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New Bioremediation by Lipase Purified from Ralstonia *mannitolilytica* for Petroleum Hydrocarbons

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Abstract

A growing issue of contaminated soil caused by oil derivers in Iraq, requires detection of new methods for bioremediation. The present work aims to purify of extracellular lipase enzyme that can eliminate oil derivers. Seven strains of lipolytic bacteria were collected from Al- Dora electricity station soil Baghdad/ Iraq that polluted with oil derivers. *Ralstonia mannitolilytica* considered higher ability in lipolytic enzymes production. Crude lipase activity gave (1.66 U/ml). The purification procedure resulted in 8.9 fold with a 23.2% final yield. The purity of lipase and molecular weight confirmed by SDS-PAGE. The efficiency of ten aliphatic hydrocarbon compounds in the soil contaminated with diesel decreases after treatment with purified enzyme (7.6 U/ml) for 24 hr. at natural environment to mimic the nature compared with control. The findings showed that the lipase enzyme, which was isolated from local isolates of *Ralstonia mannitolilytica*, was an effective (biological) therapy for the breakdown of aliphatic hydrocarbon molecules. While research on bioremediation have employed several bacterial isolates and enzymes, such as *Pseudomonas sp*., none of them have used *Ralstonia mannitolilytica* isolate and lipase enzyme for oil spill bioremediation.

Introduction

Each year, the world uses about 5.1 billion tons of crude oil, with Saudi Arabia and the United States of America producing the greatest shares [1]. Countries rely on use a variety of methods for transporting crude oil, large tankers/ vessel transport oil at sea, while oil is transported inland via trucks, pipelines, barges and railroads [2]. Small spills happen frequently, but local responders manage them. Oil biodegradation, or the decomposition of oil mediated by microorganisms, becomes a crucial process to address in this situation [3].

Petroleum hydrocarbon-containing pollutants are continually affecting our natural environment. The removal of these pollutants has been accomplished using a variety of techniques. These practices aren't efficient or environmentally friendly, either. Enzyme-based

bioremediation is a gentle approach and an easily adaptable way to get rid of these dangerous substances from our natural ecosystem compared to chemical and physical treatments. The most common microbial enzymes involved in bioremediation include cytochrome P450s, laccases, dehydrogenases, proteases, hydrolases, dehalogenases, and lipases. These enzymes have shown promise in the efficient degradation and transformation of multiple pollutants of petroleum hydrocarbons compounds [4].

Under aqueous conditions, lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyzes the hydrolysis of the carboxyl ester bonds in triacylglycerols to produce monoacylglycerols, diacylglycerols, glycerol, and fatty acids. In organic solvents, it catalyzes the synthesis of esters Lipases are capable of catalyzing a wide range of reactions under regulated circumstances. They may catalyze the reverse reaction by parallel sequential hydrolysis of carboxyl ester linkages in triacylglycerides under aqueous condition, and the production of triacylglycerols, in non-aqueous conditions [5].

Plants, animals, and microorganisms all produce lipases; many of them secrete the enzyme in the extracellular space because of their excellent stability, flexibility, and ease of production in high yields, lipases of microbial origin are of significant commercial value [6]. Many immobilized or free microbial lipases have been made available. Many different bacterial species, such as *Bacillus, Pseudomonas,* and *Burkholderia*, as well as yeast species, such as Candida *rugosa, Yarrowia lipolytica,* and *Candida antarctica*, and mold species, such as *Aspergillus, Trichoderma viride,* produce lipases with different enzymological properties and specificities [7]. The molecular weight of lipases is known to be between 19 and 60 kDa, and they are monomeric proteins [8].

A few of the industrial applications for lipases include the food industry (flavor enhancement), detergent industry (hydrolysis of oil and fats), pharmaceutical industry (synthesis of chiral drugs), paper industry (control of pitch), medical industry (triglyceride measurement), cosmetic industry (exclusion of lipids), and biodegradation industry (decomposition and removal of oil) [5]. In fact, lipase enzyme producing microorganisms is found in oil and fat, so they were found in the soil contaminated with oil derivers. Soil is one of the best places to look for new strains of microorganisms to identify and isolate.

This research aims to detect new lipases that are stable across a various range of pH and temperature, Purification of extracellular lipase enzyme and covers the natural processes that can remove oil Derivatives from contaminated soil, with a focusing on bioremediation by purified lipase from new local isolates.

Materials and methods

Sample Collection and Bacterial Strain Isolation

Petroleum Samples of soil polluted with hydrocarbons were aseptically taken 5–15 cm below the earth's surface to avoid contamination, soil samples that were polluted were taken in Iraq – Baghdad (Al- Dora Thermal Power Station GPS) from the period between the July of year 2022 to September of year 2022, before being sent to the lab for bacterial isolation, all samples were preserved in sterile plastic bags and kept at 4 °C. [9].

Isolates Identification

The isolates were identified depending on the Bergey's manual of systemic bacteriology was used to select and perform these tests in triplicates [10].The tests included: Gram staining reaction, motility, oxidase, catalase, Indol production, Methyl Red, Vogues-Proskauer test, citrate consuming , Triple Sugar Test (TSI) and VITEK 2 compact system . The cell morphology was examined by biochemical characteristics and light microscopy at 37 °C.

Screening of Strains for Extracellular Lipase Enzyme

Lipolytic activity of isolated isolates groups was screened using the modified Rhodamine B agar assay method of Devaraj and Rajalakshmi [11] with some modification. Distilled water was used to dissolve and filter-sterilize Rhodamine B (1 mg/ml). The agar plates had a pH of (7), 8 g of nutritional broth, 10 g of agar, and 4 g of sodium chloride (per Liter). Rhodamine B solution (0.001% [wt/vol]) and 10.ml of olive oil were added to the medium after it had been autoclaved and mixed briskly for one minute. The medium was then allowed to cool to 45–50°C. Before adding the medium to sterile petri dishes, it was let to stand for 10 minutes to prevent foaming. Lipase production was detected by irradiating plates with UV light at 350 nm; under UV light, the hydrolysis of substrate causes the creation of an orange fluorescence zone surrounding bacterial colonies, which indicates the potential for creating lipase.

