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Isolation and identification petroleum decomposing bacteria as the basis for management of Ambon Bays

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Abstract. The purpose of this research is to determine the bacteria that have the potential to degrade pertroleum in the waters of Ambon Bay. The research was conducted in July-September 2020 in the waters of Ambon Bay, precisely in two locations, namely PT. Perikanan Nusantara Ambon Branch Port in Galala (station 1) and Perikanan Nusantara Ambon Port in Tantui (station 2). The research method used was purposive sampling method on seawater sampling, laboratory-scale experimental method for the isolation of oil-decomposing bacteria, and morphological characterization observations using Stone Mineral Salt Solution Extract Yeast (SMSSe) media. The identification of bacteria uses macroscopic observation to observe the characteristics of the bacterial colony, while microscopic observation is used to observe the shape of the cell and the properties of the bacteria. Environmental parameters at the two stations include temperature, pH, DO, salinity, and brightness categorized as normal for bacterial growth. The results of bacterial isolation is that 7 isolates were obtained, consisting of 5 isolates found at station 1 and 2 isolates found at station 2. The highest total number of bacteria is 123.10^{-5} CFU/ml, while the lowest is 47.10^{-5} CFU/ml. In general, the characteristics of diesel oil-degrading bacterial isolates have circular and irregular colony forms, entire and lobate colony edges, raised and convex colony elevations, milky white, yellow, and cream colony colors, and bacilli and *coccus* cell shapes. Based on the results of gram isolate staining, there were 5gram negative bacterial isolates and 2gram positive bacterial isolates.

1. Introduction

Coastal and marine areas are dynamic and complex areas [1, 2]. Various activities that occur in that area indicate the existence of expansion and developmental progress in the area [3]. Several regions in Indonesia have promising coastal and marine potential [4, 5], one of them is in the Maluku Province. The utilization of coastal and marine resources potential has succeeded in advancing the development and the welfare of its people [6, 7], although still not optimally. The Maluku waters area is larger than the land area resulting in high activity in this area, one of which is boat activity [8]. Boat transportation is a mean of connection between islands which is a characteristic of the Maluku Province, because it has many small islands [9]. The high demand for products from the fisheries and marine sectors, locally, nationally, and internationally, also encourages high fishing vessel activity.

Various boat activities that have been mentioned have had positive and negative impacts on both the community and the water environment. Some of the positive impacts are that the community's needs are fulfilled and the regional income are increasing. However, negative impacts both directly and indirectly are also felt by the community and the aquatic environment. One of the real negative impacts in the waters is oil spills [10,11]. Oil spills at sea can come from tanker accidents, ship loading and unloading, docking (maintenance/reparing), and the cutting of the ship's hull. As the capital of the Maluku Province, Ambon City which is the center of development, has high shipping activity. These activities were identified in several places in the waters of Ambon Bay, including Yos Sudarso Port, Perikanan Nusantara Port, Slamet Riyadi Port, PT. Perikanan Nusantara, Pertamina Wayame Port and others.

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It is well-known, oil spills in the waters will disrupt the aquatic ecosystems, especially the important coastal ecosystems such as mangrove, seagrass, and coral reef ecosystems [12, 13, 14]. Oil spills are contaminants that can be lethal (deadly) or sublethal (inhibit reproductive growth processes and other physiological processes) for aquatic organisms. Oil spills that enter the waters do not mix with the water; the oil will form a layer on the surface which will inhibit the penetration of sunlight for photosynthesis. Oil spills will result in a lack of dissolved oxygen as a result of the photosynthesis process in waters which is needed by aquatic biota for their survival. However, the oil will mix with the water and settle on the bottom of the water for a certain period of time. Oil spills contain hydrocarbon compounds that are dangerous and detrimental to marine ecosystems. Some of the oil-forming components are known to be toxic to aquatic organisms which will also affect humans. Petroleum emissions generally contain heavy metal compounds such as Pd (lead) and Cd (cadmium) which are toxic to aquatic organisms [15, 16]. Through the food chain process, aquatic organisms that have been contaminated with chemical compounds will be consumed by humans, which indirectly causes oil spills in the waters to eventually accumulate in the human body.

One of the efforts to control water pollution due to oil spills is bioremediation [17, 18]. Compared to various technological efforts, bioremediation is considered environmentally friendly. Bioremediation is defined as the use of microorganisms to degrade an environment contaminated with hazardous waste (toxic) into a form that is not dangerous (less toxic). In general, the materials used for bioremediation process use the help of bacteria and fungi or plants that have the ability to absorb hazardous waste. The results of research in the waters of Pare-Pare Bay found three bacterial isolates that can degrade diesel oil, namely *Bacillus* sp, *Psedomonas aeruginosa*, and *Alkaligenes faecalis* [19]. The use of bacteria for bioremediation is currently developing and has the potential in the future because it is environmentally friendly and widely available in nature. The purpose of this research is to determine the bacteria that have the potential to degrade petroleum in the waters of Ambon Bay.

