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Evaluation Of Tolerance To Increasingly Higher Diesel Concentrations By Some Isolated And Characterized Indigenous Bacterial Species For Their Possible Utilization In Bioremediation Technologies

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Abstract
Diesel, a hydrocarbon-based fuel essential for transportation and power generation, is a major environmental pollutant. Fortunately, bioremediation offers a sustainable solution, as certain bacteria can metabolize diesel components, converting them into less harmful byproducts while using them as an energy source. Thus, the aims of this study were to screen, select, and characterize local bacterial strains capable of decomposing diesel from petroleum contaminated soils and to test their tolerance for increasing diesel concentrations as potentials means for bioremediation in environmental cleanup. (20) samples of soil contaminated with petroleum derivatives from different areas of southern Babylon were taken and cultured on liquid basal salt medium and Bushnell has mineral salt agar . (9) samples were found to be positive (45%), while (11) samples were found to be negative (55%) for bacterial growth. 18 bacterial isolates were obtained, and colonies with distinct phenotypes were selected and identified using cultural, microscopic, and biochemical tests by the Vitek 2 compact system. Six different bacterial species were identified, namely Enterobacter cloacae complex, Escherichia hermannii ,Staphylococcus lentus ,Citrobacter sedlakii, Aeromonas salmonicida ,Sphingomonas paucimobilis . Their tolerance to increasingly higher diesel concentrations was evaluated by measuring optical density (OD600) in liquid Bushnell has mineral salt containing different concentrations of diesel (1%, 3%, 5%, and 10%)using a UV/Vis spectrophotometer after incubation period of 5, 10, and 15 days. The results showed that the growth rates for all the tested bacterial species were higher in low diesel concentrations , while a notable decrease in growth was indicated with higher diesel concentrations. Therefore, all of the selected strains may be putative species for bioremediation of diesel contaminated environments particularly with low concentrations.

Keywords. *Diesel.biodegradation,Enterobacter.cloacae complex , Aeromonas salmonicida Sphingomonas paucimobilis, Escherichia hermannii. Staphylococcus lentus*

1. INTRODUCTION

Soil pollution by petroleum hydrocarbons is a major. global issue that has raised environmental risks in recent decades. Many industrial and urban factors have led to hydrocarbon leaks [1]. It is well known that petroleum is one of the most important sources of energy that supports the country's economy and its progress in many fields. On the other hand, these petroleum products have many types and residues of organic pollutants due to leaks from fuel storage tanks and accidents during transportation [2]. Therefore, cases of oil pollution have spread throughout the world, as it has caused major threats to the environment through its effects on food sources and soil chemistry. The emission of organic chemicals into the atmosphere has become significant. Landfills also contain a large amount of chemically manufactured organic materials[3]. These materials are certainly toxic to the health of humans, plants, and animals. Diesel is a widespread pollutant with a significant negative impact on the environment. It is used in many industries, including power generators and as a primary fuel for many modes of transportation [4]. It is also found in the waste products of. many industrial processes, such as oil.refineries and industrial plants. The use of physical and chemical processes to clean the environment of diesel pollutants is possible, but these methods are not very useful due to their high costs and the by-products they produce [5]. Therefore, biological methods have received more attention than physical and chemical methods. Numerous studies have shown that certain types of bacteria consume and decompose diesel, using it as a sole carbon source [6] [7]. Thus, the aims of this study were to: (1) Isolate and screen indigenous bacterial strains from petroleum-contaminated soils for their diesel-degrading capabilities, (2) Characterize the selected strains through phenotypic and biochemical analyses, (3) Evaluate their tolerance to progressively higher diesel concentrations to assess their potential application in bioremediation strategies for environmental cleanup.

2. MATERIALS AND METHODS

2.1. Sample Collection

(20) samples of petroleum contaminated soil from different areas of southern Babylon were collected. (10) grams were taken for each soil sample at a depth of (5-10 cm) , transported in polyethylene bags, and placed in an ice box until it arrived to the laboratory. It was placed in the refrigerator and kept at a temperature of 4°C to conduct the required experiments [8].

2.2 Preparation of growth medium

Liquid basic salt medium was used as a selective medium to isolate diesel-decomposing bacteria. It was prepared from the following materials : KH2PO4(1.36),Na2HPO4 (1.39), KNO3 (1.25), MgSO4 (0.06), CaCL2 (0.02) , (NH4)2SO4(7.7) ,NH4CL(1.5) NH4NO3(0.85), K2HPO4(0.53) and 100 ml of a trace mineral solution containing 0.01g of ZnSO4.7H2O, MnCl2.4H2O , H3BO4 , COCL2.6H2O, Fe2SO4 .2H2O, CuCL2.2H2O and NaMoO4.2H2O [9].The above materials were dissolved in a liter of distilled water and mixed well to ensure that all materials were dissolved. The top of the glass flask was closed, the pH was set to (7) , then it was sterilized by an autoclave for (20 minutes at a temperature of (121) . Diesel is also sterilized by using filters with a pore of 0.22 micrometers, after the sterilization was completed , the required concentration of sterile diesel was added [10].

