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ORIGINAL PAPER

Optimization of fat, oil, and grease (FOG) biodegradation using consortium of bacteria

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Abstract

This study investigates the potential of a bacterial consortium isolated from palm oil mill effluent (POME) to degrade Fats, Oils, and Grease (FOG). Enrichment techniques were employed to isolate a consortium capable of degrading FOG by through the incorporation of used cooking oil (UCO) into the nutrient media. The consortium, composed of three distinct bacterial strains with FOG-degrading abilities, was screened using a lipolytic activity test on Tween 20 agar media. Results identified consortium X3X4 as the most effective consortium, displaying the highest growth and FOG degradation. Optimization experiments, used a 2-level factorial design to explore the impact of bacterial inoculum concentration (2%, 6%, 10% v/v), oil concentration (1%, 3%, 5% v/v), and pH (6, 7, 8) on FOG biodegradation. Statistical analysis revealed that both oil concentration and bacterial inoculum concentration significantly influenced degradation compared to pH. Consortium X3X4, consisting of the Micrococcus lylae strain DSM 20315 and Corynebacterium aurimucosum strain, exhibited optimal FOG degradation, achieving 82.7% degradation after 20 days of incubation. From the kinetic analysis, the consortium's μ_{max} and K_s values were calculated as 0.04 h^{-1} and 4.86% v/v UCO, respectively. This study underscores the efficacy of the bacterial consortium, particularly consortium X3X4, in achieving substantial FOG biodegradation under optimal conditions. The study demonstrates the consortium's potential for wastewater treatment, though it is limited to laboratory-scale experiments. Practical industrial applications will require additional research to address scaling and operational constraints.

Keywords FOG · Bacterial consortium · Biodegradation · Used cooking oil

Introduction

Fats, oils, and grease (FOG) are ubiquitous byproducts of various food processing activities, generated in slaughterhouses, kitchens, restaurants, and even households (Long et al. 2012). This includes a wide range of food components, such as vegetable oils, cereals, butter, nuts, meats, and lard. However, food service establishments (FSEs) are the primary contributors to FOG, often due to the improper disposal of leftover food fats and cooking oil down drains (Husain et al. 2014). Kitchen wastewater can contain alarmingly high concentrations of FOG, reaching up to 21,500 mg/L (Witharana et al. 2018). Effective FOG removal is crucial, as its presence has been linked to several detrimental consequences, including sewage system overflows (SSOs), sewer blockages, and reduced oxygen levels in waterways.

The accumulation of FOG within sewer systems poses a significant threat to both environmental and public health worldwide, demanding immediate intervention (Alkhatib et al. 2015). The primary concern lies in FOG deposition on the interior walls of pipelines, leading to blockages and subsequent sanitary sewer overflows (SSOs) (Fan 2014). These events incur significant costs associated with sewer cleaning and repairs, while also causing public inconvenience due to unpleasant odors.

Triglycerides, formed by the esterification of glycerol with three fatty acids, constitute a major component of FOG (Simoneit, n.d.; Williams et al. 2012). Grease traps, designed

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with specific geometric configurations, serve as one of the primary physical methods for FOG removal from municipal wastewater. These units aim to prevent FOG from entering pipelines and are typically constructed from concrete, steel, or plastic (Weber, 1990).

However, current physical and mechanical methods for FOG removal often lack effectiveness and exhibit limitations in managing FOG levels within sewer systems. Consequently, recent research has increasingly favored biological solutions over traditional methods for treating FOG, recognizing their advantages in terms of efficiency, cost-effectiveness, safety, and environmental friendliness (Jain et al. 2011).

Biodegradation has emerged as a highly effective technique for degrading various oils. This process offers numerous benefits, including high efficiency, environmental sustainability, and faster degradation times without generating unpleasant odors (Basumatary et al. 2012). The fundamental concept relies on utilizing microorganisms within wastewater systems to break down organic compounds. Under carefully controlled conditions, these microorganisms perform a vital role in converting these compounds into safer or less harmful products (Santisi et al. 2015). Bacteria, along with algae, fungi, and plants, are commonly employed in biodegradation processes. Selecting suitable microorganisms is paramount for optimizing the biodegradation process's efficacy. For instance, (Nzila et al. 2016) successfully employed both single and mixed consortia of bacterial strains, including Sphingobacterium sp., Pseudomonas poae, Pseudomonas aeruginosa, Pseudomonas libanensis, and Stenotrophomonas rhizophila, to treat oil and grease (O&G) in sewage water. While both consortia effectively degraded cooking oil, the mixed culture demonstrated superior performance compared to single strains.

The effectiveness of biodegradation relies heavily on the biodegradation capabilities of the employed microbial population. Therefore, utilizing mixed cultures harboring a diverse range of microorganisms presents a promising strategy for complex FOG treatment. These mixed cultures exhibit greater resilience to unstable conditions and possess the ability to perform more complex tasks compared to monocultures (Kumari et al. 2017). Consequently, incorporating mixed cultures into the bioremediation of FOG waste within wastewater represents a significant step forward.

This study aims to identify the most effective bacterial consortium for FOG degradation. Three bacterial strains, Micrococcus lylae (X3), Corynebacterium aurimucosum (X4), and Novobiosepticus (X6), were selected to form the bacterial consortia.

