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Evaluation of the potential microbial degradation of diesel oil and gasoline in soil

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Abstract

Different oil products like gasoline, diesel or heavy oils can cause soil contamination. Bioremediation using microorganisms seems to be the best alternative for physicochemical treatments with fewer side effects.

This study aimed to test the ability of previously isolated microorganisms from petroleum hydrocarbon contaminated samples, to degrade gasoline and diesel oil.

Seven microbial isolates consisting in four Gram positive bacteria (C26, C31, K11, S), two Gram negative (C52, C14) bacteria and one fungus (*Aspergillus* sp.) were tested for their ability to degrade diesel oil and gasoline (10 %) in contaminated soil through the monitoring of total petroleum hydrocarbons (TPH) degradation rates during 28 days. Moreover, the growth of the tested isolates was monitored during 20 days in liquid MSM containing diesel oil or gasoline as sole source of carbon.

The obtained results demonstrated that all the tested strains have the ability to degrade both gasoil and diesel oil in soil at different rates. However, S and C52 showed the higher degradation rates in gasoline and diesel oil respectively.

Besides, in MSM all the tested isolates grew at different rates. C31 and the fungus grow better in diesel oil containing medium. On the other hand, C26, C52 and also the fungus grew better in gasoline containing medium.

Throughout this study we demonstrated that all tested microbial isolates have a great potential as bioremediation agents.

Keywords: petroleum hydrocarbons, pollution, hydrocarbonoclastic microorganisms, biodegradation, bioremediation, gasoline, diesel oil.

Résumé

Différents produits pétroliers comme l'essence, le diesel ou les huiles lourdes peuvent causer une contamination du sol. La bioremédiation utilisant des microorganismes semble être la meilleure alternative aux traitements physico-chimiques avec moins d'effets secondaires.

Cette étude visait à tester la capacité des microorganismes précédemment isolés à partir d'échantillons contaminés par des hydrocarbures pétroliers, à dégrader l'essence et le diesel.

Sept isolats microbiens comprenant quatre bactéries Gram positif (C26, C31, K11, S), deux bactéries Gram négatif (C52, C14) et un champignon (*Aspergillus* sp.) ont été testés pour leur capacité à dégrader le diesel et l'essence (10 %) dans un sol contaminé à travers le suivi des taux de dégradation des hydrocarbures pétroliers totaux (HPT) pendant 28 jours. De plus, la croissance des isolats testés a été suivie pendant 20 jours dans un milieu liquide MSM contenant du diesel ou de l'essence comme seule source de carbone.

Les résultats obtenus ont démontré que tous les isolats testés ont la capacité de dégrader le gasoil et le diesel dans le sol à des taux différents. Cependant, S et C52 ont montré les taux de dégradation les plus élevés dans l'essence et le diesel respectivement.

En outre, dans le MSM, tous les isolats testés se sont développés mais à des taux différents. C31 et le champignon ont montré une meilleure croissance sur le diesel. Alors que, C26, C52 et aussi le champignon ont montré un meilleur développement dans l'essence.

Tout au long de cette étude, nous avons pu démontrer que tous les isolats microbiens testés ont un grand potentiel comme agents de bioremédiation.

Mots clés : hydrocarbures pétroliers, pollution, microorganismes hydrocarbonoclastiques, biodégradation, bioremédiation, essence, diesel.

ملخص

يمكن أن تتسبب المنتجات البترولية المختلفة مثل البنزين والديزل والزيوت الثقيلة في تلوث التربة. يبدو أن المعالجة البيولوجية باستخدام الكائنات الحية الدقيقة هي أفضل بديل للعلاجات الفيزيائية والكيميائية مع آثار جانبية أقل.

هدفت هذه الدراسة إلى اختبار قدرة الكائنات الحية الدقيقة المعزولة سابقًا من العينات الملوثة بالهيدروكربونات البترولية على تحلل البنزين والديزل.

تم اختبار سبع سلالات ميكروبية تشتمل على أربع بكتيريا موجبة الجرام (C26 ، C31 ، K11 ، S)، واثنان من البكتيريا سالبة الجرام (C52 ، C14) وفطر واحد (*Aspergillus* sp.) . لقدرتها على تحلل الديزل والبنزين (10٪) في التربة الملوثة عن طريق مراقبة معدلات تحلل الهيدروكربونات البترولية الكلية (TPH) لمدة 28 يومًا. بالإضافة إلى ذلك ، تمت متابعة نمو السلالات المختبرة لمدة 20 يومًا في وسط سائل قليل الاملاح المعدنية MSM يحتوي على الديزل أو البنزين كمصدر وحيد للكربون.

أظهرت النتائج المتحصل عليها أن جميع السلالات المختبرة لديها القدرة على تحلل البنزين والديزل في التربة بمعدلات مختلفة. ومع ذلك ، أظهر S و C52 أعلى معدلات تحلل في البنزين والديزل على التوالي.

علاوة على ذلك ، في MSM ، نمت جميع السلالات التي تم اختبارها بمعدلات مختلفة. أظهر C31 والفطرنمو بشكل أفضل في الوسط الذي يحتوي على الديزل. من ناحية أخرى ، أظهرت C26 و C52 والفطر نموا بشكل أفضل في الوسط الذي يحتوي على البنزين.

خلال هذه الدراسة ، أثبتنا أن جميع السلالات الميكروبية المختبرة لها إمكانيات كبيرة كعوامل معالجة حيوية.

الكلمات المفتاحية: الهيدروكربونات البترولية ، التلوث ، الكائنات الدقيقة الهيدروكربونية ، التحلل الحيوي ، المعالجة الحيوية ، البنزين ، زيت الديزل.