2.3 Preparation of a medium for Selection of diesel Tolerant Bacterial Strains

Bushnell Haas mineral salt agar (BHMS) was used to obtain pure colonies of isolated bacterial species that consume diesel as the sole source of carbon and energy. This medium was prepared by dissolving the following materials: MgSO4.7H2O (0.2 g\L) , CaCL2 (0.02 g\L) , KH2PO4 (1 g\L) , K2HPO4 (1 g\L) , and NH4NO3 (1 g\L) , and two drops from 60% of FeCL3 at PH 7.2 , [11] . With the addition of (20 grams) of agar for the purpose of solidifying the medium in a liter of distilled water, then it is sterilized in an autoclave at (121) for (20) minutes. After sterilization, it was left to cool to ambient temperature, and diesel was added in the concentration 1 % after sterilization to each beaker, shaked well, then poured into dishes for the purpose of solidification by agar [12].

2.4 Isolation and selection of diesel degrading bacteria

(1 gm) of each soil sample contaminated with diesel after sifting through a sieve was weighed, then (9 ml) of saline solution were added and mixed well, (1 ml) of the mixture was added to (50 ml) of liquid basic salt medium supplemented with (1%) diesel then placed in the a shaker incubator at (30°C) for 6 days. After completing the incubation period, (0.1) ml was transferred and spread on the surface of the BHMS medium supplied with (1%) diesel. The dishes were incubated at (30°C) for 6 days. There were two control groups: the first was BHMS medium with diesel without adding the sample, and the second was BHMS medium without diesel with the addition of the sample [13].Colonies with different phenotypes were selected for further characterization.

2.5. Identification of the diesel Degrading Bacteria

Bushnell Haas Mineral Salt agar, blood agar medium, and MacConkey agar medium were used to purify bacterial isolates and study their phenotypic differences. Identification was performed by cultural characteristics, microscopic examination, as well as biochemical tests using the Vitek 2 compact system [14].

2.6. Testing the growth efficiency of the selected bacterial strains at increasing diesel concentrations

The bacterial species were activated by nutrient broth medium for 24 hours, then the samples were placed in a centrifuge at 3600 rpm for 10 minutes, the activation medium was discarded, the cells were washed 3 times with saline solution, then liquid Bushnell has mineral salt was added until OD600 = 0.5-0.8 was obtained. Then the bacterial inoculum was added to 100 ml of liquid Bushnell has mineral salt was medium containing 1% , 3% , 5%, and 10% of diesel in 250 ml flasks and placed in the shaking incubator 150 rpm at 30 degrees for 5,10 ,and 15 days. Growth (turbidity) was quantified in triplicate at OD600 using a UV/Vis spectrophotometer, with BHMS medium as the blank reference[15].

2.7. Statistical Analysis

The Least Significant .Differences Test (LSD) was used to analyze the obtained data.

3. RESULTS AND DISCUSSION

3.1. Screening and selecting diesel Degrading Strains

Table 1 shows the number of samples distributed according to the isolation areas. Among the 20 samples of petroleum contaminated soils collected in the current study, (9) samples were found to be positive (45%), while (11) samples were found to be negative (55%). 18 bacterial isolates were obtained, and table 2 shows the distribution areas of the isolates. Six bacterial isolates were selected based on differences in phenotype. Distribution of the selected and identified isolates is shown in Table 3.

Table 1 : Number of samples according to their isolation areas

Isolation area	Positive samples	Percentage	Negative samples	Percentage	Total number	Percentage
Fuel filling stations	4	%20	3	%15	7	%35
Industrial district	2	%10	3	%15	5	%25
Fuel tanks	1	%5	2	%10	3	%15
electric power generators	2	%10	3	%15	5	%25
Total number	9	%45	11	%55	20	100

Table 2 : Number of bacterial isolates according to their isolation areas

Sequence	S Sample location **ample location**	Number of isolates
1	industrial district / Al-Qasim City	4
2	Al Ajyal Mashaeda/gas station	6
3	Sunset government gas station	2
4	fuel tanks / Al-Qasim	2
5	Power generation generator Jamiya	1
6	Power generator, Al-Salam District	3

Table 3: Distribution of bacterial species isolated and identified in this study.

Sampl Sample e	Isol Isolation ation	A place of iso A place of isolation lation
AS1	Enterobacter claocae complex	Al-Ajyal Al-Mashada gas station
AS2	Escherichia hermannii	Al-Ajyal Al-Mashada gas station
AS3	Citrobacter sadlakii	Private electric power generator / Al-Salam neighborhood
AS4	Staphylococcus Ientus	Fuel tanks/Al-Qasim City
AS5	Aeromonas Salmonicida	Industrial District / Al Qasim City
AS6	Sphingomonas paucimoblis	Industrial District / Al Qasim City

In contaminated environments, microorganisms are crucial for the biodegradation (bioremediation) of hydrocarbon pollutants [16]. In the current study, bacterial isolates were obtained from soil samples—from petroleum contaminated sites—using BHMS medium supplemented with 1% diesel as the only carbon source. This approach facilitated the enrichment of hydrocarbon-degrading bacteria and evaluated their biodegradation capabilities. Six different bacterial species were selected and identified in this study, and these strains were obtained from various petroleum sites. This may be attributed to the ability of these bacteria to thrive on diverse hydrocarbon compounds, including aliphatic (e.g., diesel) and aromatic

