Omni-Akuatika, 17 (1): 60 - 69, 2021
ISSN: 1858-3873 print / 2476-9347 online

Research Article

journal homepage: http://ojs.omniakuatika.net

Viability Test of Hydrocarbonoclastic Bacteria Consortium Entrapted on Sawdust Material

Devia Hartono Puteri[1*], Aditya Hikmat Nugraha[1], Yeti Darmayati[2], Agung Dhamar Syakti[3]

[1]Marine Science Department, Raja Ali Haji Maritime University, INDONESIA
[2]Marine Microbiology Laboratory, Research Center for Oceanography, Indonesian Institute of Sciences, INDONESIA
[3]Environmental Science Department, Raja Ali Haji Maritime University, INDONESIA

**Corresponding author: deviahp4@gmail.com*

Received 15 February 2021; Accepted 2 August 2020; Available online 6 August 2021

ABSTRACT

Oil spills in the sea are generally prevented in various ways, one of them is the bioaugmentation method through bacterial entrapment on sawdust carriers. The entrapment of bacteria is one of the bacterial immobilization techniques. The potential of bacterial consortium on sawdust is still not widely used, especially in long-term storage. The purpose of this study was to obtain the viability of hydrocarbonoclastic bacterial consortium on sawdust after 0, 7, and 41 days of storage. The bacterial consortium consists of *Bacillus aquimaris, B. megaterium, B. pumilus, Halobacillus trueperi, and Rhodobacteraceae bacterium.* The samples were tested by culturing the immobilized bacterial consortium of 0, 7, and 41 days on falcon tubes containing crude oil and seawater. The viability of the immobilized bacterial consortium was tested for 28 days (days 0, 7, 14, 21, and 28) by enriched the bacterial populations periodically. The viability test was also supported by the measurement of environmental parameters on the 1st day and the 28th day such as DO, pH, salinity and, temperature. The results show that bacterial consortium in all storage periods was viable on sawdust carriers which related to the high number of bacterial populations on the 28th day. The most viable bacterial consortium on sawdust was the 0-day storage (9.59 x 10^{8} CFU/ml) which was indicated by the increases phase at the end of the day. It was proved that the bacteria are still productive and could degrade petroleum hydrocarbons.

Keywords: Bioremediation, Immobilized Bacteria, Oil Spill

ABSTRAK

Penanggulangan tumpahan minyak di laut dapat dilakukan dengan berbagai cara, salah satunya menggunakan metode bioagumentasi dengan cara *entrapment* bakteri pada serbuk kayu. *Entrapment* pada bakteri merupakan salah satu teknik imobilisasi. Penggunaan bakteri hidrokarbonoklastik dengan cara seperti ini masih belum banyak dilakukan, khususnya pada parameter masa penyimpanan. Tujuan penelitian ini adalah menguji viabilitas konsorsium bakteri hidrokarbonoklastik pada *carrier* serbuk kayu setelah 0, 7 dan 41 hari penyimpanan. Konsorsium bakteri yang digunakan terdiri dari *Bacillus aquimaris, B. megaterium*, *B. pumilus, Halobacillus trueperi* dan *Rhodobacteraceae bacterium*. Pengujian sampel dilakukan dengan membiakkan sediaan kering usia 0, 7 dan 41 hari pada *falcon tube* berisi *crude oil* dan air laut. Viabilitas sediaan kering diuji selama 28 hari (hari ke-0,7,14, 21 dan 28) dengan menghitung jumlah populasi bakteri secara berkala. Uji viabilitas juga didukung oleh pengukuran parameter lingkungan pada awal dan akhir masa pembiakan yang terdiri dari DO, pH, salinitas dan suhu. Hasil penelitian menunjukkan bahwa masing-masing sediaan kering memiliki viabilitas pada *carrier* serbuk kayu yang ditandai dari tingginya jumlah populasi bakteri pada hari ke-28. Performa sediaan kering yang paling baik ditunjukkan oleh usia ke-0 hari (*fresh*) dengan jumlah rata-rata 9.59 x 10^{8} cfu/ml dimana masih terjadinya fase peningkatan pada akhir pembiakan. Hal tersebut menandakan bahwa bakteri masih produktif dan mampu memanfaatkan hidrokarbon minyak bumi dengan baik.