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INTRODUCTION

Introduction

In ancient times, when the environment was completely pure, people lived healthier and happier life. Today the world depends on petroleum oil, and its exploitation has led to intensive economic development all over the world. However, the great necessity for this power source has conducted to the gradual reduction of the natural oil stocks (Maletić et al. 2013). Moreover, the accidental leaks and spills that occur regularly during the exploitation, production, refining, transportation and storage of petroleum and petroleum products has led to the pollution of the environment that become a major problem worldwide.

Petroleum hydrocarbons are complex organic compounds made up of carbon and hydrogen in addition to other constituents, they are known to be the most persistent organic pollutants affecting the ecosystems. The total quantity of natural crude oil spills has been evaluated at 600,000 metric tons per year with a margin of uncertainty of 200,000 metric tons per year (Das and Chandran 2011). These compounds pollute water, terrestrial and atmospheric ecosystems. Early reports estimated that about 0.08 % to 0.4 % of crude oil produced internationally escapes into the environment. It is speculated that this value is currently due to increasing activities related to the production, transportation, processing, use of oil from the reservoir, use as an energy source, accidents and military conflicts (Khanafer et al. 2017). Over 1.3 million civilians as well as military personnel are exposed at work to hydrocarbon fuels; primarily gasoline, jet fuel, diesel fuel or kerosene. Acute or chronic exposures to raw fuel, vapors, aerosols, or exhaust from fuel burning; by the dermal, inhalation, or oral routes often in conjunction with exposure to other chemicals may cause several diseases or death (Glenn and Ritchie. 2010). Pollutants can enter the human body by inhalation, skin contact or consumption of petroleum-contaminated food, causing contact skin disease, visual and auditory hypersensitivity, stomach and intestinal problems, and a high risk of childhood leukaemia (Sui et al. 2021). Moreover, it has been shown that hydrocarbons, an important constituent of petroleum (84 %), cause significant losses in soil quality due to their toxicity towards biological processes catalysed by soil microorganisms (Victor et al. 2020).

Spilled oil can be cleaned up by physicochemical methods including thermal treatment, soil washing, soil vapor extraction, solidification, and stabilization that can constitute rapid and fast solutions (Dadrasnia et al. 2013). However, the former method involves the use of adsorbents, curing agents, dispersing agents and gelling agents that are not always environmentally friendly and can even cause additional pollution (Khanafer et al. 2017). Several alternative methods are developed for disposing of such materials but the most efficient and significant disposal strategy

is bioremediation. Bioremediation is the use of living organisms (microorganisms and plants) to break down or detoxify substances that are harmful to human health and to the environment. Biodegradation of compounds is often the result of multiple biological actions (Kensa 2011). It focuses on the degradation, elimination, modification, immobilization or detoxification of various chemicals and physical wastes. Scientists are researching and developing remediation techniques to repair and recontrol the afflicted environment due to the health risks and social implications of petroleum hydrocarbon contamination. Microorganisms mostly are used for their enzymatic properties and act as biocatalysts to facilitate the biochemicals processes that degrade the contaminant based on their ability of to convert, modify and utilize toxic pollutants as energy source and for biomass production (Abatenh et al. 2017).

Bioaugmentation which is the process of introducing microorganisms into a contaminated site to facilitate degradation and/or biostimulation which is the addition of nutrient into a contaminated site to stimulate microbial degradation, have proved to be the most advantageous soil and water treatment techniques for hydrocarbon contaminated sites (Tyagi et al. 2011).

In this context, this study aims to assay the potential of previously isolated microorganisms, from petroleum hydrocarbons polluted samples, to degrade gasoline and diesel oil in contaminated soils.

LITERATURE REVIEW

Literature review

1. Petroleum hydrocarbons

Petroleum hydrocarbons are complex compounds that can be divided into four classes: The saturates, the aromatics, the asphaltenes and the resins (Das and Chandran 2011).

Petroleum products like gasoline, heavy fuel oils and diesel, are originally derived from crude oil and are a complex mixture of several hundreds of compounds, of which the petroleum hydrocarbons are one of the main ingredients. Hydrocarbons are composed only of carbon and hydrogen atoms. It is composed of organic compounds of empirical formula CnHm (C_{10}-to-C_{50} fraction). They are used for transportation industries, while the production of chemicals based on petroleum fuels can be found in plastics, pharmaceuticals, pesticides, herbicides and detergents (Canny 2002). They also are found in a variety of other elements of the modern energy system, notably solar cells, wind turbine blades, batteries, the thermal insulation of structures, and the parts of electric vehicles (Birol 2018).

When they are liberated into the environment, petroleum chemicals change because of weathering (the degradation of their compounds by light, temperature, and microorganisms) causing changes in the way they move and where they will end up (Rasoanarivony 2016).

2. Petroleum hydrocarbons pollution

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. A soil is said to be contaminated when there is an accumulation of persistent toxic compounds, chemicals, salts, radioactive materials or pathogens, capable of causing biological, physical and chemical alterations leading to an imbalance in the ecosystem and disturbances in the growth of living beings in the soil (Lamari and Yousfi 2020). Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Fig. 1) (Das and Chandran 2011). Indogesit et al. (2020) reported that the major effects of hydrocarbon pollutions are: global warming, disease and death of humans, loss of delicate species of animals that are on the verge of extinction or endangered, reduced productivity of agricultural land, the death of fishes in oceans, lakes, ponds, rivers due to toxicity and lack of oxygen when the water bodies are contaminated and economic loss.

Figure 1. Sources of petroleum hydrocarbons pollution (Sui et al. 2021).

3. Bioremediation

Bioremediation is a set of techniques using processes of biodegradation, which can be used to reduce the toxicity, mobility or volume of a contaminant in soil, subsoil, water and gaseous effluents. It cannot be used to contain a volume of polluted water or soil. It can be carried out directly in the polluted environment (in situ) or after transfer of the pollutant (ex situ) (Roger and Jacq 2000).