2. Materials and Method

2.1. Sampling method

The research was conducted in July-September 2020 in waters of Ambon Bay, precisely in two locations (Figure 1), namely at PT. Perikanan Nusantara Ambon Branch Port in Galala (station 1) and Perikanan Nusantara Ambon Port in Tantui (station 2). The selection of the two locations took into account the high activity of the fishing boats landing port. Sampling of seawater was carried out using the purposive sampling method. Seawater samples taken at the two research locations were carried out by dipping the sterilized bottles below the water surface (approximately 20 cm) with the bottles positioned opposite to the direction of the water flow. Environmental parameter data collection including temperature, pH, brightness, dissolved oxygen (DO), and salinity was carried out *in situ*. Sample testing was carried out at the Microbiology Laboratory of the Department of Biology and the Laboratory of Basic Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University.

2.2. Research procedures in the laboratory

2.2.1. The making of bacterial growth media. The sampling process up to the isolation and identification stage can be seen in Figure 2. The media used in the isolation and degradation test of petroleum using selective media, is the Stone Mineral Salt Solution Extract Yeast (SMSSe). Making SMSSe media for bacterial growth consisted of $CaCO_3$ (*Calcium carbonate*), NH_4NO_3 (*Amonium nitrat*), $Na_2HPO_4.7H_2O$ (*Sodium monohydrogen phosphate heptahydrate*), KH_2PO_4 (*monokalium phosphate fertilizer*), $MgSO_4.7H_2O$ (*Magnesium sulfat*), $MnCl_2.7H_2O$ (*Manganese* (II) *chloride heptahydrate*), with adding 0.01% (b/v) of yeast extract [20]. Making growth media consists of two, liquid media and solid media.

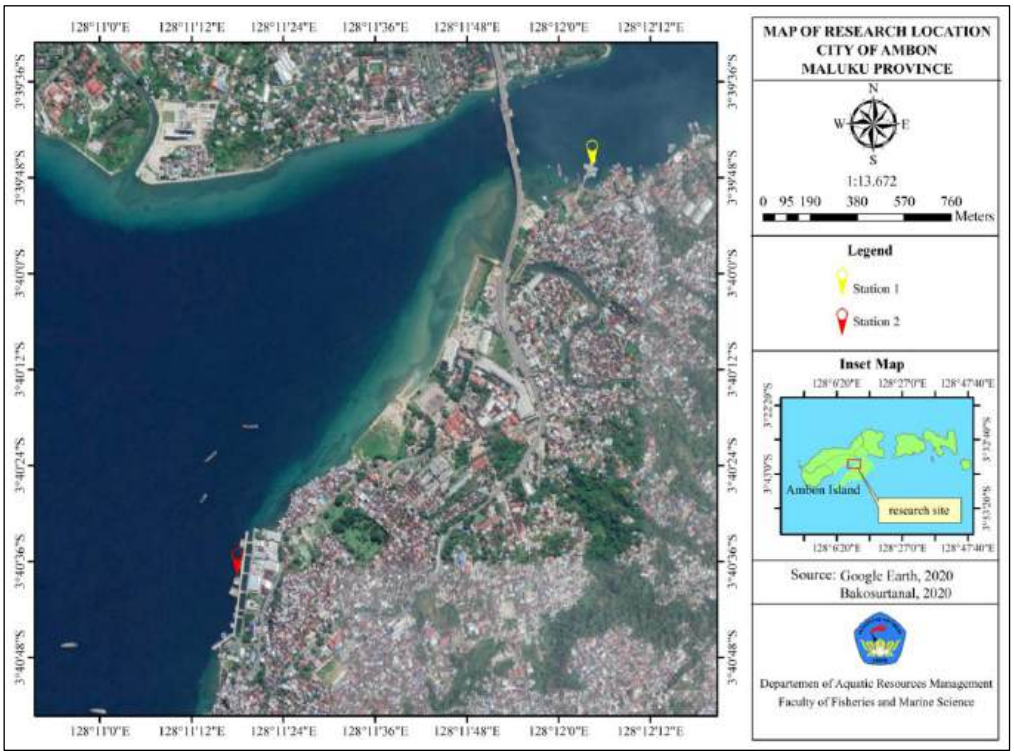


Figure 1. Map of sampling site

2.2.2. Isolation and identification of petroleum degrading bacteria. In the isolation stage, dilution is carried out by inserting a 9 ml seawater into liquid SMSSe media containing 2% (b/v) of pure oil. Next, the diluted sample was incubated in a incubator shaker at 35ºC and at the speed of 120 rpm for 3 days. Then 1 ml was taken for isolation by adding 9 ml of distilled water into the test tube for dilution. Next, it is vortexed to be homogeneous. The dilution process is carried out until the fifth dilution (10^{-5}).

