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The Existence And Characterization Of Potential Lipid-Degrading Bacteria In Palm Oil Mill Effluent

Fithri Washliyah Siregar[1], Periadnadi[2], Nurmiati[3]

[1]Mastergraduate Student, Departement of Biology
Faculty of Mathematics and Natural Science, Andalas University
Padang, West Sumatera, Indonesia

[2, 3]Faculty of Mathematics and Natural Science, Andalas University
Padang, West Sumatera, Indonesia

[1]fithriwashliyah@gmail.com

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Abstract – Palm oil mill effluent (POME) is generated from the processing of palm oil. This wastewater is a viscous, brownish liquid containing lipids and high organic content that serve as potential habitat for lipid-degrading bacteria. Lipid-degrading bacteria from ponding systems in the palm oil mill industry are capable of lysing lipids through the production of lipase enzymes by lipolytic bacteria or through the acidification pathway by fermentative bacteria. The study was conducted to determine the existence of lipid-degrading bacteria and their activity or potential character. This research was conducted using the purposive sampling method and analyzed descriptively. The results showed the existence of lipolytic bacteria (31–67×10^5 cfu/ml) and fermentative bacteria (33-51×10^5 cfu/ml) in POME. E3 has a higher lipolytic index and F2 has a higher fermentative index.

Keywords – lipid-degrading bacteria; fermentative index; lipolytic index; POME; wastewater

I. INTRODUCTION

Palm oil mill effluent (POME) is an agro-industrial organic waste from the byproducts of processing oil palm fresh fruit bunches (FFB) into crude palm oil (CPO). This palm oil processing process will produce liquid waste in large enough quantities [1]. Every ton of CPO processing produces 2,5 tons of liquid waste [2]. Typically, 1 ton of crude palm oil production requires 5-7,5 tons of water, more than 50% of which ends up as POME [3].

POME is an oil-based pollutant that is used as a potential habitat for several microorganisms. Microorganisms including bacteria are mostly able to utilize oil as a source of carbon and energy [4]. Bacteria that have this ability are known as lipid-degrading bacteria. Lipid-degrading bacteria release free fatty acids and glycerol [5].

Lipid-degrading bacteria hydrolyze lipid complex into simpler products. Reference [6] propose that lipid-degrading bacteria release free fatty acids and glycerol. The process of degradation of lipid compounds can produce various intermediate products with different reaction pathways, through acidification (fermentative bacteria) or production of lipase enzymes (lipolytic bacteria). As in [4] Bacteria that have high efficiency in collecting toxic contaminants or persistently biodegradable materials can potentially be used in treatment systems to remove pollutants such as oil and grease or heavy metals from polluted wastewater. The lipolytic properties of lipid-degrading bacteria have the potential to be biodegradable agents.

Research on lipid-degrading bacteria from palm oil industry wastewater was previously done, reported that in the palm oil industry wastewater there were 9 isolates of lipid-degrading bacteria [7]. *Micrococcus luteus* 101PB, *Stenotrophomonas*

Corresponding Author: Fithri Washliyah Siregar

maltophilia 102PB, *Bacillus cereus* 103PB, and *Bacillus subtilis* 106PB showed high lipase activity [8][9]. Five isolates of lipolytic bacteria isolated from palm oil industry wastewater [10]. In general, the study of lipid-degrading bacteria in POME has been carried out, but exploring their lipolytic and fermentative bacterial activity in POME still needs to be discovered to screen and identify those that have a high potential index. Therefore, this study aims to determine the natural existence and characterization of potential lipid-degrading bacteria, which can be used as a reference for candidate biological agents in the bioremediation process of palm oil industrial wastewater.

II. Research Methods

2.1. Sample Collection

Samples of palm oil mill effluent (POME) were collected from the site of the palm oil mill industry in West Sumatra, Agam. Sampling was carried out by purposive sampling, which are brown in color and coated with oil on the surface of the wastewater at three points on the left and right of ponding system, consisting of anaerobic ponds (pond 1-P1), facultative ponds (pond 2-P2), maturation ponds (pond 3-P3), and sedimentation ponds (pond 4-P4). The samples were placed into a sterile bottle and stored in a cool box containing ice gel for transport to the Research and Microbiology Laboratory, Faculty of Mathematics and Natural Science, Andalas University, to be prepared for analysis.

