Global J. Environ. Sci. Manage. 10(4): 1-14, utumn 2024, Serial #40

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

Podcasts

ORIGINAL RESEARCH ARTICLE

Exploration and characterization of lipid-degrading bacteria from palm oil mill effluent

P. Periadnadi[1,*], N. Nurmiati[1], F.W. Siregar[1], T.W. Edelwis[2]

[1] *Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia*

[2] *Department of Biology Education, Raja Ali Haji Maritime University, Indonesia*

ARTICLE INFO

Article History:
Received 03 December 2023
Revised 10 February 2024
Accepted 19 March 2024

Keywords:
Enzymatic
Fermentative
Isolate
Lipid-degrading bacteria
Lipolytic
Palm oil mill effluent (POME)

DOI: 10.22034/gjesm.2024.04.***

ABSTRACT

BACKGROUND AND OBJECTIVES: Lipid-degrading bacteria found in processing ponds of palm oil mill effluent are recognized for the capacity to break down lipid using lipase enzyme. Identifying novel strains of these bacteria with high bioremediation potential contributes valuable insights to the sustainable management of palm oil mill effluent. Therefore, this study aimed to identify potential bacteria, assess the in vitro lipid-degrading capabilities, characterize the traits, and evaluate lipid degradation activity of potential isolates from palm oil industry wastewater.

METHODS: The method used for exploring the potential of lipid-degrading bacteria in palm oil mill effluent entailed a survey comprising various stages including detection of bacteria presence, in vitro assessment of potential indices, characterization, lipid degradation testing, and determination of lipase activity.

FINDINGS: The results showed that several bacteria groups were present in palm oil mill effluent, including 50-74 percent lipolytic, 31-90 percent fermentative, 76-83 percent proteolytic, and 51-74 percent cellulolytic. Selected lipid-degrading isolates demonstrated significant in vitro potential, as evidenced by high lipolytic and fermentative indices. Isolate Enzymatic 3 had the highest lipolytic index, degradation value (48.72 percent), and lipase activity (0.12 units/milliliter), identified as Bacillus cereus central carbon metabolism 2010. Similarly, isolate Fermentative 2 was found to have the highest fermentative index, degradation value (22.35 percent), and lipase activity (0.01 units/milliliter), identified as Bacillus thuringiensis American type culture collection 10792.

CONCLUSION: Based on the results, isolates Enzymatic 3 and Fermentative 2 showed promising potential as biological agents for bioremediation of palm oil mill effluent. The results underscored the promising potential of specific bacteria isolates in mitigating lipid-rich effluents, advocating for the integration into sustainable wastewater management practices in palm oil industry. This study provided valuable insights for future investigations aimed at unraveling the intricate mechanisms governing lipid degradation and fostering environmentally friendly solutions for industrial waste management.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
40	6	5

*Corresponding Author:
Email: *periadnadi@sci.unand.ac.id*
Phone: +62822 8456 4481
ORCID: 0000-0002-1980-8566

Note: Discussion period for this manuscript open until January 1, 2025 on GJESM website at the “Show Article”.

INTRODUCTION

Palm oil mill effluent (POME) is a type of agro-industrial organic waste derived from by-products of processing Fresh Fruit Bunches into Crude Palm Oil. This process produces significant amounts of liquid waste (Cheah *et al.,* 2023), with each ton of Crude palm oil yielding 2.5 tons of liquid waste (Obada *et al.,* 2023). The presence of oil-fat (lipid) content and high levels of organic substances can cause pollution around palm oil processing factories (Benti *et al.,* 2023), negatively impacting the environment. POME has the potential to be an environmental pollutant due to the high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and suspended solids which potentially reduce water quality (Vijayan *et al.,* 2024). The increasing global demand for palm oil has increased production, resulting in a corresponding rise in the generation of POME (A. Aziz *et al.,* 2023; Kumaran *et al.,* 2023). As a byproduct of palm oil extraction process, POME poses significant environmental challenges due to the high lipid content and BOD (Meena *et al.,* 2023). Lipid, one of the primary constituents of POME, contributes to the recalcitrant nature and makes treatment using conventional methods difficult (Ahmad *et al.,* 2024). Furthermore, the management poses significant environmental challenges due to high organic content and pollutant load. Addressing these challenges requires innovative approaches that integrate environmental sustainability with resource recovery. Several related studies have been conducted such as "the potential of microalgae in biodiesel production and pollutant removal from POME". By isolating new microalgal strains, the study aimed to enhance the efficiency of biodiesel production while simultaneously addressing environmental concerns (Kamyab *et al.,* 2018a). Another investigation explored the dual benefits of bioenergy production and nutrient removal using green microalgae cultivated from agro-wastewater POME. A significant potential for sustainable bioenergy generation and mitigation of environmental pollution was observed (Kamyab *et al.,* 2018b). Furthermore, (Jadhav *et al.,* 2024) examined the characterization of trimetallic nanoparticles and the impact on anaerobic digestion of POME as a novel strategy for enhancing biogas production and pollutant degradation. The study was conducted to optimize the efficiency of anaerobic digestion processes while improving overall POME treatment outcomes. A study by (Abioye *et al.,* 2024) investigated the optimization of syngas production through the co-gasification of palm oil decanter cake and alum sludge using response surface methodology (RSM). The objective was to enhance syngas yields while minimizing waste generation from POME-related byproducts. In recent years, there has been growing interest in the use of microbial-based strategies for the remediation of POME (Ong *et al.,* 2021). Specifically, the exploration and characterization of lipid-degrading bacteria offer promising avenues for the development of bioremediation methods. These bacteria possess the enzymatic machinery necessary for breakdown of complex lipid into simpler compounds, thereby facilitating the remediation process (Dvořák *et al*., 2017; Chan *et al*., 2022; Narayanan, Ali, and El-Sheekh, 2023). Understanding the diversity and functional capabilities of lipid-degrading bacteria in POME is crucial for developing sustainable and efficient bioremediation strategies (Saratale *et al*., 2022). Elucidating the potential of these bacteria contributes to the growing body of knowledge on microbial-based approaches to mitigate the environmental impact of palm oil processing (Gudiukaite *et al*., 2021; Saratale *et al*., 2022; Sharma *et al*., 2022). Furthermore, the identification of effective bioremediation agents has significant implications for improving the sustainability and environmental stewardship of palm oil industry (Kahar *et al*., 2022; Ahmad *et al*., 2023). An effective method for obtaining lipid-degrading bacteria and characterization, as well as identifying lipolysis activity is isolation using specific media (Kumaran *et al.,* 2023). Characterization is used to determine the diversity and classify strains in one species making potential bacteria easily obtained and developed in POME biological processing technology. Hydrolytic enzymes are anticipated to play a significant role due to the ability to cleave glyceride bonds, thereby transforming fats into water-soluble fatty acids. These enzymes are crucial in the process of breaking down lipid efficiently. Lipid-degrading bacteria offer promising solutions to enhance the sustainability of palm oil production by mitigating environmental pollution, promoting resource recovery, reducing greenhouse gas emissions, and facilitating compliance with regulatory requirements. Incorporating bacteria into palm oil production processes represents a proactive method to addressing environmental challenges as well as promoting responsible and sustainable practices in the industry. This study serves

as an initial exploration of isolating microorganisms with comparable results. Therefore, this study aimed to examine the characterization and molecular identification of lipid-degrading bacteria in POME as a more environmentally friendly microbiological effort. The primary objective was to identify potential candidates for bioremediation of POME through various steps including detection of bacteria, in vitro testing of potential indexes, characterization, lipid degradation tests, and determination of lipase activity. The study experiment was conducted in the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia, during 2023 to 2024.