(monocyclic or polycyclic) hydrocarbons [17]. Nevertheless, native microbial communities possess only minimal inherent capacity for pollutant degradation via natural attenuation processes [18][19]. Our results also indicated that the majority of identified diesel-degrading bacteria were Gram-negative, with most isolates falling within five principal genera, Enterobacter cloacae complex , Escherichia hermannii ,Citrobacter sadlakii , Aeromonas salmonicida, Sphingomonas paucimoblis and only one gram positive bacterial species, Staphylococcus lentus. This agrees with previous studies [20][21][22].

3.2. Identification of Bacterial Isolates

3.2.1. Cultural Identification

Enterobacter claocae complex colonies appeared on bushnell haas mineral salt agar medium as white ,round, and small colonies . On Agar medium MacConkey appeared as large, round, pink colonies [23]. Escherichia hermannii appeared on bushnell haas mineral salt agar medium as round white colonies, while on MacConkey agar, they appeared as small and medium pink colonies [24]. Citrobacter sadlakii appeared on bushnell haas mineral salt agar medium as round, gray to white colonies of medium size, while on the blood agar medium, it appeared as smooth, grayish, and non-hemolytic (gamma-hemolysis) [25]. Staphylococcus Ientus appeared on bushnell haas mineral salt agar medium as shiny, round colonies of small to medium size. On blood agar medium, they appeared white in color and round in shape [26]. Aeromonas Salmonida , on bushnell haas mineral salt agar medium, they appear as medium-sized, round, gray-white colonies . On blood agar, they appear as medium-sized, gray-white colonies with a medium-sized, translucent area surrounding them [27]. Sphingomonas paucimoblis on bushnell haas mineral salt agar medium appeared as yellow colonies, but on blood agar, they appeared as white, soft, and raised colonies [28]

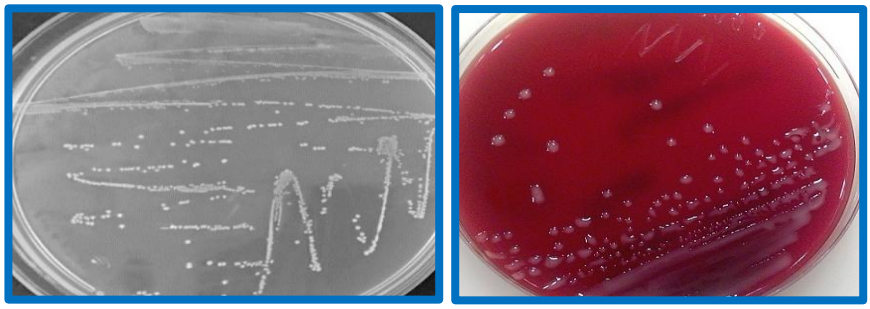


Figure 1. Phenotypic characteristics of Enterobacter claocae complex grown on bushnell haas mineral salt agar and MaCconky agar medium.

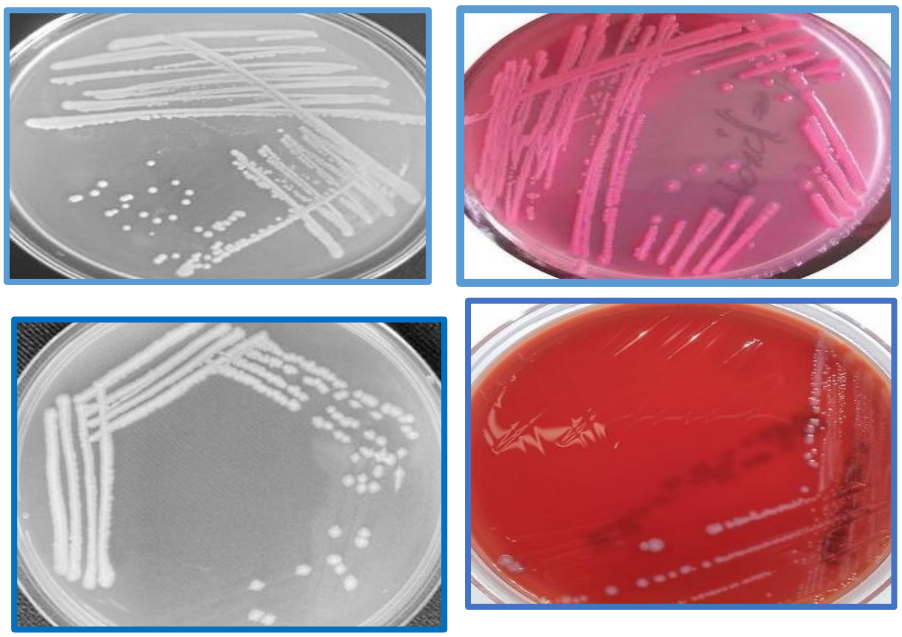


Figure 2. Phenotypic characteristics of Escherichia hermannii grown on bushnell haas mineral salt agar and MaCconky agar medium.

