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*Research article*

# Biodegradation of Oil and Grease in Synthetic Wastewater Using Lipase-Producing Bacteria Isolated from University Canteen Wastewater

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**Abstract:** This study aims to isolate lipase-producing bacteria from university canteen wastewater and assess their effectiveness in the degradation of oil and grease in synthetic wastewater. The results showed that the bacteria isolated from the grease trap wastewater of the central canteen exhibited a bacterial count of 9.3 × $10^8$ CFU/mL. In total, 11 bacterial isolates, labeled A1 to A11, were obtained, displaying six distinct colony morphologies. Lipase-producing bacteria were selected based on the size of the clear zone formed, with isolates A6 and A11 being selected for further evaluation. The effectiveness of these isolates in degrading oil and grease in synthetic wastewater was then assessed. The optimal initial concentration of oil and grease for bacterial degradation was found to be 10,480 mg/L at 32 °C over 40 hours. Bacteria A6 and A11 demonstrated average degradation efficiencies of 24.52% and 18.14%, respectively. Therefore, this study serves as a preliminary laboratory investigation aimed at generating essential data for the potential future applications in treating wastewater contaminated with oil and grease.

**Keywords:** Canteen wastewater; lipase; oil degradation

## 1. Introduction

Wastewater contaminated with cooking oil, which contains fatty acids and glycerin, presents a significant challenge for decomposition and disposal due to its low water solubility. The formation of an oil layer on the water's surface hinders the penetration of oxygen and sunlight, thereby negatively impacting aquatic ecosystems. Furthermore, oil droplets can interact with suspended particles in wastewater, leading to blockages in drainage systems and disrupting wastewater treatment processes. Additionally, when exposed to high temperatures in open environments, cooking oil undergoes oxidation, altering its properties and contributing to the formation of carcinogenic byproducts [1]. Therefore, it is essential to eliminate oil and grease from wastewater before treatment.

While physical and chemical methods for removing oil and grease are effective, they tend to be expensive and may produce secondary pollutants [1, 2]. Currently, bioremediation and biological treatment technologies have emerged as effective wastewater treatment approaches due to their high efficiency, cost-effectiveness, environmental sustainability, and other advantages [3, 4]. Bacteria play a crucial role in wastewater treatment, making

them a key microbial group in bioremediation processes. The use of bacteria capable of degrading fats and oils offers a promising alternative for wastewater treatment [5]. Numerous studies have focused on isolating and characterizing microorganisms with high efficiency in lipid degradation. Such microorganisms have been identified in various environments, including grease traps, wastewater treatment systems in industrial facilities, oil-contaminated soil, biogas digestate, and natural habitats such as mangrove sediments and wastewater-collecting canals [6–10]. Previous studies have successfully isolated various bacterial strains capable of degrading fats and oils. These include *Bacillus cereus* from effluent in a petroleum company, *Acinetobacter baumannii* from poultry processing factory effluent, *Pseudomonas aeruginosa* and *Bacillus subtilis* from crude oil, *Rhodopseudomonas* faecalis from cooking oil, *Aeromonas hydrophila* from cattle slaughterhouse effluent, and *Aeromonas jandaei* from restaurant wastewater [7–12]. These bacteria play a crucial role in wastewater treatment by producing lipase enzymes, which break down fats and oils into fatty acids and glycerol. The bacteria then utilize these byproducts as energy sources [13]. Bacterially derived lipases have broad applications in both industrial processes and wastewater treatment, including aerobic and anaerobic degradation systems [14]. Lipases are classified within the triacylglycerol ester hydrolase family (EC 3.1.1.3) and are capable of facilitating both esterification and hydrolysis reactions. Their high stereospecificity and catalytic efficiency make them valuable in a wide range of industries, such as detergents, cosmetics, food processing, leather manufacturing, biodiesel production, and wastewater treatment [8]. Moreover, lipase-producing bacteria play a significant role in the biodegradation of wastewater contaminated with oil and grease. While several studies have discovered lipase-producing bacteria from different sources, few have specifically focused on wastewater from university canteens in Thailand, which represents a locally relevant and underexplored environment. This study set the hypothesis that locally isolated bacteria from university canteen wastewater can efficiently reduce oil and grease concentration under laboratory surroundings and that strains demonstrating high lipase activity (indicated by larger clear zones) are capable of degrading higher oil and grease concentrations.

University cafeterias generate wastewater contaminated with oil and grease daily. The authors recognize that isolating and evaluating the efficiency of lipase-producing bacteria in degrading oil and grease could provide a natural and effective approach to wastewater treatment. These bacteria could be utilized to enhance the biodegradation of oil and grease, contributing to a more sustainable and efficient wastewater management system. This study aims to isolate naturally occurring local bacteria from environments contaminated with oil and grease in university canteens and investigate their efficiency in degrading fats and oils in synthetic fat-containing wastewater. The findings will provide fundamental data and guidelines for improving the efficiency of wastewater treatment systems. Utilizing bacteria isolated from their natural environment is expected to enhance treatment effectiveness compared to using microorganisms sourced from other locations, as they have already undergone natural selection.

## 2. Materials and Methods

### 2.1 Isolation of bacteria from wastewater samples

Wastewater samples were collected from the grease trap of the cafeteria at Thammasat University's Lampang Campus, located in Hang Chat District, Lampang Province, Thailand, as shown in Figure 1.