MATERIALS AND METHODS

Sample collection and preparation

Samples of POME were collected from palm oil factory located in West Sumatra, Indonesia (Fig. 1), using purposive sampling by targeting areas where the surface of the wastewater showed a brown coloration and was visibly smeared with oil. Sampling was conducted at three distinct points situated on both the right and left sides of the pond, comprising anaerobic (Pool 1 - P1), facultative (Pool 2 - P2), sensitive (Pool 3 - P3), and sedimentation ponds (Pool 4 - P4). Following collection, each sample was carefully transferred into sterile bottles and stored in a cool box containing ice gel to maintain the integrity during transportation.

The characteristics of POME with detailed information regarding the properties and composition are depicted in Table 1 showing parameters such as potential of hydrogen (pH) levels, chemical composition, nutrient content, BOD, and other relevant characteristics.

Modified nutrient agar (Himedia M001-500G) media

Nutrient agar (NA) is primarily used as a general-purpose growth media for cultivating a wide variety of microorganisms. In this study, lipolytic ability was assessed using modified NA media, an optimized formulation derived from several selective media. To prepare the media, 23 grams (g) of NA media was dissolved in distilled water to yield a total volume of 1000 milliliters. Subsequently, 10 grams of margarine and 0.5 grams of neutral red were added to the solution, followed by heating until homogeneity was achieved (Tirkey *et al*., 2014).

Carboxy methyl cellulose agar (329-500G) media

Carboxy methyl cellulose agar (CMCA) media was used to view and test the cellulolytic ability (Abdollahi *et al*., 2019).

Skim milk agar (Himedia M763-500G) media

Skim milk agar (SMA) media was used to view and test the proteolytic ability (Yee *et al*., 2019).

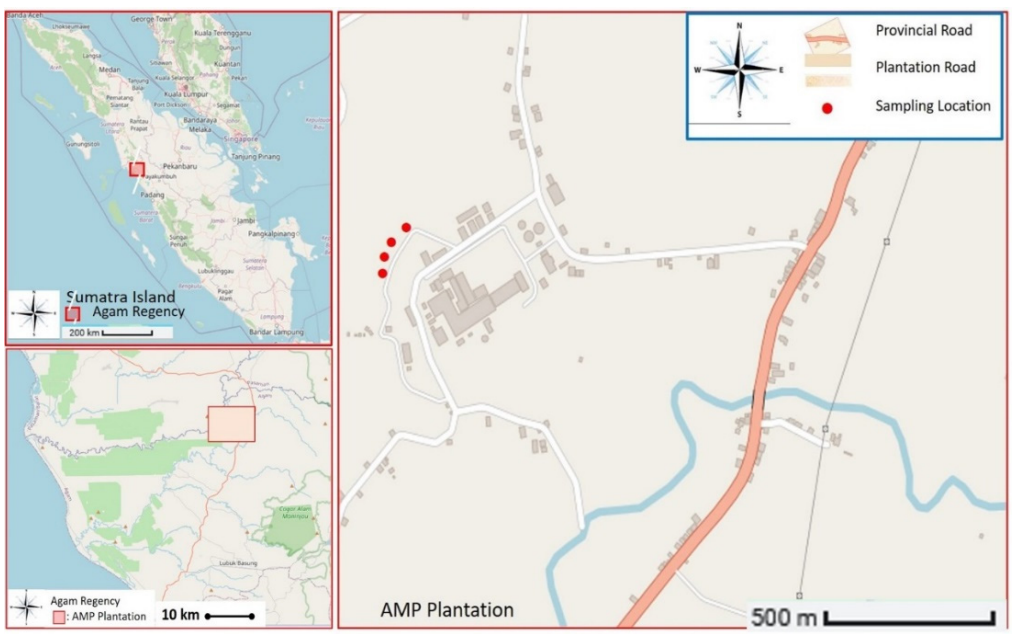

Fig. 1: Geographical location of the study area in West Sumatra, Indonesia

Table 1: The characteristics of POME (Lee *et al.*, 2019)

Water	95-96 percent (%)
Oil	4-5%
Suspendid solid (SS)	2-4%
pH	4-5
Temperature	40-90°C
COD	50.000 milligram per liter (mg/L)
BOD	25.000 mg/L
Fat	4000-6000 mg/L

Medium glucose peptone agar (Himedia M758-500G) + calcium carbonate ($CaCO_3$)

Glucose peptone agar (GPA) + $CaCO_3$ media was used to examine the activity of microbes capable of producing acid (acidification) (Nurmiati *et al*., 2018).

Isolation and cultivation methods

To isolate and cultivate lipid-degrading bacteria from POME, a meticulously designed protocol was implemented. Isolation process commenced with the use of a specifically modified NA media, carefully formulated to promote the growth and detection of lipase-producing microorganisms. This modified media was uniquely enriched with margarine, selected as a rich lipid source to mimic lipid-rich environment characteristic of POME. Furthermore, media was supplemented with neutral red, a discernible indicator used to facilitate the visual detection of microbial metabolic activity. Lipase-producing bacteria, crucial for the degradation of margarine, were monitored for distinctive ability to generate halo zones surrounding colonies. These halo zones, indicative of enzymatic activity, served as tangible evidence of lipid degradation in the microbial community. Moreover, lipid-degrading bacteria colonies demonstrated a significant propensity for absorbing the neutral red dye, substantiating metabolic functionality and active participation in the degradation process. This comprehensive method not only facilitated isolation but also provided valuable insights into the metabolic dynamics and potential applications of lipid-degrading bacteria in POME treatment and bioremediation strategies (Kumaran *et al.,* 2023).

Isolation and characterization procedures

Isolation process entailed the use of a specially modified NA media, where Margarine served as the primary lipid source, thereby facilitating the growth of lipid-degrading bacteria. The addition of neutral red acted as an indicator, enabling the visualization of bacteria activity. Following the degradation of margarine, lipid-degrading bacteria would produce halo zones surrounding colonies, indicating enzymatic activity. Simultaneously, bacteria colonies absorbed the neutral red color, providing further evidence of lipid metabolism. From isolates obtained, two distinct strains with the highest lipolytic indices were selected for further characterization. This method enabled the targeted identification and isolation of bacteria proficient in lipid degradation, laying the groundwork for subsequent investigations into metabolic pathways and enzymatic capabilities (Ilyasa *et al.,* 2024).