These strains were isolated from palm oil mill effluent (POME), chosen as a microbial source due to its high concentration of microbes adapted to oil-based waste. POME provides a favorable environment for a diverse array of microorganisms (Agualimpia et al. 2016). Also, the strains' origin from palm oil mill effluent (POME) suggests their ability to thrive in environments rich in organic matter and tolerate higher temperatures. This is particularly relevant given that POME is not only abundant in oil but is also typically discharged at elevated temperatures, often ranging from 80 to 90°C (Ismail et al. 2014).

Used palm oil cooking oil (UCO) was selected as the substrate to identify the optimal bacterial consortium. The rationale behind using UCO over fresh cooking oil (CO) lies in its similar saturated fat content compared to FOG deposits. (Ducoste 2009) reported that the saturated fat content of cooking oil and FOG deposits ranges between 7 to 20% and 20 to 90%, respectively. While various cooking oils are used in FSEs, their primary difference lies in fatty acid composition. To minimize the influence of oil type on the results, the same oil was used throughout the experiments. Additionally, a lipolytic activity screening test was conducted to identify FOG-degrading consortia and determine the optimal consortium. Finally, the performance of the selected bacterial consortium was evaluated using a 2-level factorial design, investigating various parameters such as bacterial inoculum concentration, oil concentration, and pH. Each experimental condition was performed in triplicate to ensure the reproducibility and reliability of the results. The mean values were calculated and reported accordingly.

Materials and methods

Materials

Bacterial strains

This study employed three bacterial strains isolated from palm oil mill effluent (POME): Micrococcus lylae (X3), Corynebacterium aurimucosum (X4), and Novobiosepticus (X6). These strains demonstrate FOG-degrading capabilities (Al Khatib et al. 2023) and are available upon request from the Chemical Engineering and Sustainability Department, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM). They were maintained on agar media at 4 °C.

Chemical reagents

Nutrient agar, Luria–Bertani (LB) broth, Sodium chloride (NaCl), Dipotassium hydrogenphosphate (K_2HPO_4), Ammonium Chloride (NH_4Cl), Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), Iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$), Calcium chloride dihydrate ($CaCl_2.2H_2O$).

MSM preparation

Mineral Salts Medium (MSM) was prepared by dissolving 0.1 g NaCl, 1.8 g K2HPO4, 4.0 g NH4Cl, 0.2 g MgSO4.7H2O, and 0.01 g FeSO4.7H2O in 1 L of distilled water. The solution was then autoclaved at 121 °C for 15 min (Zajic & Supplisson 1972).

Methods

Bacterial consortium preparation

To form successful bacterial consortia, compatible cultures were combined to achieve enhanced degradation (Sarkar et al. 2013). Based on a study by Al Khatib et al. (2023), strains exhibiting efficient FOG degradation were selected to prepare consortia containing two or three strains each (Table 1).

The preparation of consortia involved several steps. Agar media were prepared by dissolving 25 g agar powder in 1 L of distilled water in a conical flask until fully dissolved. The media were autoclaved at 121 °C for 15 min and poured onto sterile Petri plates.

To obtain pure colonies, sub-culturing was performed on new nutrient agar plates by streaking bacterial strains. Pure colonies were then streaked onto the prepared media. A control plate without bacterial streaks was included to ensure no contamination. Cultures and controls were incubated overnight at 37 °C.

For inoculum preparation, bacteria were inoculated into 250 mL shake flasks containing 100 mL LB broth, covered with aluminum foil and parafilm to prevent contamination, and shaken for 12 h at 250 rpm and 37 °C. Each liquid culture was transferred to a sterile Falcon tube and centrifuged for 15 min at 5000 rpm to remove the supernatant. Then, 0.5 mL of each culture (OD 600≈1) (Rahman et al. 2002) was added to 100 mL of fresh LB broth. One flask containing only LB broth without inoculation served as a blank. All flasks were shaken for 5 days at 250 rpm and 37 °C. To identify the consortium with the highest growth, the prepared broths were used to measure and monitor bacterial consortium growth using a spectrophotometer at an optical density (OD) of 600 nm.

Table 1 Bacterial consortia composition from bacterial strains

Single strain	Bacterial consortium
X3	X3 + X4
X4	X4 + X6
X6	X3 + X6
	X3 + X4 + X6

Selection of the best bacterial consortium

The prepared bacterial consortia were inoculated into 250 mL shake flasks containing 99 mL of Mineral Salts Medium (MSM) and 1 mL of palm oil-based cooking oil as the sole carbon source. These flasks were incubated at 37 °C and 250 rpm for 5 days. The growth of each consortium was monitored and assessed using a UV/vis spectrophotometer at an optical density of 600 nm (OD600). Based on the observed growth in terms of OD600 values, the consortium exhibiting the most robust growth was selected for further investigation.

Screening for lipolytic activity

A screening test was conducted to evaluate the feasibility of using the selected bacterial consortium for the biodegradation of FOG. Tween 20 peptone agar medium was prepared according to (Tzirita 2012). In 1000 mL of distilled water, 10 mL Tween 20, 5 g NaCl, 10 g peptone, 0.1 g $CaCl_2.2H_2O$, and 20 g agar were mixed. The prepared medium was autoclaved for 20 min at 121 °C before being poured into sterile Petri plates. The selected bacterial consortium was then streaked and inoculated onto the Tween 20 medium plates. These inoculated plates were incubated at 37 °C for 4 days. The formation of opaque halos around each bacterial consortium was carefully examined. The presence of such halos around the colonies would indicate the potential of the consortium to degrade lipids, supporting its potential suitability for FOG biodegradation.