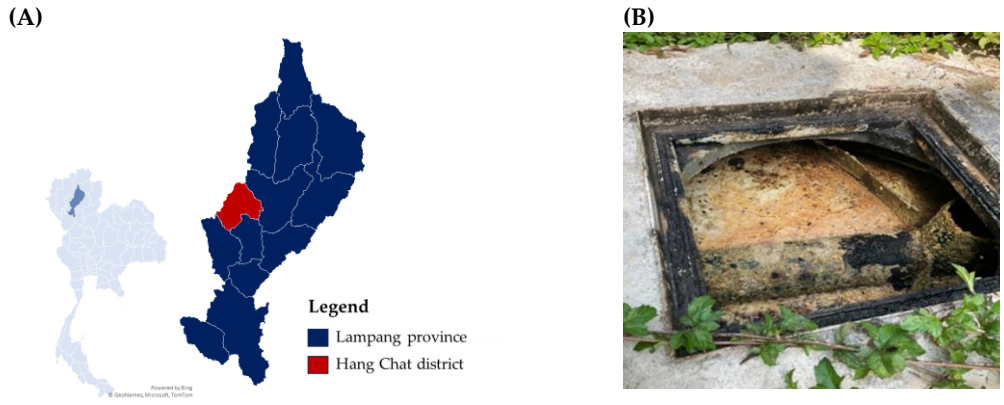


**Figure 1.** Study site and sampling point. (A) Thammasat University's Lampang Campus, Hang Chat District, Lampang Province, Thailand. (B) The cafeteria grease trap was used as the sampling point in this study.

A 5-mL sample was inoculated into 100 mL of Luria-Bertani (LB) broth in a 250 mL Erlenmeyer flask and incubated on a shaker at 150 rpm at 37 °C for 48 hours. Subsequently, a 10-fold serial dilution was performed using LB medium, and dilutions ranging from $10^{-6}$ to $10^{-10}$ were spread-plated onto solid LB agar in Petri dishes. The plates were incubated at 37 °C for 24 hours, and the colonies were counted. Bacterial concentrations in colony-forming units (CFU/mL) were calculated using Equation (1), taking the dilution factor and plated volume into account, and considering plates with 30–300 colonies:

The viable bacterial concentration was verified by plate counting. Colony-forming units (CFU/mL) were calculated using the following equation:

$$CFU/mL = \frac{\text{Number of colonies} \times \text{Dilution factor}}{Volume\ plate\ (mL)} \quad (1)$$

Colonies were then randomly selected from plates with diverse morphologies and streaked onto LB agar to obtain single, pure colonies. The plates were incubated at 37 °C for 24–48 hours until distinct colonies were obtained. The purified bacterial strains were preserved on nutrient agar slants at 4 °C for further use [15]. The resulting bacterial counts, calculated using the described dilution method, are reported in the Results and Discussion section. All environmental and microbial samples were handled following standard biosafety procedures to prevent environmental contamination. Wastewater samples were chlorinated before discharge, and bacterial cultures on plates or in flasks were autoclaved before disposal.

### 2.2 Selection of oil- and grease-degrading bacteria

Pure bacterial isolates were streaked onto solid tributyrin agar, a medium used for screening lipase enzyme production [15]. The plates were incubated at 37 °C for 48 hours, after which the size of the clear zones formed around the colonies was measured. The two bacterial isolates producing the largest clear zones on the tributyrin agar were selected and cultured in 100 mL of nutrient broth (NB) in a 250 mL Erlenmeyer flask. The cultures were incubated in a shaking incubator at 150 rpm and 37 °C for 24 hours. The clear zones forming around the bacterial growth on the surface of the medium were observed, and the size of the zones was measured. The bacteria capable of producing clear zones were then gram-stained using the gram-staining technique, and the results were examined under a microscope to assess the staining and bacterial morphology.

### 2.3 Efficiency of lipase-producing bacteria in degrading oil and grease from synthetic wastewater

#### *2.3.1. Preparation of synthetic wastewater*

The preparation of synthetic wastewater was adapted from the experiment conducted by Chitpirom and Sangaroon [6]. The synthetic wastewater consisted of 0.1 g of starch, 0.1 g of powdered milk, 0.1 g of sugar, 0.1 mL or 0.5 mL of palm oil (to obtain two initial concentrations of oil and grease), along with 40 mL of distilled water. The mixture was placed in a 250 mL Erlenmeyer flask and sterilized by autoclaving at 15 psi and 121 °C for 15 minutes.

#### *2.3.2 Efficiency of lipase-producing bacteria in synthetic wastewater*

The efficiency of lipase-producing bacteria in synthetic wastewater was tested based on the method used by Mookkhan et al. [15], with modifications. Two bacterial isolates producing the largest clear zones were selected. After increasing the bacterial concentration, the turbidity was adjusted to approximately 0.5 at $OD_{600}$. The bacterial suspension was then inoculated into three replicate Erlenmeyer flasks (250 mL) containing synthetic wastewater, while three additional flasks containing synthetic wastewater without bacteria served as controls. All flasks were incubated at 30–37 °C with shaking at 200 rpm for 40 hours. The concentration of oil and grease in both bacterial-treated and control flasks was determined using the Soxhlet extraction method with an automatic solvent extractor (VELP Scientifica, SER 158 Series), following Standard Methods AWWA 5520, Part D. The biodegradation experiments were conducted using two bacterial isolates (A6 and A11) at initial oil and grease concentrations of 2,350 and 10,480 mg/L. Each treatment was subjected to triplicate independent tests. Uninoculated flasks (medium with oil and grease but without bacterial inoculation) were included as controls. After incubation, the residual oil and grease content was quantified, and the percentage removal efficiency was calculated as in Equation (2):

$$\text{Removal efficiency (\%)} = \frac{C_{initial} - C_{final}}{C_{initial}} \times 100 \quad (2)$$

where $C_{initial}$ is the initial oil and grease concentration (mg/L), and $C_{final}$ is the concentration after incubation.