During the last three dilutions, 10^{-3}, 10^{-4}, and 10^{-5} with sterile sea water, 0.1 ml is taken and grown on a plate of SMSSe media containing 2% (b/v) of diesel oil. Then 2 % bacto agar was added as a compactor using the scatter plate method with the *hockey stick* [21]. Then, the sample is incubated at 35ºC for 24 hours. The growing bacteria were purified again based on the same morphological characteristics using the scratching method on the SMSSe media plate and added with bacto agar in order to obtain a single colony reslut. The results of the isolates that have been obtained are then identified using macroscopic observations to observe the characteristics of the bacterial colony, while microscopic observations are used to observe the shape of the cells and the properties of the bacteria [22].

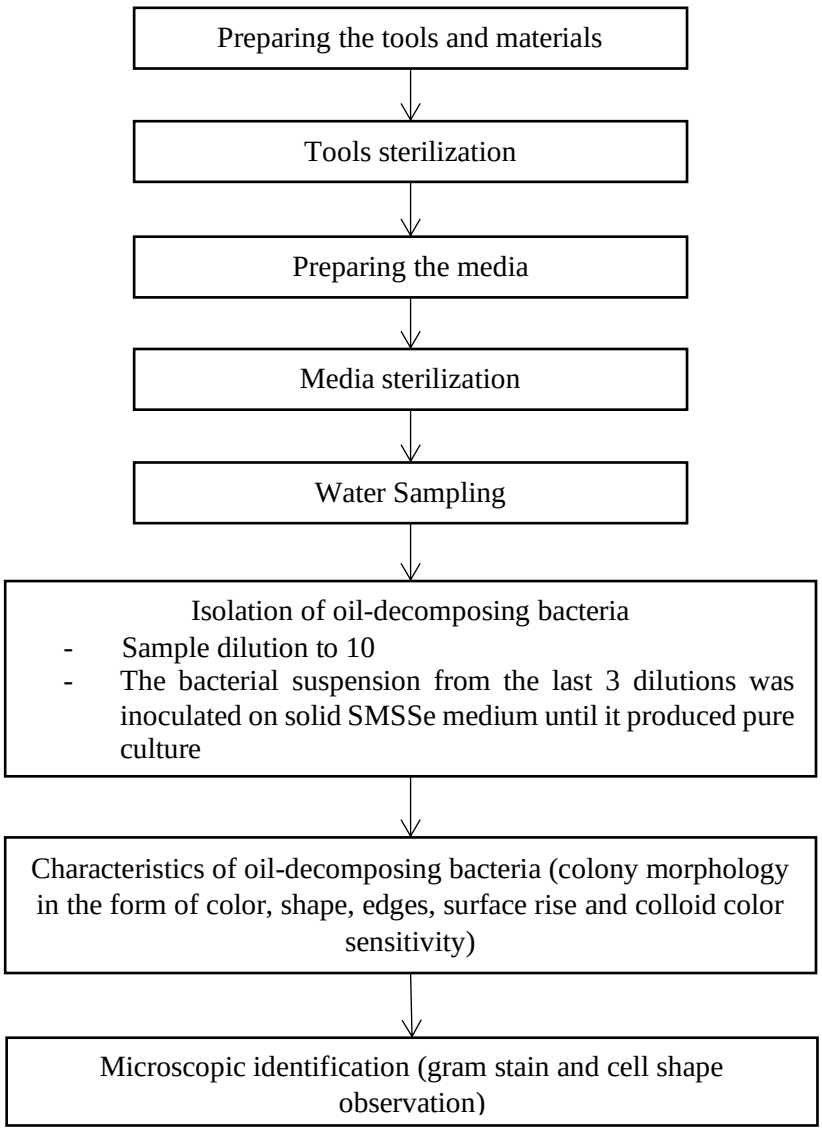


Figure 2. Research procedure flowchart

2.3. Data analysis method

The calculation of the number of bacteria was carried out using the Total Plate Count method [23], with the formula below:

$$K = \frac{(a\ x\ 10^{e}) + (b\ x\ 10^{f}) + (c\ x\ 10^{g})}{3}$$

where:
K : number of the colony (CFU/ml)^{e}
a, b, c : number of the bacteria colony
e, f, g : the dilution factor

The petroleum content analysis method can use the following formula (SNI 06 6989.10-2004):

$$\text{Oil content (mg/L)} = \frac{(A-B)}{mL\ test\ sample}\ x\ 1000$$

where:
A = The weight of flask + extract (mg)
B = The weight of empty flask (mg)

The calculation of the percentage of petroleum bidegradation uses the following formula [24]:

$$\%B = \frac{(Bmo-Bmn)}{Bmo}\ x\ 100$$

where:
%B = Biodegradation percentage
Bmo = Initial petroleum weights (g)
Bmn = Final petroleum weights (g)

3. Result and Discussion

3.1. Environmental parameter condition

The activity of degrading bacteria is influenced by the environmental factors [25]. Marine microorganisms are greatly influenced by changes in the environmental factors. The temperature has an influence on the activity of petroleum degrading bacteria in marine waters. The temperature value obtained at station 1 is 27.5^{o}C and station 2 is 27.25^{o}C. The temperature values at these two stations are still good to support the growth of marine life and microorganisms. *Psedomonas aeruginosa* bacteria can grow at temperatures reaching 30^{o}C to 50^{o}C with an optimal temperature of 45^{o}C [26]. The pH level obtained at station 1 was 6.54, while station 2 was 6.15. The pH values at both research stations are still ideal for the growth of oil-degrading bacteria. pH parameters play an important role in the bioremediation process by bacteria [27]. The optimal pH value for the bioremediation process is at pH 6, but microbes will die at pH range of 8 to 10 [28].