The process of bioremediation, is defined as the use of living organisms mainly microorganisms to detoxify or remove pollutants thanks to their diverse metabolic capabilities. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment. Bacteria have been demonstrated to the most active organisms in the degradation of petroleum hydrocarbons (Das and Chandran 2011). The metabolic processes of living organisms can make them capable of using chemical contaminants as an energy source (Sardrood 2013).

Bio-remediation technology exploits a variety of processes: natural attenuation, biostimulation and bioaugmentation. Bio-stimulation refers to the act of incorporating electron receptors like phosphorus, oxygen, carbon and/or nitrogen and some other nutrients. Bio-augmentation means the insertion of exogenous microorganisms, that can be genetically modified, able to treat the pollution (Sardrood 2013). Genetic Bioaugmentation is an in-situ bioremediation process that

motivates the horizontal transfer of catabolic plasmids of exogenous donor cells to indigenous bacteria in order to increase the biodegradation of contaminants (Ikuma and Gunsch 2012).

3.1. Microbial genes of biodegradation

In the degradation of complex hydrocarbon mixtures, such as diesel, several genes and pathways are important for microbial degradation including cytochromes P450 (CYP153), laccases, hydrolases, dehalogenases, dehydrogenases, proteases and lipases (Fig. 2). Diesel fuel is made of both saturated aliphatic (alkanes) and aromatic hydrocarbons. Some genes have been identified for the petroleum hydrocarbon metabolism, like alkB (coding for alkane monooxygenase) and ndo (codes for naphthalene dioxygenase). The genes are functional under aerobic conditions to degrade alkanes and polycyclic aromatic hydrocarbons (PAHs) respectively (Yergeau et al. 2012; Ossai 2022).

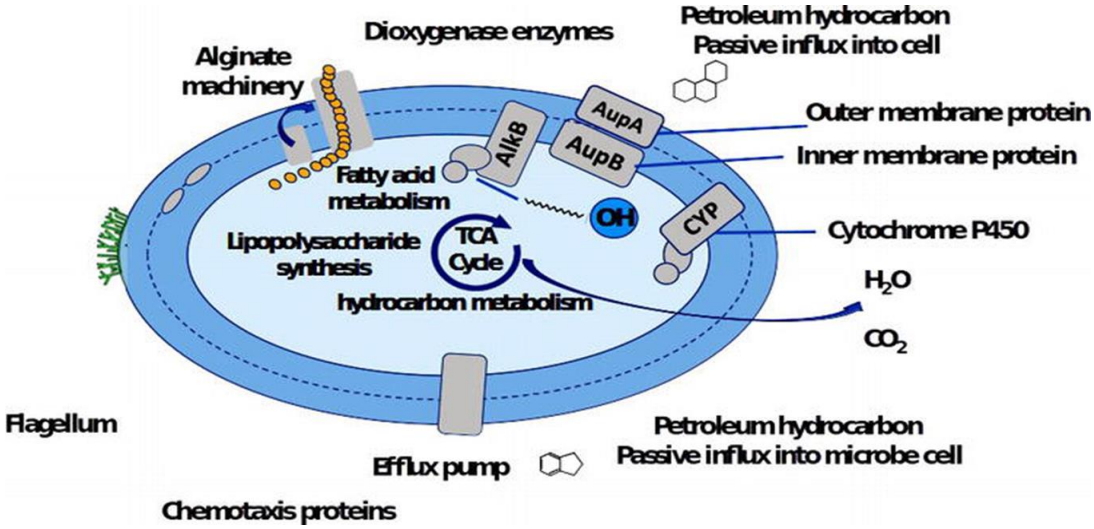


Figure 2. Microbial bioremediation of petroleum hydrocarbons (Ossai 2022).

3.2. Factors influencing bioremediation

The only way for microorganisms to act against pollutants is through access to a variety of material compounds that help them to build up energy and nutrients to increase their cell growth. The success of bioremediation depends on many factors, including the following:

3.2.1. Environmental factors

- Nutrient including, but not limited to, nitrogen and phosphorus are essential for microbial growth and activity. It has been demonstrated that "treatment of petroleum-contaminated soil with nitrogen can increase the rate of cell growth (Lambat et al. 2020). However, excessive amounts of nitrogen in the soil have also been shown to cause microbial inhibition. Walworth et al. (2005) suggest maintaining nitrogen levels below 1800 mg nitrogen/kg H_2O for optimal biodegradation of petroleum hydrocarbons.

-Soil pH is an important factor because the majority of microbial strains can only survive in a defined range of pH. Besides, soil pH can affect the appearance of nutrients. Bio-degradation of petroleum hydrocarbons is optimal at a soil pH of 7 (neutral); the allowable range is 6 to 8 (Donlan and Bauder 2007).

- Water presence affects the diffusion of water and soluble nutrients into and out of the cells of microorganisms. However, excess moisture, as in saturated soil, is undesirable since it reduces the quantity of oxygen for aerobic respiration, the presence of oxygen in most cases can enhance hydrocarbon metabolism. Anaerobic respiration, which produces less energy for the microorganisms (than aerobic respiration) and slows the rate of biodegradation, becomes the predominant process. The soil moisture content "between 12 and 30 % by weight" is optimal for petroleum hydrocarbon degradation (Donlan and Bauder 2007; Abatanh et al. 2017).

- Temperature influences rate of biodegradation, that's why most bacteria found in soil, including many hydrocarbon-degrading bacteria, are mesophiles with their optimum temperature ranging from 25 to 45ºC. The thermophile bacteria which are typically found in hot springs, are known to exist naturally in fresh soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60 degrees (Donlan and Bauder 2007).

- The pollutants can accumulate on soil particles, making some of them useless to microorganisms for bio-degradation. Under certain conditions, the bio-availability of contaminants depends not only on the nature of the contaminant but also on the type of soil. For example, hydrophobic petroleum hydrocarbons, are poorly soluble in water and are strongly adsorbed in soils with high organic matter content (Donlan and Bauder 2007).