All experiments were performed in triplicate to ensure reproducibility, with both negative and positive controls included to validate experimental outcomes. Instruments were regularly calibrated using certified standards, and standard analytical procedures were strictly followed. Measurement results were reported to a precision consistent with the method and rounded to the nearest mg/L to reflect the instrument's accuracy and reliability. Data were carefully checked for consistency to minimize experimental errors.

### 2.4 Statistical analysis

All experiments were performed in triplicate, and the data were presented as mean ± standard deviation. Removal efficiencies were compared between treatments using independent t-tests at each initial oil concentration. One-way analysis of variance (ANOVA) followed by post hoc comparisons was applied to further confirm differences among isolates at the same concentration. In addition, a two-way ANOVA was conducted to assess the main effects of isolate type, initial oil concentration, and their interaction on removal efficiency. Statistical analyses were performed using Python. A significance level of $p < 0.05$ was considered statistically significant.

## 3. Results and Discussion

### 3.1 Colony characteristics of bacteria isolated on LB medium

The plates were incubated at 37 °C for 24 hours to enumerate bacterial colonies and select isolates. The bacterial count in the grease trap was $9.3 \times 10^8$ CFU/mL. Six distinct colony morphologies were observed, comprising a total of 11 colonies. This is consistent with the study by Mookkhan et al. [15], who reported a bacterial count of $4.3 \times 10^9$ CFU/mL in cafeteria wastewater, with four different colony morphologies and 20 colonies. The bacterial count differed from previous studies on restaurant wastewater, where the highest count of lipase-producing bacteria was $1.62 \times 10^4$ CFU/mL across five samples [10]. The six distinct isolates were streaked onto LB medium and incubated at 37 °C for 24 hours to obtain single, pure colonies. Colony characteristics—including size, color, shape, elevation, surface, and edge—were recorded on LB medium, as summarized in Table 1. As noted in the table, isolates exhibiting identical colony characteristics are grouped for clarity, with further details provided in the table footnote.

**Table 1.** Colony characteristics of bacteria isolated on LB Medium

| Isolate | Colony characteristics | | | | | |
|---|---|---|---|---|---|---|
| | **Sizes** | **Color** | **Shape** | **Elevation** | **Surface** | **Edge** |
| A1 | Large | Creamy-white | Cocci | Flat | Smooth | Entire |
| A2, A9, A11 | Large | Yellow | Cocci | Convex | Smooth | Undulate |
| A3 | Small | Creamy-white | Cocci | Convex | Smooth | Undulate |
| A4 | Small | Creamy-white | Cocci | Flat | Smooth | Undulate |
| A5, A8, A10 | Large | Creamy-white | Spiral | Flat | Smooth | Undulate |
| A6, A7 | Small | Creamy-white | Cocci | Flat | Smooth | Undulate |

**Note:** Isolates grouped in the same row exhibited identical colony characteristics on LB medium.

### 3.2 Morphology of isolated bacteria and selection of lipase-producing bacteria

Gram-staining of the 11 bacterial isolates was performed to observe their shape and staining characteristics under a microscope. The results, shown in Table 2, were consistent with those of Attapong [16], who reported that bacteria isolated from restaurant wastewater were gram-negative, short rod-shaped, and randomly arranged. Notably, isolate A6 was gram-positive, whereas all other isolates were gram-negative, suggesting that A6 may belong to a different bacterial genus or species, which could also relate to its relatively strong lipase activity. This observation also aligns with the research of Sukplang et al. [10], who found that some bacteria isolated from restaurant wastewater were gram-positive, with various shapes such as bacilli, short rods, and cocci. The pure bacterial isolates were cultivated on tributyrin agar, a medium used for screening bacteria capable of degrading oils and fats. Lipase production was indicated by the hydrolysis of tributyrin, a triglyceride. The hydrolysis products were glycerol and fatty acids [14]. After incubating at 37 °C for 48 hours, five bacterial isolates produced clear zones around the colonies, namely isolates A6, A7, A8, A10, and A11, as shown in Table 2. The two isolates with the largest clear zones, A6 and A11, had clear zone diameters of 9.4 ± 0.48 mm and 9.6 ± 0.48 mm, respectively (Figure 2). The bacterial clear zone diameters on tributyrin agar were larger than those reported by Mookkhan et al. [15], who found clear zone diameters ranging from 4.5 to 7.5 mm, but smaller than those observed by Attapong [16], who reported clear zone diameters ranging from 11.5 to 20.25 mm. The presence of a wide, clear zone is an initial test indicating that the bacteria are producing lipase to degrade tributyrin.

**Table 2.** Morphological characteristics and clear zone sizes of bacterial isolates

| Isolate | Morphology of isolate bacteria | | | Size of clear zone (mm) |
|---|---|---|---|---|
| | **Gram stain** | **Shape** | **Arrangement** | |
| A1 | Negative | Short rod | Scatter | - |
| A2 | Negative | Short rod | Scatter | - |
| A3 | Negative | Short rod | Scatter | - |
| A4 | Negative | Short rod | Scatter | - |
| A5 | Negative | Short rod | Linear | - |
| A6 | Positive | Short rod | Scatter | 9.4 ± 0.48 |
| A7 | Negative | Short rod | Scatter | 9.1 ± 0.85 |
| A8 | Negative | Short rod | Scatter | 8.8 ± 0.48 |
| A9 | Negative | Short rod | Scatter | - |
| A10 | Negative | Short rod | Scatter | 8.6 ± 0.25 |
| A11 | Negative | Short rod | Scatter | 9.6 ± 0.48 |

**Note:** Size of the clear zone (mm) is the measurement of the colony's clear zone diameter, and (-) indicates no clear zone formation.