Isolate index potential test

One loop of pure isolate from a plate of modified NA media and GPA+$CaCO_3$ was grown in 20 milliliters (mL) of GPB media with the same cell density of 10^7 colony-forming units/milliliters (cfu/mL) for 24 hours (h). Subsequently, 1 mL was taken to be grown on modified NA, CMCA, and SMA media to test the potential of isolate, as well as fermentative activity with GPA+$CaCO_3$ media using the pour plate method and incubated at 37 degrees Celsius (°C) for 48 hours. The percentage of isolates was calculated from bacteria that grew on the modified media with a halo zone (Krishnapriya, Venkatesh Babu and G., 2015).

POME degradation test

Oleic acid standard curve was prepared with several variations in concentration made using a solution of 0.07 M oleic acid. The solution was taken as 0.5; 1; 1.5; 2; 2.5 mL, and diluted with 96 percent (%) ethanol to 10 mL. Subsequently, 2 mL mixture was taken, added with 0.5 mL copper(II) (Cu) acetate reagent, and the mixture was vortexed for 1 minute. Absorbance measurements were carried out using a spectrophotometer at a wavelength of 715 nm (Krishnapriya, Venkatesh Babu and G., 2015).

Molecular identification of the 16S ribosomal ribonucleic acid (rRNA) gene of lipid-degrading bacteria

Molecular identification referred to a scientific method used to identify and classify bacteria based on genetic material, specifically the 16S rRNA gene. This method is valuable for studying lipid-degrading bacteria in various environments, including soil, water, and industrial waste streams. It provides insight into the diversity of bacteria capable of degrading fats and oils, as well as potential applications in bioremediation, waste treatment, and other environmental processes (Wan *et al*., 2023).

Isolation of bacteria genomic deoxyribonucleic acid (DNA)

Bacteria isolates were cultured in NB media for 16 hours at 37°C then placed in a 2 mL microcentrifuge tube and centrifuged at 4°C for 5 minutes at 14,000 revolutions per minute (rpm). The supernatant formed was discarded, the cell pellet obtained was added with 1 mL TAE (Tris Acetate- Ethylenediaminetetraacetic acid) buffer and homogenized using a vortex. The sample was centrifuged at 10,000 rpm for 15 minutes, the resulting supernatant was discarded, and the cell pellet was resuspended in 75 microliters (µL) of lysozyme solution. The samples were incubated at 36°C for 60 minutes and after every 10 minutes, the tube was inverted. Subsequently, 250 µL Glycerol Ethanolamine Solution (GES) and 125 µL of 7.5 M ammonium acetate were added (Cheau Chin *et al*., 2020).

Amplification of the 16S rRNA gene by PCR

DNA sample of 0.5 µL was placed into Polymerase chain reaction (PCR) tube containing mixed PCR reagents. The reagents included 17.5 µL double-distilled water (ddH_2O), 2.5 µL deoxyribonucleotide triphosphate (dNTP), 2.5 millimolar (mM), 2.5 µL Thermus aquaticus (Taq) buffer, 0.2 µL Taq Polymerase DNA enzyme, and 1 µL primer. In general, bacteria DNA samples used primer 27 F: 5`-- AGA GTT TGA TCC TGG CTC AG– 3` and Primer 1492 R: 5`-- GGT TAC CTT GTT ACG ACT TT -- 3`. Primers were PCR subjected to the following conditions: Pre-start at 94 °C for 2 minutes, followed by denaturation at 92°C for 2 minutes, primer annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute 20 seconds in 25 cycles. The Post PCR was performed at 72°C for 10 minutes, then the temperature was lowered and ended at 4°C. PCR products were analyzed by electrophoresis in line with a previous study (Barak *et al*., 2023).

Gel electrophoresis

The amplification results were fractionated by electrophoresis using Mupid Mini Cell (ex) on a 1% agarose gel in TAE buffer for 30 minutes at 100 V. The electrophoresis gel was soaked in ethidium bromide solution with a concentration of 1 µL/100 mL for 20 minutes. The separation results were visualized on Gel Doc Printgraph (Bioinstrument, advanced technology of the third order (ATTO)). Ultraviolet (UV) transilluminator with a standard 100 base pair (bp) DNA ladder (Promega) was used to determine the results and size of the amplified DNA bands. Purification of PCR products was carried out using the Polyethylene Glycol (PEG) precipitation method. A sample of 25 µL PCR product was added to 15 µL PEG solution (40% PEG 6000 and 10 mM $MgCl_2$) and 6 µL of 3 M sodium acetate, then inverted for 10 minutes and centrifuged at 16,000 rpm for 25 minutes. The supernatant formed was discarded by pipetting carefully. DNA pellet was washed with 50 µL of 70% ethanol 2 times and dissolved in 20 µL ultra-pure dH_2O. Subsequently, pure 16S (Small subunit) Recombinant DNA (rDNA) samples were stored at –20°C (Packirisamy and Pandurangan, 2023). The photographic view of the experimental setup (Fig. 2) provides a visual representation for the exploration and characterization of lipid-degrading bacteria from POME.

RESULTS AND DISCUSSION

In vitro potential of lipid-degrading bacteria

Bacteria can form a halo zone depending on the activity, indicating the presence of extracellular lipase enzyme and breakdown of polymer molecules through acidification. The degradation of lipid content is caused by hydrolysis into glycerol and free fatty acids, a process known as lipolysis (Mamtani, Shahbaz, and Farid, 2021). Isolates with the highest index values from each specific media (modified NA and GPA+$CaCO_3$) in each pool were selected for further in vitro testing. The calculation of lipolytic, cellulolytic, proteolytic, and fermentative index used modified NA, CMCA, SMA, and GPA+$CaCO_3$ media. Isolate E3 had the highest lipolytic index (IL) of 2.40, F2 showed the highest fermentation index (IF) of 3.25, while E10 and F8 had the highest IP and IS values of 3.00 and 3.00, respectively (Table 2). These results implied that potential isolates had high