Growth curve of the selected bacterial consortium

To determine the growth curve of the selected consortium, a loopful of its liquid culture was inoculated into a 250 mL shake flask containing 99 mL of MSM liquid medium and 1 mL of palm oil-based used cooking oil. The MSM liquid medium cultures were cultured for 4 days at 250 rpm and 37 °C. Throughout this period, the growth of the selected consortium was monitored at regular intervals (every 6–8 h) using a spectrophotometer to measure its optical density (OD600). This procedure provided a detailed representation of the growth dynamics of the chosen consortium under the specified conditions.

Growth kinetics for the selected bacterial consortium

To assess the growth kinetics of the selected bacterial consortium, it was cultured at varied concentrations of used cooking oil (UCO) ranging from 0.1 to 2.0% v/v in the MSM medium. These cultures were observed for 5 days to monitor UCO degradation and bacterial growth. For each UCO concentration, the optical density (OD600) was measured using

a UV/vis spectrophotometer. Additionally, the starting and final bacterial concentrations for each UCO concentration were determined over the 5-day period using a standard cell counting method. Based on this data, the rate of growth (μ) was calculated using the following equation:

$$\mu = \frac{\ln X_f - \ln X_i}{time} \quad (1)$$

where μ is the rate of growth (d^{-1}), X_i and X_f are the initial and final bacterial concentration (CFU/mL), respectively.

The saturation constant (K_s) and the maximum growth rate (μ_{max}) were calculated using Monod Eq. (2) by plotting the FOG concentrations against their growth rates.

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}}\frac{1}{C} + \frac{1}{\mu_{max}} \quad (2)$$

where C is the substrate concentration.

FOG degradation optimization

Several parameters can significantly influence the selected bacterial consortium's efficiency in FOG degradation. These include the concentration of bacterial inoculum, the concentration of oil, and the pH of the culture medium. To determine the optimal conditions for maximizing the consortium's enzymatic degradation of FOG, a 2-Level Factorial Design (2 LFD) was employed. Table 2 summarizes the range of values chosen for these variables:

The FOG degradation process was optimized using Design-Expert software (version 7.0.0, STAT-EASE, US), with oil degradation percentage being the key metric. First, inoculum was prepared by adding the selected bacterial consortium to individual shake flasks containing fresh LB broth. These flasks were stoppered with cotton wool, covered with aluminum foil (for sterility and aerobic conditions), and shaken for 24 h at 30 °C and 200 rpm to promote bacterial growth. After incubation, cells were harvested by centrifugation and washed to remove residual carbon sources. Finally, the washed cell suspension was standardized and introduced into fresh flasks containing minimal salts medium and used cooking oil (UCO) as the sole carbon source. UCO concentration and pH varied according to the experimental design. Following 20 days of incubation, the remaining oil in each sample was quantified using gravimetric analysis (Latha & Kalaivani 2012). This involved extracting oil with n-hexane and calculating the percentage of oil degradation based on the separated water and oil phases. Overall, this meticulous optimization process aimed to identify the most effective conditions for FOG degradation by the selected bacterial consortium.

Table 2 Experimental variables used in 2-level factorial design

Component	Level		
	-1	0	**1**
Oil concentration (% v/v)	1	3	5
Inoculum concentration (% v/v)	2	6	10
pH	6	7	8

Gravimetric analysis for oil degradation assessment

The gravimetric method established by (Latha & Kalaivani 2012) was used to determine the percentage of oil degradation in each sample. After 15–20 days of incubation, the sample contents were transferred to separating funnels. Then, 5 mL of n-hexane, a solvent for hydrophobic lipids, was added, creating two distinct layers: a lower water phase and an upper layer containing oil and n-hexane. The upper layer was evaporated at 70 °C to remove the n-hexane, leaving behind the recovered oil. Finally, the following equations were used to calculate the percentage of fat and oil degradation.

$$\text{FOG degradation (\%)} = \frac{\text{amount of oil degraded}}{\text{amount of oil added}} \times 100 \quad (3)$$

$$\text{Amount of oil degraded} = \text{weight of oil added in the media} - \text{weight of residual oil} \quad (4)$$

$$\text{Weight of residual oil} = \text{weight of tube holding the extracted oil} - \text{weight of empty tube} \quad (5)$$

Results and discussion

Preparation of bacterial consortia

Pure colonies of the bacteria isolated from palm oil mill effluent (POME) were used to create the bacterial consortia. These colonies were obtained through repeated subculture on fresh agar plates using an inoculation loop (Fig. 1).

During the enrichment process, the initially translucent yellowish LB broth became visibly turbid after 12 h of shaking at 250 rpm and 37 °C. This increased turbidity suggests active growth and synergistic interactions within the bacterial consortia formed for this experiment.

Selection of bacterial consortium

To identify the consortium with the most efficient lipid-degrading potential, its growth activity was monitored over 5 days using a UV/vis spectrophotometer at 600 nm (OD600). The results are presented in Table 3.