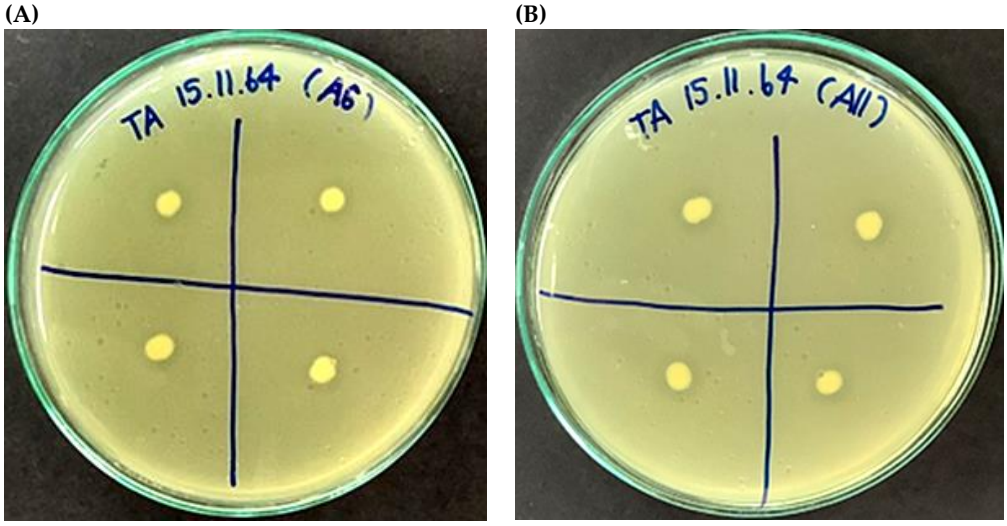


**Figure 2.** Clear zone formation on tributyrin agar indicates extracellular lipase production. Yellow bacterial colonies are surrounded by distinct halos (clear zones). (A) Isolate A6 and (B) isolate A11.

### 3.3 Preliminary efficiency of bacteria in degrading oil and grease in synthetic wastewater

Bacteria capable of producing lipase enzymes were selected to test their efficiency in degrading oil and grease. Lipase-producing bacteria degrade oil and grease by enzymatically binding to their molecules. This results in a hydrolysis reaction, in which lipase breaks the ester bonds between the hydroxyl group of glycerol and the carboxyl group of fatty acids, producing glycerol and fatty acids as the final products [17]. The bacterial isolates A6 and A11 were selected for the degradation test due to their ability to produce the largest clear zones, indicative of lipase activity, on tributyrin agar. Palm oil was used as the substrate for the degradation test, as numerous studies have demonstrated that most lipase-producing bacteria degrade palm oil more effectively than other oils. To study the efficiency of these lipase-producing bacteria in degrading oil and grease, the initial concentrations of oil and grease in the synthetic wastewater were 2,350 and 10,480 mg/L, respectively. A broader concentration range could be explored in future studies to further optimize the treatment conditions. The current study focused on a selected range based on preliminary experiments, which provided initial insights into treatment efficiency. The bacterial concentration of isolates A6 and A11 was adjusted to approximately 0.5 based on turbidity measured at a wavelength of 600 nm. The control group consisted of synthetic wastewater with identical initial concentrations of oil and grease, 2,350 and 10,480 mg/L, respectively. The results of the degradation test revealed that, at an initial oil and grease concentration of 2,350 mg/L, isolates A6 and A11 exhibited degradation efficiencies of 11.26% and 18.01%, respectively, resulting in remaining concentrations of 2,086 and 1,927 mg/L. At an initial oil and grease concentration of 10,480 mg/L, isolates A6 and A11 showed degradation efficiencies of 24.52% and 18.14%, respectively, resulting in remaining concentrations of 7,911 and 8,579 mg/L, as shown in Figure 3 and Table 3. The reported oil and grease degradation efficiencies of 24.52% and 18.14% for isolate A6 and A11, respectively, were relatively low compared with removal efficiencies achieved in well-optimized biodegradation systems. For example, previous studies have found that the biodegradation of oil and grease under favorable conditions ranges from 70–95% with average values around 88% in well-tuned activated sludge systems [18]. The limitation of removal in the present study suggests the microbial community may have been constrained in hydrophobic degradation and recalcitrant oil and grease fractions. The affected factors likely include toxicity from high oil concentrations, obstructing microbial growth and enzyme function [19]. Poor availability caused by oil and grease droplet formation or adsorption onto solids and insufficient activity of key hydrolytic or oxidative enzymes (e.g., lipases and monooxygenases) are needed to disrupt complex molecules [20]. To improve the degradation performance, several enhancement strategies can be considered. Effective oil and grease mineralization typically requires a balanced supply of nitrogen, phosphorus, trace elements, and, in many cases, a co-substrate to stimulate cometabolic pathways [21-22]. Co-culturing approaches, for instance,

combining bacteria with fungi, can also be effective since fungi are capable of breaking down complex hydrophobic fractions, thereby increasing substrate availability for bacterial degraders. Another promising approach is the immobilization of microbial cells or enzymes on carriers such as alginate beads, activated carbon, or synthetic polymers, which not only protect cells from toxic effects but also maintain high local biomass and enzyme concentrations. Taken together, these limitations highlight the need for targeted enhancement strategies to overcome biological and operational blockages and raise oil and grease removal above the relatively low levels currently observed.