- The presence of metals ions in small quantities affects bacteria and fungi, but in big quantities they inhibit cells' metabolic activity. Metal compounds have a direct and indirect impact on the rate of degradation (Abatanh et al. 2017).

-When toxic compounds are found on high concentrations, the toxic nature of some contaminants can create toxic effects to microorganisms and slow down the remediation process. Toxicity levels and mechanisms vary depending on the specific toxicants, their concentration, and the microorganisms that are exposed (Donlan and Bauder 2007).

3.2.2. Biological factors

Competition for limited carbon sources between microorganisms, antagonistic reactions, or the predation of microorganisms by protozoa and bacteriophage affect the degradation of organic compounds. The rate of contaminant degradation frequently depends on the contaminant concentration and the amount of "catalyst" present. In this context, the amount of "catalyst" represents both the number of organisms capable of metabolizing the contaminant and the amount of enzymes produced by each cell. The expression of specific enzymes by the cells can

increase or decrease the rate of contaminant degradation. In addition, the degree of metabolism is dependent on the use of specific enzymes whose "affinity" for the contaminant and availability of that particular product are largely essential (Madhavi and Mohini 2012).

- The major biological factors are included here: mutation, horizontal gene transfer, enzyme activity, interaction (competition, succession, and predation), its own growth until critical biomass is reached, population size and composition (Boopathy 2000).

3.3. Hydrocarbonoclastic micoorganisms

Hydrocarbonoclastic means degrading hydrocarbons or simply ‘eating’ or ‘breaking’ hydrocabons molecule. A characteristic feature of hydrocarbonoclastic microorganisms is their ability to use hydrocarbons as the only source of carbon and energy. They have hydroxylases (also called oxygenases) that introduce oxygen atoms from molecular oxygen into the corresponding fatty acids. The latter are biodegraded to a key intermediate metabolite; acetyl-CoA (Khanafer et al. 2017).

Several species belonging to different genera have been reported to be able to degrade hydrocarbons, these include: *Pseudomonas, Nocardia, Vibrio, Corynebacterium, Candida, Arthrobacter, Rhodotorula, Brevibacterium, Flavobacterium, Sporobolomyces, Achromobacter, Bacillus, Aeromonas, Thiobacillus, Acinetobacter, Lactobacter, Staphylococcus, Penicillium, Articulosporium, Halomonas, Klebsiella, Proteus, Aspergillus, Micrococcus, Neurospora, Rhizopus, Mucor* and *Trichoderma*. These organisms have been identified in large quantities in many oil-polluted waters and soils, but are found in lesser quantities in uncontaminated environments (Victor et al. 2020).

In the case of oil spills the key may be a tiny microscopic bacterium called *Alcanivorax borkumensis*, a non-pathogenic aquatic bacterium. Its genome has the code for several enzymes and it is considered to be a "hydrocarbonoclastic" (Kadri et al. 2018).

3.4. Benefits of bioremediation

- It is a fast, natural process and eco-friendly waste treatment process for polluted environments. The micro-organisms are able to breakdown the contaminant and grow in number while the contaminant is present.

- It requires very little effort and can be done on site, often without causing major disruption to normal operations.

- It does not use hazardous chemicals. Nutrients, especially fertilizers, are added to make microbial growth active and rapid. Bioremediation converts hazardous chemicals into harmless water and gases.

- Ecological and sustainable technique (Abatenh et al. 2017; Sui et al. 2021).

3.5. Disadvantages of bioremediation

- It is limited to compounds that are biodegradable and not all compounds are likely to degrade quickly and completely.
- The products of biodegradation may be more persistent or toxic than the original compound.
- Biological processes are often very specific and take longer than other treatment option.
- It is difficult to extrapolate from laboratory and pilot-scale studies to large-scale field operations (Abatenh et al. 2017).

METHODOLOGY

Methodology

1. Objective

This study aims to evaluate the ability of previously isolated microorganisms from petroleum hydrocarbons contaminated samples to degrade gasoline and diesel in soil.

2. Material and methods

2.1. Material

2.1.1. Microbial strains

Seven microbial strains previously isolated from petroleum contaminated soils were assessed in this study. Before use, their purity was checked by means of microscopic observations. These were given code names as follows: C14 (Gram- rods), C26 (Gram- cocci), C31 (Gram+ rods), C52 (Gram- rods), K11 (Gram- cocci), S (Filamentous Gram + bacteria) and Fungus (belongs to the genus *Aspergillus*).

2.1.2. Soil sample

Soil sample was collected from the region of Sougueur (south Tiaret), Algeria.

2.1.3. Diesel and gasoline oil

Gasoline and diesel samples were purchased from local gas station (Tiaret, Algeria).

2.1.4. Chemicals and culture media

- n-Hexane

- MSM (Minimal salts medium): Culture medium composed only of mineral salts without any source of carbon. It is used for the enrichment, isolation and growth of microorganisms with specific nutritional types. The medium consists of 5.0 g NaCl, 5.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g $(NH_4)2SO_4$, 0.25 g $MgSO_4 \cdot 7H_2O$, 2.0 g $NaNO_3$, 0.02 g $FeCl_2 \cdot 4H_2O$, 0.02 g $CaCl_2$, per litre of distilled water (Obi et al. 2016).

- Sabouraud Agar: Culture media used for the isolation and cultivation of fungi.

2.2. Methods

2.2.1. Microcosm preparation and inoculation with microbial isolates

The first step in this study was the preparation of soil for the assay by autoclaving it at 121ºC for 15 min three time (24 h apart) to remove the indigenous microorganisms. The soil was supplemented with 250 $mg.kg^{-1}$ of $(NH_2)2SO_4$ and 100 $mg.kg^{-1}$ of K_2HPO_4 to biostimulate the growth of microbial inocula (Bento et al. 2004).