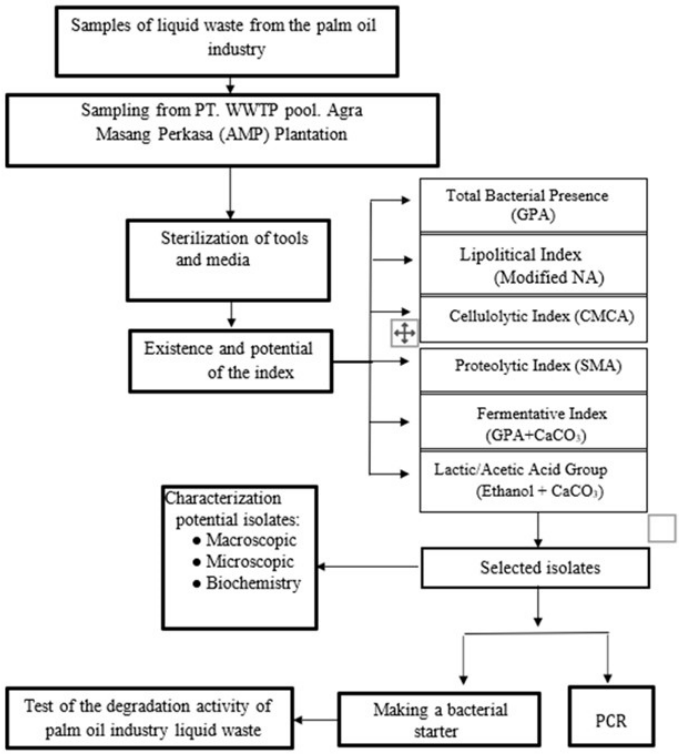

Fig 2: The photographic view

adaptability to various substrate types or possessed dual functionality. Variations in available substrates in specific media significantly influence bacteria adaptation. The potential index value validates the capability of lipid-degrading bacteria to metabolize other compounds, such as proteins and carbohydrates. The determination of this index value also relies on the ratio between the diameter of the halo zone formed and bacteria colony. Specifically, colonies with a smaller halo zone diameter yielded a lower index value, and vice versa. This assessment was conducted using selective media, as shown in Fig. 3.

The size of halo zone formed indicated the sensitivity level of bacteria in converting the substrate in the test media. Variations in the halo zone size occurred because each isolate produced hydrolysis products at different concentrations (Rathakrishnan and Gopalan, 2022). Halo zone size was influenced by the growth rate of bacteria, the rate of diffusion for active ingredients in the media, the sensitivity to active substances, as well as the thickness and viscosity of the media (Ruan *et al*., 2023).

Morphological and biochemical characterization of potential isolates

The morphological characterization of isolates was conducted both macroscopically and microscopically. As shown in Table 3, the macroscopic characterization entailed observing the colony morphology of bacteria, including shape, margin, color, and colony elevation. Microscopic characterization comprised gram staining aimed at observing cell morphology, which categorized bacteria into gram-positive and gram-negative groups, as well as endospore staining (Fig. 4).

The potential isolates obtained were subjected to biochemical tests for further identification. Biochemical tests conducted include catalase, 3% potassium hydroxide (KOH), motility, triple sugar iron agar (TSIA) to observe glucose, lactose, and sucrose fermentation, as well as hydrogen sulfide (H_2S) and gas formation. The results obtained for potential isolates are presented in Table 4.

Based on morphological and biochemical characterization, the potential isolates obtained were identified with reference to Bergey's manual

Table 2: In vitro potential of lipolytic, fermentative, proteolytic, and cellulolytic indices of potential lipid-degrading isolates

Isolate	In vitro potential			
	IL	IF	IP	IS
E3	2.40	1.40	2.00	2.75
E4	2.25	2.50	2.50	2.00
E7	2.33	1.75	2.00	2.60
E10	2.20	1.30	3.00	2.16
F2	1.50	3.25	2.50	1.75
F4	1.30	2.80	2.00	2.00
F8	1.40	2.67	2.67	3.00
F10	1.60	2.50	1.50	2.40

IL (Lipolytic index), IF (Fermentative index), IP Proteolytic index), and IS (Cellulotic index)

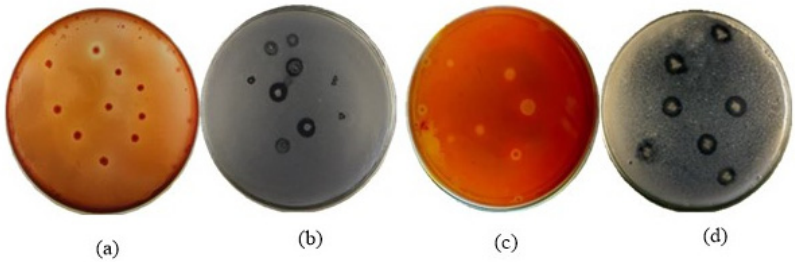

Fig. 3: In vitro potential of potential isolates in specific media: a) Lipolytic, b) Fermentative, c) Cellulolytic, d) Proteolytic

of determinative bacteriology (Bergey and Holt, 1994). Isolates E3, E4, E7, F2, F4, F8, and F10 had characteristics classified under the genus Bacillus, belonging to the group of gram-positive bacteria (Leprince and Mahillon, 2023).

Potential isolate with lipid degradation activity

The assessment of lipid degradation activity was conducted on two selected isolates, E3 and F2, by measuring the formation of free fatty acid levels quantified using oleic acid standard curve. The concentration of oleic acid formed due to lipid degradation can be determined based on the absorbance value at a wavelength of 715 nm. Simultaneously, lipase enzyme activity value was also determined, correlating with the influence of liquid waste as a substrate in lipid lipolysis process of selected isolates. Lipid degradation values and lipase enzyme activity are presented in Table 5.

Table 5 showed differences in degradation values between the two isolates, presumably due to the distinct characteristics. Isolates E3 and F2 follow different lipolysis pathways, as influenced by the primary specific media, namely modified NA (enzymatic) and GPA+$CaCO_3$ (acidifying). Lipolysis process is categorized into two groups based on the cleavage of glycerol bonds, specifically and non-specifically. The specific cleavage of the glycerol bond occurs at positions one and three, resulting in 1, 2-diacylglycerol and monoacylglycerol. On the other hand, non-specific cleavage occurs at three positions, producing three fatty acids and one glycerol or triglyceride molecule. In addition to heating, lipolysis process is closely associated with lipase activity. Based on the results, isolate with the highest lipase activity was produced by E3, specifically at 0.12 U/mL. The higher the degradation value, the greater lipase activity, enhancing lipolysis process. Isolates with high lipase activity and degradation values are considered optimal for waste degradation applications (Alabdallal *et al*., 2021). Consequently, it can be concluded that isolate E3 is a more suitable bacteria compared to F2 as a candidate in the biodegradation of POME from Palm Kernel Shells (PKS).