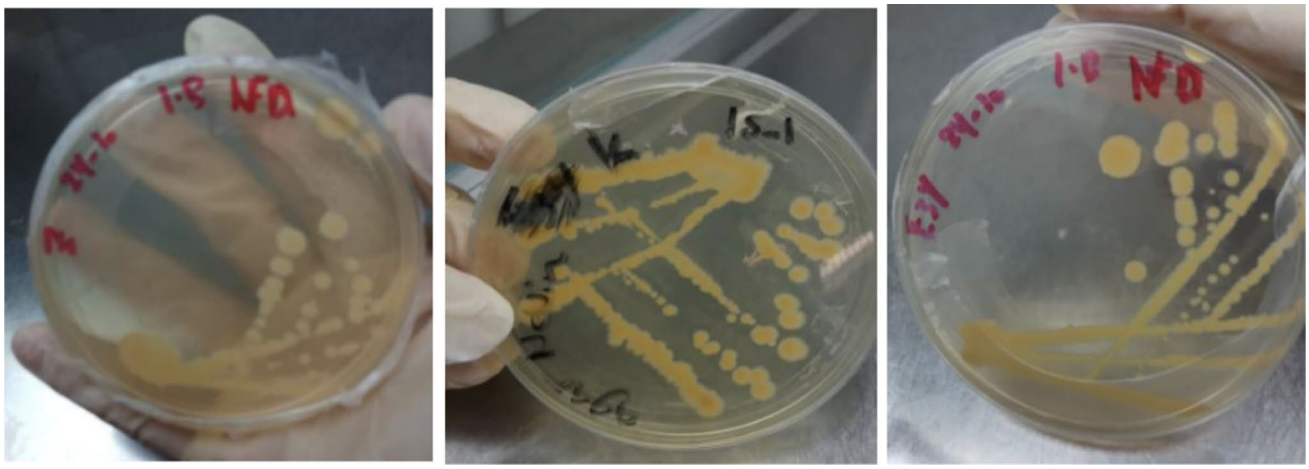

Fig. 1 Examples of purified colonies of isolated bacteria from POME

Table 3 The OD 600 readings of the bacterial consortia for 5 days

No	Consortium	OD 600 readings				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	X3X6	0.052	1.729	1.68	0.59	0.251
2	X6X4	0.048	2.001	1.902	0.351	0.221
3	X3X4	0.096	2.313	2.064	0.98	0.609
4	X3X4X6	0.031	1.684	1.635	0.26	0.052

The OD600 method relies on the measuring light scattering within a bacterial culture. As bacteria grow and multiply, their cells scatter more light, leading to higher OD values. Therefore, OD600 serves as an indirect indicator of bacterial growth within a medium.

The aim was to assess whether the growth rate of a consortium is superior to that of its individual member strains. This difference can arise due to adaptations and cooperative interactions among strains, potentially enhancing their collective growth. However, competition between strains may also occur, hindering the consortium's overall growth.

Interestingly, consortium X3X4X6, despite comprising the three fastest-growing individual strains, exhibited the slowest overall growth rate. This may suggest incompatibility between the three strains. On the other hand, consortium X3X4 achieved the highest growth rate (2.313) after two days. Compared to the consortium growth observed by (Husain et al. 2011) (less than 1.00), X3X4 demonstrated a considerably higher growth rate, potentially indicating a stronger capability for FOG degradation.

Screening of bacterial consortium

While a consortium's growth rate may not directly translate to superior FOG degradation, evaluating its degradation activity is crucial. Therefore, the selected consortium (X3X4) was screened using Tween 20 peptone agar medium to assess its efficiency in FOG degradation.

The rationale behind this screening lies in the fact that effective FOG degradation by the consortium results in Tween 20 breakdown through lipase enzyme activity. This breakdown leads to the formation of insoluble calcium salts, manifesting as opaque halos around bacterial colonies on the agar medium. These halos serve as visual indicators of the consortium's lipolytic activity and potential for FOG biodegradation.

During the screening, bacteria in the medium relied solely on Tween 20 as their carbon source. Figure 2d clearly shows a pronounced halo around the X3X4 colony, reflecting a high intensity of precipitate surrounding it. This precipitate signifies the formation of calcium salts due to Tween 20 degradation by the consortium.