The sterilized soil was distributed over 48 pots where 24 pots were contaminated with 10 %

diesel oil and the other 24 pots were contaminated with 10 % gasoline (Fig. 3).

Theneafter, soils were inoculated with 3 ml volume of the standardized microbial suspensions (0.5 Mac Farland) (Ghazali et al. 2004). After homogenization with a sterile spatula, the mixture is incubated for 7 days at room temperature. Sterile distilled water was added every 48 hours with homogenization to maintain the humidity and O_2 level in the medium in order to allow biodegradation of the pollutants. Three repeats are made for each microbial isolate and for the control (without microbial suspension). Total petroleum hydrocarbons (TPH) degradation kinetics was followed by sampling at time intervals after 7 days, 14 days, 21 days and 28 days.

Figure 3. Preparation of microcosms.

2.2.2. Extraction and determination of total petroleum hydrocarbon (TPH)

One gram from each soil pot was collected and placed in a 100 ml flask, then 2 ml of n-hexane was added. The mixture was agitated vigorously on a magnetic stirrer for 30 min to permit for hexane to extract the oil out of the soil sample. The solution was then filtered using a paper filter and 0.1 ml of the filtrate was diluted by adding 5 mL of hexane. The absorbance of the extract was measured spectrophotometrically at 400 nm using n-hexane as a blank. The TPH in the soil samplewas calculated with respect to a standard curve obtained from testing several concentrations of diesel oil and gasoline (separately) diluted with n-hexane (Agarry and Latinwo 2015). Percent degradation (D) was calculated using the following formula:

$$D = \frac{\mathrm{TPHi} - \mathrm{TPHr}}{\mathrm{TPHi}} \times 100$$

Where TPHi and TPHr are the initial and residual TPH concentrations, respectively

2.2.3. Evaluation of the growth of microbial isolates in liquid medium MSM

The test was performed in tubes containing 8 mL of MSM supplemented with the diesel and gasoline (0.1ml), separately, that were inoculated by 2 mL of standardized microbial isolates suspensions (Fig. 4). Tubes were incubated at 30 °C for 20 days. An abiotic control was used and consisted in the MSM supplemented with diesel and gasoline separately but devoid of microorganisms. Optical density (OD) was measured at 625 nm using UV Spectrophotometer (Nwinyi et al. 2014).

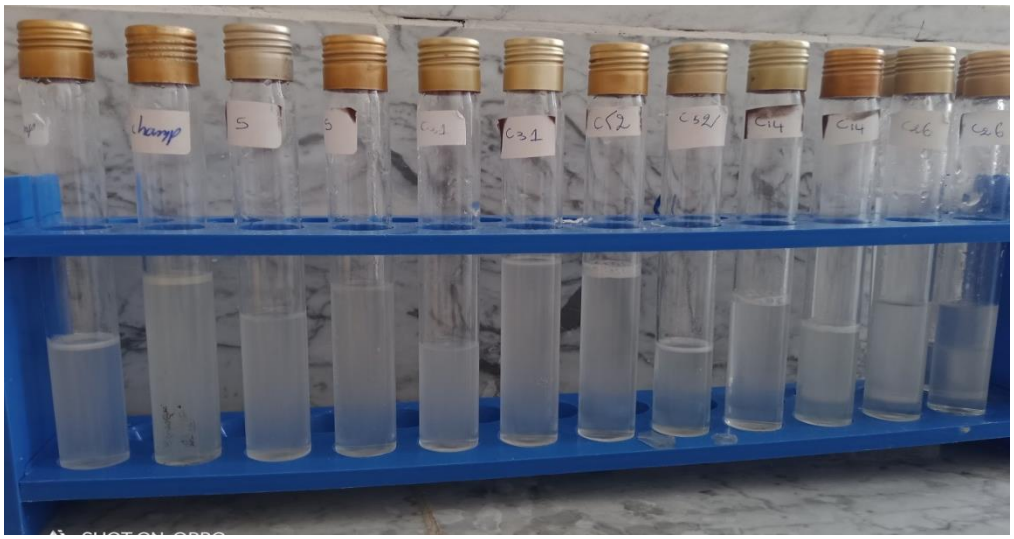


Figure 4. Liquide medium (MSM) test of microbial growth.

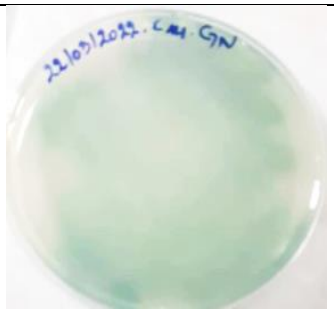
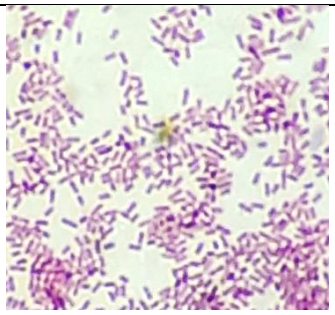
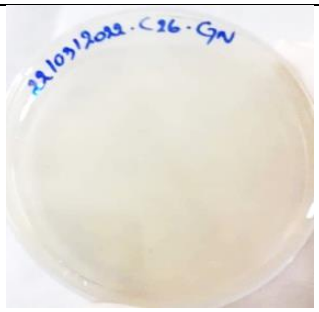
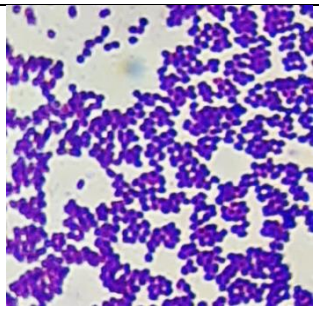
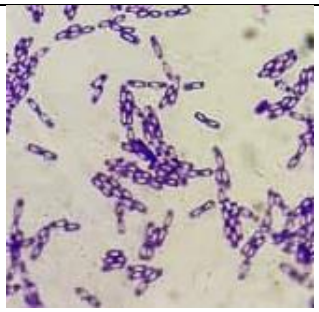
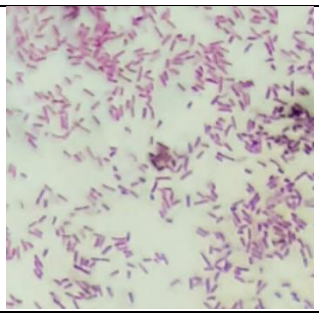
RESULTS

