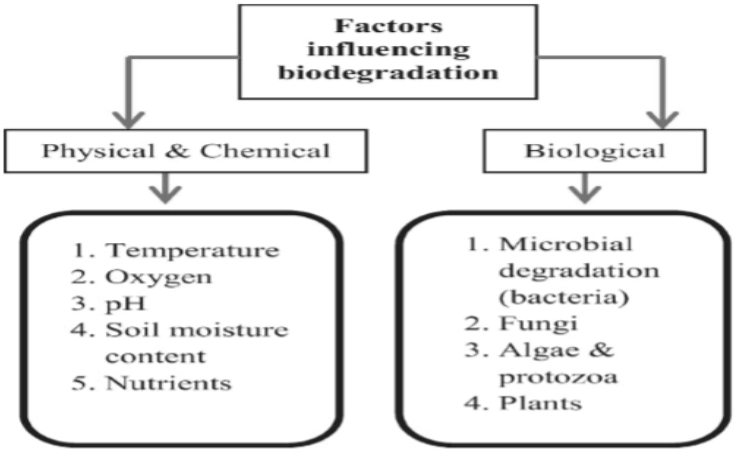
They prevent the growth of plants and animals, cause human health hazards due to genetic mutation. Additionally, oil-polluted effluents have high oil content and hue and have an increased need for chemical oxygen, all of which can significantly harm to marine and terrestrial ecosystems. **(Ghanavati *et al.*, 2008).** The use of petroleum hydrocarbon products raises the possibility of soil contamination with diesel and used motor oil, which becomes one of the main environmental issues. The International Tanker Association performed statistical analysis. Owners Pollution Federation Ltd. (ITOPF) since the year Three oil spills of at least 700 tonnes happened between 1970 and 2013, according to data 2013 itself.

Biodegradation

Due to their catastrophic effects on the environment, large-scale maritime oil spills and oil leak catastrophes have drawn a lot of attention globally. Several physical and Chemical treatments are used all throughout the world, however bioremediation offers the most economical and environmentally friendly solution.

Even some of the components that make up hydrocarbons are extremely toxic, mutagenic, or cancer-causing in nature. The study of various methods that might be used to clean up the environment has, however, dramatically increased as a result of heightened awareness of the negative effects of environmental pollution. Hydrocarbon pollution removal from the environment eventually involves physical, chemical, and biological alternatives, and this process has been well documented. According to reports, the primary mechanism causing the purification of hydrocarbon-polluted environments is the biodegradation of the hydrocarbons by natural populations of microorganisms **(van Hamme *et al.*, 2003).**

For the clean-up of petroleum-contaminated soil and water to restore its original environment. The effectiveness of stress-tolerant hydrocarbon-degrading microorganisms is less successful despite the substantial research that has been done on microbial bioremediation of oil pollutants over the past ten years studied. In situ biodegradation, which involves removing petroleum and other hydrocarbons from a culture by using widely dispersed microbes, is one of the innovative, cost-effective, and environmentally friendly treatment methods for petroleum biodegradation **(Schinner *et al.*, 1997).** Biodegradable petroleum hydrocarbons lighting is dependent on the localised bacteria to alter or mineralize the toxic organic substances. Petroleum hydrocarbons and bacterial contamination in the soil are influenced by a variety of circumstances.



Alcaligen sp, Bacillus sp, Chromobacterium *sp, Corynebacterium sp, Pseudomonas sp, Aeromonas sp*, and *Serratia* were the eight hydrocarbon-degrading bacteria that were explicitly identified together with *Flavobacterium sp* **(Gayathiri *et al.*, 2017).**

As a widespread issue, the release of petroleum components into the environment mainly arises from petroleum exploration, accidents, transportation, and leaks fromas well as from enterprises; there are locations for waste storage or disposal. This is a big problem in oil-producing countries like Iran.

According to a source 1,500,000 m3 of soil near the Tehran Oil Refinery had been contaminated with crude oil. Due to petroleum includes elements like benzene, toluene, and ethyl that could be harmful Substances like naphthalene, benzene, and xylene may harm plants, both human and animal health. Because hydrocarbons in the soil lower oxygen tension and have a harmful effect on soil ecosystems, oil spills have a negative influence on such ecosystems **(Kebria DY *et al.*, 2009).**

Increasing the plant-harmful phenomenon known as anaerobiosis roots. Petroleum pollution is handled physically, techniques using chemistry and biology. Invoking the first two techniques, Limitations such high costs, inefficiency, and changing the natural environment ecosystem. The approach of biological therapy to get rid of petroleum pollution is more intriguing today **(Sarkar D *et al.*, 2005).**

Bioremediation

A bioremediation technique the process by which living things, including bacteria, are used to convert (degrade) or eliminate dangerous substances. In Recent years have seen the use of microbes that degrade hydrocarbons to clean a Petroleum-contaminated soil has emerged as a widespread, effective, and a method that is inexpensive for transforming toxic trash into non-toxic products. The detrimental consequences on ecological and human health have been lessened by this method. Additionally, it allows for in situ therapy to be carried out without significantly disrupting natural ecosystems, as opposed to remediation techniques, both chemical and physical. In bioremediation, indigenous microorganisms from contaminated sites or new microorganisms can be used **(Mirdamadian SM *et al.*, 2010).**

Most microorganisms are unable to utilise hydrocarbons due to the hydrophobic property of petroleum compounds to be used in bioremediation. Characterization of bacterial strains found in petroleum-contaminated areas can be beneficial.

Increasing the Efficiency of Bioremediation

Microorganisms such as Pseudomonas spp., Bacillus spp.and Micrococcus. Enterobacter spp., Rhodoccucos spp. Petroleum compounds can be degraded by Mycobactena spp., Modococci spp., and Acinetobacter spp. These, however, are degraded by such microorganisms.

Different compounds are formed at different rates. As a result, evaluation is required their ability to biodegrade. Inputs for various industries, automobiles, and other devices include hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs), benzene, kerosene, and diesel. Energy sources from household activities. Among for engines, fuel, and industrial purposes, diesel is widely used.