Dissolved oxygen at the two research stations are 7.55 mg/l and 9.7 mg/l, respectively. Nitrifying bacteria require a minimum of 2 mg/l dissolved oxygen for respiration [29]. The Decree of the State Minister for the Environment No. 51 of 2004 states that the value of dissolved oxygen that is good for marine life is > 5 mg/l. Dissolved oxygen that supports the activity of degrading bacteria ranges from 5.83 mg/l to 6.83 mg/l [30].

The salinity values at the research stations were 29‰ and 29.25‰, respectively. These values are still considered normal for marine waters. Salinity greatly affects the growth of oil-degrading bacteria such as *Bacillus* sp [31]. Bacterial diversity will be negatively impacted if the salinity increases because it can inhibit the growth of bacterial colonies and reduce the level of bacterial activity [32]. The brightness levels at each research station are still relatively good, at 5 m and 4 m. Brightness affects the presence of bacteria in the water. If the level of turbidity is high, it will inhibit the penetration of sunlight entering the waters so that it impacts the photosynthesis process.

3.2. Isolation of Bacteria, Purification and Selection of Diesel Oil Degrading Bacteria from PT. Perikanan Nusantara Port and Perikanan Nusantara Ambon Port

The isolation results show that there are bacteria that have the potential to decompose pertroleum at the two research stations. Based on the results of bacterial colonies growing on SMSSe media, 7 bacterial isolates were found. The bacterial isolates obtained were each given the sample codes A1.1, A1.2, A1.3, A1.4, A2.1, T1.1, T1.2. Isolates with sample codes A1.1, A1.2, A1.3, and A1.4 were obtained from

isolating sample 1 at PT. Perikanan Nusantara, Galala, sampling point 1, while isolate A2.1 was obtained from sampling point 2. Isolates T1.1 and T1.2 were obtained from isolating sample 2 at Perikanan Nusantara Ambon Port, sampling point 1. Based on the purification of the 7 isolates, the single colonies that grew on marine agar media were similar to the isolates found on SMSSe media (Figure 3). In the sample coded T2, no bacterial isolates were found. Pure isolates scratched on marine agar media are bacteria that have the potential to decompose diesel oil.