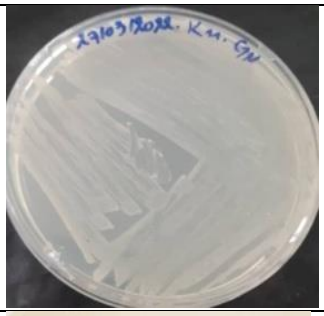
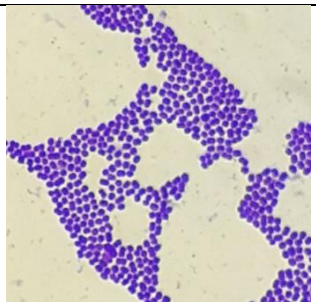
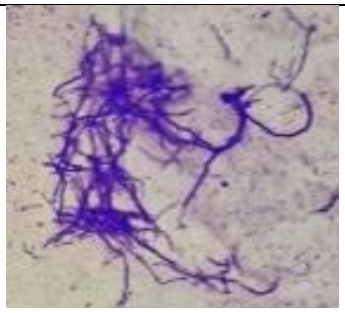
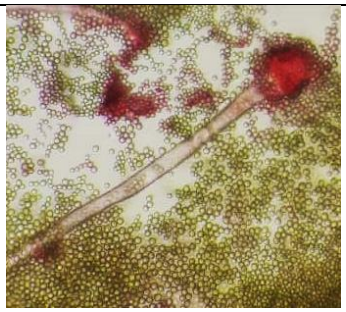
Results

1. Purity of the microbial isolates

Results of the macroscopic and microscopic observations of the microbial isolates are reported on table 1.

Table 1. Macroscopic and microscopic observations of the microbial isolates.

Isolates	**Macroscopic observation**		**Microscopic observation**	
C14	Green colonies		-Thin short rods, in clusters - Gram negative	
C26	Translucent colonies		- Cocci in clusters - Gram positive	
C31	White colonies		- Rods with terminal spores - Gram positive	
C52	Green-yellow colonies		- Small rods - Gram negative	

K11	White colonies		Gram negative Cocci	
S	Translucent colonies with black dots		- Filaments of bacilli in clusters. - Gram positive	
Fungus	Yellow- green colony		*Aspergillus* sp.	

2. Rate of degradation of diesel oil and gasoline in soil

After 28 days incubation of the microbial isolates in soils contaminated with diesel oil and gasoline, we noted that all the tested strains have the ability to degrade both contaminants, though at different rates.

The strains named S and C52 demonstrated the higher rates of degradation regarding diesel oil 82 ± 23.4 % and 71 ± 25.7 % respectively. Whereas, the strain K11 demonstrated the lower rate 11.03 ± 3.8 % (Fig. 5).

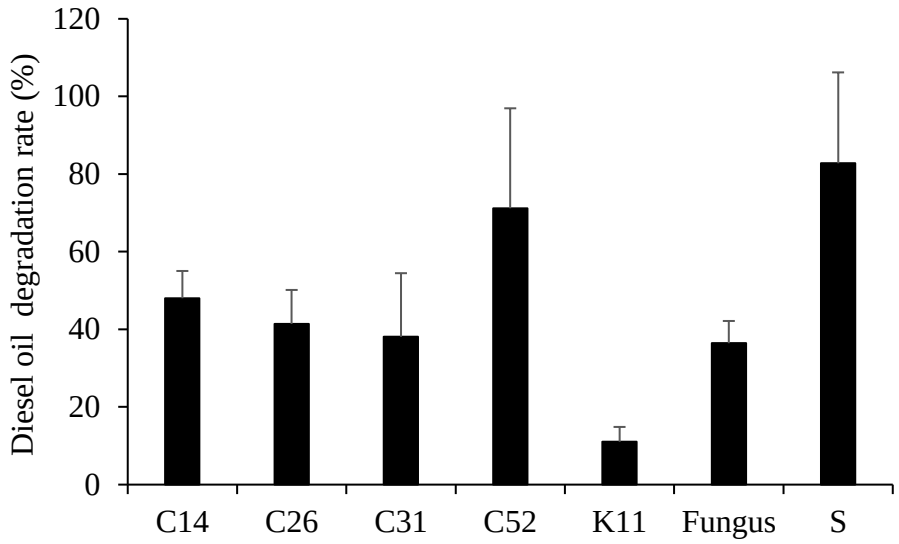


Figure 5. Percentage degradation of diesel oil in soil by microbial isolates after 28 days.

Besides, the same strains S and C52 in addition to K11 demonstrated the higher rates of degradation regarding gasoline 94.36 ± 7 %, 48.7 ± 9.5 % and 68.5 ± 25.8 % respectively (Fig. 6).