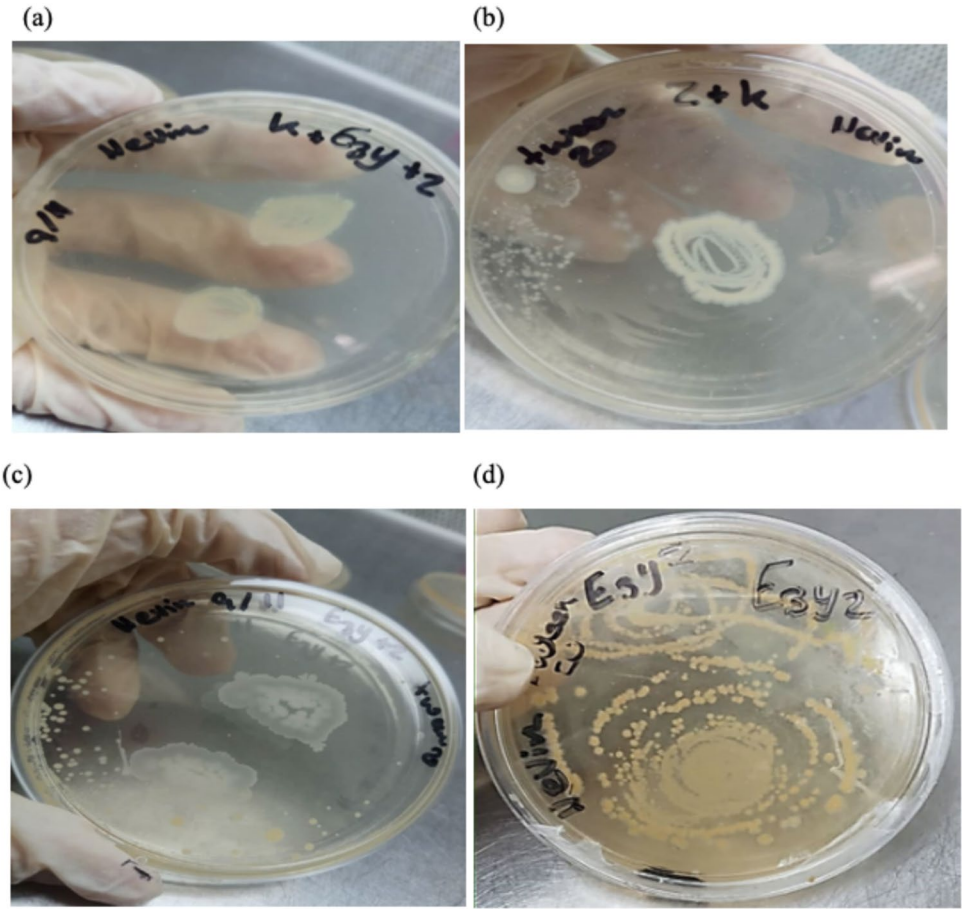

Fig. 2 Screening of lipid degradation by bacterial consortia on Tween 20 media. **a** X3X4X6, **b** X4X6, **c** X3X6 and **d** X3X4

The lipase enzyme released by the bacterial cells interacts with Tween 20, triggering the release of free fatty acids. These acids then combine with calcium ions present in the medium, ultimately resulting in the formation of insoluble calcium salts, visible as the observed precipitates (Al Khatib et al. 2023; Gopinath et al. 2013; Husain et al. 2011). The pinpoint particles of calcium salts appeared on day 2 and steadily intensified until day 4.

In contrast, the other consortia displayed minimal to moderate levels of precipitates around their colonies (Fig. 2.a, b, and c). These observations, along with the intense calcium salt formation by X3X4, suggest its significantly higher FOG degradation potential compared to the other consortia. This finding even surpasses the results obtained in the study by (Al Khatib et al. 2023) on single bacterial strain screening.

Based on these compelling results, the X3X4 consortium was chosen for further investigation and optimization of its FOG degradation capabilities.

Micrococcus (X3) and Corynebacterium (X4) bacteria are well-known for their production of lipases, also identified as triacylglycerol acylhydrolases. These powerful enzymes promote the hydrolysis of ester bonds within triglycerides, the primary constituents of fats and oils. This enzymatic process effectively breaks down complex fat and oil molecules into their simpler components: fatty acids and glycerol. (Muthukamalam et al. 2017). Many Corynebacterium species are known for synthesizing biosurfactants, which are surface-active molecules. These compounds play a crucial role by emulsifying hydrophobic substances, such as oils and hydrocarbons, thereby boosting their solubility and enhancing their availability for microbial uptake. By effectively lowering surface tension, biosurfactants improve the interaction between microbial cells and oil droplets,

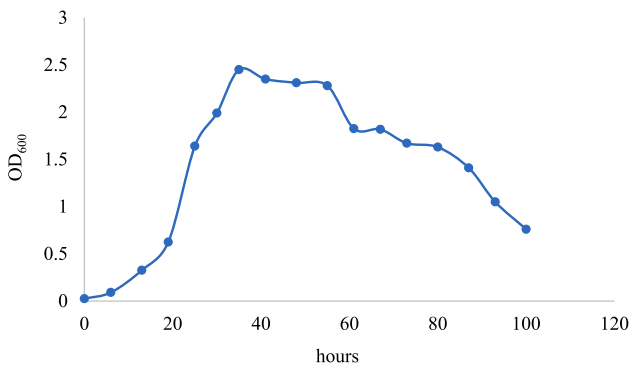

Fig. 3 The growth pattern of the consortium X3X4

consequently accelerating the degradation process (Markam et al. 2024). On the other hand, corynebacterium species show a remarkable ability for breaking down diverse hydrocarbons, including substances like crude oil and diesel. Their high effectiveness in this regard is attributed to their capacity to produce both biosurfactants and a variety of enzymes essential for the degradation process (Muthukamalam et al. 2017).

Growth curves of the selected bacterial consortium

The selected X3X4 consortium exhibited a typical bacterial growth curve with four distinct phases (Fig. 3). During the first 12 h (lag phase), the microorganisms acclimated to their new environment. Subsequently, the exponential phase (12–48 h) showed a rapid increase in bacterial numbers, indicated by a rise in absorbance from 0.326 to 2.45. This growth slowed significantly in the stationary phase (40–55 h), reaching a plateau around 2.3. Finally, nutrient depletion and/or waste accumulation triggered the death phase beyond 55 h, characterized by a decline in bacterial viability.

Interestingly, comparing the X3X4 consortium's growth curve to those of the best individual strains identified by (Al Khatib et al. 2023) revealed a noteworthy difference. While the single strains (X3 and X4) achieved maximum growth below 1.5, the X3X4 consortium reached a peak of 2.45 at 35 h. This enhanced growth likely stems from synergistic interactions between the two strains within the consortium (Jing et al. 2007), highlighting the potential benefits of consortium formation for improved FOG degradation.