Kata kunci: Bioremediasi, Imobilisasi Bakteri, Tumpahan Minyak

http://dx.doi.org/10.20884/1.oa.2021.17.1.879

1. Introduction

The International Tanker Owners Pollution Federation (ITOPF) noted that 164.000 tons of petroleum hydrocarbons entering the world's ocean waters due to the case of oil spills in 2010-2019 (ITOPF 2020). Indonesian waters which are included in international shipping lanes are one of the waters affected by the oil spill in the sea (Andilas & Yanggana 2017). Oil spills that occur every year give a negative impact on the utility value of coastal natural resources and huge losses to various marine economic activities, especially in the marine tourism sector. It is certainly caused by the ecological disaster of oil spill on the coastal water (Suwedi 2017).

The ecological disaster by oil spills occurs by its toxicity which give a negative impact on the sustainability of marine life (Thamer et al., 2013). Setyonugroho (2019) reported that the short-term impact of oil pollution is the harm of cell membranes of marine biota, while the long-term impact is disruption of the food chain in the sea. The layer of crude oil on the surface blocks the exchange of gases from the atmosphere and reduces the solubility of oxygen in the water, thus it interferes with the process of respiration and photosynthesis in phytoplankton, which is a food producer in the sea. Furthermore, oil that is adsorbed and eaten by marine life will accumulate in protein compounds. This accumulated trait can be transferred from one organism to another through the food chain.

Oil spill treatment that environmentally friendly was generally carried out by bioremediation method through bioaugmentation technique. Bioaugmentation technique is the process of restoring an area such as soil, water, or beaches that utilize microorganisms such as bacteria as degrading oil. Carbon, which is the largest content in crude oil, is a source of energy for microorganisms, thus bioremediation is an alternative in efforts to restore the environment from pollution activities. One of the weaknesses in the bioaugmentation technique it requires a very large number of stocks of bacteria to minimize the costs of the process (Podorozhko et al., 2008). A breakthrough is needed in providing bacterial biomass, including the process of microbial consortium preparations in dry form using sawdust which is practical and effective in the use (Thapa et al., 2012). In this case, sawdust is used as a carrier of bacterial consortium due to its advantages such as low-cost price, ease to handle, and long shelf life. Furthermore, it resistant to toxic chemicals, pH, temperature, solvents, and heavy metals (Bayat et al., 2015). According to Dzionek et al., (2016), Arthrobacter sp. which immobilized on sawdust did not lose their enzymatic activity after 6 weeks of storage (at 25°C and 45°C) and was still able to degrade similar quantities of crude oil.

The immobilized bacterial consortium aims to reduce the workload in the maintenance of bacterial liquid culture, extend shelf life and facilitate distribution of bacterial consortium in dry form without losing viability and performance of decomposing bacteria (Triana et al., 2006). One of the challenges of immobilized bacteria is that the long-term storage to see the viability of living bacteria in the carrier. Currently, the main obstacle to this process s the lack of research and engineering related to the immobilized bacterial consortium, especially to increase the viability of bacteria in a long-term period to degrade petroleum contamination. Thus, immobilizing bacteria onto the carrier (sawdust) is the alternative of choice.

2. Material and Methods

Preparation of Inoculums

Bacterial which was isolated from sediment collected from the Cilacap coast in Indonesia was described previously by Syakti et al., (2013). The bacterial consortium consists of five strains [*Bacillus aquimaris* (S2), *Bacillus megaterium* (S3), *Bacillus pumilus* (S6), *Halobacillus trueperi* (S4), *Rhodobacteraceae bacterium* (S5)]. These bacteria possess the ability to degrade petroleum hydrocarbon compounds such as phenothiazine, fluorene, fluoranthene, dibenzothiophene, phenanthrene, and pyrene. Each bacterial from the freezer was activated in marine agar and incubated at 37°C. Furthermore, each activated bacteria then cultivated twice in marine broth and incubated on an orbital shaker (120 rpm) for 24 h. These activated bacteria were subsequently grown in one erlenmeyer containing marine broth and incubated on an orbital shaker (120 rpm) for 24 h (Al-wasify & Hamed 2016). This solvent served as the starting inoculum for all subsequent experiments.