Extraction of Lipase

We modified the procedure outlined by Ali *et al.* [7] as follows: Lipase extraction and screening medium made from dissolved tween-80 agar, 5.0g of NaCl, 10.0g of peptone, 0.1g of CaCl2.2H2O, and 15.0g of agar-agar (per litre) with pH 7. The chosen isolate of *Ralstonia mannitolilytica* was cultured for 48 hours at 37°C in a shaking incubator with 500 cc of medium tween-80 at 150 rpm. The supernatant was collected after cool centrifugation at 4 °C for 15 minutes at 10,000 rpm. It was then used to measure the amount of protein and lipase in the sample and to purify the enzyme.

Assay for Lipolytic Activity

Titrimetric analysis was used to measure the lipase activity based on the hydrolysis of olive oil. The reaction mixture comprised 2.5 millilitres of deionized water in addition to one millilitre of the lipase enzyme, one millilitre of 0.1 M Tris-HCl buffer (pH 8.0), and three millilitres of olive oil. The reaction mixture was then incubated at 37 C for 30 minutes, with both the test (in which all the reaction mixture were added with enzyme) and the blank (in which all the reaction mixture were added without enzyme) being carried out. The test solution was transferred to a 50 ml Erlenmeyer flask after 30 minutes. The reaction was paused by added three millilitre of 95% ethanol. Utilizing three drops of phenolphthalein as an indicator, liberated fatty acids were titrated against NaOH (0.05 M). The final result was a pink colour [12]. The amount of enzyme required to produce one micromole of fatty acid per mint under the assay conditions is known as a unit lipase.

The lipase activity was calculated using:

$$\text{Lipase activity(U/ml)} = \frac{(\text{Milliliter NaOH for sample} - \text{Milliliter NaOH for blank})\text{X}0.005}{\text{Milliliter of lipase x Reaction time}} * 1000$$

Where:

0.005 = Normality of NaOH

Lipase Purification

After obtaining the supernatant, it was utilised for purification and to measure the content of protein and lipase. The cell-free supernatant being saturated (0-70%) with ammonium sulfate while being continuously stirred at 4°C. Dialysis was performed on the ammonium sulfate fraction using a dialysis bag with a 10-KDa cutoff against a 50 mM Tris-HCl buffer at 4°C for 24 hours. Following dialysis, the concentrated enzyme was loaded onto. A DEAE-cellulose chromatographic column (3x25cm) was used for ion exchange chromatography, and Sephadex G-100 column (2×41cm) and eluted with 50 mM Tris–HCl buffer pH 8. At a flow rate of 1 ml/min, the lipase was eluted from the column. The protein content of enzyme fractions (5 ml each) was measured spectrophotometrically at 280 nm. The highest concentrations of the fractions were used for the lipase activity assay.

Extracellular Protein Content

Using the Lowry method and bovine serum albomin (BSA) as the reference material, the total extracellular protein content was ascertained [13].

Determination of Enzyme Molecular Weight

According to Laemmli [14], the molecular weight of the lipase enzyme was determined using SDS-PAGE. Include protein content.

Characterization of Purified Lipase

-Temperature Effects on the Stability and Activity of Lipase

The optimum temperature for lipase activity was measured at different temperatures (25, 35, 45, 55 and 65) °C for 30 min by using standard assay condition. The purified Lipase was pre-incubated in water bath at (25, 35, 45, 55 and 65) °C in 50 mM Tris-HCL buffer pH 8 for 30 min, and immediately transferred into an ice bath. Enzymatic activity was determined and the residual activity (%) for lipase was plotted against the temperature.

-Effect of pH on Lipase Stability and Activity

To determine the optimum pH for lipase activity using 50 mM concentration of Tris-HCL buffer pH range (5-6-7-8-9-10), the enzyme activity was tested for 30 min at 45°C by using standard assay condition. The enzyme was pre-incubated for 30 minutes at 45°C in buffers with different pH values (6, 7, 8, 9, and 10). The tubes were cooled in an ice bath. Following that, the residual activity (%) for lipase was calculated, and it was plotted against the pH value.

Experiments on the Biodegradation of Diesel

Su *et al.* with a few adjustments [15] state that the reaction mixture for the degradation of diesel consists of five millilitres of diesel, half a millilitre of pure enzyme solution (7.6 U/ml), and fifty millilitres of Tris-Hcl pH 8., the investigations were repeated using the same enzymes and different dosages of lipase (100%, 50%, and 25%), and the reaction mixture was incubated at 37°C. Heat-inactivated enzyme was used in the control experiment. A 24-hour incubation time was used to calculate the rate of deterioration of a specific diesel, every experiments was carried out three times. Gas chromatography (GC) was utilised by Al-Nuaimi *et al.* [16] to test samples and measure the amount of hydrocarbon biodegradation. The following equation estimates how the hydrocarbons in diesel will biodegrade [17].

$$Csam = \frac{\text{Cst x Asam}}{\text{Ast}}$$

Where: C sam = Concentration of sample, C st = Concentration of stander, A sam= Area of sample
A st = Area of stander, While calculating the percentage of hydrocarbon biodegradation using the formula below [15]

$$Hydrocarbon\ degradation\ (\%) = \frac{\mathrm{C0} - Ct}{\mathrm{C0}} * 100$$

Where: C0= Concentration before degradation, Ct = Concentration after degradation.

Aliphatic Hydrocarbon Degradation in Diesel-Contaminated Soil

The modified Su *et al.* technique [15] was used to degrade the aliphatic hydrocarbons that were present in the contaminated soil sample of the AL-Dora power station in Bagdad. Ten grammes of contaminated soil, one millilitre of pure lipase enzyme (containing 7.6 U/ml), one millilitre of tris Hcl buffer 0.1 M, and two millilitres of diesel were used as the substrate in the reaction mixture. In place of purified Lipase enzyme, a blank contained 1 millilitre of distilled water (DW). The sample and blank were both incubated at room temperature as natural in situ conditions. After 24 hours, the hydrocarbon biodegradation of the samples was measured using gas chromatography (GC-drive- Shimadzu 2010, Japan)[16]. The following equation was used to estimate the hydrocarbon biodegradation process [17].

$$Csam = \frac{\mathrm{Cst\ x\ Asam}}{\mathrm{Ast}} * 2$$

Where: C sam = Concentration of sample, C st = Concentration of stander, A sam= Area of sample
A st = Area of stander, 2 = To find the final concentration per ppm.