2.2. Existence of Indigenous Microflora from POME

The existence of indigenous microflora was observed by colony bacteria that grew on Glucose Peptone Agar (GPA) medium [11], the existence of lipolytic bacteria was observed on modified Nutrient Agar (NA) medium, and fermentative bacteria was observed on Glucose Peptone Agar+Calcium Carbonate (GPA+$CaCO_3$) medium. Each tested was carried out with serial dilutions for POME up to 10^{-5} and two replication (duplo). The existence of bacteria is confirmed by formation of a clear zone around the colony after 24 hours of incubation. The smallest with the largest clear zone from each different colony grew on modified NA medium and GPA+$CaCO_3$ were selected to be cultured using streak method in GPA [12]. The isolates obtained were coded with the category's initial (E for enzymatic and F for fermentative), and the number of isolates.

2.3. Evaluation of Lipolytic Index from Selected Isolates

Selected isolates were grown in 10 ml of GPB media with the same cell density of 10^7 cfu/g for 24 hours. 1 ml culture isolates were grown on modified NA medium with pour plate method and incubated at 37°C for 48 hours. Observations of the halo zone were carried out after 48 hours. Lipolytic index of indigenous bacteria was calculated by comparing the diameter of the clear zone formed by the colony of bacteria and the diameter of the colony of bacteria [13]. Determination of qualitative criteria of degradation index was based on assumptions if less than ≤1 given low criteria, equal to 1-2 given the criterion of medium and above ≥2 given high criteria [14].

2.4. Evaluation of Fermentative Index from Selected Isolates

Selected isolates were grown in 10 ml of GPB media with the same cell density of 10^7 cfu/g for 24 hours. 1 ml culture isolates were grown on GPA+$CaCO_3$ medium with pour plate method and incubated at 37°C for 48 hours. Observations of the halo zone were carried out after 48 hours. Fermentative index of indigenous bacteria was calculated by comparing the diameter of the clear zone formed by the colony of bacteria and the diameter of the colony of bacteria [12]. Determination of qualitative criteria of degradation index was based on index criteria [14]. To differentiate and confirm the colony bacteria lactate or acetate group used Ethanol+$CaCO_3$ Agar. Only acetic bacteria can grow and form clear zone around the colony [12].

2.5. Characterization of Potential Lipolytic and Fermentative Bacteria

The isolates were characterized morphologically by observing the colony shape, elevation, color, margin, and surface of each isolates. Characterization was also carried out by observing cell morphology with Gram staining and biochemical tests (catalase test with H_2O_2 3%, KOH 3%, glucose fermentation and gas formation test with Triple Sugar Iron Agar (TSIA), and motility test in Sulfid Indol Motility (SIM) Agar medium).

2.6. Data Analysis

The data in this study were the existence of bacteria, morphological character (microscopic and macroscopic), biochemical characters, lipolytic and fermentative index. The data was analyzed with descriptive and qualitative method.

III. Results And Discussion

3.1. The Existence of Indigenous Microflora from POME

The bacteria existence in POME was taken from four ponding system in the palm oil mill industry. The results of the enumeration of lipolytic, fermentative, and total bacteria in each sample can be seen in Table I.

TABLE I. THE EXISTENCE OF BACTERIAL GROUPS FROM POME

Bacteria	Total colony (…×10^5cfu/ml)			
	P1	P2	P3	P4
Lipolytic (modified NA)	67	49	35	31
Fermentative (GPA+$CaCO_3$)	33	51	44	38
Total bacteria (GPA)	106	94	71	42

The highest total bacteria were sequentially at locations P1, P2, P3, and P4. This difference is caused by decrease in the amount of substrate (organic content) flowing from pond to pond. The P1 location which has the highest organic matter concentration is the best substrate for the growth of various types of bacteria, followed by the K2, K3, and K4 locations. According to [15], it is known that there is a biosolid content decrease along with the overhaul of organic matter in the lagoon. The P1 is the initial holding pond for wastewater treatment, receiving a high load of organic matter and solids, flowing directly from the final liquid waste pre-treatment tank. Organic content will decompose into simpler compounds through the facultative pond (P2), maturation pond (P3), to sedimentation pond (P4).