Figure 3. Phenotypic characteristics of Citrobacter sadlakii grown on bushnell haas mineral salt agar and blood agar medium.

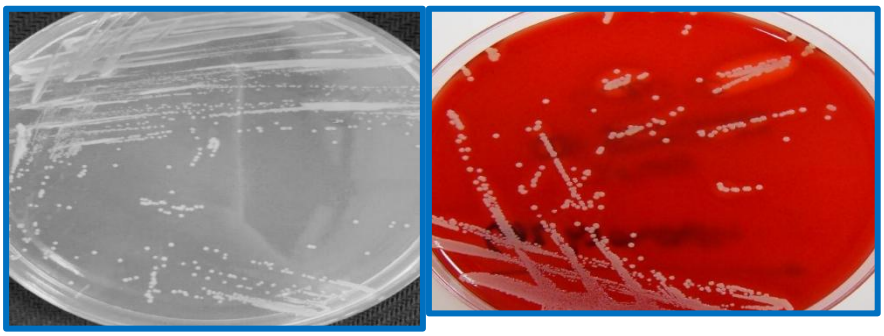


Figure 4 . Phenotypic characteristics of Staphylococcus lentus grown on bushnell haas mineral salt agar and blood agar medium.

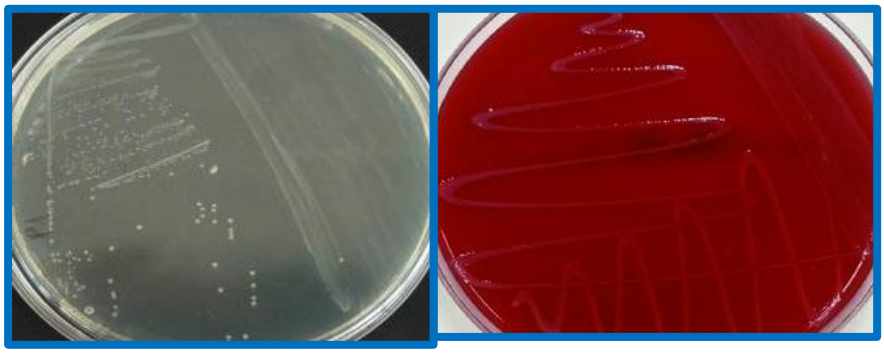
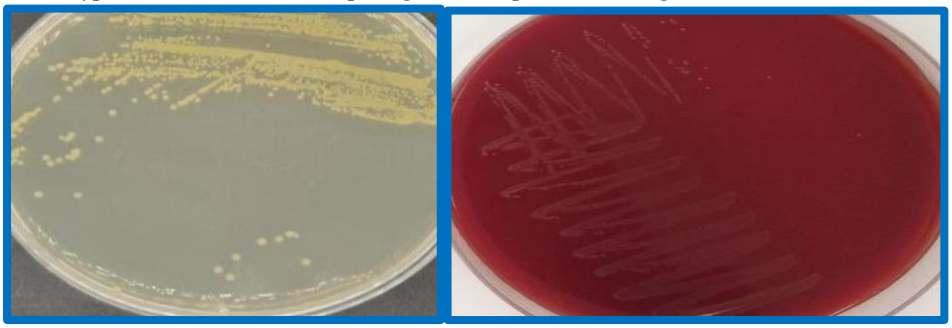


Figure 5 . Phenotypic characteristics of Aeromonas Salmonida grown on bushnell haas mineral salt agar and blood agar medium

Figure 6 . Phenotypic characteristics of Sphingomonas paucimoblis grown on bushnell haas mineral salt



agar and blood agar medium

3.3. Identification by VITEK 2 System

This test was using the VITEK compact ,maccording to the method followed [29].

3.3.1 Testing the growth efficiency of isolated and identified bacterial strains at different concentrations of diesel

The results of Enterobacter cloacae complex showed when measuring its growth density by OD600 in the liquid mineral salt medium, to which 1% diesel was added during the incubation period of 5 days OD600 0.312, while during the 10 days there was a noticeable increase in OD600 measurement was recorded 0.462 and at the incubation period of 15 days was higher 0.482. On the other hand, at a concentration of 3% diesel for this bacteria during the 5 days period, which was 0.239 and after 10 days 0.265, while at the incubation period of 15 days the was 0.291, at a 5% diesel concentration, OD600 measurement recorded a greater decrease than the previous two concentrations. After 5 days, was 0.119, at 10 days, 0.15, and after 15 days, (0.174). At a 10% diesel concentration, OD600 measurement recorded a decrease compared to the previous concentration. During 5 days, was 0.105, at 10 days, 0.113, and after 15 days,

0.171. These results are similar to previous studies conducted on hydrogenated compounds, including phenol [30] . As shown in Table 4.