Growth kinetics of the selected bacterial consortium

To explore the growth dynamics of the selected consortium under various used cooking oil (UCO) concentrations, different UCO levels were tested, and the specific growth rate (μ) was determined for each (Table 4). The highest growth rate (0.058 h^{-1}) was observed with 1% v/v UCO, aligning with previous research suggesting 1% as the optimal concentration for bacterial consortia growth (Abioye et al. 2009; Nwaogu et al. 2008). This finding aligns with (Husain et al. 2011) observation of optimal growth at 1% v/v

Table 4 Specific growth rates of the selected consortium X3X4 at different UCO concentrations

UCO (v/v %)	Specific growth rate μ (hr^{-1})
0.1	0.003
0.5	0.007
1.5	0.0077
2.0	0.0082

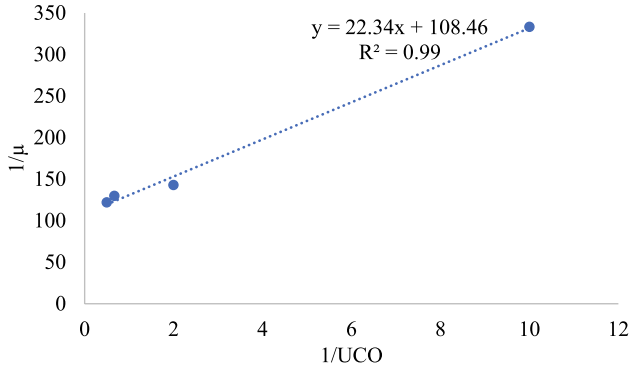

Fig. 4 The reciprocals of growth rates against the reciprocals of UCO concentrations

total petroleum hydrocarbon (TPH) concentration. Notably, increasing UCO concentrations beyond 1% led to reduced growth rates. This can be attributed to complex chemical reactions during frying, which generate inhibitory byproducts that hinder bacterial growth (Alsulaiman & Nizam 2018).

Furthermore, the linear relationship between 1/C (UCO concentration) and 1/μ (growth rate) presented in Fig. 4 enabled the calculation of the saturation constant (K_s) and the maximum growth rate (μ_{max}) as 4.86% v/v and 0.04 h^{-1}, respectively. The K_s and μ_{max} values are significantly higher than those reported by (Abbassi & Shquirat 2008; Benyahia et al. 2005) ($K_s < 1\%$ v/v) and (Abubakar 2018), who reported μ_{max} of 0.74 day^{-1} (0.03 h^{-1})and K_s of 1.23 palm oil (% v/v). Higher K_s indicates better substrate availability for bacterial growth within the chosen consortium, and higher μ_{max} implies that the microbial consortium can multiply rapidly when exposed to the substrate (UCO). This advantage translates to potentially superior FOG degradation performance compared to consortia with lower K_s values, as suggested by (Husain et al. 2011).

Optimization of FOG degradation

The FOG biodegradation process was optimized through 20 day experiments in an incubator shaker, with daily monitoring of palm oil reduction. The selected X3X4 consortium effectively degraded the palm oil, utilizing it as a carbon and energy source. This degradation was visually evident by the disappearance of large oil droplets that were initially present in the cultures.

Following the designed experimental approach, the percentage of biodegradation, residual oil, and oil degraded by X3X4 were calculated for each run using the provided equations (Eqs. 3, 4, and 5). Table 5 summarizes the obtained results.

Table 5 Biodegradation results of the selected consortium for optimum FOG degradation

Run	pH	Oil conc. (%v/v)	Inoculum conc. (%v/v)	Degraded Oil (ml)	Residual Oil (ml)	Biodegradation (%)
1	6	5	10	2.86	2.14	57.2
2	7	3	6	2.41	0.59	80.33
3	6	1	2	0.43	0.58	42.5
4	8	1	2	0.6	0.4	60
5	8	1	10	0.75	0.25	74.6
6	6	5	2	3.57	1.43	71.4
7	8	5	2	3.58	1.22	75.6
8	8	5	10	2.64	2.36	52.8
9	7	3	6	2.37	0.63	78.93
10	7	3	6	2.38	0.62	79.33
11	6	1	10	0.83	0.17	82.7

As shown in Table 5, the oil breakdown efficiency by the consortium varied between 42.5% and 82.7%. Run 11, conducted at pH 6, 1% oil, and 10% inoculum, achieved the highest oil removal (82.7%). This can be attributed to the higher bacterial inoculum concentration, suggesting the consortium's enhanced capacity for palm oil degradation under these conditions. It is noteworthy that optimum values were not observed at the center points, possibly due to an increase in oil content as inoculum concentration decreased. This implies insufficient secreted enzymes to reach and effectively degrade the palm oil substrate. These results demonstrate that the selected consortium outperforms individual strains in palm oil degradation, achieving up to 82.7% degradation in 20 days compared to 68% achieved by individual strains in the same period, as reported by (Al Khatib et al. 2023). However, the degradation level is lower compared to certain other studies (Sarkar et al. 2013).

The efficiency of oil degradation is influenced by oil concentration due to its effect on substrate availability. Increased oil levels provide more substrates for enzymes to act upon, resulting in higher degradation rates. However, excessive oil concentrations can hinder bacterial growth by producing toxic byproducts during the breakdown process (Peekate Lekiah et al., 2023; Sharma & Schiewer, 2016). This highlights the need to determine the optimal oil concentration for effective bioremediation of cooking oil contamination.