The removal efficiencies of isolate A6 and A11 were compared at two initial oil and grease concentrations (2,350 and 10,480 mg/L). Independent t-tests indicated significant differences between isolates at both concentrations. At 2,350 mg/L, isolate A11 achieved significantly higher removal efficiency than isolate A6 ($t = -7.04$, $p = 0.011$). In contrast, at 10,480 mg/L, isolate A6 exhibited significantly higher removal efficiency than isolate A11 ($t = 16.47$, $p < 0.001$). One-way ANOVA confirmed that removal efficiencies differed significantly between isolates at both concentrations ($F = 49.61$, $p = 0.002$ at 2,350 mg/L; $F = 271.42$, $p < 0.001$ at 10,480 mg/L). A two-way ANOVA was conducted to evaluate the effects of isolate type, initial oil concentration, and their interaction on removal efficiency. The analysis showed that isolate type ($F = 0.096$, $p = 0.765$) had no significant main effect, while initial concentration had a highly significant effect ($F = 166.03$, $p < 0.001$). Importantly, the interaction effect between isolate type and initial concentration was also highly significant ($F = 162.24$, $p < 0.001$), indicating that the performance of each isolate depended on the initial oil concentration. The statistical analyses revealed that oil removal efficiency was strongly influenced by both the initial concentration of oil and grease and the microbial isolate used. Although no overall main effect of isolate type was observed when concentrations were pooled, the significant interaction demonstrates that removal performance is context-dependent. At lower initial oil concentrations (2,350 mg/L), isolate A11 showed superior performance compared to isolate A6, suggesting that isolate A11 may be more effective under moderate contamination levels. Conversely, at higher concentrations (10,480 mg/L), isolate A6 exhibited significantly higher removal efficiency than isolate A11. This reversal implies that isolate A6 possesses adaptive or enzymatic mechanisms, allowing it to cope better with elevated oil loads. Such interaction effects highlight the importance of considering environmental conditions, particularly pollutant concentration, when evaluating the bioremediation potential of microbial isolates. The findings suggest that while isolate A11 could be preferable in systems with moderate oil contamination, isolate A6 may be more advantageous for environments with high pollutant loads. This concentration-dependent performance provides valuable insight into the optimization of isolate selection for wastewater treatment applications.

The results indicate that as the initial oil and grease concentration increases, the degradation efficiency of the bacteria reduces because of various correlated physicochemical and biological constraints. Excessive oil and grease in synthetic wastewater can form a continuous film or thick layer on the water surface, hindering oxygen transfer and limiting aerobic microbial respiration [23]. The resulting reduction in oxygen availability reduces bacterial growth and declines lipase enzyme production, thereby decreasing degradation efficiency. Furthermore, at high oil loads, hydrophobic compounds tend to combine and adsorb onto solid surfaces, deteriorating their bioavailability to bacteria [24]. Elevated concentrations of oil and grease can also exert toxic effects, disrupting cell membranes, enzyme structures, and overall metabolic activity. Collectively, these factors inhibit microbial growth and lipase activity, leading to lower biodegradation performance under high initial oil and grease concentrations. These findings align with the study by Attapong [16], who observed that at an initial oil and grease concentration of 1,500 mg/L, *Bacillus pumilus* exhibited an average degradation efficiency of 86.13%. Furthermore, a study by Thongkrua et al. [25] found that at an initial concentration of 1,500 mg/L, *Bacillus pumilus* showed an average degradation efficiency of 85.19%, while *Serratia aquinivoran* showed a degradation efficiency of 76.5%. The experimental results indicate that the incubation duration significantly influences the oil and grease degradation efficiency of lipase-producing bacteria. The incubation period was set to 40 hours based on the research by Kotlakome et al. [26], who found that *Bacillus pumilus* exhibited the highest oil and grease degradation efficiency at 40 hours, with an average degradation efficiency of 99.47%. According to Attapong [16], the initial concentration of oil and grease was 1,500 mg/L, with incubation at 35 °C for 40 hours. The degradation efficiencies of *Bacillus pumilus* LWW2, *Bacillus pumilus*

LWW8, *Bacillus pumilus* LWW9, and *Serratia quinivorans* LWW13 were 86.13 ± 0.24, 86.79 ± 0.13, 91.24 ± 0.10, and 76.50 ± 0.36%, respectively. This time period was identified as the phase when *Bacillus pumilus* experienced stable growth. The results suggest that the duration of oil and grease degradation is dependent on the bacterial strain, as different strains produce lipase enzymes under varying conditions and time frames. The findings show that isolates A6 and A11 demonstrate lower oil degradation efficiency, suggesting that these isolates may require a longer duration to degrade oil and grease more effectively. Furthermore, the experimental results reveal that temperature affects the efficiency of oil and grease degradation in lipase-producing bacteria. The incubation temperature was maintained at an average of 32 °C, falling within the optimal range for bacterial growth and lipase production, as most bacteria thrive at temperatures between 30 and 37 °C [27]. Temperatures that are either too low or too high can adversely affect bacterial growth, resulting in reduced lipase production. In addition, temperature extremes can affect the stability of lipase enzymes, leading to structural changes and decreasing their catalytic efficiency [28]. Consequently, bacteria are less efficient at degrading oil and grease in synthetic wastewater. Overall, these findings demonstrate that while isolates possess oil and grease degrading capability, their performance under the setting conditions was less efficient than that of *Bacillus pumilus.* This may reflect variation in enzyme kinetics, regulatory mechanisms, and environmental adaptability. Future optimization of culture parameters such as temperature, incubation period, and initial oil and grease concentrations may increase the degradation efficiency of these isolates.