Table 4: Measurement of OD600 of Enterobacter cloacae complex in 1%, 3%, 5%, and 10% diesel concentrations

Concentration / Time	Control	1%	3%	5%	10%	p-value
	Mean±S.D					
5 day	0	0.312±0.02	0.239±0.04	0.119±0.01	0.105±0.03	0.013*
10 day	0	0.462±0.03	0.265±0.05	0.153±0.02	0.113±0.04	0.002**
15 day	0	0.482±0.01	0.291±0.04	0.174±0.06	0.171±0.01	0.0001**

Table 5 shows the results of Escherichia hermannii . At a concentration of 1% diesel, OD600 was 0.177 after 5 days and 0.278 after 10 days, and at an incubation of 15 days ,OD600 value was 0.365. Also, at a 3% concentration, there was a clear decrease compared to the previous concentration ,recorded 0.111 during 5 days, 0.209 through 10 days, and 0.297 period 15 days. On the other hand, OD600 value at a 5% concentration was lower than the previous two concentrations, it was 0.107 after 5 days, 0.139 period 10 days, and 0.204 during 15 days. The decrease in OD600 value was greater at a 10% diesel concentration, was 0.097 after 5 days, 0.192 period 10 days, and 0.198 through 15 days These results are also similar to previous studies conducted on hydrogenated compounds [31].

Table 5: Measurement of OD600 of Escherichia hermannii in 1%, 3%, 5%, and 10% diesel concentrations

Concentration / Time	Control	1%	3%	5%	10%	p-value
	Mean±S.D					
5 day	0	0.177±0.003	0.111±0.022	0.107±0.004	0.097±0.033	0.047
10 day	0	0.278±0.029	0.209±0.016	0.139±0.066	0.192±0.018	0.018*
15 day	0	0.365±0.051	0.297±0.032	0.204±0.016	0.198±0.055	0.043*

The results of OD600 measurements for Citrobacter sedlakii is shown in the table 6. At a concentration of 1% diesel and incubation for 5 days were 0.367, and after 10 days there was an increase in the OD600 value, which was 0.503, and the increase was more at a 15-day incubation of 0.575, but at a concentration of 3% diesel the OD600 value began to decrease slightly at a period of 5 days, the value was 0.209, after 10 days 0.314, and at a period of 15 days 0.415, and the decrease also continued at a concentration of 5% diesel, and during a 5-day incubation 0.194, after 10 days 0.263, and at 15 days 0.283, and at a concentration of 10% diesel the decrease was greater and greater, where at a concentration of 0.075 at 5 days, at 10 days 0.084, and at 15 days 0.098, Similar to previous studies [33].

Table 6: Measurement of OD600 of Citrobacter sadlakii, in 1%, 3%, 5%, and 10% diesel concentrations

Concentration / Time	Control	1%	3%	5%	10%	p-value
	Mean±S.D					

5 day	0	0.367± 0.082	.209±0.042	0.194±0.02 2	0.075±0.01 1	0.022*
10 day	0	0.503± 0.044	0.314±0.03 3	0.263±0.01 8	0.084±0.02 1	0.0001**
15 day	0	0.575± 0.016	0.415±0.06 1	0.283±0.01 3	0.098±0.00 8	0.0001**

As in the table 7,Staphylococcus lentus recorded OD600 value at a concentration of 1% at 5 days of 0.244, at 10 days of 0.261, and at 15 days of incubation, 0.263. This bacteria recorded an increase at a concentration of 3%, unlike other species, where at 5 days it was 0.382, at 10 days, 0.449, and after 15 days, 0.497. However, at a concentration of 5% diesel, OD600 value decreased again, at 5 days it was 0.064, at 10 days it was 0.107, and during 15 days it was 0.115. This decrease continued at a concentration of 10%, at 5 days it was 0.061, at 10 days it was 0.075, and at 15 days it was 0.109,as mentioned [34] .

Table 7: Measurement of OD600 of Staphylococcus lentus , in 1%, 3%, 5%, and 10% diesel concentrations

Concentration / Time	Control	1%	3%	5%	10%	p-value
	Mean±S.D					
5 day	0	0.244±0.015	0.382±0.054	0.064±0.011	0.061±0.012	0.0001**
10 day	0	0.261±0.032	0.449±0.019	0.107±0.004	0.075±0.006	0.0001**
15 day	0	0.263±0.031	0.497±0.068	0.115±0.013	0.109±0.014	0.0001**

OD600 values of Aeromonas salmonicida are shown in the table 8 . At a concentration of 1% diesel, and at an incubation period of 5 days, were 0.315, and after 10 days, 0.387, and at 15 days, 0.468. At a concentration of 3%, OD600 values were close to the previous concentration values, as which were 0.284 during 5 days, 0.302 at 10 days, and 0.457 at the fifteenth day. However, at a concentration of 5%, OD600 values began to decrease, as which were recorded at an incubation period of 5 days, 0.179 after 10 days, and 0.198 at 15 days. The decrease continued at a concentration of 10% diesel, as at the fifth day of incubation, 0.101, the tenth day, and 0.141 at the fifteenth day, similar to previous studies conducted on hydrogenated compounds, including phenol [35].