The results of related studies previously confirmed some differences in the bacterial counts. Total population in the range of 7.4×105–2.0×10^6 cfu/ml as in [16]. POME bacteria from a holding tank, namely 9.5×105−7.9×10^6 cfu/ml with NA medium [17]. Bacteria from a contact pond obtained 17×10^5 cfu/ml [18], 26×10^5 cfu/ml of bacteria in NA medium from the pond outlet [19]. Another study [20], monitored the number of bacteria from POME in water bodies on different release days using NA medium, ranging from 1.9–7.1×10^6 cfu/ml.

In POME there were lipolytic bacteria that detected in modified NA medium. The results indicated the existence of lipolytic bacteria in a range of 31–67×10^5 cfu/ml. The existence of fermentative bacteria was detected using GPA+$CaCO_3$ medium showing a range of 33-51 × 10^5 cfu/ml. The existence of lipolytic and fermentative bacteria caused by POME consisting carbohydrates compounds, fats, minerals.Variations in the range of bacterial populations can be influenced by various factors, including substrate composition and environmental conditions such as temperature and pH. Based on [17], concentration of oil and fat and sugar in POME affected variations in the number of bacterial counts. Reference [21] showed that nutritional factors, minerals, temperature, oxygen levels, degree of acidity, and volume of waste water determine the population of bacteria. In addition, [22] other influential factors such as the process of handling POME by the factory, differences in environmental conditions around the factory, isolation techniques, isolation media, calculation methods, calculation bias, selected dilution index, and number of samples. Storing or preparing POME samples may also result the loss of some non-viable bacteria.

3.2. Evaluation of Lipolytic Index

Twelve isolates of lipolytic bacteria are characterized lipolytic index (Table II).

TABLE II. THE LIPOLYTIC INDEX OF POTENTIAL ISOLATES FROM POME

Isolates	Pond	Lipolytic Index
E1	1	1,25
E2		1,80
E3		**2,40**
E4	2	**2,25**
E5		1,40
E6		1,67
E7	3	**2,33**

E8		1,75
E9		1,50
E10	4	**2,20**
E11		1,60
E12		2,00

The lipolytic index of potential isolate candidates obtained from the detection of clear zone formation on modified NA media reached 1,25−2,40. The highest bacterial lipolytic index from each pond sequentially, isolate E3 of P1: 2,40; E7 of P3: 2,33; E4 of P2: 2,25; and E10 of P4:2,20 (Figure 1). The calculation of the lipolytic index was obtained from 12 total isolates selected from 3 potential candidates for each pond which were successfully isolated with bacterial colony morphology that differed from one another and grew dominantly on modified NA medium.

Modified NA medium consist of NA+margarine+neutral red is selective lipolytic medium. Bacteria on this medium are able to convert lipid substrates in the form of fat in margarine into a carbon source needed for bacterial growth. The neutral red indicator shows a positive reaction to lipolytic bacteria, as indicated by the growing bacterial colonies which are darker in color than the color of the medium. Margarine is added to lipolytic selective media as a lipid substrate. [23] cit to [24], states that margarine is a water emulsion containing lecithin which plays a role in dispersing water molecules into the oil. Selective lipolytic media added dye indicators such as neutral red. The presence of fatty acids from the hydrolysis of lipids will be absorbed by the dye indicator, causing a clearer area around the colony to form which is usually yellow, while the colony turns red [10].