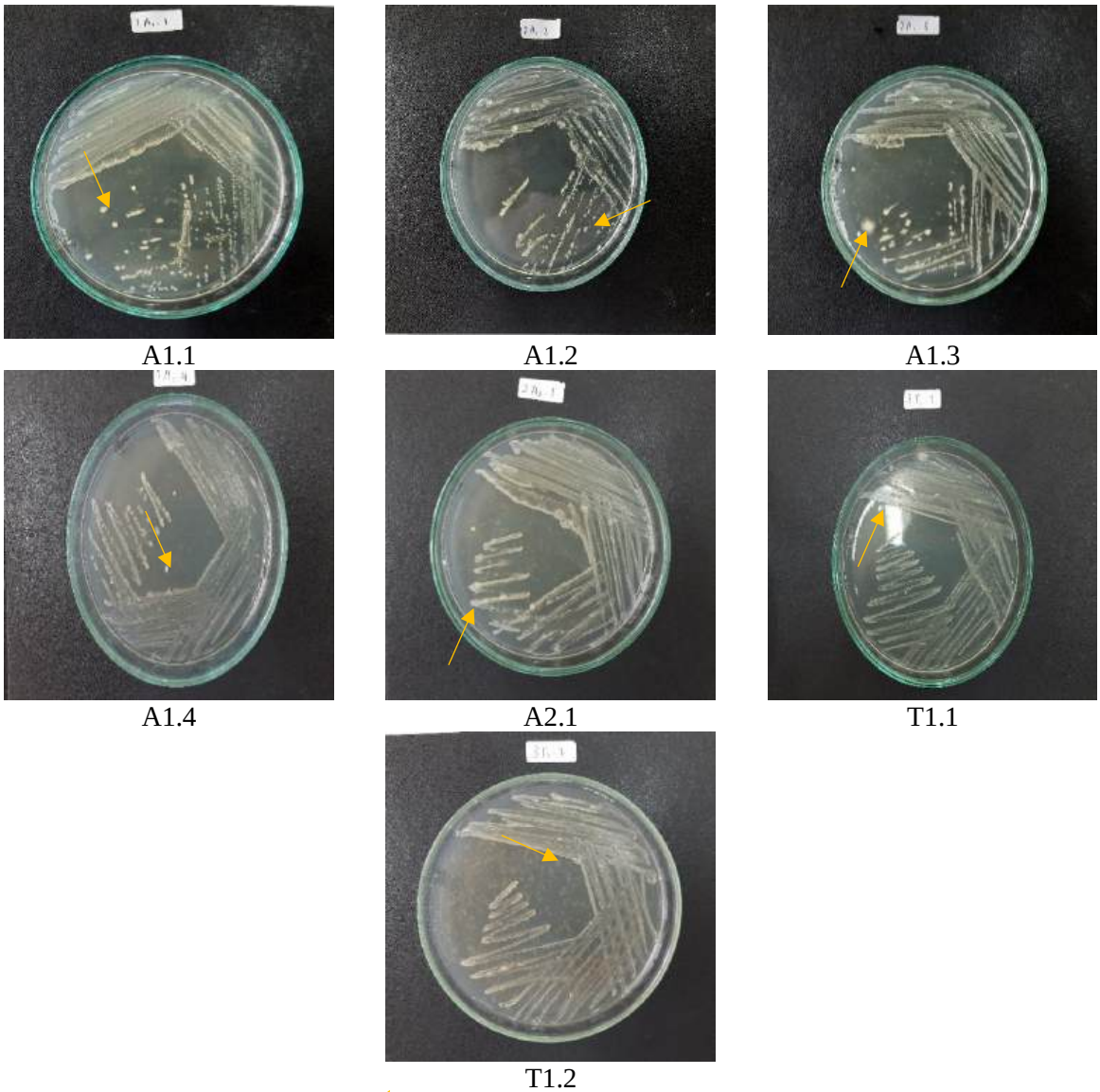


Figure 3. The results of scratching the diesel oil degrading bacterial isolate on Marine Agar media

3.3. The number of bacterial colonies using the TPC Method based on the dilution

Based on the results of the research, it was found that the highest average total number of bacteria was 123.10^{-5} CFU/ml originating from the waters of PT. Perikanan Nusantara Port, Galala, while the lowest is 47.10^{-5} CFU/ml from Perikanan Nusantara Ambon Port (Table 1). There are samples that are not overgrown by bacteria, this is suspected to be caused by the presence of Calcium Carbonate ($CaCO_3$) which is still deposited in the media. The concentration of $CaCO_3$ causes variations in the pH of the

media, which causes changes in bacterial enzyme activity, therby affecting the reaction rate [33]. Moreover, there was the clumping of diesel oil that becomes a factor for bacteria not growing. The number of colonies that grew in samples A1, A2, and T1 at 2 % diesel oil concentration on the third day was related to the degradation percentage. In general, the greater the number of bacterial colonies that grow, the higher the percentage of degradation [34].

Table 1. The number of oil decomposing bacteria at the PT. Perikanan Nusantara Port and Perikanan Nusantara Ambon Port

No.	Sample Code	Samples taken (ml)	Σ Bacteria			Σ TPC CFU/ml
			10^{-3}	10^{-4}	10^{-5}	
1	A1	0.1	-	65.10^{-5}	180.10^{-5}	123.10^{-5}
2	A2	0.1	83.10^{-5}	-	-	83.10^{-5}
3	T1	0.1	20.10^{-5}	40.10^{-5}	80.10^{-5}	47.10^{-5}
4	T2	0.1	-	-	-	-

Note: A = Station 1 PT. Perikanan Nusantara Port; T = Station 2 Perikanan Nusantara Ambon Port