Results and Discussion

Isolation and Screening of Strains for Lipolytic Bacteria

Soil samples were collected from Al- Dora electricity station Iraq /Baghdad, seven strains of lipolytic bacteria was isolated from soil that had been polluted with oil derivatives: (*Ralstonia mannitolilytica,Ralstonia insidiosa, Sphinggomonas poucimoilis, Ralstonia insidiosa, Acinetobacter baumannii, Pseudomonas putida, Burkholderia cepacia)*. The bacterium that formed the largest halo on Rhodamine B-olive oil plates was selected for this study. Rhodamine-B plates with *Ralstonia mannitolilytica* strain were used to test the strain's ability to produce lipase activity. When orange was seen under UV light, it indicated that *Ralstonia mannitolilytica's* lipase activity was active. (Figure 1). Based on the biochemical characteristics stated and VITEK2 compact system (AST-No. 12 cards-BioMerieux), It was then determined to be *Ralstonia mannitolilytica.*

Figure 1: Colonies with lipase activity on a Rhodamine B agar plate with olive oil as the substrate.

Assay of Lipase Activity

The bacterial strain that showed positive results on the Rhodamine-B agar plate test. Lipase activity is often first screened using a plate test [18]. The Rhodamine B test was utilized in this study. Lipase activity cannot be determined only using the Rhodamine B assay because it is not specific way for doing. Also olive oil was used as a substrate for the titrimetric determination of lipase activity. A unit lipase is defined as the quantity of an enzyme that, under specific assay conditions, releases one micromole fatty acid per mint. crude Lipase activity by *Ralstonia mannitolilytica* gave (1.66 U/ml).

Purification of the Extracellular Lipase from Ralstonia Mannitolilytica

The procedures for extracellular lipase purification that *Ralstonia mannitolilytica* secretes are listed in Table 1 and Figures 2 A and B. The content of ammonium sulphate determines when lipase activity increases. The lipase produced by *Pseudomonas aeruginosa* VSJK-R9 isolated from restaurant wastewater was purified by ammonium sulfate precipitation, dialysis and gel exclusion chromatography-Sephadex G-100, with 11.45-fold purification to obtain a yield of 35.08%. [19]. Tripathi et al. [20] purified lipase from *Microbacterium sp*. by sequential methods of ammonium sulfate precipitation and Sephadex G-75 gel column chromatography. This purification procedure resulted in 2.1-fold purification of lipase with a 20.8% final yield.

Table 1: Purification step of lipase production by *Ralstonia manitoliytica.*

Purification steps	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Total activity (U)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude enzyme	150	1.5	4.12	225	0.36	1	100
Ammonium Sulphate	30	4.5	3.92	135	1.14	3.1	60
Ion exchange chromatography on DEAE Cellulose	35	3.6	3	126	1.2	3.3	56
Gel filtration chromatography Sephadex G-100	24	4.5	2.5	108	1.8	5	48