Molecular identification of the 16S rRNA gene of lipid-degrading bacteria

PCR electropherogram was conducted using the

Table 3: Colony morphological characteristics of potential isolates

Character	Isolate	Morphology			
		Form colonies	Edge	Color	Elevation
Macroscopic	E3	Irregular	Lobate	Yellowish white	*Umbonate*
	E4	*Irregular*	*Undulate*	White	*Raised*
	E7	*Irregular*	*Undulate*	Yellowish white	*Flat*
	E10	*Punctiform*	*Entire*	Milk white	*Convex*
	F2	*Circular*	*Entire*	Yellowish white	*Convex*
	F4	*Circular*	*Entire*	White	*Flat*
	F8	*Irregular*	*Undulate*	Milk white	*Flat*
	F10	*Irregular*	*Undulate*	Yellowish white	Raised

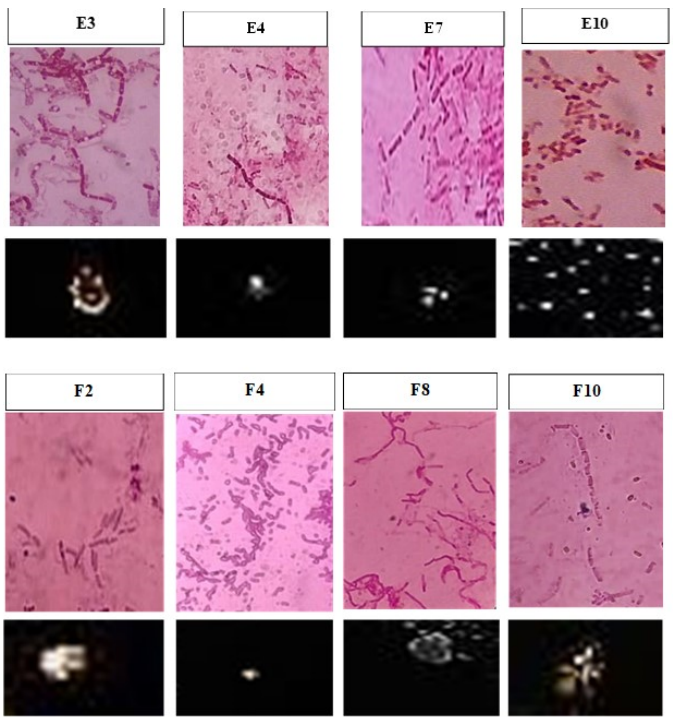

Fig. 4: Macroscopic morphology of potential lipid-degrading isolates on GPA media and microscopic morphology with gram staining

16S rRNA primers on isolates E3 and F2. The observed results of DNA sample amplification from bacteria isolates were distinguished by the presence of bands on the gel. The size of DNA bands was approximately 1300 and 1400 base pairs, as shown in Fig. 5.

The presence of a DNA band at this position indicated that the amplification was specific to the universal gene primer marker used. Specific bacteria primers can generate gene fragment amplicons (Leprince and Mahillon, 2023). The 16S rRNA gene contains a conserved region typically ranging from 500 to 1550 base pairs, characterized by a high guanine and cytosine (G+C) content. The amplification products obtained require sequencing to determine the nucleotide sequence comprising the detected DNA fragment. The nucleotide sequence used for the 16S rRNA gene

Table 4: Biochemical test of potential isolates

Test	Isolate							
	E3	E4	E7	E10	F2	F4	F8	F10
Catalase	+	+	+	+	+	+	+	+
KOH 3%	+	-	-	-	-	-	-	-
Motility	-	-	-	+	+	+	-	-
TSIA	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
Glucose	+	+	+	+	+	+	+	+
Lactose/ Sucrose	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-
Gas	+	+	-	-	-	+	-	-

"+" = positive reaction result "-" = negative reaction result

Table 5: Lipid degradation values and lipase enzyme activity of potential isolates

Lipid degradation					
Isolate treatment	Test	Absorbance (Å)	Concentration	Degradation value	Average
Control	1	0.111	0.389	4.469	3.576
	2	0.109	0.382	2.681	
E3	1	0.169	0.582	56.320	48.721
	2	0.152	0.525	41.123	
F2	1	0.125	0.435	16.985	22.347
	2	0.137	0.475	27.713	
Lipase activity					
Isolate treatment	Test	Absorbance (Å)	Concentration	Activity Lipase	Average
Control	1	0.024	0.099	0.031	0.033
	2	0.027	0.109	0.035	
E3	1	0.111	0.389	0.123	0.116
	2	0.097	0.342	0.108	
F2	1	0.083	0.296	0.094	0.099
	2	0.094	0.332	0.105	

analysis was compared for sequence similarity in the database. The primary method used was basic local alignment search tool (BLAST). BLAST analysis of gene sequences showed several species with high similarity to isolates E3 and F2. Analysis results derived from DNA sequence identified isolate E3 as *Bacillus cereus* strain CCM 2010, with 100% homology or similarity level with E-value of 0. Meanwhile, isolate F2 corresponded to *Bacillus thuringiensis* strain American type culture collection (ATCC) 10792, with a homology or similarity level of 99% and an E-value. According to Leprince and Mahillon (2023), the E-value represents an estimation of two statistically significant terms. A higher value suggests a lower level of homology between sequences, and vice versa, while a value of 0 signifies identical sequences. The similarity or relationship between isolates and species, presumed to share a level of similarity in line with nucleotide base sequences, can be illustrated through a cladogram (Fig. 6). This refers to a diagram depicting kinship comparisons among equivalent genes from various species, reconstructing a tree of life (genealogical tree) to discern relative relationships (Kin *et al*., 2023). A cladogram delineates the evolutionary histories of species and elucidates the diversity of living organisms.

The cladogram, constructed through a comparison of the 16S rRNA gene sequences, indicated that isolate E3 was proximate to species namely *Bacillus*

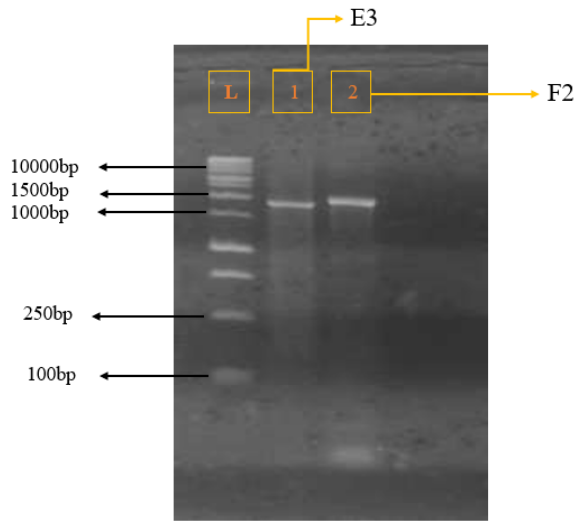

Fig. 5: Visualization of PCR amplification results for isolates E3 and F2.