3.4. Identification of diesel oil degrading bacteria

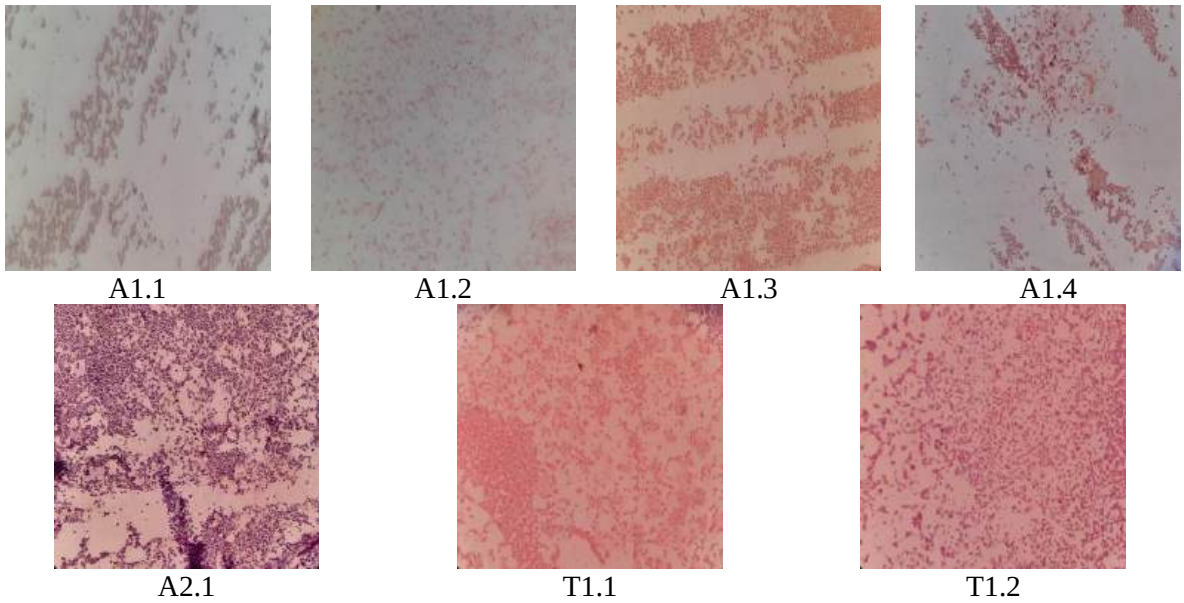
For the isolates found from the purification results, the identification process was then carried out in the form of direct colony observation through color, shape, edge, and colony elevation. Meanwhile, to see the forms of bacterial cells and to determine the group of bacteria from the resulting isolate, gram staining was performed. There are 4 isolates (A1.1, A1.2, A1.3, and A1.4) which have circular colony shapes, entire colony edges, raised elevation forms, and a bacillus cell shape which macroscopically have the same characteristics (Table 2). Isolate A2.1 has a circular colony shape, an entire colony edge, a convex elevation form, and a bacillus cell shape. The colors of isolates A1.1, A1.2, and A1.3 are milky white, A1.4 is yellow, and isolate A2.1 is cream. At station 2, which is located at Perikanan Nusantara Ambon Port, there is 1 bacterial isolate with code T1.1 which has an irregular colony shape, a lobate colony edge, a raised elevation form, a coccus cell shape, and milky white color. Morphological differences in the types of bacteria that grow on Marine Agar media are thought to be influenced by environmental factors around the research station.

Microscopic identification of bacteria was also carried out using gram staining. Gram staining is used in the identification of bacteria to determine whether the bacteria found are gram-positive or gram-negative. Based on the results of gram staining on 7 isolates, 5 isolates of gram-negative bacteria were obtained (A1.1, A1.2, A1.3, A1.4, and T1.1) and 2 gram positive (A2.1 and T1.2). The results of gram-negative isolates were shown by the appearance of red cells, while isolates that were included as gram-positive were shown by the appearance of purple cells (Figure 4). The difference in color of bacterial cells is caused by the difference in the composition of the cell walls of each bacterial isolate. Gram negative bacteria generally have cell wall with high lipid content. Lipids are dissolved by acetone alcohol, so the crystal violet dye on the bacterial cell wall cannot be maintained and instead binds the red safranin substance at the time of staining [35]. Furthermore, the cell wall in gram-positive bacteria consists of peptidoglycan which is insoluble in acetone alcohol, so the blue color of crystal violet dye complex is maintained at the time of staining. Gram positive bacteria have a larger cell size because they contain thick peptidoglycan on the cell wall, whereas gram-negative bacteria have a smaller cell size because they have thin peptidoglycan on the cell wall. Bacteria from the class g-proteobacteria and a-protebacteria dominate and play an important role in the bioremediation process of oil, especially in the degredation of poliaromatic hydrocarbons (PAHs) and alkanes [36]. The degrading bacteria also come from *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus insolitus* and *Bacillus marinus* which have the potential to be developed in petroleum bioremediation [37].

Table 2. The results of characterization of diesel oil degrading bacterial isolates

Station	Sampling Point	Isolate Code	Colony Form	Colony Edges	Colony Eleveation	Colony Color	Cell Form	Size	Gram Stain
1	1	A1.1	*Circular*	*Entire*	*Raised*	Milky white	Bacillus	Medium	Negative
		A1.2	*Circular*	*Entire*	*Raised*	Milky white	Bacillus	Small	Negative
		A1.3	*Circular*	*Entir*	*Raised*	Milky white	Bacillus	Small	Negative
		A1.4	*Circular*	*Entire*	*Raised*	Yellow	Coccus	Small	Negative
	2	A2.1	*Circular*	*Entire*	*Convex*	Cream	Bacillus	Large	Positive
2	1	T1.1	*Circular*	*Entire*	*Raised*	Cream	Bacillus	Small	Negative
		T1.2	*Irregular*	*Lobate*	*Raised*	Milky white	Coccus	Large	Positive

Note: Station 1 = PT. Perikanan Nusantara Port
Station 2 = Perikanan Nusantara Ambon Port



Note: Gram-negative = A1.1, A1.2, A1.3, A1.4, T1.1, C.2, Gram-positive = A2.1, T2.1

Figure 4. The result of gram staining of bacterial isolates

4. Conclusion

Based on the results and discussion stated, it can be concluded that there are seven isolates of petroleum degrading bacteria obtained from the research location, namely five isolates found in the waters at the PT. Perikanan Nusantara Port and two isolates at the Perikanan Nusantara Ambon Port.