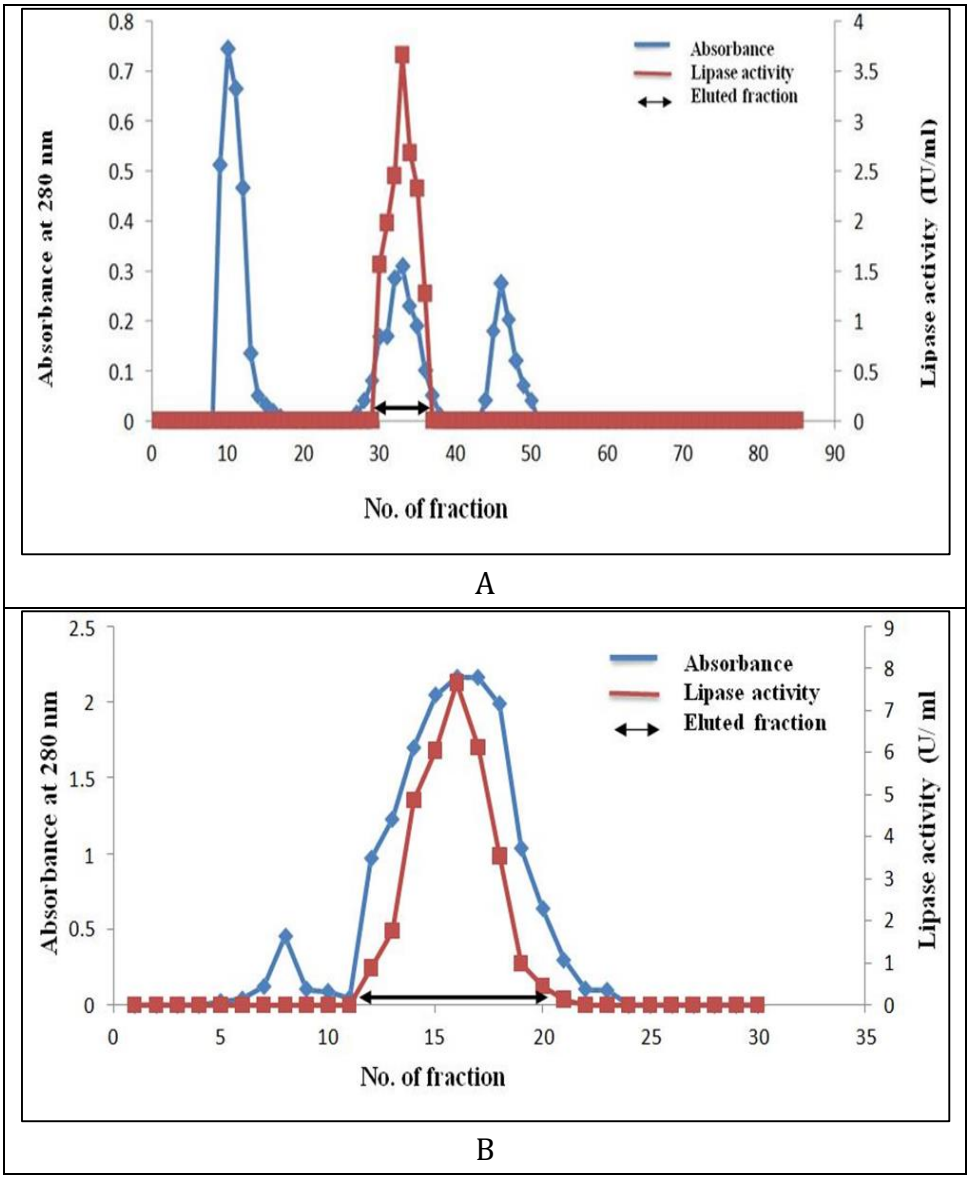


Figure 2 A: Lipase generated by *Ralstonia manitolilytica* was subjected to ion exchange chromatography using a DEAE-Cellulose column measuring 3 by 10 cm and flowing at a rate of 1 millilitres per minute. B: *Ralstonia manitolilytica*-produced lipase was separated by gel filtration chromatography on a SephadexG-100 column measuring 2 cm by 41 cm and equilibrated with 50 mM Tris HCl buffer pH 8.3.3.

Enzyme Molecular Weight

The purity of lipase and molecular weight were confirmed by SDS-PAGE (Figure 3). The molecular mass of purified lipase after Coomassie Brilliant Blue staining was estimated to be approximately 39 kDa. This finding Somewhat asymptotic agree with most of the *Bacillus* lipases have molecular weight in the range of 11–70 kDa. [21]. Although there is disagreement, the isolated lipase from *Burkholderia metallica* has a molecular mass of 55–60 kDa. [22].

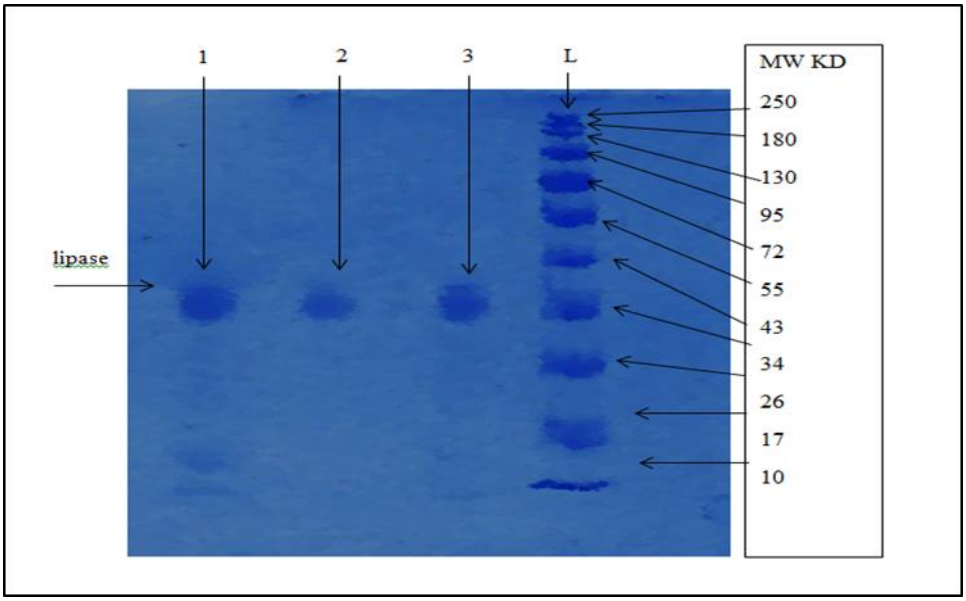


Figure 3: SDS-PAGE of *Ralstonia manitolilytica* lipase at various stages of purification. Lane L molecular weight ladder; Lane 1: crude; Lane 2: gel filtration G-100; Lane 3: ion exchange

Characterization of Purified Lipase Enzyme

Effect of Thermal Activity and Stability

The temperature has a significant impact on enzyme activity. The temperature range of (25-65)° C was used to assess lipase activity, and 45°C was shown to have the highest activity levels. Lower or higher incubation temperatures resulted in a decrease in activity. The thermal stability of the purified lipase was evaluated by letting it stand at pH 8.0 for 30 minutes at various temperatures (25, 35, 45, 55, and 65 °C), following which residual activity was calculated (Figure 4A). These results conflict with the hypothesis that *Ralstonia sp.* prefers a pH of 37 °C [23].

Similar to a number of *Pseudomonas fluorescens* isolates, lipases were shown to function at their peak at 45 °C [24]. Which are at odds with the isolated lipase enzyme from *Psychrotrophic Bacterium*, which has a temperature range of 5 to 65°C and an activity temperature of 37°C [25]. On the other hand, lipase that has been isolated from *Bacillus cereus* is most active at 55°C [26].

When the enzyme was incubated at 45°C, it maintained 100% of its activity; however, it started to lose activity. The residual activity reached 40–60% at 65°C and 55°C, respectively, and 70–85% at 25°C and 35°C, respectively (Figure 4B). These findings suggest that extracellular lipase's stability is comparable to other lipases *from Pseudomonas fluorescens* that have been documented [24]. Although greater than other lipases that have been previously documented [27].