Immobilization of bacterial consortium onto sawdust

The sawdust was filtered by sediment sieve shaker <106 µm and then sterilized by autoclaving at 121°C for 20 min and cooled down before use. 0.5 milliliter of bacterial consortium inoculum was pipetted into a sterile petri dish that contained 5 g sterilized sawdust and the egg white was then added to sawdust that contains consortium inoculum to obtain a slightly solid texture and formed into pellets. Those immobilized bacterial consortiums were then incubated at room temperature for a certain period; 0, 7, and 41 days (Table 1). This

immobilized bacterial consortium was used as the first treatment for further measurement of the bacterial population (Hazaimeh et al., 2014).

Treatment of Immobilized Microbial Consortium

Microbial consortium treatment on sawdust was carried out twice. The immobilized microbial consortium on the second treatment was added by crude oil and seawater in a 45-milliliter falcon tube, whereas the first treatment was the contrary. Each falcon tube on the second treatment was filled with 10-milliliter seawater, 2% crude oil, and 2% immobilized bacterial consortium (Table 1). These solvents were then incubated on an orbital shaker (120 rpm) (Al-Wasify and Hamed 2014) at room temperature for a certain period; 0, 7, and 41 days.

Measurement of Immobilized Microbial Consortium Population

One pellet of each day-storage on the first treatment was granulated by mortar and alu approximately 5 min at 10^{-1}. The granule was then diluted by serial dilution method 10^{-3} until 10^{-5} depending on each day-storage. Furthermore, the solvent on each-day storage on the second treatment was directly diluted by serial dilution method 10^{-5} until 10^{-8} depending on each day-storage as well. The third-last dilution on each treatment was then grown in Marine Agar and directly incubated at 37°C (Hazaimeh *et al*. 2014) which were enriched every week (0, 7th, 14th, 21st, and 28th) for 1 month.

Environmental Measurement

The sample for population and environmental measurement were measured separately to prevent contamination. The solvent was dipped by a refractometer and DO meter to measure salinity, pH, DO, and temperature to obtain the value. Each solvent for environmental parameters was measured twice a month (0 and 28th).

Immobilized Bacterial Consortium Analysis

The morphology of bacterial colonies from each strain was identified visually such as margin, size, color, elevation, texture, and shape. The cultivated bacterial of each strain and bacterial consortium for treatment preparation was counted to obtain a result of the activated bacteria. It was due to the important role of activated bacterial in the success of the experiment. Furthermore, the immobilized bacterial consortium of each treatment was counted to obtain the results of viable bacteria. All the grown colonies were counted by colony counter and analyzed by TPC (Total Plate Count) method according to the rules of SNI 2332.3: 2015. The obtained data were then processed by Ms. Excel and tabulated in tables or graphs and discussed descriptively. The total bacterial cell was calculated by:

$$N = \frac{\sum C}{[(1 \times n1) + (0{,}1 \times n2)] \times (d)}$$

where N is the number of colonies, reported as CFU per m, Σ is the number of colonies on all the plates counted, n1 is the number of plates at the first dilution counted, n2 is the number of plates at the second dilution counted, d is the first dilution calculated

Table 1. Treatment Variation

No.	Sample	Variations
1.	A	0-day storage (fresh)
2.	B	7 days storage
3.	C	41 days storage

Statistical Analyses

The total number of bacterial cells was calculated to obtain the bacterial growth of each treatment for 28 days. The results of TPC (Total Plate Count) of each treatment was then further analyzed by two-way ANOVA to see the most influenced immobilized bacterial consortium by adding crude oil and seawater for 28 days. Results are reported as mean ± standard deviation (n =5).

3. Results and Discussion

Bacterial Morphology

Bacterial activation was carried out to get active bacteria. It was due to the condition of bacteria which was inactive and the use of inactive bacteria condition was less optimal to degrade petroleum hydrocarbon compounds (Sopiah et al., 2016). The Bacterial Morphology characteristics are presented in Table 2.

Bacterial morphology was observed visually to obtain the characteristics such as size, form, elevation, colour, margin, and texture. Tables 2 shows a variation of bacterial morphology characteristics in each treatment. The characteristics of bacterial colony were normal as it stated by Komarawidjaja (2016) that he characteristics of the bacteria, in general, are white/yellow, small/medium size, circular in shape, have flat and convex elevations, have flat/jagged edges, smooth, and shiny surfaces.

Total Number of Bacterial Cultivation

Bacterial cultivation was carried out twice to enrich and multiply the number of bacterial populations and ensure the activeness of each species for further experiment. One of the factors that affect bacterial growth is the availability of nutrients (Okoro 2010). The bacterial growth of the second cultivation is presented in Figure 1.