Applications

- It is one among the petroleum chemicals produced when fractional distilling crude oil.
- Contains a variety of carbon chains between 9 and 25.
- Aromatic and aliphatic carbon atoms may both be present.
- Hydrocarbon substances.
- The environment (groundwater, soil, and air) may be exposed to these hydrocarbon components through a variety of sources.

Example of Bioremediation

- Garages
- Gas station services
- Chemicals and petrochemicals
- Agricultural waste
- Spilled gasoline from automotive exhaust
- Asphalt runoff road surfaces
- Vehicle emission
- Fossil fuel combustion

This occurrence might be deliberate or due to manmade activity, namely, accidentally civilisation, industrialisation and urbanisation also, to a certain extent, by natural disasters consequently, contaminants produced from hydrocarbons are harmful to human immunity, mutagenic, and carcinogenic the natural ecosystem's ability to function in both humans and animals.

One of the most harmful types of water contamination are oil spills, which are a major issue in industrialised regions the present-day developing world. The term "biodegradation "utilised in several hospitals as a therapy option location with hydrocarbon contamination which makes use of organisms to break down and/or detoxify toxic organic substances. Crude petroleum (petroleum) is a complicated mixture of millions of chemicals.

The Main Ingredients of Hydrocarbons

Alkenes	20-50%
Crude oil	50-98%

Microbes include bacteria, fungus, yeast, and microalgae can break down petroleum hydrocarbons. Nevertheless, bacteria are crucial to degradation of hydrocarbons. Microorganisms are equipped with metabolism-related equipment to utilisation of petroleum products as a carbon and energy supply. Size of the biodegradation in polluted sites is the amount depending on a number of elements; including atomic structure of the hydrocarbons, what kind of microbe population there is, and ideal environmental circumstances for increase the body's bioavailability by pollutants that affect bacteria.

Environmental concerns are especially high when petroleum products are accidentally released. Currently, using traditional disposal techniques like burning or burying waste in unsafe landfills can become prohibitively expensive when levels of toxins are high and large. Bioremediation mostly relies on biodegradation, which could mean mineralization of organic pollutants completely into carbon dioxide, water, and inorganic cell protein, chemicals, and the conversion of intricate organic pollutants to by biological agents like microorganisms, such as other simpler organic molecules. Many native microorganisms in soil and water have the ability to degrade a variety of hydrocarbons.

Estimated to be 600,000 gallons of natural crude oil seepage 200,000 metric tonnes per year, with a 200,000 metric tonnes per year uncertainty range. Whether by accident or as a result of human activity, hydrocarbons entering the environment are a problem. Common soil remediation technologies include mechanical, burying, evaporation, dispersion, and washing3 methods. However, the cost of using these technologies can result in incomplete decomposition of impurities. The use of microorganisms during the bioremediation procedure due to their varied metabolic capacities, to detoxify or eliminate contaminants developing techniques for the deterioration and removal of several environmental contaminants containing petroleum industry by products **(Medina-Bellver JL *et al.*, 2005).**

Furthermore, the bioremediation technologyis thought to be non-invasive and somewhat economical. The soil and water ecosystems are endangered by petroleum contamination, which results from leaking above- and underground storage tanks, spills during the transport of petroleum products, abandoned manufactured gas sites, and various industrial processes. Remediation is also expensive. In the process of cleaning up oil-polluted places, encouraging environmentally friendly techniques. These approaches use lesscost-prohibitive and do not add new chemicals to the environment.

Bioremediation is a viable alternative to physicochemical methods for oil spill decontamination. This technique is regarded as an effective technology for treating oil pollution. Bioremediation is a cost-effective, environmentally friendly treatment method that employs microbes to degrade pollutants hydrocarbons. Soil moisture, temperature, and population density are all factors influencing the rate of microbial growth **(Malatova K *et al.*, 2005).**

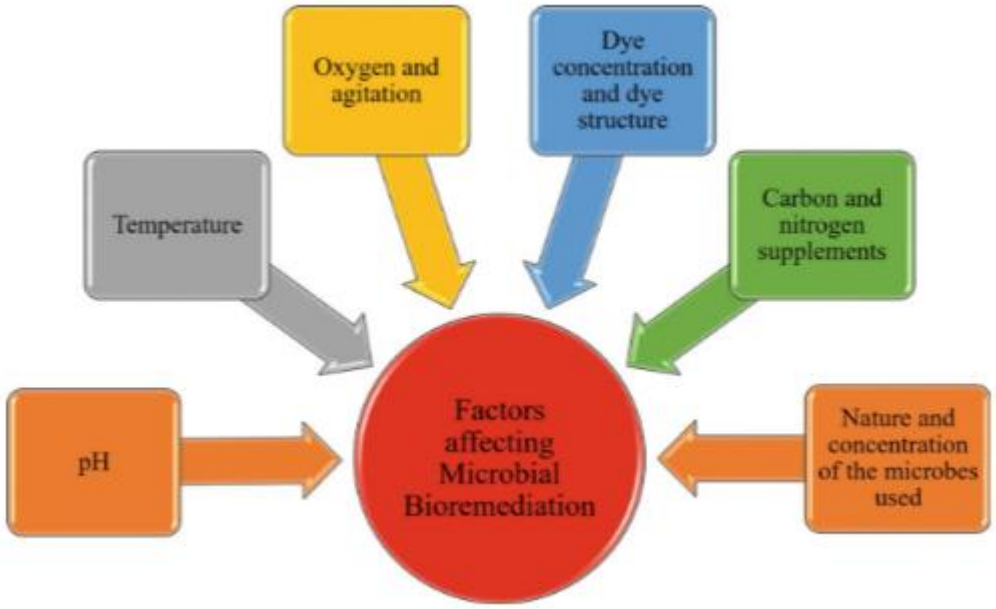
Using certain microbial strains that have been genetically altered or isolated to boost their capacity for reducing pollution is known as "bioaugmentation." An example of this would be using these organisms to clean up oil spills in polluted marine habitats.

The ability of bacteria to metabolise contaminants into less harmful and toxic chemicals is essential to this process.
Native microorganisms that typically have a limited ability to breakdown these chemicals perform biodegradation naturally in contaminated areas.