The pH level has impact on the active sites of lipase, as well as the movement of substrates through the enzyme during cooking oil biodegradation. This affects both the enzyme's structure and its catalytic effectiveness (Baena et al., 2022). The pH influences how amino acid residues in the enzyme's active site ionize, potentially altering the enzyme's three-dimensional configuration and its substrate-binding capability (Mander et al., 2014). The study's finding of an optimum pH of 6 aligns with previous research, which indicates that most commercial lipid-degrading bacteria struggle to grow efficiently at pH levels below 5 (Nzila et al. 2016).

Although the study was performed under controlled conditions, investigating pH levels (6, 7, 8) and oil concentrations (1%, 3%, 5% v/v), while the temperature was maintained at a constant temperature of 30 °C, the isolation of the used strains from POME implies that these strains may still function at higher concentrations and temperatures.

Level Factorial Design

A 2-level factorial design (2LFD) was employed to identify the key factors impacting palm oil biodegradation by the selected consortium. This design involved eleven experiments, including three center points, evaluating three

Table 6 Regression analysis (ANOVA) of the amount of degraded oil

Source	Sum of squares	DF	Mean of squares	*F*-Values	*p*-values Prob > F	Status
Model	14.68	3	4.89	33.23	0.0004	significant
A-pH	0.018	1	0.014	0.015	0.9079	not significant
B-Oil conc	14.31	1	2.23	612.67	0.0016	significant
C-Inoculum conc	0.352	1	0.11	29.12	0.0327	significant
Pure Error	5.181E-003	2	2.590E-003			not significant
Lack of Fit	0.38	1	0.38	261	0.1571	not significant

variables: palm oil concentration, inoculum concentration, and pH.

The observed increase in oil degradation with higher oil concentration can be attributed to enhanced substrate availability. An increased substrate concentration allows for improved enzyme–substrate interaction, maximizing the utilization of the enzymes' limited active sites and accelerating the hydrolysis reaction (Shuler et al. 2001). Similarly, a higher inoculum concentration translates to more bacterial cells, leading to greater enzyme secretion and ultimately a faster rate of lipid breakdown (Campbell & Farrell 2016).

An analysis of variance (ANOVA) was conducted on the results of optimized biodegradation for residual oil (Table 6). The model's F-value of 33.23 implies its statistical significance, with a very low *P*-value (0.0004) falling below the threshold of 0.050. This indicates the model's strong ability to predict significant patterns influencing oil degradation by the consortium. The R-squared value of 0.916 further supports the model's applicability and reliability. Additionally, the "Curvature F-value" (2.61) and "Lack of Fit F-value" being non-significant suggest that the model adequately captures the curvature and lacks major fitting issues.

The agreement between "Pred R-Squared" (0.879) and "Adj R-Squared" (0.915) reinforces the model's validity. Furthermore, the model identifies oil concentration (factor B), inoculum concentration (factor C), and their