This research is an early stage research to isolate and identify biosurfacants producing and diesel oil degrading bacteria. The suggestions that can be recommended based on the results of this research is that there is a need for further research on the effect of environmental conditions (pH, temperature, and nutrients) on increasing the percentage of oil biodegradation, on molecular identification of biosurfactant-producing bacteria, and on simulations of oil pollution management models using dynamic systems.

References

[1] Ramesh R *et al* 2015 Land–Ocean Interactions in the Coastal Zone: Past, present & future. *Anthopocene* **12**: 85-98http://dx.doi.org/10.1016/j.ancene.2016.01.005

[2] Yulianto F, Suwarsono, Maulana T and Khomarudin M R 2019 The dynamics of shoreline change analysis based on the integration of remote sensing and geographic information system (GIS) techniques in Pekalongan coastal area, Central Java, Indonesia. *Jorunal of Degraded and Mining Lands Management* **6**(3): 1789-802 DOI:10.15243/jdmlm.2019.063.1789

[3] Trinanda T C 2017 Management of Indonesia's coastal areas in the framework of environmental conservation-based development. *Matra Pembaruan* **1**(2): 75-84

[4] Stacey N, Gibson E, Loneragan N R, Warren C, Wiryawan B, Adhuri D, and Fitriana R 2019 Enhancing coastal livelihoods in Indonesia: an evaluation of recent initiatives on gender, women and sustainable livelihoods in small-scale fisheries. *Maritime Studies* **18:** 359–71 https://doi.org/10.1007/s40152-019-00142-5

[5] Arianto M F 2020 The Potential of Coastal Area in Indonesia. *Jurnal Geografi: Geografi dan Pengajarannya* **XX** (XX): 1-7

[6] Peng C, Yamashita K, and Kobayashi E 2016 Effects of the coastal environment on well-being *Journal of Coastal Zone Management* **19**(2): 1-7 DOI: 10.4172/2473-3350.1000421

[7] Stanford R J, Wiryawan B, Bengen D G, Febriamansyah R, and Haluan J 2014 Improving livelihoods in fishing communities in West Sumatra: More than just boats and machines *Marine Policy* **45**: 16–25 DOI: 10.1111/faf.12220

[8] Sihaloho A, Wunas S, Jinca M Y, and Parung H 2012 Ferry transport of Trans Maluku interislands cluster in Indonesia. *Internasional Journal of Civic & Environmental Engineering* **12**(5): 9-14

[9] Syamsumarlin and Ofu M H M 2015 Boat activities in the voyage on the Surya Indah motor boat, Kapala Madan Distric, South Buru Regency, Maluku Province *Etnoreflika* **4**(2): 831-47

[10] Chang S E, Stone J, Demes K and Piscitelli M 2014 Consequences of oil spills: a review and framework for informing planning. *Ecology and Society* **19**(2): 26 http://dx.doi.org/10.5751/ES-06406-190226

[11] Latif B, Kurniawan A, and Ayubi I M 2018 The Legal Review of Petroleum Spill Case (Pertamina Oil Spill in Kalimantan). *Udayana Master Law Journal* **7**(2): 133-53 DOI: 10.24843/JMHU.2018.v07.i02 .p1

[12] Santos H F A *et al* 2015 Impact of oil spills on coral reefs can be reduced by bioremediation using probiotic microbiota. *Sci Rep* **5,** 18268 https://doi.org/10.1038/srep18268

[13] Duke N C 2016 Oil spill impacts on mangroves: Recommendations for operational planning and action based on a global review. *Marine Pollution Bulletin* **109**: 700-15 http://dx.doi.org/10.1016/j.marpolbul.2016.06.082

[14] Hernawan U and Risdianto R K 2017 Coastal Protection of Southern Part of the Bintuni Bay from Oil Spill: An Environmental Sensitivity Index Approach. *Bulletin of the Marine Geology* **32**(2): 77-88

[15] Mustafa A D, Juahir H, Yunus K, Amran M A, Hasnam C N C, Azaman F, Abidin I Z, Azmee S H, and Sulaiman N H 2015 Oil spill related heavy metal: a review. *Malaysian Journal of Analytical Sceince* **19**(5): 1348-60

[16] Zhang S, Guo H, and Zhang S 2020 Are oil spills an important source of heavy metal contamination in the Bohai Sea, China *Environ Sci Pollut Res* 27**,** 3449–361 https://doi.org/10.1007/s11356-019-06913-1