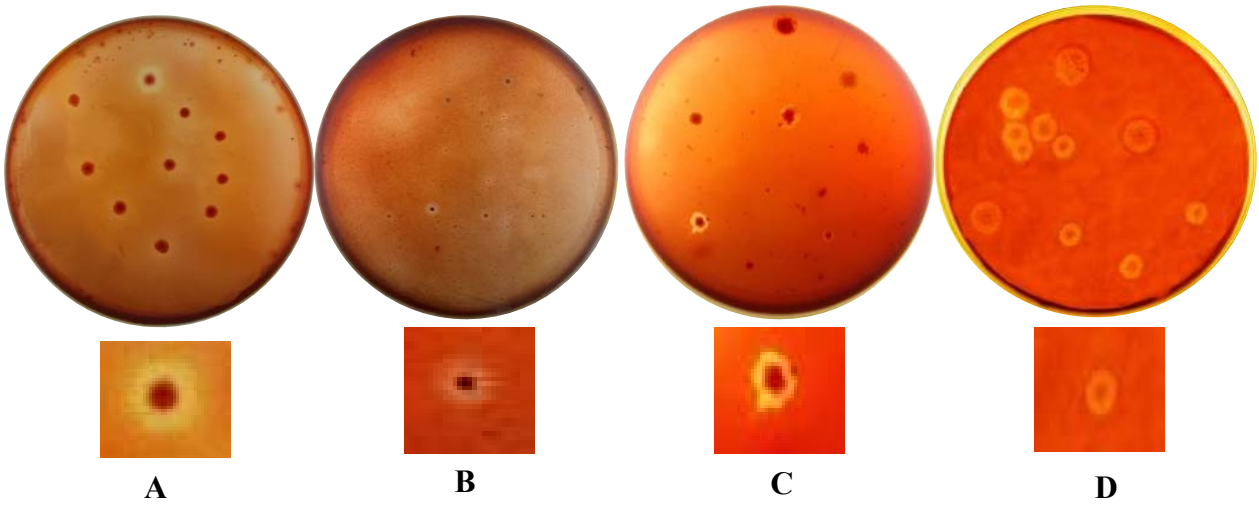

Figure 1. The clear zone area produced by each potential lipolytic isolates on modified NA medium

A. E3, B. E4, C. E7, D.E10.

3.3. Evaluation of Fermentative Index

Twelve isolates of fermentative bacteria are characterized fermentative index (Table III).

TABLE III. THE FERMENTATIVE INDEX OF POTENTIAL ISOLATES FROM POME

Isolates	Pond	Fermentative Index
F1		2,67
F2	1	**3,25**
F3		1,25
F4		**2,80**
F5	2	1,67
F6		1,83

F7	3	1,50
F8		**2,67**
F9		1,33
F10	4	**2,50**
F11		1,33
F12		2,00

The fermentative index of potential isolate candidates obtained from the detection of clear zone formation on GPA+CaCO3 medium reached 1,25–3,25. The highest bacterial fermentative index from each pond sequentially, isolate F2 of P1: 3,25; F4 of P2: 2,80; F8 of P3: 2,67; and F10 of P4: 2,50 (Figure 2). Calculation of the fermentative index was obtained from 12 total isolates selected from 3 potential isolate candidates in each pond which were successfully isolated with different bacterial colony morphology and growing dominantly on GPA+$CaCO_3$ media.

Bacteria on GPA+$CaCO_3$ medium are basically fermenting bacteria that produce acid from the hydrolysis of carbon compounds. Medium of GPA+$CaCO_3$ was used to detect bacteria that were capable to carrying out the acidification activity. Stated by [12] fermentative bacteria produce acid in GPA+$CaCO_3$ medium from the breakdown of glucose which is secreted out by the bacteria and will be neutralized by adding $CaCO_3$. This addition is triggers the reaction of forming calcium lactate from calcium and acid produced by the bacteria, so that a clear area is formed around the colony.

Fermenting bacteria produce acid based on their main metabolic results, which are divided into two groups, Lactic Acid Bacteria (LAB) and Acetic Acid Bacteria (BAA). This bacterial group can be identified by confirming the ability of bacteria to hydrolyze carbon sources into certain organic acids, using Ethanol+$CaCO_3$ medium. Confirmation tests were carried out to see which bacteria were able to grow on the media by utilizing alcohol and being oxidized to acetic acid.

The results of the classification of acidifying bacteria on isolates with the highest fermentative index from each pond, isolates F2, F4, F8, F10 showed that there was no bacterial activity in oxidizing ethanol to acetic acid, characterized by the absence of bacterial colonies on Ethanol+$CaCO_3$ medium, so it can be categorized as LAB. This is supported by the opinion [25], bacteria that have a high ability to oxidize alcohol, aldehydes, and sugars to acetic acid and gluconic acid under aerobic conditions are in the LAB group. Acidification activity can be seen in the formation of a clear zone around the colony due to the production of acetic acid by bacteria which dissolves $CaCO_3$ in the media. Reference [26] added that BAA is usually found in substrates that are rich in sugars, acids, and alcohol such as fruits and fermented foods and drinks.