Biostimulation is defined as an environmental modification procedure that promotes the proliferation of existing bacteria as well as the biodegradation of pollutants to be addressed in the modified environment. In practise, this process is used by adding nutrients or substances, such as carbon, nitrogen, and phosphorus, to the environment to be treated, as well as ensuring appropriate environmental conditions, such as moisture and oxygen content, to promote microbial growth and natural biodiversity of microorganisms.

Nitrogen, phosphorus, and molecular oxygen concentrations in seawater are examples of abiotic factors. As a result, nitrogen and phosphate are used to reduce the limit of these nutrients, which promotes the growth of degrading organisms. Bacteria that consume petroleum hydrocarbons. **(Rosenberg, E *et al.*, 2014).**

Factors of Petroleum Hydrocarbons Remediation in Soil:



Microorganisms that biodegrade petroleum hydrocarbon components such as polynuclear aromatic hydrocarbons (PAHs), naphthalene, and benzene, toluene and other monoaromatic hydrocarbons, as well as aliphatic hydrocarbons such as n-alkanes, can be readily converted.

Isolating oneself from the environment, particularly from petroleum-contaminated sites. Microorganisms are capable of be obtained initially through enrichment culture procedures in which the highest specific growth rate or maximum. As a selection criterion, final cell concentration can be used. Petroleum hydrocarbons can be degraded through the use of Bacteria, fungi, yeast, and microalgae are all examples of microorganisms. Bacteria, on the other hand, play a crucial role in hydrocarbon degradation.

Energy for industry and daily living is primarily derived from petroleum-based goods. Aliphatic and aromatic hydrocarbons are mixed in a highly sophisticated way to create petroleum products. The unavoidable result of oil use is environmental pollution from petroleum products. Activities related to manufacturing, shipping, and distribution

Oil waste from vehicles is released into a significant cause of oil pollution in mechanic shops is the use of vehicles or motorbikes, and its various petroleum products. Environments lubricating oils, gasoline, diesel, and engine oil in mechanics workshops, among others. The consistency of these products might alter or harden the microbiological and physicochemical makeup of the soil may be impacted by the texture of the soil **(Udeani TKC *et al.*, 2009).**

The ability to create and maintain conditions that favour enhanced oil biodegradation rates in the contaminated environment is critical to the success of oil spill bioremediation. Numerous scientific review articles have addressed various factors that influence the rate of oil biodegradation. One important requirement is the presence of microorganisms with the necessary metabolic capabilities.

Characteristics of the Contaminated Soil

- There is a need to [take action] because of the growing number of repair businesses and their careless environmental disposal of old oil. As a result of the environmental risks, take into account options for their removal from the environment.
- Inextricably linked to petroleum products. One of the most promising strategies for removing contaminants is bioremediation, which pollutants that contain hydrocarbons.

- The removal of microorganisms from a hydrocarbon-contaminated site is carried out with the assistance of a wide range of microorganism
- , including the native In-soil microbial life.
- The microbial population serves as the first line of defence against oil pollution in the environment.
- Many bacteria from more than 70 genera are capable of degrade one or more types of petroleum hydrocarbons and gain energy.

Hence to overcome the investigation to evaluate the hydrocarbon degrading microorganisms present in the contaminated soil with petroleum along with study consisting of morphological, biochemical, gelatine hydrolysis method and degradation of gravimetric method.

MATERIALS AND METHODOLOGY

Collection of Samples

The oil contaminated soil samples were collected from the petroleum bunk, around the places of Tiruvannamalai District, Tamilnadu, India. In the lab, stores and other unwanted soil debris were removed by using 2mm sieves and stored in sealed polythene bag at 4°c for further analysis.

Physiochemical Parameters of Soil Samples

Soil samples were dried, sieved, homogenised, and stored at 4°C for analysis. Physiochemical properties such as pH, organic carbon, calcium, magnesium, sulphur manganese, zinc, copper, and boron were determined. With minor modifications, the physiochemical properties of soil samples were determined. Titrimetric analysis was used to determine the calcium and magnesium parameters.

Isolation of Bacteria from Soil

The bacterial strains were isolated from the soil through serial dilution technique. Serial dilution carried out in peptone water in Aseptic conditions. The Nutrient agar plates were prepared and inoculated with the sample which was serially diluted.

Identification and Screening Of Hydrocarbon Degradation By Dcpip

Bacterial isolates with growth > 1.000 at 660nm were inoculated in 5ml of BHM medium containing petrol (50μl), diesel (50μl), and engine oil (50µl) (111:1) as hydrogen substrate. Then, 40µl of 2,6-Dichlorophenol Indophenol (DCPIP) was added and incubated at 37°C for 5 days. The medium was observed for blue colour decolorization. After 5 days of incubation, the medium was centrifuged to separate the biomass, and the absorbance of the supernatant was measured at 600 nm with a uv vis Spectrometer.

Identification of Hydrocarbon Depredating Bacteria

The Primary Screened HDB isolate was chosen and identified using morphological and biochemical characteristics from Bergey's manual of determinative bacteriology.

BIODEGRADATION STUDIES

Degradation Analysis

The isolated bacterial strain was inoculated on a plate containing crude petroleum. The hydrocarbon degradation on the plates was observed. The bacterial isolates were inoculated in 50ml of broth and incubated at 37°C -80rpm until the OD Value at 600nm reached 1.00. The bacterial cultures were centrifuged at 10,000 rpm for 10 minutes, washed twice with sterile Saline, and suspended in 10ml of Saline. 0.1ml of the inoculum was added to the broth containing crude oil and incubated at 37°C, 85-110rpm for 24 hours. The supernatant solution was collected for absorbance. The percentage of degrationwas calculated as follows:

$$\text{Percentage of Degradation} = \frac{\text{Initial OD} - \text{final OD}}{\text{Initial OD}} \times 100$$

Hydrocarbon Degradation by Gravimetric Method

The isolated bacteria were tested for the degradation of various hydrocarbons such as petrol, diesel, and engine oil. The isolate was grown for 30 days at room temperature on BHM medium supplemented with 2g/L of petrol, diesel, and engine oil separately. The content was transferred to a separating funnel and extracted with 5ml of benzene twice and organic phase was centrifuged at low speed for 10 minutes at regular intervals of 10 days to assess residual concentrations of each oil. The amount of each residual oil was measured gravimetrically after benzene evaporated, and the percentage of residual oil and hydrocarbon degradation percentage were calculated.