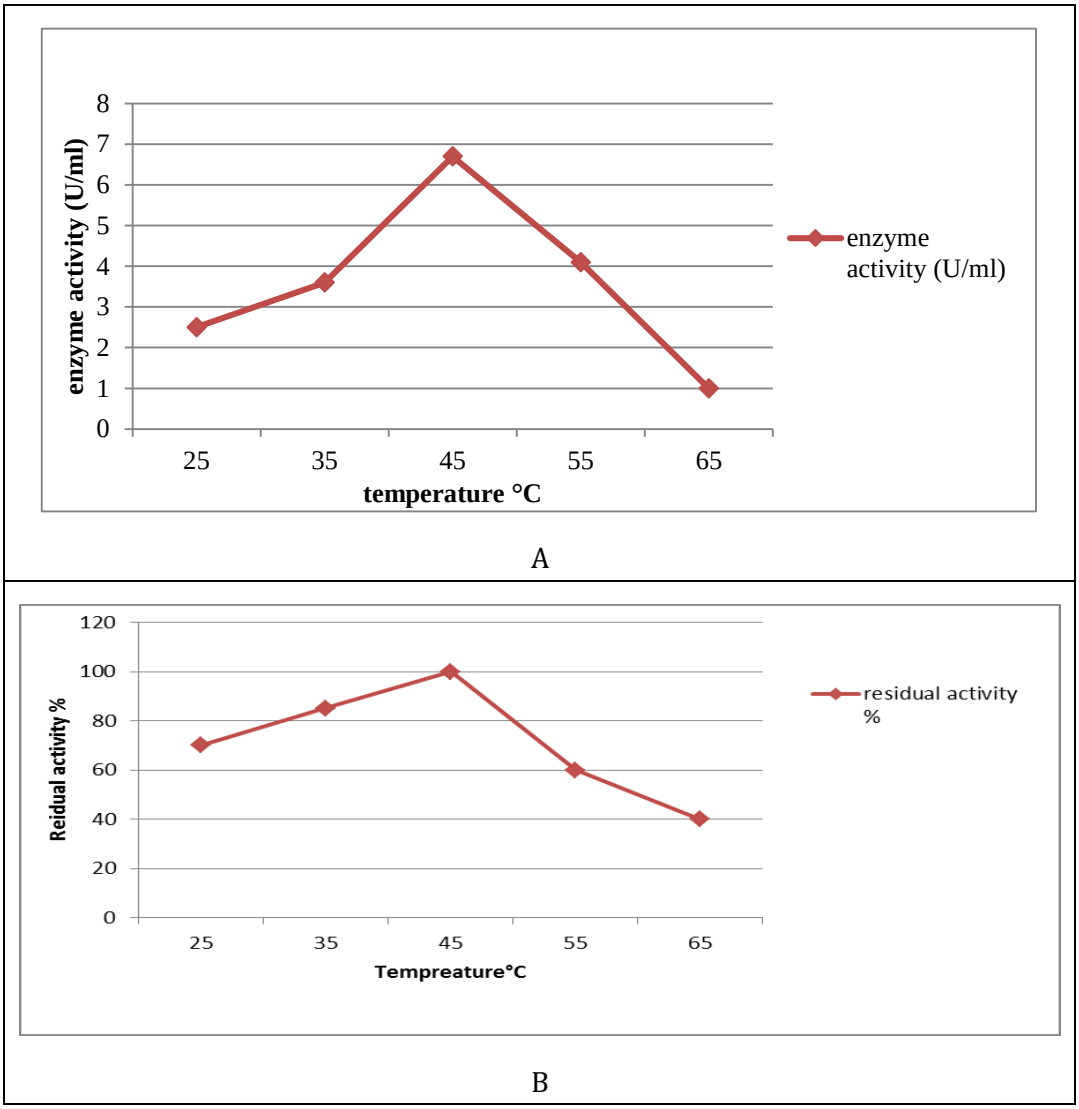


Figure 4 A: temperature's impact on *Ralstonia mannitolilytica's* pure lipase activity. **B:** temperature's impact on the stability of *Ralstonia mannitolilytica's* purified lipase.

Effect of pH Activity and Stability

pH significantly affects the activity of enzymes because of its effects on protein structures. The pH range in which the lipase activity was evaluated was 5.0–10.0, with pH 8.0 revealing the greatest activity. The pH stability of the enzyme was assessed at 45 °C for a duration of 30 minutes, throughout a range of pH values (pH 5.0–10.0). As seen in (Figure 5A). Additionally, it was noted by Akhter *et al.* [26] that pH 8.0 was ideal for *Bacillus cereus* lipase. These findings conflict with the finding that *Ralstonia sp.* prefers a pH of 7.5 [23]. On the other hand, Phukon *et al*. [28] discovered that *Pseudomonas helmanticensis* lipase preferred a pH of 7.0. The enzyme showed 100% activity at pH 8.0, 76% activity at pH 7.0, 15% activity at pH 6.0, and 0% activity at pH 5.0. The enzyme also quickly lost much of its activity, with pH 9.0 and pH 10.0 having 50% and 23% of the enzyme's original activity left, respectively (Figures 5B). These findings suggest that extracellular lipase's stability is comparable to other lipases from *Pseudomonas fluorescens* that have been described [24].