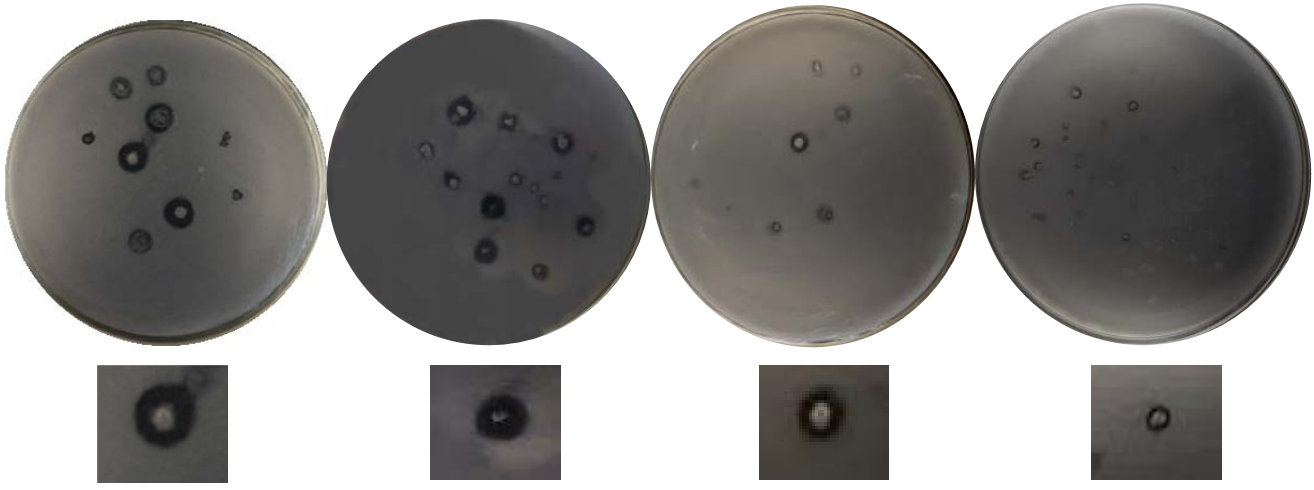

Fig. . The clear zone area produced by each potential fermentative isolates on GPA+$CaCO_3$ medium

A. F2, B. F4, C. F8, D. F10

3.4. Characterization of Potential Lipolytic and Fermentative Bacteria

Selected lipolytic and fermentativen isolates continued to be characterized by partial morphology, such as macroscopic, microscopic, and biochemical (Figure 3) (Table IV).

TABLE IV. PARTIAL CHARACTERS OF POTENTIAL LIPOLYTIC AND FERMENTATIVE BACTERIA FROM POME

Characteristics	**Isolatas**	**Morphology**			
		Shape	**Margin**	**Color**	**Elevation**
Macroscopy	E3	*Irregular*	*Lobate*	Yellowish white	*Umbonate*
	E4	*Irregular*	*Undulate*	White	*Raised*
	E7	*Irregular*	*Undulate*	Yellowish white	*Flat*
	E10	*Punctiform*	*Entire*	Milky white	*Convex*
	F2	*Circular*	*Entire*	Yellowish white	*Convex*
	F4	*Circular*	*Entire*	White	*Flat*
	F8	*Irregular*	*Undulate*	Milky white	*Flat*
	F10	*Irregular*	*Undulate*	Yellowish white	*Raised*

Microscopy	**Isolates**	**Cell shape**	**Gram**		**Endospore**
			Positive	**Negative**	
	E3	*Streptobacil*	+	-	+
	E4	*Streptobacil*	+	-	+
	E7	*Streptobacil*	+	-	+
	E10	*Coccobacili*	-	+	—
	F2	*Bacil*	+	-	+
	F4	*Coccobacili*	+	-	+
	F8	*Streptobacil*	+	-	+
	F10	*Streptobacil*	+	-	+

Biochemical	**Isolates**							
	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, (-) : negative, A/A:*acid/acid*