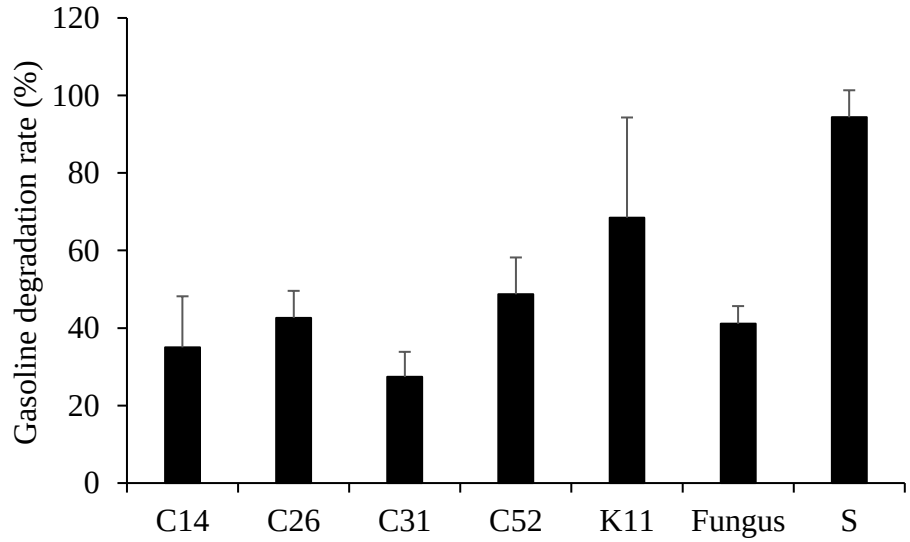


Figure 6. Percentage degradation of gasoline in soil by microbial isolates after 28 days.

3. Growth rate of the microbial isolates in MSM supplemented with gasoline and diesel oil

After 20 days incubation of the microbial isolates in liquid medium with gasoline or diesel oil as the sole carbon source, we noticed that all the tested strain showed ability to grow in such media.

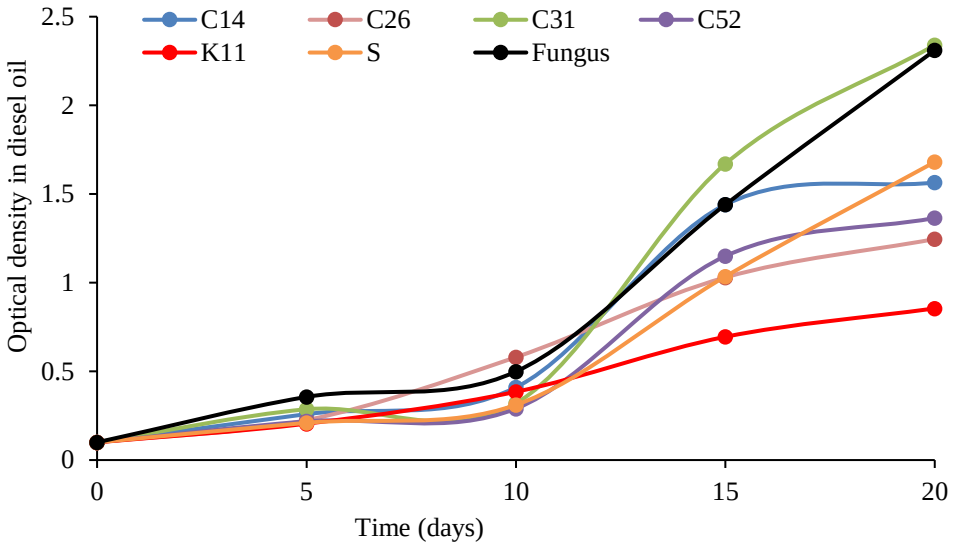


Figure 7. Growth rate of the microbial isolates in MSM supplemented with diesel oil.

The microbial isolates C31 and Fungus demonstrated the higher growth rates after 20 days incubation with diesel oil and the strain K11 demonstrated the lower growth rate (Fig. 7). On the other hand, C26, C52 and Fungus showed the higher growth rates with gasoline, whereas, S showed the lower rate (Fig. 8).

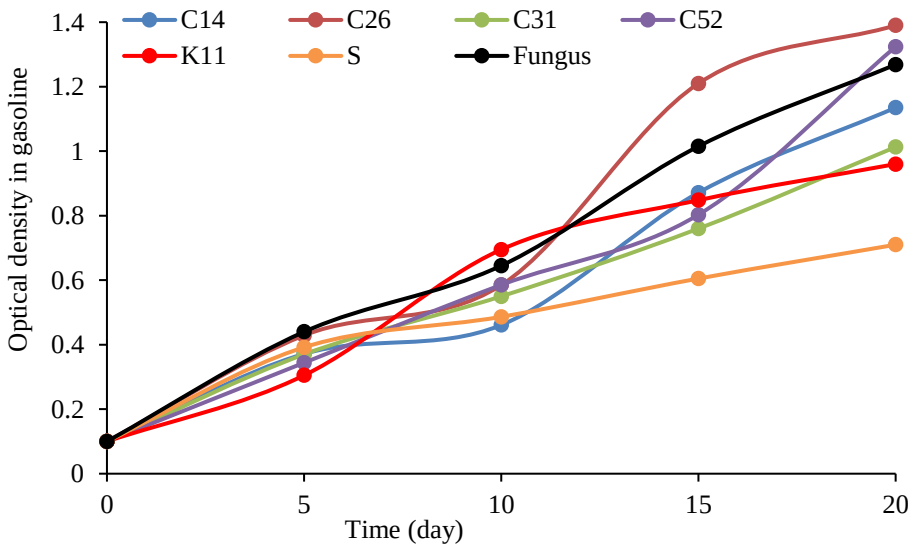


Figure 8. Growth rate of the microbial isolates in MSM supplemented with gasoline.

DISCUSSION

Discussion

Petroleum hydrocarbons are toxic environmental pollutants. Bioremediation process using microorganisms is now recognized as environment friendly and economical for the efficient conversion of toxic, recalcitrant compounds into non-toxic products especially in case of contaminated land and water (Deshmukh et al. 2016).

Many scientists reported that mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil and marine environments (Das and Chandran 2011).