RESULT AND DISCUSSION

Result

Petroleum is the primary energy source for daily life and business around the world. However, crude oil a dark-brown, viscous liquid mixture comprised of petroleum and the other naturally hydrocarbons with different molecular weight that serve as the primary fuel source used in the production of several petroleum products. Crude poly-aromatic hydrocarbon (PAHs) makes up more than 30% of oils composition. Additional hundreds of different hydrocarbons including paraffins, naphthene, sulphur, organic nitrogen compounds and aromatic compounds hydrocarbon that contain oxygen and compounds (Phenol).

The use of petroleum hydrocarbon products raises the possibility of soil contamination with diesel and used motor oil, which becomes one of the main environmental issues. The International Tanker Association perfumed statistical analysis. Owners' pollution Federation Ltd. (ITOPF) since the year oil spill of at least 700 tonnes happened between 1970 and 2013, according to data 2013 itself.

The present investigation was made to prove that Invitro biodegradation of petroleum hydrocarbon using microorganism from contaminated soil. The oil contaminated soil samples were analysed for the physiochemical composition. It shows that slightly acidic to neutral pH as the reading. The results were shown in **table 1.**

The soil samples were found to contain the highest volume of organic carbon, sulphur, iron, manganese zinc and copper. The lowest value of calcium. The results were shown in **table 2.**

The bacterial isolates were isolated from the soil samples collected from petrol bunk of, Tiruvannamalai district. Bacterial isolates were isolated from the crude oil contaminated soil sample causing nutrient agar medium. The total bacterial count rages 1.52×10^7 CFU/ml, 7.24×10^7 CFU/ml. The result was shown in **table 3** the selected bacterial isolates were identified based on their cultural, morphological and biochemical characters.

Isolates A characterized with rod, white creamy, circular flat colony. Isolates B noted as cylindrical rod, white irregular large colonies.Isolates C identified as creamy white, flat circular with gram positive feature. The results were shown in **table 4.**

The three bacterial isolates were screened for the petroleum hydrocarbon degradation by spectrophotometric method using DCPIP as an indicator. The results were shown that the percentage of degradation it 27.5%, 8.75% and 20%. The hydrocarbon degrading bacteria was identified by Gram stanning, motility test, IMVIC test, and oxidase and catalase test.

The biochemical analysis shows that the isolates A as positive for oxidase while negative for catalase, indole and urease. Isolates B shown as oxidase and catalase positive but negative for indole, urease. Isolates C as negative to oxidase, indole and unease but positive to catalase. The result were shown in **table 5**

The isolates were checked for the extent of crude oil degradation in solid and liquid medium. All the isolates were found to be the highest degradation ratio. Visual degradation of the sample shows that it was higher in case of basal broth.

The isolated microorganism having ability to degrade petrol was determined by gravimetric method. The results were noted as percentage of residual oil and degradation of petrol after 10, 20 and 30 days of incubation. It noted as 75%, 25%, 60%,40% and 24% towards isolate A. Isolate C shows that 80%, 20%, 62%, 37%, 31% and 69%. Isolate B as 68%, 32%, 45%, 55%, 17% and 83%. The results were shown in **chart 1.**

However, it was reported that the mixed bacterial culture gave the maximum degradation percentage because there is no single isolate of bacteria with the metabolic capacity to degrade all the components found in the crude oil.

Table 1 Ph Reading

PARAMETER	SAMPLE 1	SAMPLE 2	SAMPLE 3
pH	5.60	6.15	5.87

Table 2 Physiochemical Parameters of Soil Sample

PARAMETERS	SAMPLE 1	SAMPLE 2	SAMPLE 3
Organic carbon	2.33	1.77	2.57
Calcium	279	314	280
Sulphur	11.66	10.33	16.68
Iron	226.33	280.59	274.44
Manganese	30.55	6.34	26.50
Zinc	58.77	3.44	44.55
Copper	36.77	0.65	15.55

Table 3 Estimation of Total Bacterial Population Using Cfu

Sample	No. of. Colonies	No of Cells/ml
Sample 1	144	5.73×10^7 CFU/ml
Sample 2	345	7.24×10^7 CFU/ml
Sample 3	24	1.52×10^7CFU/ml

Table 4 Morphological Colony Features for Three Bacterial Isolates

ISOLATES	SIZE	GRAM STAIN	COLOR	FORM	MARGIN
A	Rods	+	Creamy white	Circular flat	Entire
B	Cylindrical	-	White	Irregular large	Undulate
C	Rods	+	Creamy white	Circular flat	Entire

Table 5 Three Bacterial Isolates Biochemical Tests Result

Isolates	Oxidase	Catalase test	Simmons citrate test	Indole test	Methyl red (MR) test	Urease test	Gelatin hydrolysis
***Bacillus subtilis*(A)**	Variable	+	+	-	-	-	+
***Pseudomonas aeruginosa* (B)**	+	+	+	-	-	-	+
***Bacillus cereus* (C)**	-	+	+	-	-	-	-

Table 6 Analysis of Degradation

S.NO	Bacterial cultures	Percentage of degradation (%)
1	*Bacillus subtilis*	85.6%
2	*Pseudomonas aeruginosa*	94%
3	*Bacillus cereus*	70.6%