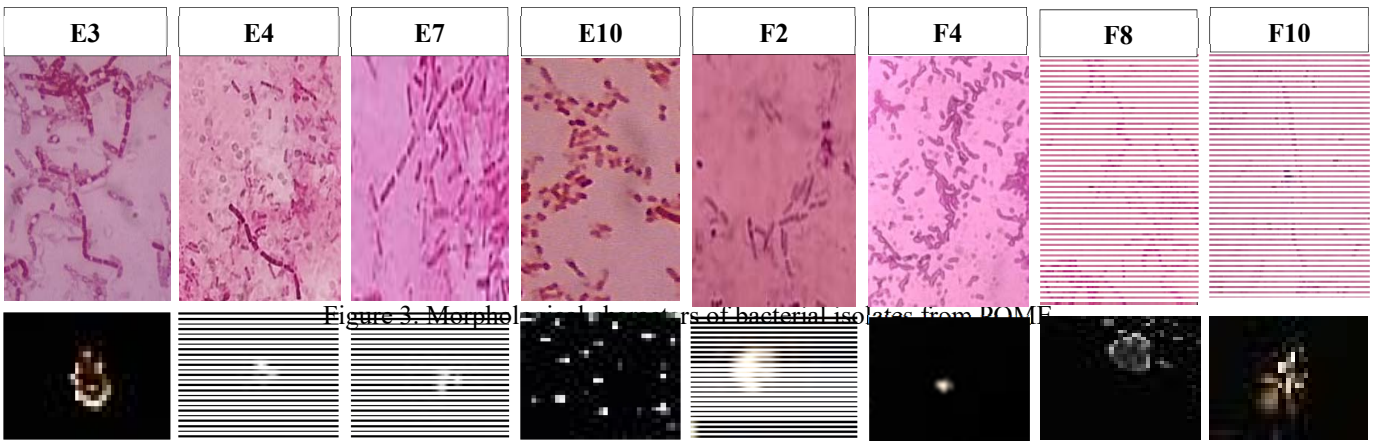

Figure 3. Morphological characters of bacterial isolates from POME

Based on morphological and biochemical characterization, the isolates potential was identified based on Bergey's Manual of Determinative Bacteriology. Isolates E3, E4, E7, F2, F4, F8, and F10 have characteristics that belong to the bacteria of the genus *Bacillus*. According to [27], *Bacillus* bacteria belong to the group of Gram-positive bacteria. The *Bacillus* genus exhibits a wide range of colony morphology, the composition of the medium and the incubation conditions have a strong influence. Stated in [28], bacteria of the genus *Bacillus* are usually not motile, if motile with peritrichous flagella, catalase positive, oxidase negative, grow optimally at temperatures of 30-37°C, and form endospores at extreme temperatures. As in [8], the genus *Bacillus* are aerobic or facultative-anaerobic, very sensitive to heat, pH, and salinity, so they are widespread in various habitats including POME treatment stabilization ponds with high temperatures.

The characteristics obtained from isolate E10 are similar to the group of bacteria from the genus *Pseudomonas*. *Pseudomonas* is a bacilli-shaped Gram-negative bacterium that is commonly found in polluted habitats with high temperatures. According to [27], the bacteria of this genus are generally non-motile, catalase positive, oxidase negative, which normally live in almost all aquatic environments. Stated in [29], *Pseudomonas* is naturally related to palm oil raw materials and palm oil degradation. This happens because of the ability of these bacteria to utilize fat as a carbon source.

The bacteria obtained are in accordance with several previous studies. Several types of bacteria from the genus *Bacillus* in POME, *Bacillus subtilis*106PB and *Bacillus cereus*103PB from POME [17]. Isolates of POME bacteria in water bodies on different release days, including *Bacillus subtilis* and *Pseudomonas aeruginosa* [20]. Suggested in [30], that differences in environmental conditions are the cause of the different types of lipid-degrading bacteria that grow. The level of diversity of lipid-degrading bacteria is also influenced by abiotic factors. The chemical composition of hydrocarbons, temperature, oxygen, nutrients, and acidity (pH) can be determining factors.