The aim of this study was to test the ability of previously isolated microorganisms from petroleum hydrocarbon contaminated samples, to degrade gasoline and diesel oil in soil. As well, their ability to grow in liquid medium containing either gasoline or diesel oil as sole source of carbon was estimated.

Seven microbial isolates were tested in this study, four of them are Gram positive bacteria, two are Gram negative bacteria and one fungus belonging to the genus *Aspergillus*.

After 28 days incubation of the microbial isolates in soils contaminated with either diesel oil or gasoline, all the tested strains demonstrated ability to degrade both contaminants at different rates. The isolates S (actinobacteria), and C52 (Gram negative rods forming a green pigment in the medium and suspected of belonging to *Pseudomonas* genus), demonstrated the higher rates of TPH degradation regarding diesel oil. Besides, the same strains S and C52 in addition to K11 (Gram positive cocci) demonstrated the higher rates of degradation regarding gasoline in soil. Moreover, all the tested microbial isolates showed ability to grow in the liquid medium (MSM).

Microbial degradation of hydrocarbons is based on many factors which include the level of dissolved oxygen, pH, and microbial population in the environment (Adeniji et al. 2017). The isolation of such organisms in these environments also indicates that they have developed adaptive strategies to the environment and/or use those substances as energy sources (Ebakota et al. 2017). The first strains that were isolated as hydrocarbon degrading are Gram-negative bacteria such as *Pseudomonas putida* or closely related *Pseudomonas sp*. Gram-positive bacteria such as *Bacillus, Rhodococcus, Staphylococcus, Exiguobacterium* have also been found to be hydrocarbon-tolerant, although mechanisms of tolerance are not well understood (Lazaroaie 2010). Several studies demonstrated that *Pseudomonas* spp. have a remarkable ability to degrade a wide range of organic pollutants, including polycyclic aromatic hydrocarbons, halogenated derivatives, and recalcitrant organic residues. In a study, it was mentioned that *Pseudomonas* showed TPH degradation ranging from 65 % to 96 % (Bhattacharya et al. 2003).

Moreover, actinobacteria are an important group of soil microorganisms. Their metabolic diversity and specific growth characteristics make them well suited as agents for bioremediation. They produce a variety of extracellular enzymes, which metabolize a variety of compounds and spores resistant to desiccation. In addition, their filamentous growth allows them to colonize soil particles (Polti et al. 2014).

Besides, a broad range of microorganisms can produce biosurfactants involved in hydrocarbons degradation using different substrates such as oils, alkanes, sugars, and agro-industrial wastes. Microorganisms frequently produce biosurfactants during proliferation on water immiscible substrates, to make easy utilization of the substrates by the cells. As well, many enzymes are involved in the hydrocarbon degradation such as monooxygenase, dehydrogenase, hydrolase and laccase. A wide range bacterial and fungal strains have been studied for their ability to produce these degradative enzymes during the biodegradation of hydrocarbons (Parthipan et al. 2017).

Likewise, enzymes like cytochrome P450 have an imperative role in the degradation of oily hydrocarbons, and degradation is enhanced in the aerobic condition in gasoline contaminated soil (Ramsay et al. 2000). Schneiker (2006), found that Actinobacteria was the most abundant phylum associated with cytochrome P450 alkane hydroxylases genes in contaminated soils. The presence of cytochrome P450 has been previously linked to an expanded hydrocarbon degradation capacity, which might confer a selective advantage to Actinobacteria in gasoline-contaminated soils especially in the later steps of the bioremediation process. Laccases, transferase, cytochrome P450 monooxygenase and various enzymes are used as basic process of microflora activities in contaminated soil (Jadhav et al. 2019). Currently, *Pseudomonas* sp., *Acinetobacter* sp., and *Rhodococcus* sp. are bacteria that have the most effect on the degradation of petroleum pollutants (Sui et al. 2021). Van Beilen (2006), showed that many alkane-degrading yeasts and fungi also contain cytochrome P450, they are likely to contribute to oil degradation in polluted marine and soil environments. Fungal strains have the capacity to adapt to petroleum compounds and degrade a wide range of these pollutants. *Aspergillus terreus* was demonstrated to decrease the contamination with petroleum by 44 % in a laboratory test with 10 % petroleum contaminated soils (Mohsenzadeh et al. 2012). In fact, fungi play a major role as decomposers in all ecosystems thanks to their robust morphology and diverse metabolic properties. There has been growing interest in the unique capacity of fungi to degrade toxic and recalcitrant compounds by employing a variety of extracellular and intracellular enzyme systems including prominent fungal enzymes: catalases, laccases, peroxidases and cytochrome P450 monooxygenases (Deshmukh et al. 2016).

CONCLUSION AND PERSPECTIVES

Conclusion and perspectives

Quite a lot of research has been carried on hydrocarbon degradation. Due to the large oil spills that have occurred in the past decades, the need for an effective strategy to deal with the harmful effect of oil on marine life, and also to understand the risk to humans and its adverse effect on the entire biosphere, has been constantly felt. The importance of microbial methods for hazardous waste remediation is evident with the evolution of environmental regulations and social acceptance of biological technologies. Microbial degradation can be considered as a key component in the clean-up strategy for petroleum hydrocarbon bioremediation.

Laboratory microcosm experiment showed that all tested seven microbial isolates are able to degrade diesel oil and gasoline in the soil, taking into account that C52 and S have the best potential in TPH degradation. Moreover, all the microbial isolates showed ability to grow in the liquid medium MSM with gasoline and diesel oil as sole source of carbon with C26 and fungus showing the higher growth rates.

These results indicate that the tested microbial isolates may be potential bioremediation agents which constitutes a basis for further studies ‘‘in situ’’ for contaminated soils and aquatic environments.

This work should be followed-up by the identification of the isolates and genes implicated in the biodegradation of petroleum hydrocarbons.

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