Table 8 : Measurement of OD600 of Aeromonas salmonicida in 1%, 3%, 5%, and 10% diesel concentrations

Concentration / Time	Control	1%	3%	5%	10%	p-value
	Mean±S.D					
5 day	0	0.315±0.017	0.284±0.012	0.179±0.021	0.101±0.005	0.002**
10 day	0	0.387±0.104	0.302±0.016	0.198±0.033	0.141±0.018	0.001**
15 day	0	0.468±0.033	0.457±0.051	0.246±0.019	0.151±0.024	0.001**

The results of the last sixth bacterial species, Sphingomonas paucimobilis is illustrated in the table 9. It showed a decrease in OD600 values at all concentrations, where at a concentration of 1% diesel and in a period of 5 days of incubation, 0.064 and at 10 days 0.185, and at the fifteenth day 0.241, and at a concentration of 3% and in a period of 5 days incubation 0.082 and on the tenth day 0.105, and on the fifteenth day 0.143, and at a concentration of 5% and in 5 days 0.077, and on the tenth day 0.121, and

after 15 days 0.135, and the decrease increased more at a concentration of 10% after a period of 5 days incubation 0.042, and on the tenth day 0.067 and at the fifteenth day 0.082, as mentioned [36].

Table 9:Measurement of OD600 of Sphingomonas paucimoblis in 1%, 3%, 5%, and 10% diesel concentrations

Concentration / Time	Control	1%	3%	5%	10%	p-value
	Mean±S.D					
5 day	0	0.064±0.009	0.082±0.022	0.077±0.006	0.042±0.005	0.044*
10 day	0	0.185±0.017	0.105±0.061	0.121±0.016	0.067±0.012	0.001**
15 day	0	0.241±0.013	0.143±0.017	0.135±0.007	0.082±0.023	0.001**

In this study, we observed an increase in the growth of most of these isolated bacterial species in media with lower diesel concentrations, such as 1% concentration, and a decrease in growth in media with higher diesel concentrations, such as 10% concentration. This is consistent with previous studies on some hydrocarbon compounds[37].

The decrease in bacterial growth with increasing diesel concentrations can be attributed to several toxic and inhibitory effects of diesel on bacterial cells, which include (1)Toxicity of Hydrocarbons: diesel consists of complex hydrocarbons (alkanes, aromatics, and polycyclic aromatic hydrocarbons (PAHs)), many of which are toxic to microorganisms at high concentrations. These compounds can disrupt cell membranes, leading to loss of membrane integrity, leakage of cellular contents, and cell death [38]. (2)Solvent Effects on Cell Membranes: Diesel acts as an organic solvent, dissolving lipids in bacterial cell membranes. This compromises membrane fluidity and function, affecting nutrient uptake, energy production, and cell signaling [39]. (40)Limited Bioavailability and Physical Barriers: High diesel concentrations can form a hydrophobic layer, restricting oxygen and nutrient diffusion to bacterial cells. This creates an anoxic or nutrient-deprived environment, inhibiting growth [41].(42) Metabolic Overload and Accumulation of Toxic Intermediates :Some bacteria can degrade diesel, but excessive concentrations may lead to : overproduction of toxic metabolic intermediates (e.g., alcohols, aldehydes, organic acids) and Overwhelming of detoxification pathways (e.g., oxidative stress from cytochrome P450 enzymes)[43][44]. (5)Osmotic Stress & Water Activity Reduction: High diesel concentrations reduce water activity, making it harder for bacteria to maintain osmotic balance. This can lead to dehydration and metabolic inhibition. (6) Oxygen Depletion (for Aerobic Degraders): Aerobic diesel-degrading bacteria require oxygen for hydrocarbon breakdown. High diesel levels increase biological oxygen demand (BOD), leading to hypoxia and growth inhibition [45]. (7) Inhibition of Enzymes & Essential Cellular Processes: certain hydrocarbons may directly inhibit key enzymes involved in respiration, DNA replication, or protein synthesis[46].

Therefore, these bacterial species can be used in the bioremediation processes of soils contaminated with low concentrations of petroleum derivatives, and replace complex chemical and physical methods in this way to reduce or eliminate soil pollution, as it is an easy, modern, environmentally friendly, inexpensive method that does not have harmful effects on the ecosystem.

CONCLUSION

Strains of Enterobacter cloacae complex , Escherichia hermannii ,Citrobacter sadlakii ,Staphylococcus lentus,Aeromonas salmonicida, Sphingomonas paucimoblis may be considered as potential means for the treatment of various diesel contaminated environments.

REFERENCES

[1]. Ossai, I. C., Ahmed, A., Hassan, A., & Hamid, F. S. (2020). Remediation of soil and water contaminated with petroleum hydrocarbon: A review. Environmental Technology & Innovation, 17, 100526.

[2]. Sharma, B., & Shrestha, A. (2023). Petroleum dependence in developing countries with an emphasis on Nepal and potential keys. Energy Strategy Reviews, 45, 101053.

[3]. Al-Abadi, N. J. A. (2022). The role of the oil industry in environmental pollution-effects and suggested solutions. Journal of International Management, 28(4), 100899.