Table 2. Bacterial morphology characteristics

Treatment	Characteristic	Number of Strain				
		S2	S3	S4	S5	S6
A	Size	Small	Small	Medium	Small Medium	Small Medium
	Form	Round	Round	Round	Round	Round
	Elevation	Flat	Raised	Convex	Convex	Raised
	Colour	White	White	Milky	Milky	Milky
	Margin	Entire	Entire	Entire	Entire	Entire
	Texture	Smooth	Smooth	Smooth	Smooth	Smooth
B	Size	Small	Small	Medium	Medium	Small Medium
	Form	Round	Round	Round	Round	Round
	Elevation	Flat	Flat	Convex	Raised	Raised
	Colour	White	White	Milky	Milky	White
	Margin	Entire	Entire	Entire	Entire	Entire
	Texture	Smooth	Smooth	Smooth	Smooth	Smooth
C	Size	Small	Small	Small	Small	Small Medium
	Form	Round	Round	Round	Round	Round
	Elevation	Flat	Flat	Convex	Raised	Convex
	Colour	White	White	Milky	White	Milky
	Margin	Entire	Entire	Entire	Entire	Entire
	Texture	Smooth	Smooth	Smooth	Smooth	Smooth

Figure 1 shows the growth of each strain. Each strain was cultivated for the treatment preparation of immobilized microbial consortium. The highest and lowest average numbers were respectively S6 1.27 x 10^9 CFU/ml and S2 1.87 x 10^8 CFU/ml. The bacterial growth variation can be affected by the availability of nutrients, temperature, and physiology of microorganisms in the previous media (Nugroho 2010). The number of bacterial growth was relatively more than 10^6 Cell Forming Units (CFU)/ml so it was prepared to live well on the media and be able to degrade petroleum hydrocarbons (Okoro 2010).

Total Number of Bacterial Consortium (CC)

The bacterial consortium was used as the main material on sawdust. The growth of the bacterial consortium is presented in Figure 2.

Figure 7 shows the growth of the bacterial consortium for each further treatment. The highest and lowest average numbers were respectively 3.40 x 10^8 CFU/ml (C) and 2.22 x 10^8 CFU/ml (B). The bacterial growth variations depend on several factors as well such as the availability of nutrients, temperature, and physiology of microorganisms in the previous media (Nugroho 2007). The number of bacterial growth was relatively more than 10^6 Cell Forming Units (CFU)/ml so it was prepared to live well on the media and be able to degrade petroleum hydrocarbons (Okoro 2010).

Total Number of Immobilized Bacterial Consortium on Sawdust

Microbial consortium treatment on sawdust was carried out twice. The immobilized microbial consortium on the second treatment was added by crude oil and seawater, whereas the first treatment was the contrary. The first treatment of each storage period was measured only on day-0 to obtain the data of withstanding microbial which still alive on sawdust, whereas the second treatment was measured the average of an immobilized microbial consortium which still alive for 1 month.

Table 3 shows the significant difference between the first and the second treatment. The bacterial populations on the first treatment reached only 10^2 to 10^3 while the second treatment reached 10^7 and 10^8. These growths were drastically changed due to the availability of crude oil. Nugroho (2006) reported that bacteria need molecules of carbons as a source of nutrition and energy to carry out metabolism and reproduction. Hydrocarbon compounds in petroleum are a source of carbon for the growth of certain microorganisms, while non-hydrocarbon are complementary nutrients needed for their growth. Through a unique hydrocarbon degradation mechanism, this carbon source can be utilized to carry out the metabolic process and its reproduction.