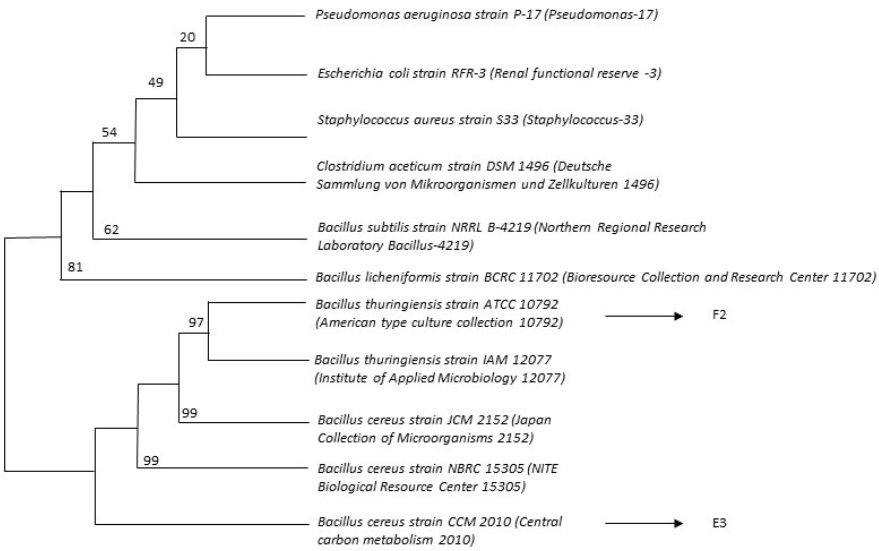

Fig. 6: The cladogram of E3 and F2 isolates using the Neighbor-Joining Tree method

cereus strain NBRC 15305 *and* JCM 2152, as well as *Bacillus thuringiensis* strain IAM 12077. Additionally, this isolate demonstrated a close relationship with F2, which showed similarities to *Bacillus thuringiensis* strain IAM 12077 and *Bacillus cereus* strain JCM 2152. The bootstrap values depicted were 99 and 97 respectively for species sharing close strain types. When the bootstrap value is low, it is essential to exclude the sequence from the analysis to ensure the reliability of the cladogram (Post *et al*., 2023). According to a previous study, a cladogram is deemed acceptable in biosystematic analysis when the results are monophyletic, dichotomous, free of polytomies, have high bootstrap values, as well as possess stable and robust clades (Lucas, 2023). Monophyletic traits refer to groups with only one descendant. Members of a group are assumed to share identical genetic traits, patterns, and biochemical characteristics.

CONCLUSION

In conclusion, the study into POME from processing ponds showed a rich diversity of bacteria communities,

including lipolytic, fermentative, proteolytic, and cellulolytic species. Through targeted exploration, potential lipid-degrading bacteria in POME were successfully identified, underscoring the enzymatic capacity driven by lipase production. The identified isolates demonstrated significant in vitro potential, characterized by high lipolytic and fermentative indices. Lipid-degrading bacteria present in POME use lipase enzyme to break down lipid, originating from processing ponds in palm oil industry. This study focused on identifying, characterizing, and evaluating potential bacteria capable of lipid degradation in POME. Survey method was used including detection, assessment, characterization, and determination of lipase activity. The results showed that POME contains significant percentages of lipolytic, fermentative, proteolytic, and cellulolytic bacteria, contributing to the degradation and transformation of lipid. Among the evaluated isolates, E3 had the highest lipolytic index, while F2 showed the peak fermentative index. Further molecular characterization through the 16S rRNA gene evaluated the taxonomic identity of isolates. Based on the results, E3 and F2 were identified as *Bacillus cereus* strain CCM 2010 and *Bacillus thuringiensis* strain ATCC 10792, respectively. E3 showcased a substantial capacity in POME degradation, attaining a degradation rate of 48.72% alongside a significant lipase activity measuring 0.12 U/mL. Conversely, F2 demonstrated a degradation rate of 22.35% and lipase activity measuring 0.01 U/mL. The results underscored the promising potential of specific bacteria isolates in mitigating lipid-rich effluents, advocating for the integration into sustainable wastewater management practices in palm oil industry. This study provided valuable insights for future investigations aimed at unraveling the intricate mechanisms governing lipid degradation and fostering environmentally friendly solutions for industrial waste management. It was concluded that specific isolates, namely Enzymatic and Fermentative, were effective biological agents for the remediation of POME. These isolates have promising characteristics, attributed to enzymatic activities, which could contribute significantly to breakdown of lipid in the wastewater. Furthermore, the main lipid substrates targeted by the identified bacteria include triglycerides, free fatty acids, and other lipid derivatives commonly found in POME. The efficiency of bacteria in degrading lipid varies depending on several factors such as bacteria species, environmental conditions, and substrate availability. Through comprehensive exploration and characterization, the identified bacteria demonstrated promising potential for bioremediation applications and environmental sustainability efforts. Lipid-degrading bacteria offer promising solutions to enhance the sustainability of palm oil production by mitigating environmental pollution, promoting resource recovery, reducing greenhouse gas emissions, and facilitating compliance with regulatory requirements. Incorporating these bacteria into palm oil production processes represents a proactive approach to addressing environmental challenges as well as promoting responsible and sustainable practices in the industry.

AUTHOR CONTRIBUTIONS

P. Periadnadi, the corresponding author, contributed to supervising the first author in collecting data, analyzing data, and interpreting the results. N. Nurmati drafted or wrote the manuscript, study design, execution, acquisition of data, analysis, and interpretation. F.W. Siregar prepared test data and images and interpreted the results. T.W. Edelwis participated in the interpretation of the results and manuscript preparation.

ACKNOWLEDGEMENT

The authors are grateful to the Biology Department and LPPM of Andalas University for facilitating the researchers in this research and thanks also to all members of the drafting team who have contributed to assisting this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this manuscript. In addition, ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

PUBLISHER'S NOTE

GJESM Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

ABBREVIATION	DEFINITION
%	Percent
µL	Microliters
°C	Degree Celsius
A	Adenine
ATCC	American-type culture collection
ATTO	Advanced technology of the third order
B-4219	Bacillus-4219
BOD	Biochemical oxygen demand
BLAST	Basic local alignment search tool
bp	Base pair
C	Cytosine
$CaCO_3$	Calcium carbonate
CCM	Central carbon metabolism
cfu/mL	Colony forming units/milliliters
CMCA	Carboxy methyl cellulose agar
COD	Chemical oxygen demand
Cu	Copper
ddH_2O	Double-distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
E	Enzymatic
et al.	And others
F	Fermentative
Fig.	Figure
G	Guanine
g	Gram
GES	Glycerol ethanolamine solution
GPA	Glucose peptone agar
h	Hours
H_2S	Hydrogen sulfide
IAM	Institute of Applied Microbiology
IF	Fermentation index
IL	Lipolytic index
JCM	Japan collection of microorganisms
KOH	potassium hydroxide
MA	Milk agar
mg/L	Milligram per liter
mL	Milliliter
mM	Millimolar
NBRC	NITE Biological Resource Center
NA	Nutrient agar
NRRL	Northern regional research laboratory
P	Pool
P-17	Pseudomonas-17
PEG	Polyethylene Glycol
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PKS	Palm kernel shells
POME	Palm oil mill effluent
rpm	Revolution per minute
rDNA	Recombinant DNA
RFR-3	Renal functional reserve -3
rRNA	Ribosomal ribonucleic acid
RSM	response surface methodology
S	Small subunit
S33	*Staphylococcus-33*
SMA	Skim milk agar

SS	Suspended solid
T	Thymine
TAE	Tris acetat-ethylenediaminetetraacetic acid
Taq	Thermus aquaticus
TSIA	Triple sugar iron agar
U/mL	Units per milliliter
UV	Ultraviolet

REFERENCES

Abdollahi, M.; Damirchi, S.; Shafafi, M.; Rezaei, M.; Ariaii, P., (2019). Carboxymethyl cellulose-agar biocomposite film activated with summer savory essential oil as an antimicrobial agent. Int. J. Biol. Macromol., 126: 561–568 **(8 pages)**.