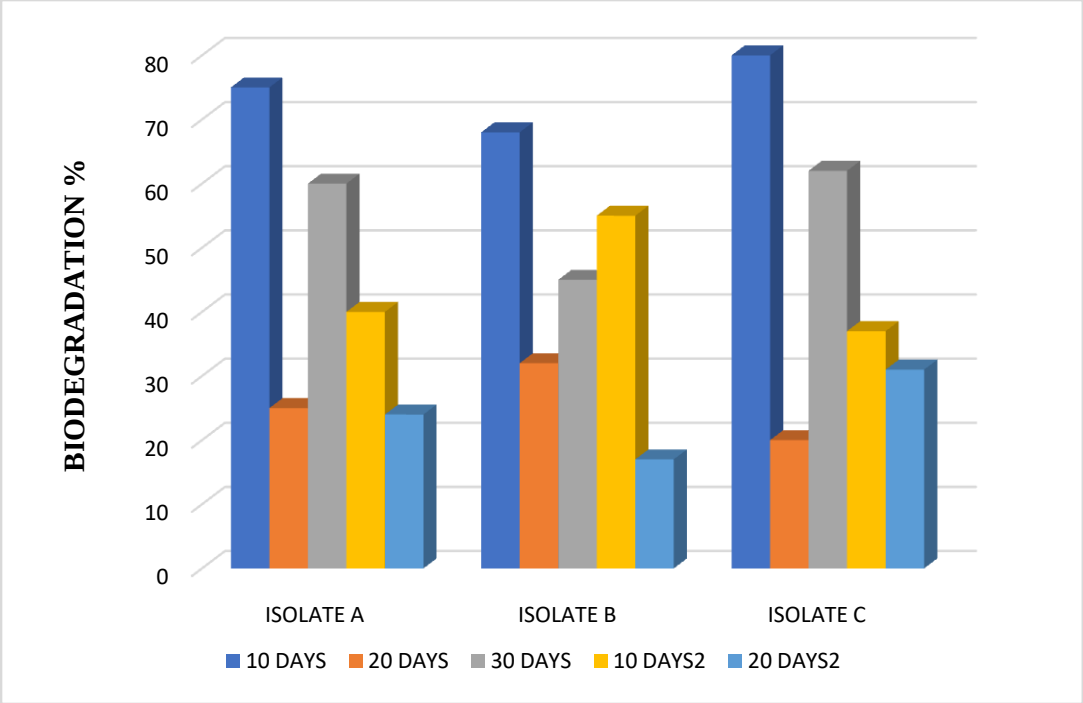


Chart 1 Hydrocarbon Degradation by Gravimetric Method (Biodegradation %, Residual Oil %)

DISCUSSION

The use of petroleum raises the possibility of soil contamination with diesel and used engine oil, which becomes one of the main environmental issues. Even some of the components the makeup hydrocarbons are extremely toxic, mutagenic or cancer causing in nature.

The study of various methods that might be used to clean up the environmental, however, dramatically increased as a result of heightened awareness of the negative effects of environmental pollution. Hydrocarbon pollution removal from the environmental eventually involves physical, chemical and biological alternatives.

The present study was invitro biodegradation of petroleum hydrocarbon using microorganism from contaminated soil.

The result of physiochemical composition shows the average value of 6.194.**Similar to our study,** These are similar to the results obtained by Atlas (1981). Who reported that neutral pH enables biodegradation activity of bacteria in soil. Bacteria have limited tolerance for acid conditions and fungi are more tolerant. Since the pH in this study was low, it could be assumed that fungi were more involved in the biodegradation of oil due to its ability to adopt acidic condition and secretion of acidophilic enzymes. The number of culturable bacteria decreased gradually under acidic conditions, while the number of culturable fungi remained relatively constant over the acidic pH range. The ratio of culturable bacteria to culturable fungi was greater than one at pH 6; in contrast, the bacteria-to-fungi ratios were less than one at acid pH.

The soil sample rich in mineral it had the ability to give nutrients to the bacterials.**Similar to our study,** Many studies reveal that the increasing total carbon content of the soil with increasing concentration of crude oil may be attributed to the high the release of carbon in the oil. In soil, oil can rapidly decay and minerals leading to the release of cations and trace elements **(Nnaji et al., 2005).**

Oil products not only modify physiochemical and biological properties of the soil, but also contribute to limiting the productive ability of crop. Microorganism posses' mechanism by which they degrade the crude oil compounds by utilizing them as carbon and energy sources.

The three isolates were isolated from the soil is 1.52×10^7 CFU/ml to 7.24×10^7 CFU/ml. the presence of organism shows that the soil bacteria have the properties to constrain the biodegradation rate of bioremediation of petroleum contaminated soil. **Similar to our study,** indeed, the presence of such a significant number of bacteria in the contaminated soil reflects

their adaptive ability to survive even in the presence of various petroleum products. Previous studies show the oil pollution can change the composition and diversity of soil bacteria and this change of soil properties will greatly constrain the biodegradation rate of bioremediation of petroleum contaminated sites **(Liu et al., 2005).**

The three isolates character shows that two positive and one negative bacterium. It may note as *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cerus.* **Similar to our study,** three different oil degrading bacteria were selected and isolated from contaminated soil samples from Khurais oil fields, 250 km southwest of Dhahran and 150 km east-northwest of Riyadh, Saudi Arabia initially labelled as A, B and C illustrated morphological characteristic for isolates. Isolate A was distinguished with rod, creamy white, circular flat and entire margin. By contract, B isolates characterized by cylindrical rods size white, irregular large form, negative stain and undulate margin. Rod size, creamy white, circular flat, entire margin and positive gram stain features characterize isolates **(C. F.A. AL-Dhabaan/Saudi journal of biological science).**

The three isolates showed that maximum level of degradation upto 20%. **Similar to our study,** screened hydrocarbon degrading efficiency of bacterial isolates by DCPIP test and reported that 28 isolates able to degrade PAHs. Similarly, the degrading potential of bacteria cultures occurs in the complete reduction of DCPIP in 75 hrs. for mineral oil, 87 hrs for used oil, 125 hrs. Semi-synthetic oil, and 138 hrs. for synthetic oil. Bacteria belonging to genera *burkholderia, pseudomonas, and Serratia* have ability to degrade different heavy molecular weight hydrocarbon premium motor spirit (PMS), Dual purpose kerosene (LPFO). Different species of bacillus, burkholderia, micrococcus, proteus, pseudomonas, have been reported by several authors to utilize hydrocarbon through the oxidation of DCPIP. Ability of the isolates to degrade the hydrocarbon was confirmed by the colour change from blue to colourless. **(Bidoia E *et al*., 2010).**