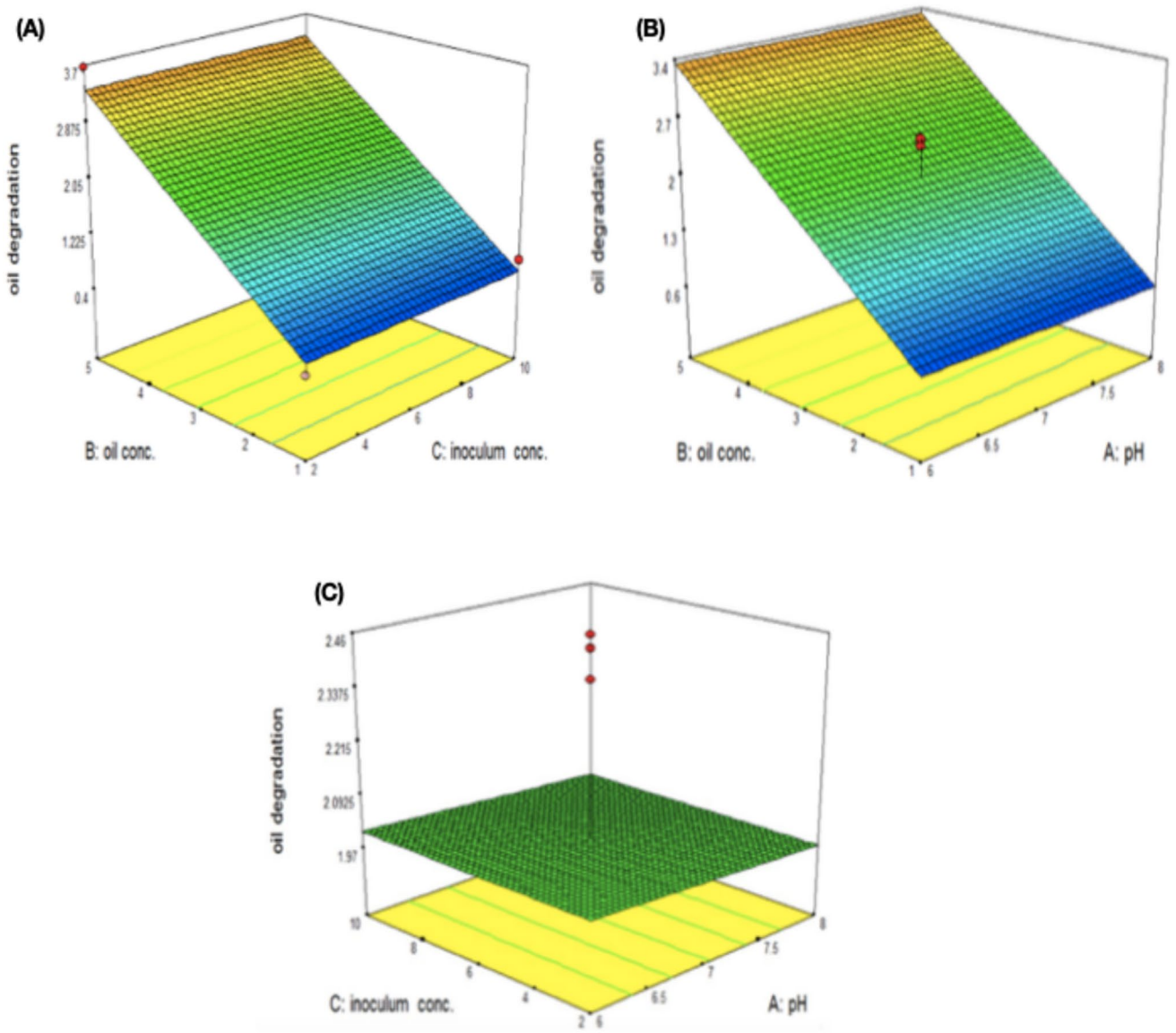

Fig. 5 The 3D surface plot of the interaction between, **A** Oil Concentration and Bacterial Inoculum, **B** Oil Concentration and pH, **C** Bacterial Inoculum and pH

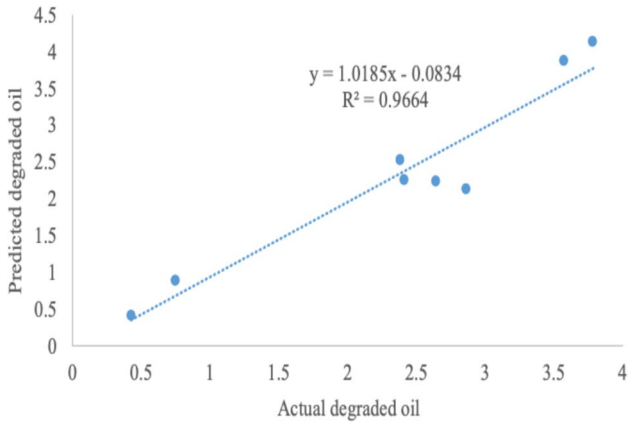

Fig. 6 The actual values versus predicted values of biodegraded oil

interaction (BC) as significant factors impacting oil degradation.

Effects of optimization variables on FOG biodegradation

The graphical capabilities of Design Expert 7.0.0 software facilitated the visualization of the model equation, offering deeper insights into the influence of optimization factors on FOG biodegradation. Figure 5 showcases three-dimensional response surface curves depicting the interactive effects of the three key parameters on the consortium's degradation efficiency.

The 3D surface plot in Fig. 5 clearly reveals that palm oil concentration and bacterial inoculum concentration exert the most significant impact on the amount of degraded oil. In contrast, the observed influence of the pH level appears to be less pronounced. This observation aligns with the mathematical prediction model for biodegradation presented in Eq. 6.

$$Oil\ Degradation = 1.023 - 0.034A + 0.35B - 0.11C - 0.052AB - 0,0042BAC + 0.0015ABC \quad (6)$$

where A, B, and C are pH, concentration of oil, and bacterial inoculum concentration, respectively.

Figure 6 further strengthens the model's validity by comparing the actual (experimental) and predicted (calculated using Eq. 6) values of oil degradation percentage. The excellent agreement between the two datasets is reflected by the high R-squared value of 0.966.

Based on these combined analyses, the optimal conditions for maximizing biodegradation are identified as 1% oil concentration, 10% bacterial inoculum concentration, and pH 6. This information provides valuable insights for future efforts to optimize the consortium's effectiveness in real-world FOG treatment applications.

The results showed the potential use of the bacterial strains in degrading UCO. This gives these strains the potential to be used in multiple applications such as oil treatment in wastewater from residential areas, hotels and restaurants as well as in being used as a catalyst in wastewater treatment plants where FOG could be causing disturbance to the treatment process.

Conclusion

This study investigated the potential of a bacterial consortium (X3X4) composed of Micrococcus lylae DSM 20315 and Corynebacterium aurimucosum for used cooking oil (UCO) biodegradation. The X3X4 consortium exhibited superior growth characteristics compared to individual strains, highlighting the benefits of consortium formation. Subsequent kinetic studies and optimization experiments focused on maximizing UCO degradation efficiency. The optimization process, using a two-level factorial design (2LFD), identified 1% v/v UCO concentration, 10% v/v bacterial inoculum concentration, and pH 6 as the optimal conditions, achieving a significant biodegradation rate of 82.7%. This performance surpassed the degradation achieved by individual strains, demonstrating the enhanced potential of the consortium approach. These findings support the feasibility of utilizing the X3X4 consortium for effective UCO biodegradation. The optimized conditions provide valuable insights for practical applications, potentially contributing to the sustainable management of oily wastewater streams. However, in order to transform the optimized results to industrial scale, further research is needed to address challenges such as their performance in large-scale reactors, inoculum production economics and economic feasibility from a cost and sustainability perspective.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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