[4]. Ogunkunle, O., & Ahmed, N. A. (2021). Overview of biodiesel combustion in mitigating the adverse impacts of engine emissions on the sustainable human–environment scenario. Sustainability, 13(10), 5465.
[5]. Xiaojie, Z., Mukherjee, K., Manna, S., Das, M. K., Kim, J. K., & Sinha, T. K. (2022). Efficient management of oil waste: Chemical and physicochemical approaches. In Advances in Oil-Water Separation (pp. 439-467). Elsevier.
[6]. Imron, M. F., Kurniawan, S. B., Ismail, N. I., & Abdullah, S. R. S. (2020). Future challenges in diesel biodegradation by bacteria isolates: a review. Journal of Cleaner Production, 251, 119716.
[7]. Oyediran, O. O. (2022). Assessment of Physicochemical Properties and Microbial Community of Diesel Contaminated Soil (Master's thesis, Kwara State University (Nigeria)).
[8]. Eze, V. C., Onwuakor, C. E., & Orok, F. E. (2014). Microbiological and physicochemical characteristics of soil contaminated with used petroleum products in Umuahia, Abia State, Nigeria. Journal of Applied & Environmental Microbiology, 2(6), 281-286.
[9]. Bekele, I., Lulie, B., Habte, M., Boke, S., Hailu, G., Mariam, E. H., ... & Sileshi, G. W. (2022). Response of maize yield to nitrogen, phosphorus, potassium and sulphur rates on Andosols and Nitisols in Ethiopia. Experimental Agriculture, 58, e11.
[10]. Chavan, P. S. (2017). Methods of Sterilization.
[11]. Hein Alvial, G. A. (2024). Biological Oxidation of Refractory Sulfidic Ores at Circumneutral pH (Doctoral dissertation).
[12]. Jain, A., Jain, R., Jain, S., Jain, A., Jain, R., & Jain, S. (2020). Sterilization of Glassware; Preparation and Sterilization of Media. Basic Techniques in Biochemistry, Microbiology and Molecular Biology: Principles and Techniques, 93-99.
[13]. Khan, A. L., Numan, M., Bilal, S., Asaf, S., Crafword, K., Imran, M., ... & Lee, I. J. (2022). Mangrove's rhizospheric engineering with bacterial inoculation improve degradation of diesel contamination. Journal of hazardous materials, 423, 127046.
[14]. Książczyk, M., Kuczkowski, M., Dudek, B., Korzekwa, K., Tobiasz, A., Korzeniowska-Kowal, A., ... & Bugla-Płoskońska, G. (2016). Application of routine diagnostic procedure, VITEK 2 compact, MALDI-TOF MS, and PCR assays in identification procedure of bacterial strain with ambiguous phenotype. Current microbiology, 72, 570-582.
[15]. G. Zafra, B.-E. A. Valderrama, R. Regino, and F. Aguilar, "Molecular characterization and evaluation of oil-degrading native bacteria isolated from automotive service station oil contaminated soils," Chemical Engineering Transactions, vol. 49, 2016.
[16]. A. S. Ayangbenro, "Biodegradation of natural bitumen by Providencia stuartii isolated from heavy oil contaminated soil," Global NEST Journal, vol. 19, no. 2, pp. 353–358, 2017.
[17]. 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.
[18]. S. Sihag, H. Pathak, and D. P. Jaroli, "Factors affecting the rate of biodegradation of polyaromatic hydrocarbons," Interna tional Journal of Pure & Applied Bioscience, vol. 2, no. 3, pp. 185–202, 2014.
[19]. B. M. Macaulay, "Understanding the behaviour of oil degrading micro-organisms to enhance the microbial remediation of spilled petroleum," Applied Ecology and Environ mental Research, vol. 13, no. 1, pp. 247–262, 2015.
[20]. K. Patel Nilesh and H. Pethapara, "Isolation and screening of hydrocarbon degrading bacteria from soil near Kadi (Gujarat) region," International Journal of Research in BioSciences, vol. 2, no.4, pp. 10–16, 2013.
[21]. T. Hall, "BioEdit: a user-friendly biological sequence align ment editor and analysis program for Windows 95/98/NT," Nucleic Acids Symposium Series, vol. 41, pp. 95–98, 1999.
[22]. M. K. Subathra, G. Immanuel, G. Immanuel, and A. H. Suresh, "Isolation and Identification of hydrocarbon degrading bacteria from Ennore creek," Bioinformation, vol. 9, no. 3, pp. 150–157, 2013.
[23]. Murray, M. J., DeBlock, H., Erstad, B., Gray, A., Jacobi, J., Jordan, C., ... & Mehta, S. (2016). Clinical practice guidelines for sustained neuromuscular blockade in the adult critically ill patient. Critical care medicine, 44(11), 2079-2103.
[24]. Makri, A., Vlachopoulos, D., & Martina, R. A. (2021). Digital escape rooms as innovative pedagogical tools in education: A systematic literature review. Sustainability, 13(8), 4587.
[25]. Habib, S., Kamran, M., & Rashid, U. (2015). Impact analysis of vehicle-to-grid technology and charge ng strategies of electric vehicles on distribution networks–a review. Journal of Power Sources, 277, 205-214.
[26]. Anila, A. M. (2008). Characterization of Staphylococci and Evaluation of Methods for Detection of Methicillin Resistant Staphylococcus Aureus (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).
[27]. Retnaningrum, E., Rizqullah, M. F., & Wilopo, W. (2020, September). Biocatalysts characters of novel bacteria from crude oil-contaminated river. In AIP Conference Proceedings (Vol. 2260, No. 1). AIP Publishing.
[28]. Marmo, P., D'Andrea, M., Casu, F., DI LALLO, G., Migliore, L., & Thaller, M. (2018). Sphingomonas turrisvirgatae a new agar-degrading Sphingomonas species. In Abstract Book Congresso.
[29]. Wang, Y., Chen, X. F., Xie, X. L., Xiao, M., Yang, Y., Zhang, G., ... & Xu, Y. C. (2019). Evaluation of VITEK MS, Clin-ToF-II MS, Autof MS 1000 and VITEK 2 ANC card for identification of Bacteroides fragilis group isolates and antimicrobial susceptibilities of these isolates in a Chinese university hospital. Journal of Microbiology, Immunology and Infection, 52(3), 456-464.
[30]. Sahib, Z. Y., & Al-Saadi, A. G. M. (2023). Isolation and Identification of some Phenol Degrading Bacterial Species from some Contaminated Environments. IOP Conference Series: Earth and Environmental Science, 1259(1), 012039. doi: 10.1088/1755-1315/1259/1/012039
[31]. Sahib, Z. Y., & Al-Saadi, A. G. M. (2023). Phenol Biodegradation by some Bacterial Species Isolated from Oil Contaminated Environments. IOP Conference Series: Earth and Environmental Science, 1259(1), 012038. doi: 10.1088/1755-1315/1259/1/012038
[32]. Dankaka, S. M., Muhammad, J. B., Usman, S., Jagaba, A. H., & Abdullahi, N. (2023). Phenol biodegradation by Acinetobacter baumanii and Citrobacter sedlakii isolated from petroleum products contaminated environment. Case Studies in Chemical and Environmental Engineering, 8, 100468.
[33]. Duman, Z. E. Enhanced large scale production of recombinant staphylococcus simulans lysostaphin and its characterization (Master's thesis, Biyoteknoloji Enstitüsü).