Based on the biochemical characters and colony morphology the organism identified as isolate A as *bacillus substilis*, B as *pseudomonas aeruginosa*, C as *bacillus cereus*. The organism considered as the traditional degrader of hydrocarbon. **Similar to our study,** Performed morphological colony characteristics examination (Gram stain, 250 mobility and motility, shape and color) and biochemical tests (catalase, urease, oxidase activities, nitrate reduction, Idol production, acid/gas production from carbohydrates and fermentation of sugars) to identify our Hydrocarbons degradation isolates were considered a traditionally identification methods. **(Udgire *et al*., 2015).**

The organism shows high level of degradation based on the nature of environment. It shows the high result of degradation in basal broth. **Similar to our study,** Temperature plays very important roles in biodegradation of petroleum hydrocarbons, primarily by its direct effect on the chemistry of the pollutants, and secondly on its effect on the physiology and diversity of the microbial population. Ambient temperature of an environment affects both the properties of spilled oil and the activity or population of microorganisms. At low temperatures, the viscosity of the oil increases, while the volatility of toxic low-molecular weight hydrocarbons is reduced, delaying the onset of biodegradation (Atlas, 1981). In the crude contaminated soil, the temperature ranges at 50° C that favours the growth of the microorganisms and the degradation process. **(Venosa A.D.et al., 2003).**

The ability of petrol degradation shows higher percentage noted as isolate A *bacillus subtilis* shows 85.6%, isolate B *pseudomonas aeruginosa* 94% and isolate C *bacillus cereus* 70.6% **Similar to our study,** Kumar and Manjunatha observed that degradation of engine oil and diesel by isolate CSN-1 was 58.21% and 69.64% and 52.45% and 63.50% by isolate OK-6. *P. aeruginosa* and *B. subtilis* isolated from crude oil contaminated site exhibited maximum crude oil degrading ability 88.75% and 87.41% by gravimetric analysis. It has been reported in several literatures that *Pseudomonas sp*. have potential to degrade many different PAHs.

From this investigation the organism *pseudomonas aeruginosa* have the highest efficient to degrade the hydrocarbon and eco-friendly for the degradation of hydrocarbon.Further studies are carried out to justify the potential of *pseudomonas aeruginosa* for the degradation of petroleum hydrocarbon.

CONCLUSION

All living things that enter the impacted region after an oil spill suffer terrible effects. Numerous microorganisms that break down oil can be found in a variety of settings. These bacteria may aid in the quick removal of the hydrocarbons. This study intends to find out whether the bacteria isolated from different sources can break down different kinds of oils. Different oil samples were used to expose the bacteria that had been obtained from various sources. Oil deterioration was evident in all of the test tubes that had been infected after 30 days. Thus, it can be said that after a few more thorough examinations, the bacteria that were isolated from various soil samples can successfully breakdown any sort of oil and be employed in bioremediation**.**

BIBILOGRAPHY

[1]. **Abioye, P.O., Abdul Aziz, A., Agamuthu, P.,** 2010. Enhanced biodegradation of used engine oil in soil amended with organic wastes. Water, Air & Soil Pollution, **209**: 173–179. https://doi.org/10.1007/s11270-009-0189-3.
[2]. **Al-Wasify, RS., Hamed, SR.,** 2014. Bacterial biodegradation of crude oil using local isolates. International Journal of Bacteriology, 1-8.
[3]. **April TM, Foght JM, Currah RS.** (2000). Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. Canadian Journal of Microbiology.46 (**1**):38–49
[4]. **Atlas, R. M., A. Horowitz, and M. Busdosh.** 1978. Prudhoe crude oil in Arctic marine ice, water, and sediment ecosystems: degradation and interactions with microbial and benthic communities. J. Fish. Res. Board Can. **35**:585-590
[5]. **Atlas, R.,** 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbial Reviews. **45**: 180-209
[6]. **Atlas, R.M.,** 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbial. Rev. 45 (**1**), 180–209.
[7]. **BOROZAN AB** SOIL POLLUTION WITH HEAVY METALS AND BIOREMEDIATION METHODS. Algae 104(105): **32**
[8]. **Bossert, I., and R. Bartha.** 1984. The fate of petroleum in soil ecosystems, p. 434-476. In R. M. Atlas (ed.), Petroleum microbiology. Macmillan Publishing Co., New York.
[9]. **Camacho Montealegre CM, Edmo Montes R, Daniel Kumazawa M, Marcos Rogério T** (2021) Prokaryotic community diversity during bioremediation of crude oil contaminated oilfield soil: effects of hydrocarbon concentration and salinity. Brazilian Journal of Microbiology 52(**2**): 787-800.
[10]. **Chandra, S., Sharma, R., Singh, K., Sharma, A.,** 2013. Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. Ann. Microbiol. **63**, 417–431.
[11]. **Cheesbrough, M.,** 1991. Medical Laboratory Manual for Tropical Countries. University Press, Cambridge
[12]. **Chikere, CB., Okpokwasili, GC., Chikere, BO.,** 2011. Monitoring of microbial hydrocarbon remediation in the soil. 3 Biotech, 1(**3**): 117–138.
[13]. **Cooney, J. J., S. A. Silver, and E. A. Beck**. 1985. Factors influencing hydrocarbon degradation in three freshwater lakes. Microb. Ecol. **11**:127-137.
[14]. **Costa, A.S., Romao, L.P., Araujo, B.R., Lucas, S.C., Maciel, S.T., Wisniewski Jr., A., Alexandre, M.R.,** 2012. Environmental strategies to remove volatile aromatic fractions (BTEX) from petroleum industry wastewater using biomass. Bioresour. Technol. **105**, 31–39.
[15]. **Cui J, Hong Chen, Mingbo Sun, Jianping Wen** (2020) Comparison of bacterial community structure and function under different petroleum hydrocarbon degradation conditions. Bioprocess and biosystems engineering 43(**2**): 303-313.
[16]. **Das D, Baruah R, Roy A S, Singh A K, Boruah H P D, Kalita J, Bora T C** (2015) Complete genome sequence analysis of Pseudomonas aeruginosa N002 reveals its genetic adaptation for crude oil degradation. Genomics. 105(**3**), 182 190.
[17]. **Das, N., Chandran, P.,** 2011. Microbial degradation of petroleum hydrocarbon contaminants: An overview, SAGE-Hindawi Access to Research. Biotechnology Research International, **13**, 941-810.
[18]. **Daxini N, Mistry K.** Bio-surfactant assistance in crude oil degradation by halophilic Bacillus cereus ND1. Indian Journal of Geo Marine Sciences. 2018; 47(8):1640-1647.
[19]. **Dibble, J. T., and R. Bartha.** 1979. Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol. **37**:729-739.
[20]. **Edison, T.E., Cruz, D., Martin, J., Torres, O.,** 2012. Gelatin hydrolysis test protocol. Am. Soc. Microbiol.
[21]. **Etaware PM** (2021) Biomineralization of toxicants, recalcitrant and radioactive wastes in the environment using genetically modified organisms In **Swamini, C. et al.** Novel application of carbon-based nanomaterials CRC Press. Taylor and Francis.
[22]. **Farber, R., Rosenberg, A., Rozenfeld, S., Benet, G., Cahan, R.,** 2019. Bioremediation of artificial diesel-contaminated soil using bacterial consortium immobilized to plasma-pretreated wood waste. Microorganisms, 7(**11**): 497.
[23]. **Fedorak, P. M., and D. W. S. Westlake.** 1981. Microbial degradation of aromatics and saturates in Prudhoe Bay crude oil as determined by glass capillary gas chromatography. Can. J. Microbiol. **27**:432-443.
[24]. **Feng L, Xiupeng Jiang, Yanning Huang, Dongdong Wen, Tianyu Fu, et al.** (2021) Petroleum hydrocarbon-contaminated soil bioremediation assisted by isolated bacterial consortium and sophorolipid. Environmental Pollution **273**: 116476.
[25]. **G. Bengtsson, P. Zerhouni, J.** Appl. Microbiol. 94 (2003) 608– 617.