[17] Giwa O M and Ibiyote F O Bioremediation of heavy metal in crude oil contaminated soil using isolated Indigenous microorganism cultured with E coli DE3 BL21. *International Journal of Engineering and Applied Sciences* **4**(6): 67-70
[18] Ismail A S, El-Sheshtawy H S, and Khalil N M 2019 Bioremediation process of oil spill using fatty-lignocellulose sawdust and its enhancement effect. *Egyptian Journal of Petroleum* **28**(2): 205-11 https://doi.org/10.1016/j.ejpe.2019.03.002
[19] Hasyimuddin A, Djide M N, and Samawi M F 2016 Isolation of Diesel Oil Degrading Bacteria from the Waters of Pare-Pare Bay. *Biogenesis* **4**(1): 41-46. DOI: https://doi.org/10.24252/bio.v4i1.1119
[20] Sharpley J M 1996 *Elementary Petroleum Microbiology*. (Texas: Gulf Publishing Company)
[21] Ilham K N, Meidyansyah A S, and Arrozi W M 2017 Reduction of oil spills using the bacterial culture method at TLP West Seno, Makassar Strait. *Pros. Sem. Masy. Biodiv. Indoensia* 3(2): 258-265. DOI: 10.13057/psnmbi/m030217
[22] Buchanan R E and Gibbons N E 1974 *Bergey's Manual of Determinative Bacteriology*. Eighth Edition. (Baltimore: Williams & Wilkins Company) p 1246
[23] Cappucino J G and Sherman N 1983 *Microbiology*: A Laboratory Manual. (Massachusetts: Addison-Wesley Publishing) p 466
[24] AOAC. 1984 *Official Methods of Analysis*. 15th Ed. (Arlington Virginia: Association of Official Analytical Chemistry, Inc)
[25] Lew S, Glińska-Lewczuk K, and Lew M 2019 The effects of environmental parameters on the microbial activity in peat-bog lakes. *PLoS ONE* 14 (10): e0224441. https://doi.org/10.1371/journal. pone.0224441
[26] Safitriani, Thontowi A, Yetti E, Suryani, and Yopi 2017 Optimal Growth of Marine Bacteria Pseudomonas aeruginosa LBF-1-0132 in Piren Compounds. *Indonesia Journal of Biology* **13**(1): 107-16
[27] Ajoku G A O and Oduola M K 2013 Kinetic Model of pH effect on Bioremediation of Crude Petroleum Contaminated Soil. 1. Model Development. *American Journal of Chemical Engineering* **1**(1) 6-10. doi: 10.11648/j.ajche.20130101.12
[28] Obahiagbon K O, Amenaghawon A N, and Agbonghae A O 2014 Effect of Initial pH on the Bioremediation of Crude Oil Polluted Water Using a Consortium of Microbes. The Pacific Journal of Science and Technology **15**(1): 452-7 http://www.akamaiuniversity.us/PJST.htm.
[29] Nainggolan T A, Khotimah S, and Turnip M 2015 Ammonia degrading bacteria from rubber liquid waste, Pontianak, West Kalimantan. *Protobiont* **4**(2) 69-76
[30] Puspitasari I, Trianto A, and Suprijanto J 2020 Exploration of oil-degrading bacteria from the waters of Tanjung Mas Port, Semarang. *Journal of Marine Research* **9**(3): 281-8. DOI: 10.14710/jmr.v9i3.27606
[31] Ahamed F, Dhar K, Ferdouse J, and Anwar M N 2014 Effect of salinity on the growth of petroleum hydrocarbons degrading Bacillus sp. isolated from chronically polluted ship breaking yards. *Elixir Bio Technology* **68**: 22280-83.
[32] Yulma, Ihsan B, Sunarti, Malasari E, Wahyuni N, and Mursyban 2017 Identification of Bacteria in Mangrove Leaf Litter Decomposed in the Mangrove and Proboscis Monkey Conservation Area (KKMB) of Tarakan City. *Journal Tropical Biodiversity and Biotechnology* **2**: 28-3. DOI: 10.22146/jtbb.27173
[33] Ferdaus F, Wijayanti O M, Retnonigtyas E S, and Irawati W 2008 Effect of pH, Substrate Concentration, Addition of Calcium Carbonate, and Fermentation Time on the Acquisition of Lactic Acid from Banana Peels. *Widya Teknik Journal* **7(**1): 1-14. DOI: https://doi.org/10.33508/wt.v7i1.1256

[34] Charlena, Mas'ud Z A, Yani M, Sjahriza A, and Tarigan J G 2011 Biodegradation of Heavy Oil Waste Using Single and Mixed Isolates with the Addition of Linear Alkylbenzene Sulfonate. Proc of the Nat Sem on Indonesian Applied Chemistry. The Potential of Applied Chemical Researh in Supporting Innovation-Based Science and Technology Development". 182-9
[35] Fitri L and Yasmin Y 2011 Isolation and observation of the morphology of chitinolytic bacterial colonies. *Scientific Journal of Biology Education*. **3**(2): 20-5
[36] Thontowi A and Yopi 2013 Diversity of Alkane and Poliaromatic Hydrocarbon Degradation Marine Bacteria in Pari Island, Jakarta. *Indonesia Journal of Biology* **9**(1): 131-40
[37] Sopiah N, Oktaviani A N, Sulistia S, Suciati F, and Aviantara D M 2011 Isolation and identification of hydrocarbon degrading bacteria from petroleum contaminated oil. *Tek. Lingk. Journal* **12**(3): 291-8