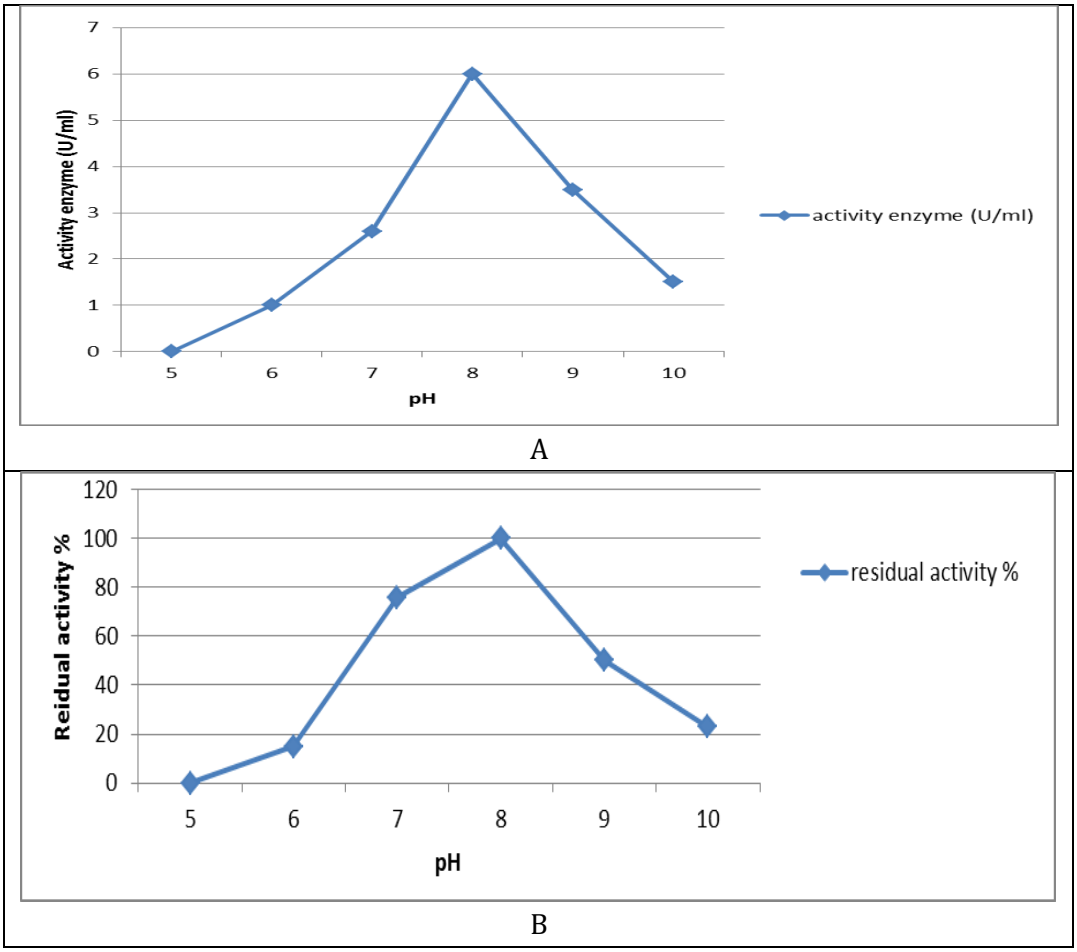


Figure 5 A: The impact of pH on *Ralstonia mannitolilytica's* pure lipase activity. **B:** The stability of pure lipase produced by *Ralstonia mannitolilytica* is affected by pH.

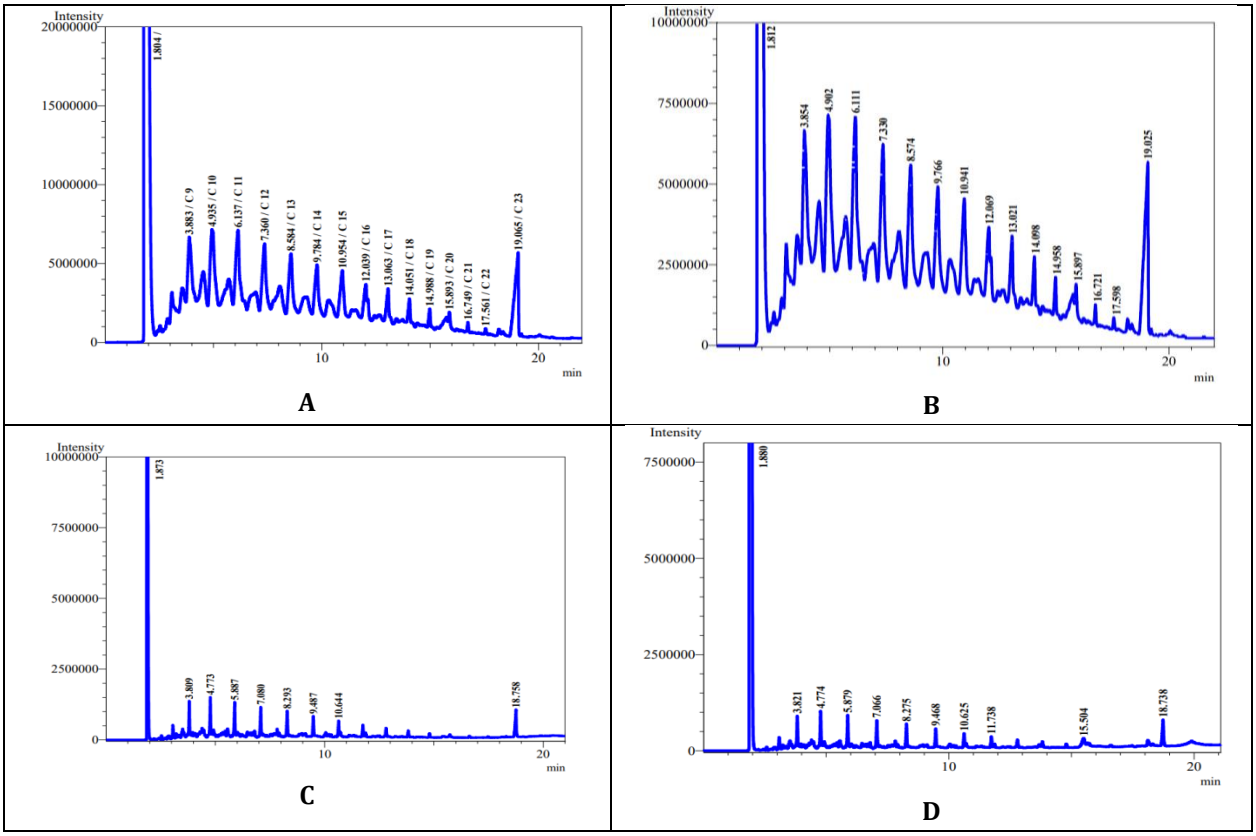
Biodegradation of Diesel Experiments

Diesel fuel, which generally has a mixture of C9 to C23 (this hydrocarbons components in diesel depending on analyzed using gas chromatography (GC-drive)) and has a boiling range of 200–350 °C, is among the crude oil's intermediate distillates products [29]. The reaction mixture was then incubated at 37°C while the diesel was treated with lipase enzyme, which was obtained from a local strain known as *Ralstonia mannitolilytica*. The same enzymes and different dosages of lipase (100%, 50%, and 25%) were used in subsequent experiments. Enzyme that had been inactivated by heat was utilized as a control. After 24 hours of incubation, samples were analyzed using gas chromatography (GC-drive) to evaluate hydrocarbon biodegradation Figure (6 A,B,C,D and E). The results in Table 2.