[34]. Di Pierro, F., Sagheddu, V., Galletti, S., Forti, M., Elli, M., Bertuccioli, A., & Gaeta, S. (2021). Antibacterial activity of a fractionated Pistacia lentiscus oil against pharyngeal and ear pathogens, alone or in combination with antibiotics. Frontiers in Microbiology, 12, 686942.
[35]. Coppotelli, B. M., Ibarrolaza, A., Dias, R. L., Del Panno, M. T., Berthe-Corti, L., & Morelli, I. S. (2010). Study of the degradation activity and the strategies to promote the bioavailability of phenanthrene by Sphingomonas paucimobilis strain 20006FA. Microbial ecology, 59, 266-276.
[36]. Sikkema, J., de Bont, J. A., & Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews, *59*(2), 201-222.
[37]. Heipieper, H. J., Neumann, G., Cornelissen, S., & Meinhardt, F. (2007). Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. Applied Microbiology and Biotechnology, *74*(5), 961-973.
[38]. Harayama, S., Kishira, H., Kasai, Y., & Shutsubo, K. (1999). Petroleum biodegradation in marine environments. Journal of Molecular Microbiology and Biotechnology, *1*(1), 6370.
[39]. Van Beilen, J. B., & Funhoff, E. G. (2007). Alkane hydroxylases involved in microbial alkane degradation. Applied Microbiology and Biotechnology, *74*(1), 13-21.
[40]. Rojo, F. (2009). Degradation of alkanes by bacteria. Environmental Microbiology, *11*(10), 2477-2490.
[41]. Atlas, R. M., & Philp, J. (2005). Bioremediation: Applied microbial solutions for real-world environmental cleanup. ASM Press.
[42]. Ron, E. Z., & Rosenberg, E. (2014). Enhanced bioremediation of oil spills in the sea. Current Opinion in Biotechnology, *27*, 191-194.
[43]. Singh, S. N., & Tripathi, R. D. (Eds.). (2013). Microbial Degradation of Xenobiotics.Springer.
[44]. Bento, F. M., Camargo, F. A. O., Okeke, B. C., & Frankenberger, W. T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation, and bioaugmentation. Bioresource Technology, *96*(9), 1049-1055.
[45] Athmar Ahmad Abdul Hussain and Aamal Ghazi Mahdi Al-Saadi .(2021). Isolation and identification of some chromium resistant bacteria from some contaminated sitesJ. Phys.: Conf. Ser. 1999 012022
[46] Noor Mohammad Ali, Aamal Ghazi Mahdi Al-Saadi.(2021). Molecular, Biochemical, and Phenotypic Identification of Phenol and Cresol Degrading Acinetobacter baumanni Strain Selected from Al-Rumaila Oil Well. Indian Journal of Forensic Medicine & Toxicology,. 15(1): p. 2480-2489.