[26]. **Gayathiri, E., Bharathib, B., Selvadhas, S., Kalaikandhan, R.,** 2017. Isolation, Identification and molecular characterization of hydrocarbon degrading bacteria and its associated genes - a review. Int. J. Pharm. Bio. Sci. 8 (2), 1010–1019 (B).
[27]. **Haderlein A, Legros R, Ramsay BA.** Pyrene mineralization capacity increased with compost maturity. Biodegradation. 2006; 17:293-302
[28]. **Holt JG, Krieg NR,Sneath PHA, Staley JT, Williams ST.** Bergeys Manual of Determinative Bacteriology, 9th edn Baltimore: Williams & Wilkins, 1994.
[29]. **Imam A** (2021) Biological Machinery for Polycyclic Aromatic Hydrocarbons Degradation: A review. Bioresource Technology **343**: 126121.
[30]. **Ite AE, UJ Ibok** (2019) Role of plants and microbes in bioremediation of petroleum hydrocarbons contaminated soils. International Journal of Environmental Bioremediation & Biodegradation 7(**1**): 1-19.
[31]. **Jacqueline Michel and Merv Fingas** (2015) Oil Spills: Causes, Consequences, Prevention and Countermeasures. Fossil Fuels. 16(**44**), 159 201.
[32]. **Jain PK, Gupta VK, Gaur RK, Lowry M. Jaroli DP**. Bioremediation of petroleum oil contaminated soil and water. Research journal of environmental toxicology. 2011; 5(1):1-26.
[33]. **Jobson, A., F. D. Cook, and D. W. S. Westlake.** 1972. Microbial utilization of crude oil. Appl. Microbiol. **23**:1082-1089.
[34]. **Jobson, A., M. McLaughlin, F. D. Cook, and D. W. S. Westlake.** 1974. Effect of amendments on the microbial utilization of oil applied to soil. Appl. Microbiol. 27:166-171
[35]. **K. A. Kvenvolden and C. K. Cooper** Natural seepage of crude oil into the marine environment, Geo-Marine Letters, vol. **23**, no. 3-4, pp. 140– 146, 2003.
[36]. **Kebria DY, Khodadadi A, Ganjidoust H, Badkoubi A, Amoozegar MA.** Isolation and characterization of a novel native Bacillus strain capable of degrading diesel fuel. Int J Environ Sci Technol 2009; **6**:435e42.
[37]. **Kurnaz, S.Ü., Büyükgüngör, H.,** 2016. Bioremediation of total petroleum hydrocarbons in crude oil contaminated soils obtained from southeast Anatoli. Acta Biologica Turcica, **29**: 57– 6
[38]. **Kumar PSV, Manjunatha BK.** Isolation of Diesel, Engine Oil and Crude Oil Degrading Fungi from Oil Contaminated Soil for their Bioremediation Potential. J
[39]. Agric. Food Nat. Resource. 2017; 1(**1**):65-70.
[40]. **Li Q, J Liu, GM Gadd** (2020) Fungal bioremediation of soil co-contaminated with petroleum hydrocarbons and toxic metals. Applied Microbiology and Biotechnology **104**: 8999-9008.
[41]. **Li Q, Yanning Huang, Dongdong Wen, Rongbing Fu, Leiyu Feng, et al.** (2020) Application of alkyl polyglycolides for enhanced bioremediation of petroleum hydrocarbon-contaminated soil using Sphingomonas changbaiensis and Pseudomonas stutzeri. Science of The Total Environment **719**: 137456.
[42]. **Li, X.W., Liu, Z.P.,** 2002. Microbial biodegradation of petroleum hydrocarbon. Acta Microbiol. Sin. **42**, 764–767.
[43]. **Macaulay BM, D Rees** (2014) Bioremediation of oil spills: a review of challenges for research advancement. Annals of environmental Science **8**: 9-37.
[44]. **Mancera-Lopez ME., Esparza-Garcia F., Chavez-Gomez B., Rodriguez-Vazqueza R., SaucedoCastaneda G., Barrera-Cortes J.,** 2008. Bioremediation of an aged hydrocarbon-contaminated soil by a combined system of biostimulation–bioaugmentation with filamentous fungi. International Biodeterioration & Biodegradation. **61**: 151–160.
[45]. **Mandree P** (2021) Bioremediation of Polycyclic Aromatic Hydrocarbons from Industry Contaminated Soil Using Indigenous Bacillus Spp. Processes 9(**9**): 1606.
[46]. **Markiewicz A, AM Strömvall, K Björklund** (2020) Alternative sorption filter materials effectively remove non-particulate organic pollutants from stormwater. Science of the Total Environment **730**: 139059
[47]. **Meckenstock, R.U., Boll, M., Mouttaki, H., Koelschbach, J.S., Tarouco, P.C., Weyrauch, P., Dong, X., Himmelberg, A.M.,** 2016. Anaerobic degradation of benzene and polycyclic aromatic hydrocarbons. J. Mol. Microbiol. Biotechnol. **26**, 92–118
[48]. **Mihelcic, J. R., and R. G. Luthy.** 1988. Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Appl. Environ. Microbiol. **54**: 1188-1198
[49]. **Montagnolli, R.N., Lopes, P.R.M., Bidoia, E.D.,** 2014. Assessing Bacillus subtilis biosurfactant effects on the biodegradation of petroleum products, Springer International Publishing Switzerland, 8: 4114-4116.
[50]. **Moreno Ulloa A** (2020) Chemical Profiling Provides Insights into the Metabolic Machinery of Hydrocarbon-Degrading Deep-Sea Microbes. M-Systems 5(**6**): e00824-20.
[51]. Muthal AP (2017) Study of Selectivity in Extraction, Separation, and Detection on Drugs Using Gas Chromatography. Seton Hall University
[52]. **Nikolopoulou M, Pasadakis N, Kalogerakis N.** Enhanced bioremediation of crude oil utilizing lipophilic fertilizers. Desalination 2007; **211**:286e95.