Abioye, K.J.; Harun, N.Y.; Sufian, S.; Yusuf, M.; Jagaba, A.H.; Waqas, S.; Kang, K., (2024). Optimization of syngas production from co-gasification of palm oil decanter cake and alum sludge: An RSM approach with char characterization. Environ. Research. 246: 118027 **(16 pages)**.

Ahmad, I.; Ibrahim, N.N.B.; Abdullah, N.; Koji, I.; Mohamad, S.E.; Khoo, K.S.; Cheah, W.Y.; Ling, T.C.; Show, P.L., (2023). Bioremediation strategies of palm oil mill effluent and landfill leachate using microalgae cultivation: An approach contributing towards environmental sustainability. Chinese Chem. Lett., 34, 107854 **(15 pages)**.

Alabdalall, A.H.; Al-Anazi, N.A.; Aldakheel, L.A.; Amer, F.H.; Aldakheel, F.A.; Ababutain, I.M.; Al-Khaldi, E.M., (2021). Application and characterization of crude fungal lipases used to degrade fat and oil wastes. Sci. Rep., 11(1): 19670 **(11 pages).**

Aziz, M.M.A.; Kassim, K.A.; ElSergany, M.; Anuar, S.; Jorat, M.E.; Yaacob, H.; Imteaz, M.A., (2020). Recent advances on palm oil mill effluent (POME) pretreatment and anaerobic reactor for sustainable biogas production. Renew. Sustainable Energy Rev., 119: 109603 **(14 pages).**

Barak, N.; Fadeev, E.; Brekhman, V.; Aharonovich, D.; Lotan, T.; Sher, D., (2023). Selecting 16S rRNA Primers for Microbiome Analysis in a Host–Microbe System: The Case of the Jellyfish Rhopilema nomadica. Microorganisms. 11, 11(4): 955-965 **(10 pages)**.

Benti, N.E.; Aneseyee, A.B.; Geffe, C.A.; Woldegiyorgis, T.A.; Gurmesa, G.S.; Bibiso, M.; Asfaw, A.A.; Milki, A.W.; Mekonnen, Y.S., (2023). Biodiesel production in Ethiopia: Current status and future prospects. Sci. Afr., 19: e01531 **(12 pages)**.

Bergey, D.H.; Holt, J.G., (1994). Bergey's manual of determinative bacteriology. Williams and Wilkins Publisher, Baltimor **(787 pages)**.

Chan, S.S.; Khoo, K.S.; Chew, K.W.; Ling, T.C.; Show, P.L., (2022). Recent advances biodegradation and biosorption of organic compounds from wastewater: Microalgae-bacteria consortium - A review. Bioresour. Technol., 344: 126159 **(12 pages)**.

Cheah, W.Y.; Siti-Dina, R.P.; Leng, S.T.K.; Er, A.C.; Show, P.L., (2023). Circular bioeconomy in palm oil industry: Current practices and future perspectives. Environ. Technol. Innov., 30: 103050 **(10 pages)**.

Cheau, C.Y.; Yi, J.C.; Soh, K.L.; Christina, V.S.; Aik, C.S.; Mei, F.C.; Chien, L.C.; Lian, K.L., (2020). Comparison of different industrial scale palm oil mill effluent anaerobic systems in degradation of organic contaminants and kinetic performance. J. Clean. Prod., 262: 121361 **(11 pages)**.

Dvořák, P.; Nikel, P.I.; Damborský, J.; de Lorenzo, V., (2017). Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. Biotechnol. Adv., 35: 845–866 **(21 pages)**.

Gudiukaite, R.; Nadda, A.K.; Gricajeva, A.; Shanmugam, S.; Nguyen, D.D.; Lam, S.S., (2021). Bioprocesses for the recovery of bioenergy and value-added products from wastewater: A review. J. Environ. Manage., 300: 113831 **(12 pages)**.

Ilyasu, N.S.; Adams, N.H.; Umar, R.; Ishaya, S.; Nweke, O.D.; Usman, S.; Yakasai, H.M., (2024). Palm oil mill effluent degradation by a novel strain of Bacillus sp. isolated from contaminated environment. Case Studies Chem. Environ. Eng., 9: 100637 **(8 pages)**.

Jadhav, P.; Krishnan, S.; Kamyab, H.; bin Khalid, Z.; Bhuyar, P.; Zularism, A.W.; Nasrullah, M., (2024). Characterisation of synthesised trimetallic nanoparticles and its influence on anaerobic digestion of palm oil mill effluent. Chemosphere. 346: 140512 **(9 pages)**.

Kahar, P.; Rachmadona, N.; Pangestu, R.; Palar, R.; Triyono Nugroho Adi, D.; Betha Juanssilfero, A.; Yopi, Manurung, I.; Hama, S.; Ogino, C., (2022). An integrated biorefinery strategy for the utilization of palm-oil wastes. Bioresour. Technol., 344: 126266 **(9 pages)**.

Kamyab, H.; Chelliapan, S.; Din, M.F.M.; Lee, C.T.; Rezania, S.; Khademi, T.; Bong, C.P.C., (2018). Isolate new microalgal strain for biodiesel production and using FTIR spectroscopy for assessment of pollutant removal from palm oil mill effluent (POME). Chem. Eng. Transactions., 63: 91-96 **(6 pages)**.

Kamyab, H.; Friedler, F.; Klemes, J.J.; Chelliapan, S.; Rezania, S., (2018). Bioenergy production and nutrients removal by green microalgae with cultivation from agro-wastewater palm oil mill effluent (POME)-a review. Chem. Eng. Transactions. 70: 2197-2202 **(6 pages)**.

Kin, K.; Chen, Z. hui, Forbes, G.; Lawal, H.; Schilde, C.; Singh, R.; Cole, C.; Barton, G.J.; Schaap, P., (2023). The protein kinases of Dictyostelia and their incorporation into a signalome. Cell. Signal., 108: 110714 **(11 pages)**.

Krishnapriya, S.; Venkatesh Babu, D.L.; Galop, P.A., (2015). Isolation and identification of bacteria to improve the strength of concrete. Microbiol. Res., 174: 48–55 **(8 pages)**.

Kumaran, M.; Palanisamy, K.M.; Bhuyar, P.; Maniam, G.P.; Rahim, M.H.A.; Govindan, N., (2023). Agriculture of microalgae Chlorella vulgaris for polyunsaturated fatty acids (PUFAs) production employing palm oil mill effluents (POME) for future food, wastewater, and energy nexus. Energy Nexus., 9: 100169 **(11 pages)**.

Lee, Z.S.; Chin, S.Y.; Lim, J.W.; Witoon, T.; Cheng, C.K., (2019). Treatment technologies of palm oil mill effluent (POME) and olive mill wastewater (OMW): A brief review. Environ. technol. innov, 15: 100377 **(19 page)**.