Table 2: Show the concentration and percentage of degradation of hydrocarbons components in diesel.

Sr.	Name of hydrocarbon component	Control per ppm	treatment by 100% concentration of lipase		treatment by 50% concentration of lipase		treatment by 25% concentration of lipase	
			concentration of degradation per ppm	%	concentration of degradation per ppm	%	concentration of degradation per ppm	%
1	C9	206	5	97.5	3	98.5	3.3	98.3
2	C10	932.9	4.9	99.4	1.7	99.8	12.6	98.6
3	C11	1169	5.3	99.5	3.1	99.7	8.6	99.2
4	C12	1310	1.4	99.8	3.4	99.7	12	99
5	C13	1563	4	99.7	3	99.8	15.8	98.9
6	C14	2143	2	99.9	1.5	99.9	0.7	99.9
7	C15	3064	6	99.8	2.4	99.9	12.5	99.5
8	C16	2471	UDL	100	3.6	99.8	13.8	99.4
9	C17	4576	UDL	100	6	99.8	18.2	99.6
10	C18	9818	UDL	100	UDL	100	30.5	99.6
11	C19	17028	UDL	100	UDL	100	UDL	100
12	C20	7189	UDL	100	UDL	100	206.9	97.1
13	C21	27462	UDL	100	UDL	100	UDL	100
14	C22	21625	UDL	100	UDL	100	UDL	100
15	C23	163.5	4.3	97.3	3	98.1	19.5	88

(UDL= Under Detection Limited)



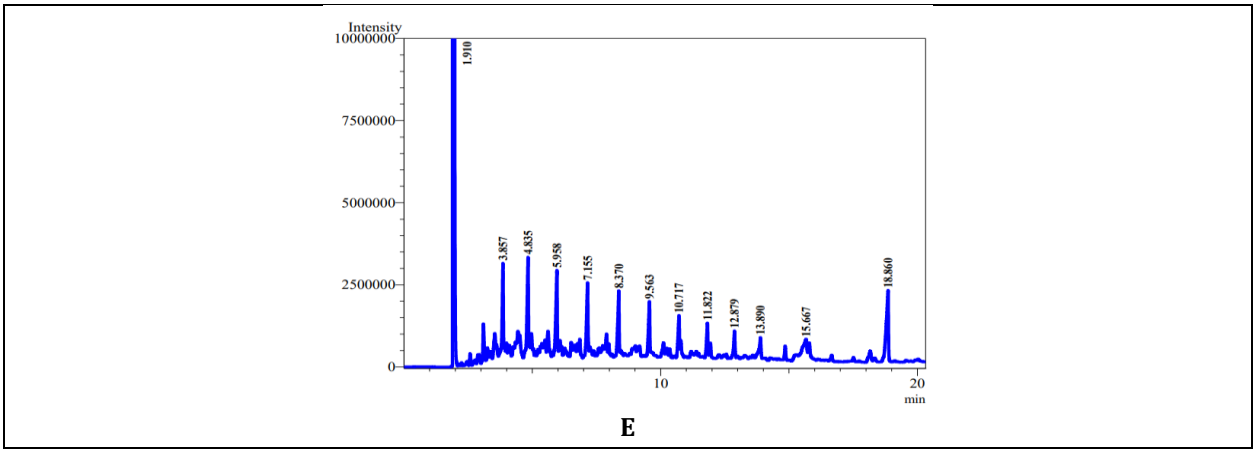


Figure 6 A: Chromatogram of the Diesel standard sample mixture of hydrocarbons in GC-Drive method. **B**: Chromatogram of the Diesel Control sample mixture of hydrocarbons in GC-Drive method. **C**: Chromatograms analysis for diesel after treated with 100% concentration of lipase in GC-Drive method. **D**: Chromatograms analysis for diesel after treated with 50% concentration of lipase in GC-Drive method. **E**: Chromatograms analysis for diesel after treated with 25% concentration of lipase in GC-Drive method.

Lipase Enzyme Reduces Diesel (Aliphatic Hydrocarbon Compounds) in Contaminated Soil

Following the identification of ten aliphatic hydrocarbon components in the diesel-affected soil samples, 10 grammes of contaminated soil samples were combined with 1 millilitre of pure lipase enzyme (7.6 U/ml). When aliphatic hydrocarbon compounds were incubated with lipase for 24 hours at natural environment to maintain natural conditions, the findings in (Table 3) demonstrated the removal effectiveness of these compounds based on the reduction in aliphatic hydrocarbon concentration following lipase treatment and comparison with control (Figure 7 A and B).

Based on the observed results, it can be stated that the breakdown of aliphatic hydrocarbon compounds by lipase enzyme, which was isolated from local bacterial isolates, was an effective biological therapy. *Ralstonia mannitolilytica* considered one of the microorganisms that have the higher ability in lipolytic enzymes production, As a result, the most efficient lipase isolation was selected, the enzyme was purified from it and used in the biodegradation of aliphatic hydrocarbons in diesel-contaminated soils, not only the previous studies showed the use of such bacterial isolates and lipase enzyme for oil spill bioremediation; nonetheless, research has been done on bioremediation employing other bacterial isolate types and enzymes. This explains the increased availability of carbon in soils contaminated with petroleum derivatives as a result of oil spills makes more carbon available to bacteria. In contrast, three isolates of *Pseudomonas aeroginosa* were recovered and identified from the Al-Gharraf oil field by Rasheed et al. [30]. They came to the conclusion that the isolated microorganisms may be considered a viable medium for the advancement of better techniques for oil recovery.

New bacterial strains were isolated and identified in a research by Hamzah *et al.* [31] from crude oil at the Al-Rafidain oil field in Basrah, Iraq. The researchers found that many different

kinds of bacteria from different genera were recovered from crude oil, along with isolates of *Pseudomonas sp*. Prior studies have demonstrated that the majority of microbial species involved in the degradation of petroleum in soil samples are bacteria. These bacterial isolates were found in surface soil, indicating that they were able to survive in the oil-contaminated environment, while the oil's unfavourable conditions eliminated the isolates that were unable to thrive in this environment [32].