IV. CONCLUSIONS

Palm oil mill effluent has indigenous lipolytic and fermentative bacteria. Isolate E3, which refers to *Bacillus* genus was chosen as the potential isolate of the indigenous bacteria with the greatest lipolytic, while isolate F2, which refers to *Bacillus* genus was chosen as the potential isolate of the indigenous bacteria with the greatest fermentative index.

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REFERENCES

[1] Y. D. Nasution. “Pengolahan Limbah Cair Pabrik Kelapa Sawit yang Berasal dari Kolam Akhir (Final Pond) dengan Proses Koagulasi Melalui Elektrolisis”. Jurnal Sains Kimia, vol. 8(2), pp. 38-40, 2004.

[2] M. R. Taha, and A. H. Ibrahim. “COD Removal from Anaerobically Treated Palm Oil Mill Effluent (AT-POME) via Arated Heterogeneous Fenton process: Optimization Study”. Journal of Water Process Engineering, (1), pp. 8-16, 2014.

[3] G. M. Adegbola, A. O. Adeoye, and S. K. Olatunde. “A Review of Biodegradation as Panacea for Palm Oil Mill Effuents (POME) Pollution”. International Journal of Current Microbiology and Applied Sciences, vol. 9(11), pp. 2506-2516, 2020.

[4] A. T. Jameel and A. A. Olanrewaju. “Aerobic Biodegration of Oil and Grease in Palm Oil Mill Effluent Using Consortium Microorganisms”. Current Research and Development in Biotechnology Engineering at International Islamic University Malaysia, vol. 3, pp. 43-51, 2011.

[5] N. C. Bestari dan Suharjono. “Uji Kualitatif dan Kuantitatif Isolat Bakteri Lipolitik dari Limbah Cair Pabrik Pengolahan Ikan Kecamatan Muncar, Banyuwangi”. Jurnal Biotropika, vol. 3(3), pp. 151-155, 2015.

[6] A. Ahmad, T. Setiadi, M. Syafila, O. B. Liang. “Studi Kinetika Reaksi Hidrolisis Senyawa Kompleks Organik dalam Proses Biodegradasi Anaerob”. Jurnal Biosains, vol. 6(1), pp. 1-9, 2001.

[7] W. Januar, S. Khotimah, dan A. Mulyadi. “Kemampuan Isolat Bakteri Pendegradasi Lipid dari Instalasi Pengolahan Limbah Cair PPKS PTPNXIII Ngabang Kabupaten Landak”. Jurnal Protobiont, vol. 2(3), pp. 136-140, 2013.

[8] J.D. Bala, J. Lalung, and N. Ismail. “Biodegradation of Palm Oil Mill Effluent (POME) by Bacteria”. International Journal of Scientific and Research Publications, vol. 4(3), pp. 502-511, 2014.

[9] J. D. Bala. Aerobic Treatment and Biodegradation of Palm Oil Mill Effluent by Indigenous Microorganism. [Dissertation]. Environmental Technology Division, School Of Industrial Technology, University Sains Malaysia. 2016.

[10] Periadnadi, Nurmiati, dan M. K. "Swandi. Potensi Bakteri Lokal dalam Mendegradasi Limbah Cair Kelapa Sawit”. Prosiding Semirata Bidang MIPA BKS-PTN Barat, Jambi. pp. 2641-2647, 2017.

[11] Periadnadi dan Nurmiati. Mikroflora Indigenous pada Buah-Buahan Tropis. Padang: Jurusan Biologi FMIPA UNAND, 2010.

[12] Periadnadi. Vorkommen und Stoffweschelleistungen von Bakterien der Gattungen Acetobacter und Gluconobacter whrend der Weinbereitung unter Berucksichtigung des Zucker-Sure-Stoffweschsels. [Disertasi]. Vorgelegt beim Fachbereich Biologie und Informatik der Johan Wolfgang Goethe-Universitat in Frakfurt am Main, Frankfurt, 2003.

[13] M. Faizah, T. Ardyati, and Suharjono. “Isolation and Identification of Indigenous Cellulolytic Bacteria from Sago Pith Waste at Palopo, South Sulawesi, Indonesia”. J. Exp. Life Science, vol. 10(2), 2020.