Leprince, A.; Mahillon, J., (2023). Phage Adsorption to Gram-Positive Bacteria. Viruses., 15(1): 196-206 **(13 pages)**.

Lucas, S.G., (2023). Cladistics and Stratigraphy. Geosci., 13(3): 86-97 **(11 pages)**.

Mamtani, K.; Shahbaz, K.; Farid, M.M., (2021). Glycerolysis of free fatty acids: A review. Renew. Sustainable Energy Rev., 137: 110501 **(11 pages)**.

Meena R, A.A.; Banu J, R.; Bhatia, S.K.; Kumar, V.; Piechota, G.; Kumar, G., (2023). A review on the pollution assessment of hazardous materials and the resultant biorefinery products in Palm oil mill effluent. Environ. Pollut., 328: 121525 **(11 pages)**.

Narayanan, M.; Ali, S.S.; El-Sheekh, M., (2023). A comprehensive review on the potential of microbial enzymes in multipollutant bioremediation: Mechanisms, challenges, and future prospects. J. Environ. Manage., 334: 117532 **(12 pages)**.

Nurmiati, N.; Periadnadi, P.; Alamsyah, F.; Sapalina, F., (2018). Characterization and Potential of Acid Fermentative and Proteolytic Natural Microflora in Several Products of Traditional Dadih from Lembah Gumanti District West Sumatra, Indonesia. Int. J. Curr. Microbiol. Appl. Sci., 7: 3151–3163 **(12 pages)**.

Obada, D.O.; Kekung, M.O.; Levonyan, T.; Norval, G.W., (2023). Palm oil mill derived empty palm fruit bunches as a feed stock for renewable

energy applications in Nigeria: A review. Bioresour. Technol. Reports. 24: 101666 **(10 pages)**.

Ong, E.S.; Rabbani, A.H.; Habashy, M.M.; Abdeldayem, O.M.; Al-Sakkari, E.G.; Rene, E.R., (2021). Palm oil industrial wastes as a promising feedstock for biohydrogen production: A comprehensive review. Environ. Pollut., 291: 118160 **(15 pages)**.

Packirisamy, V.; Pandurangan, P., (2023). Polyacrylamide gel electrophoresis: a versatile tool for the separation of nanoclusters. Biotechniques. 74: 51–62 **(13 pages)**.

Post, N.W.; Gilbert, C.C.; Pugh, K.D.; Mongle, C.S., (2023). Implications of outgroup selection in the phylogenetic inference of hominoids and fossil hominins. J. Hum. Evol., 184: 103437 **(12 pages)**.

Rathakrishnan, D.; Gopalan, A.K., (2022). Isolation and characterization of halophilic isolates from Indian salterns and their screening for production of hydrolytic enzymes. Environ. Challenges., 6: 100426 **(10 pages)**.

Ruan, H.; Aulova, A.; Ghai, V.; Pandit, S.; Lovmar, M.; Mijakovic, I.; Kádár, R., (2023). Polysaccharide-based antibacterial coating technologies. Acta Biomater., 168: 42–77 **(33 pages)**.

Saratale, R.G.; Cho, S.K.; Bharagava, R.N.; Patel, A.K.; Varjani, S.; Mulla, S.I.; Kim, D.S.; Bhatia, S.K.; Ferreira, L.F.R.; Shin, H.S.; Saratale, G.D., (2022). A critical review on biomass-based sustainable biorefineries using nanobiocatalysts: Opportunities, challenges, and future perspectives. Bioresour. Technol., 363: 127926 **(12 pages).**

Sharma, P.; Gaur, V.K.; Gupta, S.; Varjani, S.; Pandey, A.; Gnansounou, E.; You, S.; Ngo, H.H.; Wong, J.W.C., (2022). Trends in mitigation of industrial waste: Global health hazards, environmental implications and waste derived economy for environmental sustainability. Sci. Total Environ., 811: 152357 **(12 pages)**.

Tirkey, B.; Pal, U.S.; Bal, L.M.; Sahoo, N.R.; Bakhara, C.K.; Panda, M.K., (2014). Evaluation of physico-chemical changes of fresh-cut unripe papaya during storage. Food Packag. Shelf Life., 10: 190–197 **(7 pages)**.

Vijayan, V.; Joseph, C.G.; Taufiq-Yap, Y.H.; Gansau, J.A.; Nga, J.L.H.; Li Puma, G.; Chia, P.W., (2024). Mineralization of palm oil mill effluent by advanced oxidation processes: A review on current trends and the way forward. Environ. Pollut., 342: 123099 **(12 pages)**.

Wan, L.; Li, L.; Zhang, X.; Li, B.; Harro, J.M.; Shirtliff, M.E., (2023). Effects of palm olein and palm stearin on cecal and fecal microbiota of C57BL/6J mice under low and high fat intakes. Food Chem., 404: 134693 (**10 pages**).

Yee, W.; Tang, S.G.H.; Phua, P.S.P.; Megawarnan, H., (2019). Long-term maintenance of 23 strains of freshwater microalgae on solid microbiological culture media: A preliminary study. Algal Res., 41: 101516 **(12 pages)**.

AUTHOR (S) BIOSKETCHES

Periadnadi, P., Ph.D., Associate Professor, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia.
- Email: periadnadi@sci.unand.ac.id
- ORCID: 0000-0002-1980-8566
- Web of Science ResearcherID: NA
- Scopus Author ID: NA
- Homepage: https://sinta.kemdikbud.go.id/authors/profile/6026055/?view=wos

Nurmiati, N., Ph.D., Associate Professor, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia.
- Email: nurmiati@sci.unand.ac.id
- ORCID: 0000-0002-3713-6417
- Web of Science ResearcherID: NA
- Scopus Author ID: NA
- Homepage: https://sinta.kemdikbud.go.id/authors/profile/6026040

Siregar, F.W, M.Sc., Lecturer, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia.
- Email: fihriwashliyahsiregar@gmail.com
- ORCID: 0000-0002-8167-0833
- Web of Science ResearcherID: NA
- Scopus Author ID: NA
- Homepage: http://scholar.unand.ac.id

Edelwis, T.W, M.Sc., Lecturer, Department of Biology Education, Raja Ali haji Maritime University, Indonesia.
- Email: triwidyaedelwis@gmail.com
- ORCID: ORCID: 0000-0003-1791-4311
- Web of Science ResearcherID: NA
- Scopus Author ID: 57191976133
- Homepage: https://biologi.fkip.umrah.ac.id

HOW TO CITE THIS ARTICLE

Periadnadi, P.; Nurmiati, N.; Siregar, F.W.; Edelwis, T.W., (2024). Exploration and characterization of lipid-degrading bacteria from palm oil mill effluent. Global J. Environ. Sci. Manage., 10(4): 1-14.

DOI: 10.22034/gjesm.2024.04.***

URL: ***