Table 3: The Lipase enzyme breaks down the components of aliphatic hydrocarbons in diesel-contaminated soil.

Sr.	Name of hydrocarbon component	Control per ppm	treatment by 100% concentration of lipase for 24 hr.	
			concentration of degradation per ppm	%
1	C9	35.7	0.75	98.
2	C10	32.8	1.4	95.7
3	C11	30.7	3.4	88.9
4	C12	38.1	2.6	93.1
5	C13	43.4	2.8	93.5
6	C14	38.5	1.5	96.1
7	C15	54.5	UDL	100
8	C16	113.9	UDL	100
9	C17	4.8	UDL	100
10	C23	48.6	2.1	95.6

(UDL= Under Detection Limited)

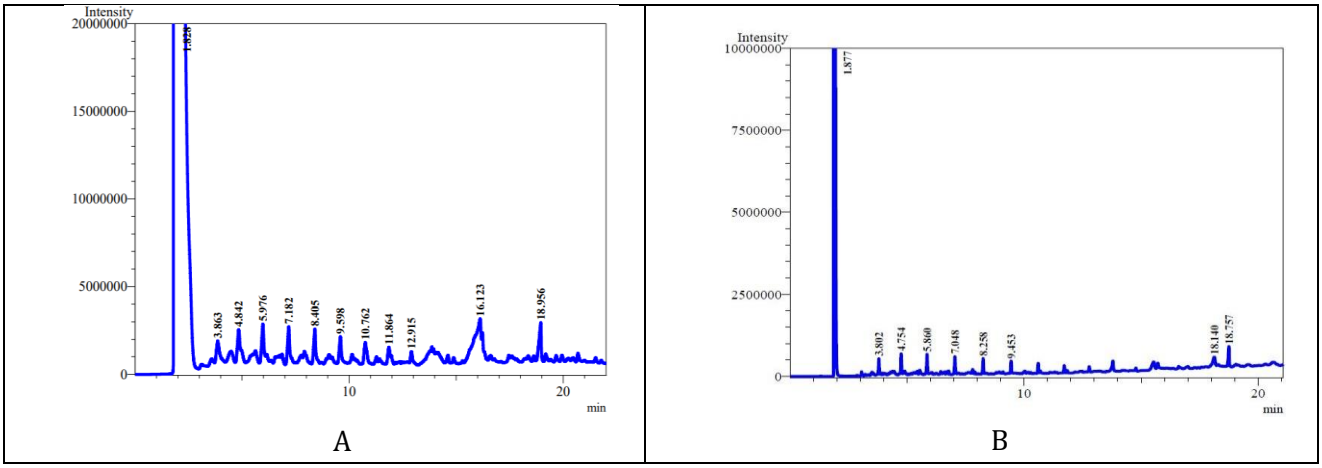


Figure 7. A: Chromatogram of soil contaminated by diesel sample mixture of aliphatic hydrocarbons in dichloromomethans solvent by GC-Drive method. **B:** Chromatograms analysis for soil contaminated by diesel after treated with lipase in dichloromomethans.

Conclusion

The most effective isolate for generating lipase enzyme with crude lipase activity (1.66 U/ml) and purified lipase activity (7.6 U/ml) is *Ralstonia mannitolilytica*. After treating soils polluted with diesel due to oil spills, concentration 100% produced outstanding bioremediation outcomes. It is determined that the biological treatment of aliphatic hydrocarbon compounds degradation by isolated lipase enzyme from novel local *Ralstonia*

mannitolilytica was effective. Such outcomes provide most cost-effective remediation's strategy by removing operating and transportation costs at both local oil-contaminated and environmental friendly sites. When used in conjunction with other treatments, it may also be seen as a simple, safe approach because it produced little waste, disrupted the environment only slightly, degraded a wide range of toxins, and required just a moderate amount of funding.

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المعالجة الحيوية الجديدة بواسطة اللايبيز المنقى من رالستونيا مانيتوليتيكا للهيدروكربونات البترولية

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معلومات المؤلف

الايميل:

الخلاصة:

تعد مشكلة التربة الملوثة بسبب مشتقات النفط مشكلة متزايدة في العراق. تحتاج الكشف عن طرق جديدة للمعالجة البيولوجية. يهدف العمل الحالي إلى تنقية إنزيم اللايبيز خارج الخلية الذي يمكنه التخلص من الملوثات النفطية. تم جمع سبع سلالات من البكتيريا المحللة للدهن من تربة محطة كهرباء الدورة بغداد / العراق الملوثة بالمشتقات النفطية. تعتبر رالستونيا مانيتوليتيكا ذات قدرة أعلى في إنتاج الإنزيمات المحللة للدهون. أعطت فعالية اللايبيز الخام (1.66 وحدة / مل). نتج عن إجراء التنقية 8.9 أضعاف مع عائد نهائي بنسبة 23.2٪. تم تأكيد نقاوة اللايبيز والوزن الجزيئي بواسطة SDS-PAGE. تنخفض كفاءة عشرة مركبات هيدروكربونية أليفاتية في التربة الملوثة بالديزل بعد معالجتها بالإنزيم المنقى (7.6 وحدة / مل) لمدة 24 ساعة. لمحاكاة البيئة الطبيعية قياساً بعينة المقارنة. لخصت النتائج إلى أن تحلل مركبات الهيدروكربون الأليفاتية بواسطة إنزيم اللايبيز المنقى من عزلات رالستونيا مانيتوليتيكا المحلية كان علاجًا فعالًا (بيولوجيًا). على الرغم من وجود دراسات متعلقة بالمعالجة الحيوية استخدمت أنواعًا وأنزيمات بكتيرية معزولة مختلفة مثل اجناس الزائفة لكن لم يستخدم مسبقاً إنزيم اللايبيز كعلاج حيوي ضد ملوثات المشتقات النفطية المعزول من رالستونيا مانيتوليتيكا.