[14] Y. W. Choi, I. J. Hodgkiss, and K. D. Hyde. “Enzyme Production By Endophytes of *Brucea javanica*”. Journal Agric Tech, vol. 1, pp. 55-66, 2005.

[15] Kementerian Lingkungan Hidup dan Kehutanan. Penanganan Air Limbah Pabrik Kelapa Sawit (PKS) Sebagai Hasil Studi Kebijakan Bersama Indonesia-Jepang (2011-2013). KLH RI Deputi Bidang Pembinaan Sarana Teknis Lingkungan dan Peningkatan Kapasitas, 2013.

[16] E. I. Ohimain, C. D. Olukole, S. C. Izah, R. A. Eke, A. C. Okonkwo. “Microbiology of Palm Oil Mill Effluents”. J Microbiol Biotech Res, vol. 2(6), pp. 852-857, 2012.

[17] J.D. Bala, J. Lalung, A. Al-Gheethi, H. Kaizar, N. Ismail. “Microbiota of Palm Oil Mill Wastewater in Malaysia”. Tropical Life Sciences Research. pp. 1-31, 2018.

[18] L. Widiastuti, Y. Sulistiyanto, A. Jaya, Y. Jagau, L. Neneng. “Potensi Mikroorganisme Sebagai Biofertilizer dari Limbah Cair Pabrik Kelapa Sawit”. Jurnal Surya Medika, vol. 5(1), 2019.

[19] Chairunnisa, Riyanto, dan A. Karim. “Isolasi dan Uji Bakteri Lipolitik dalam Mendegradasi Minyak pada Limbah Cair Kelapa Sawit di Kebun Marihat, Pematang Siantar”. Jurnal Ilmiah Biologi UMA (JIBIOMA), vol. 1(2), pp. 44-52, 2019.

[20] E. O. Imo and C. E. Ihejirika. “Microbial Load and Biodegradation of Palm Oil Mill Effluent (POME) by Microorganisms at Different Stages of Discharge”. EQA-International Journal of Environmental Quality, vol. 44, pp. 9-17, 2021.

[21] J. N. Okereke, S. O. Obiekezie, and K. O. Obasi. “Microbial Flora of Oil-Spilled Sites in Egbema, Imo State, Nigeria”. Afr J Biotechnol, vol. 6(8), pp. 991- 993, 2007.

[22] K.L Hii, S.P. Yeap, and M.D. Mashitah. “Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives”. Eng. Life Sci, vol. 12(1), pp. 7–28, 2012.

[23] M. K. Swandi, Periadnadi, dan Nurmiati. “Isolasi Bakteri Pendegradasi Limbah Cair Industri Minyak Sawit”. J. Bio. UA, vol. 4(1), pp. 71-76, 2015.

[24] S. Fardiaz. Analisis Mikrobiologi Pangan. Jakarta: Raja Grapindo Persada, 1993.

[25] J. M. Lynch and D. M. Barbano. “Kjeldahl Nitrogen Analysis as Reference Method for Protein Determination in Dairy Products”. J AOAC Intl, vol. 82(6), pp. 1389-1392, 2019.

[26] K.B. Maal dan N. Shafiee. “Isolation and Identification of a Novel Strain of Acetobacter Ghanensis KBMNS-IAUF-6 From Banana Fruit, Resistant to High Temperature and Ethanol Concentration”. Iranian Journal of Medical Microbiology. vol. 13(4), 2019.

[27] J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, S. T. William. Bergey's Manual of Determinative Bacteriology 9th ed. Baltimore: William and Wilkins, 1994.

[28] S. T. Cowan and K. J. Steel. Manual for The Identification of Medical Bacteria, 3rd ed. London: Cambridge University Press, 1993.

[29] O. L. Okwute and U. J. J. Ijah. "Bioremediation of Palm Oil Mill Effluent (POME) Polluted Soil Using Microorganisms Found in Organic Waste". The International Journal of Biotechnology, vol. 3(3), pp. 32-46, 2014.

[30] I. B. G. Darmayasa. "Isolasi dan Identifikasi Bakteri Pendegradasi Lipid (Lemak) pada Beberapa Tempat Pembuangan Limbah dan Estuari DAM Denpasar". Jurnal Bumi Lestari, vol. 8(2), pp. 122-127. 2008.