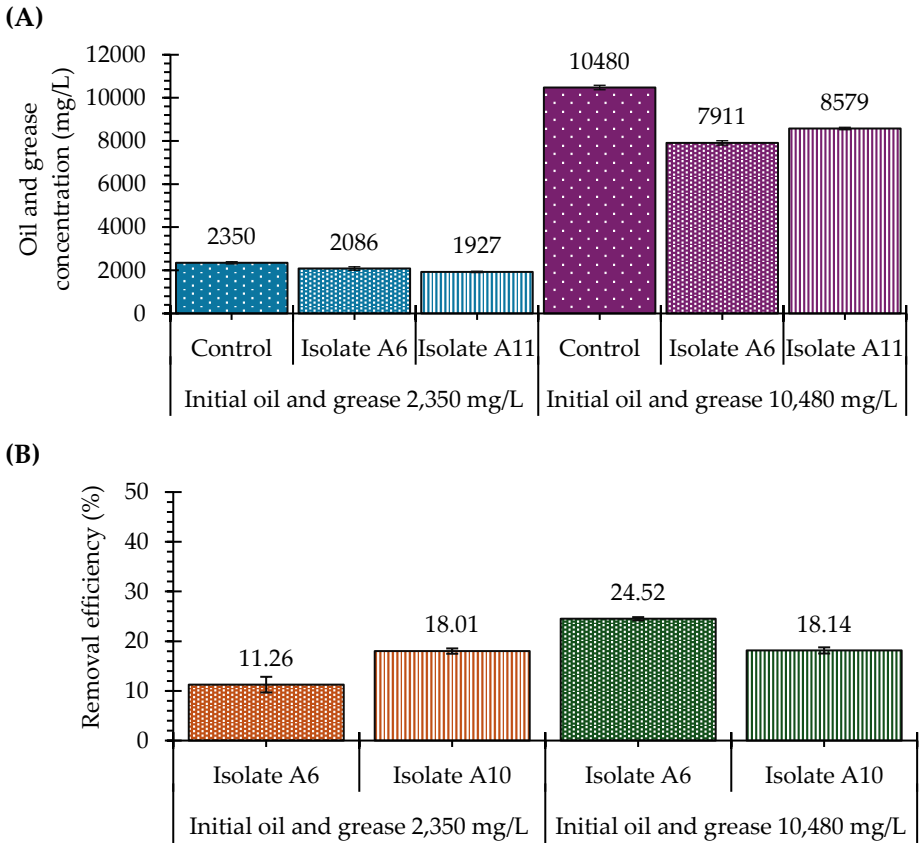


**Figure 3.** Comparison of (A) oil and grease concentrations and (B) removal efficiencies of oil and grease by isolate A6, isolate A11, and the uninoculated control at different initial concentrations.

**Table 3.** Comparison of oil and grease degradation efficiency with previous research

| Inoculum | Effluent/ isolation condition | Type of tested oil | Initial oil and grease concentration | Degradation temperature (°C) | Time (hour) | Oil and grease removal (%) | Reference |
|---|---|---|---|---|---|---|---|
| *Aeromonas jandaei* | Restaurant wastewater/ 1 g of immobilized cells on a scrub pad ($10^8$ CFU/g) in 100 mL of effluent, 30 °C, 150 rpm | Various cooking oils | 3% (W/V) | 30 | 48 | 98.37 | [10] |
| WA5 | Canteen University/ Streak pure culture on tributyrin agar, 37 °C, 48 hours | Vegetable oil | - | 37 | 168 | 20.44 | [15] |
| | | Lard | - | | | 52.84 | |
| *Bacillus pumilus. strain* UBU5 | Fresh market/0.1 mL culture on screening medium, 37 °C, 24 hours | Palm oil | 1,500 mg/L | 30 | 40 | 86.11 | [19] |
| *Bacillus pumilus* | Restaurant/ 10 µL pure culture on tributyrin agar, 37 °C, 48 hours | Palm oil | 1,500 mg/L | 30 | 32 | 85.19 | [18] |
| *Meyerozyma quilliermondii* | Restaurants and canteens/ Streak on Tween 20 medium, 72 hours | - | - | Room temperature | 168 | 83.03 | [29] |
| A6 | Canteen University/ Streak pure culture on tributyrin agar, 37 °C, 48 hours | Palm oil | 2,350 mg/L | 30-37 | 40 | 11.26 ± 1.57 | This study |
| A11 | | | | | | 18.01 ± 0.53 | |
| A6 | | | 10,480 mg/L | | | 24.52 ± 0.32 | |
| A11 | | | | | | 18.14 ± 0.62 | |

### 3.4 Limitations of the research and further studies

This study has several limitations and serves as an initial exploration of the effectiveness of oil and grease degradation. The experimental conditions were restricted, with only two oil concentrations tested without the systematic optimization of key parameters such as pH, temperature, or incubation time. In

addition, the bacterial isolates (A6 and A11) were only characterized morphologically and not taxonomically identified; modern molecular approaches such as 16S rRNA sequencing should be employed for accurate classification. The assessment of lipase activity was also limited, relying mainly on qualitative observations of clear zones without reporting enzyme activity in standard quantitative units or providing data on enzyme kinetics. Furthermore, the study did not examine the broader environmental factors influencing lipid degradation, including nutrient availability and physicochemical parameters such as temperature range, pH levels, salinity, oil types, oil and grease concentrations, and duration of the degradation process [30]. These limitations reduce the applicability of the findings to real wastewater systems. Future research should therefore prioritize the molecular identification of lipase-producing bacterial strains, alongside the systematic characterization and quantification of lipase enzyme activity, before assessing their efficiency under a wider range of environmental conditions. Additionally, the future work should focus on optimizing culture conditions to enhance lipase production and oil–grease degradation and on assessing the process at pilot scale or in real wastewater treatment systems to determine its practical applicability. Table 4 summarizes the factors affecting lipase enzymes in reducing oil and grease concentrations in wastewater, adapted from previous studies [1, 30] and the findings of this present study.