[53]. **Ojuederie OB, OO Babalola** (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. International journal of environmental research and public health 14(**12**): 1504
[54]. **P. J. J. Alvarez and T. M. Vogel,** "Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries," Applied and Environmental Microbiology, vol. 57, no. 10, pp. 2981– 2985, 1991
[55]. **Patrick, W. H., Jr., and R. D. DeLaune.** 1977. Chemical and biological redox systems affecting nutrient availability in coastal wetlands. Geosci. Man 18:131-137.
[56]. **Perez-Cadahia, B., Lafuente, A., Cabaleiro, T., Pásaro, E., Méndez, J., Laffon, B.,** 2007. Initial study on the effects of Prestige oil on human health. Environ. Int. 33 (**2**), 176–185.
[57]. **Pirnik et al. J** Bacteriol. 1974 119: 868 [PMID: 4852318]
[58]. **S. J. Geetha, S. J. Joshi, and S. Kathrotiya,** "Isolation and characterization of hydrocarbon degrading bacterial isolate from oil contaminated sites," APCBEE procedia, vol. **5**, pp. 237–241, 2013.
[59]. **Sarkar D, Ferguson M, Datta R, Birnbaum S.** Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. Environ Pollut 2005; **136**:187e95
[60]. **Sharma N, M Lavania, B Lal** (2019) Microbes and their Secondary Metabolites: Agents in Bioremediation of Hydrocar-bon Contaminated Site. Arch Pet Environ Biotechnol **4**: 151
[61]. **Trejos Delgado C, Gloria E Cadavid R, Angelina Hormaza A, Edison AA, Leonardo Barrios Z** (2020) Oil bioremediation in a tropical contaminated soil using a reactor. Anais da Academia Brasileira de Ciências 92(**2**): 20181396.
[62]. **Udgire, M., Shah, N., Jadhav, M.,** 2015. Enrichment, isolation and identification of hydrocarbon degrading bacteria. Int. J. Curr. Microbiol. Appl. Sci. 4 (**6**), 708–713. ISSN: 2319-7706
[63]. **Varjani, S.J., Rana, D.P., Jain, A.K., Bateja, S., Upasani, V.N.,** 2015. Synergistic ex-situ biodegradation of crude oil by halotolerant bacterial consortium of indigenous strains isolated from on shore sites of Gujarat, India. Int. Biodeterior. Biodegrad. **103**, 116–124
[64]. **Venosa A.D.,** and **Zhu X.,** Biodegradation of Crude Oil Contaminating Marine shorelines and Freshwater Wetlands. Spill Sci. Tech. Bull. 8 (**2**): 163-178 (2003).
[65]. **VS, Millioli, ELC, Servulo, LGS Sobral & DD De Carvalho,** Bioremediation of crude oil-bearing soil: evaluating the effect of Rhamnolipid addition to soil toxicity and to crude oil biodegradation efficiency, Global NEST Journal, 11 (**2**) (2009), 181.
[66]. **Walker, C.H.,** 2006. Principles of Ecotoxicology. CRC, Taylor and Francis, Boca Raton, U.S.A
[67]. **Ward, D. M., and T. D. Brock**. 1978. Hydrocarbon biodegradation in hypersaline environments. Appl. Environ. Microbial. **35**:353-359.
[68]. **Widdel, F., Rabus, R.,** 2001. Anaerobic biodegradation of saturated and aromatic hydrocarbons. Curr. Opin. Biotechnol. **12**, 259–276
[69]. **Wilkes, H., Buckel, W., Golding, B.T., Rabus, R.,** 2016. Metabolism of hydrocarbons in n-Alkane utilizing anaerobic bacteria. J. Mol. Microbiol. Biotechnol. **26**, 138– 151.
[70]. **Wu C, Feng Li, Shengwei Yi, Fei Ge** (2021) Genetically engineered microbial remediation of soils co-contaminated by heavy metals and polycyclic aromatic hydrocarbons: Advances and ecological risk assessment. Journal of Environmental Management **296**: 113185
[71]. **Zafra, G., A.E. Absalón, M.D.C. Cuevas and D.V. Cortés-Espinosa.** 2014. Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soils. Water Air Soil Pollute., **225**: 1826.
[72]. **Zanaroli, G., Toro, S.D., Todaro, D., Varese, G.C., Bertolotto, A., Fava, F.,** 2010. Characterization of two diesel fuel degrading microbial consortia enriched from a non-acclimated, complex source of microorganisms. Microbe. Cell Fact. 9 (**10**), 1–13 (10.1186/1475-2859-9-10).
[73]. **Zhang et al. J Zhejiang** Uni Sci. 2005 6: 725 [PMID: 16052704] .