**Table 4.** Factors affecting lipase enzymes to reduce oil and grease concentrations in wastewater, adapted from [1, 30]

| Aspects | Factors |
|---|---|
| Source | Plant |
| | Microbe |
| | Animal |
| Target microorganism | Choice of microbial strain |
| | Population size |
| | Natural or inoculated microorganism |
| Substrate characteristics | Concentration |
| | Type of substrate |
| | Nutrient availability |
| | Salinity |
| Environmental conditions | Temperature |
| | pH |
| | Metal ions and inhibitors |
| | Electrostimulation |
| Immobilization | Free enzyme |
| | Immobilized enzyme |
| Time interval | Duration of the degradation |

## 4. Conclusions

This preliminary study investigated lipase-producing bacteria isolated from oil- and grease-contaminated wastewater collected from a university cafeteria grease trap. Six distinct bacterial strains were obtained, of which five exhibited lipase activity. Among them, isolate A6 showed the highest degradation efficiency, reducing oil and grease by approximately 24.52% under laboratory conditions. These findings provide baseline data on the lipase activity and biodegradation potential of bacteria from a locally relevant source. The results serve as a foundation for future research focusing on molecular identification, optimization of culture and degradation conditions, and evaluation of performance in pilot-scale or real wastewater treatment systems.

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**Author Contributions:** Conceptualization, P.K. and Y.S.; methodology, Y.S., P.S. and A.P.; formal analysis, Y.S., P.S., S.S. and A.P.; investigation, P.S. and A.P.;. writing—original draft preparation, P.K. and Y.S.; writing—review and editing, P.K. and Y.S.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## References

[1] Nimkande, V. D.; Bafana, A. A Review on the utility of microbial lipases in wastewater treatment. *J. Water Process Eng*. **2022**, *46*, 102591. https://doi.org/10.1016/j.jwpe.2022.102591

[2] Montagnolli, R. N.; Lopes, P. R. M.; Bidoia, E. D. Applied models to biodegradation kinetics of lubricant and vegetable oils in wastewater. *Int. Biodeterior. Biodegrad*. **2009**, *63(3)*, 297–305. https://doi.org/10.1016/j.ibiod.2008.06.002

[3] Wang, T.; Wang, W.; Hu, H.; Khu, S.-T. Novel quantitative evaluation of biotreatment suitability of wastewater. *Water* **2022**, *14*(7), 1038. https://doi.org/10.3390/w14071038

[4] Zahri, K. N. M.; Zulkharnain, A.; Sabri, S.; Gomez-Fuentes, C.; Ahmad, S. A. Research trends of biodegradation of cooking oil in Antarctica from 2001 to 2021: A bibliometric analysis based on the scopus database. *Int. J. Environ. Res. Public Health* **2021**, *18*(4), 2050. https://doi.org/10.3390/ijerph18042050

[5] Prasad, M. P.; Manjunath, K. Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacteria species. *Indian J. Biotechnol.* **2011**, *10*(1), 121–124.

[6] Chitpirom, K.; Sangaroon, P. Detection of lipolytic bacteria from environmental samples. *J. Public Health* **2012**, *42*(3), 3–18.

[7] Hassan, S. W. M.; Abd El Latif, H. H.; Ali, S. M. Production of cold-active lipase by free and immobilized marine *Bacillus cereus* HSS: Application in wastewater treatment. *Front. Microbiol.* **2018**, *9*, 2377. https://doi.org/10.3389/fmicb.2018.02377

[8] Bunmadee, S.; Teeka, J.; Lomthong, T.; Kaewpa, D.; Areesirisuk, P.; Areesirisuk, A. Isolation and identification of a newly isolated lipase-producing bacterium (*Acinetobacter baumannii* RMUTT3S8-2) from an oily wastewater treatment pond in a poultry processing factory and its optimum lipase production. *Bioresour. Technol. Rep.* **2022**, *20*, 101267. https://doi.org/10.1016/j.biteb.2022.101267

[9] Haselroth, K. J.; Wilke, P.; Costa, I. M. D.; Rotta, V. M. L.; Rosado, A. F.; Hermes, E. Effectiveness of *Aeromonas hydrophila* for the removal of oil and grease from cattle slaughterhouse effluent. *J. Clean. Prod.* **2021**, *287*, 125533. https://doi.org/10.1016/j.jclepro.2020.125533

[10] Sukplang, G.; Narasettiti, C.; Chunhachart, O.; Srithaworn, M.; Lorliam, W.; Krajangsang, S.; Panyachanakul, T. Isolation and identification of lipase-producing bacteria from a wastewater treatment pond for the removal of oil and grease in wastewater treatment. *Chiang Mai J. Sci.* **2024**, *51*(6), e2024096. https://doi.org/10.12982/CMJSci.2024.0096

[11] Abubakar, A.; Abioye, P. O.; Aransiola, S. A.; Raju, M. N.; Prasad, R. Crude oil biodegradation potential of lipase produced by *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated from hydrocarbon contaminated soil. *Environ. Chem. Ecotoxicol.* **2024**, *6*, 26–32. https://doi.org/10.1016/j.enceco.2023.12.001

[12] Saejung, C.; Phonaiam, S.; Kotthale, P.; Chaiyarat, A. Bacterial cellulose as a reinforcement material of alginate beads improves effectiveness and recycling potential of immobilized photosynthetic bacteria for cooking oil waste removal. *Carbohydr. Polym.* **2024**, *324*, 121532. https://doi.org/10.1016/j.carbpol.2023.121532

[13] Zulaika, A.; Rahman, H.; Ningrum, S. S.; Maulida, A. F. Exploring microbial lipases: Screening and identification for biocatalyst potential in bioethanol synthesis from glycerol-based biodiesel waste. *Results Eng.* **2024**, *23*, 102427. https://doi.org/10.1016/j.rineng.2024.102427

[14] Chandra, P.; Enespa; Singh, R.; Arora, P. K. Microbial lipases and their industrial applications: A comprehensive review. *Microb. Cell Fact.* **2020**, *19*, 169. https://doi.org/10.1186/s12934-020-01428-8

[15] Mookkhan, P.; Asa, P.; Noisamdaeng, P. Isolation and lipid decomposition efficiency of bacteria isolated from canteen wastewater. *Sci. Technol. RMUTT J.* **2017**, *7*(2), 241–250.

[16] Attapong, M. Study on lipolytic bacteria efficiency for fat, oil, and grease degradation in synthetic wastewater. M.Sc. Thesis, University of Phayao, Phayao (Thailand), **2017**.

[17] National Center for Biotechnology Information (NCBI). Biochemistry, Lipase. https://www.ncbi.nlm.nih.gov/books/NBK537346/ (accessed 2025-10-02).

[18] Cisterna-Osorio, P.; Arancibia-Avila, P. Comparison of biodegradation of fats and oils by activated sludge on experimental and real scales. *Water* **2019**, *11*(6), 1286. https://doi.org/10.3390/w11061286

[19] Al Khatib, M. F. R.; Alqedra, F.; Alam, M. Z. Biodegradation of fats, oil and grease using microorganisms isolated from palm oil mill effluent. *IIUM Eng. J.* **2023**, *24*(2), 1–10. https://doi.org/10.31436/iiumej.v24i2.2590

[20] Cui, J.; Dörmann, P. Microbial degradation of hydrocarbons from petroleum assisted by biosurfactants: Pathways and bioremediation potential. *Biochimie* (in press, journal pre-proof). https://doi.org/10.1016/j.biochi.2025.09.011

[21] Leys, N. M.; Bastiaens, L.; Verstraete, W.; Springael, D. Influence of the carbon/nitrogen/phosphorus ratio on polycyclic aromatic hydrocarbon degradation by *Mycobacterium* and *Sphingomonas* in soil. *Appl. Microbiol. Biotechnol.* **2005**, *66*(6), 726–736. https://doi.org/10.1007/s00253-004-1568-8

[22] Feng, J. Y.; Zhou, X. D. Effects of co-substrates and inorganic ions on biodegradation of oil-degrading strains. *Adv. Mater. Res.* **2011**, *179–180*, 781–786. https://doi.org/10.4028/www.scientific.net/AMR.179-180.781

[23] Aziz, Z. S.; Jazaa, S. H.; Dageem, H. N.; Banoon, S. R.; Balboul, B. A.; Abdelzaher, M. A. Bacterial biodegradation of oil-contaminated soil for pollutant abatement contributing to achieve sustainable development goals: A comprehensive review. Results Eng. 2024, 102083. https://doi.org/10.1016/j.rineng.2024.102083

[24] Usman, M.; Salama, E.; Arif, M.; Jeon, B.-H.; Li, X. Determination of the inhibitory concentration level of fat, oil, and grease (FOG) towards bacterial and archaeal communities in anaerobic digestion. Renew. Sustain. Energy Rev. 2020, 131, 110032. https://doi.org/10.1016/j.rser.2020.110032

[25] Thongkruea, S.; Attapong, M.; Sanpha, S. The efficiency of bacteria in degradation of fat, oil, and grease in synthetic wastewater. *Acad. J. Sci. Technol. Nakhon Sawan Rajabhat Univ.* **2018**, *10*(11), 75–88.

[26] Kotlakome, R.; Suttipanta, N.; Thongplew, N. Enhancing efficiency of grease traps through the addition of lipase-producing bacteria for wastewater management in the fresh markets. *Sci. Essence J.* **2020**, *36*(2), 41–54.

[27] Ali, S.; Khan, S. A.; Hamayun, M.; Lee, I.-J. The recent advances in the utility of microbial lipases: A review. *Microorganisms* **2023**, *11*(2), 510. https://doi.org/10.3390/microorganisms11020510

[28] Al-Rehaili, T. T.; Danial, E. N.; Ayaz, N. O. Improvement of lipase thermal stability using immobilization techniques. *Res. J. Pharm. Biol. Chem. Sci.* **2018**, *9*(4), 519.

[29] Nguyen, N. T.; Cao, N. D. Isolation and identification of lipid-degrading yeast from wastewater of canteens and restaurants in Ninh Kieu District, Can Tho City. *CTU J. Innov. Sustain. Dev.* **2017**, *7*, 27–32. https://doi.org/10.22144/ctu.jen.2017.045

[30] Gupta, R.; Gupta, N.; Rathi, P. Bacterial lipases: An overview of production, purification, and biochemical properties. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 763–781. https://doi.org/10.1007/s00253-004-1568-8