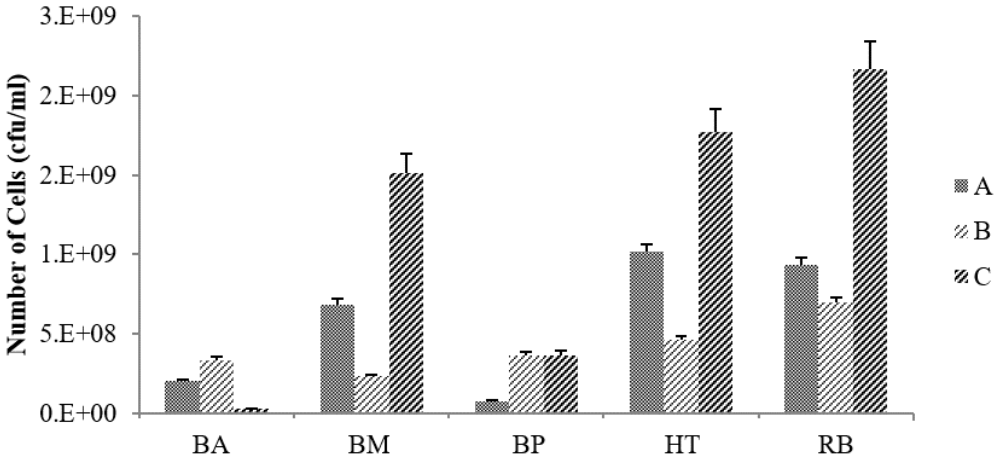

Figure 1. Growth of bacterial populations on the second cultivation; (A) cultivated bacteria for 0 day storage (B) cultivated bacteria for 7 days storage (C) cultivated bacteria for 41 days storage.

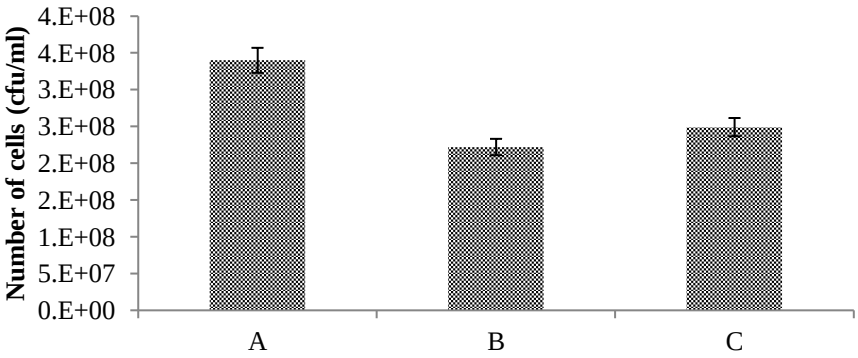

Figure. 2. Growth of bacterial consortium populations; (A) bacterial CC for 0 day storage (B) bacterial CC for 7 days storage (C) bacterial CC for 41 days storage.

Table 3. The bacterial population of immobilized microbial consortium

Treatments	Total Number of Bacteria (CFU/ml)	
	First Treatment	The Average of Second Treatment
A	5.33×10^2	9.59×10^8 b
B	1.04×10^3	9.72×10^7 a
C	1.37×10^3	2.71×10^8 a

Nb. A = 0 day storage (fresh); B = 7 days storage; C = 41 days storage.

The growth of the immobilized bacterial population in each treatment was relatively varied. The highest average was 9.59×10^8 CFU/ml (A), 2.71×10^8 CFU/ml (C), and 9.72×10^7 CFU/ml (C). The highest average growth of treatment A was affected by the condition of the immobilized bacterial consortium which still fresh and productive in degrading oil. However, the average growth of treatments B and C was inverse proportion (Figure 3). This case was caused by the growth of fungi in treatment B which has grown on the surface of the immobilized bacterial consortium. This case was presumably affected by carrier humidity on treatment B. The emersion of carrier humidity was caused by the inconsistent volume of egg whites added in the making of the immobilized microbial consortium. Podorozhko *et al.* (2008) reported that the appearance of fungi could inhibit the performance of oil-decomposing microorganisms. Therefore, to increase the absorption ability of oil decomposing microbes, it is necessary to modify the carrier to increase better resistance to fungal growth.

Figure 3 shows the variation of bacterial growth on 0 day storage which respectively the highest number was 1.44×10^8 CFU/ml (A), 2.06×10^5 CFU/ml (B), and 2.74×10^3 CFU/ml (C). The high number of cells on treatment A was due to the short-term storage. Thus, the fresh immobilized bacterial consortium was still active in degrading crude oil. However, the lack of

bacterial population on treatment C was due to the long-term storage that reached more than 1 month. Thus, the bacterial metabolic activity was not efficient (Obuekwe *et al.* 2001).

In general, bacterial growth is the result of cell multiplication. Thus, bacterial growth is frequently expressed a cell reproduction. Bacteria multiply regularly through exponential growth that the rate of cell division increases as the increase of growth period (Safitriani et al., 2017). The growth of the bacterial population of treatment B and C on day-0 shows a lag phase which was a period of adjustment for microorganisms, whereas treatment A immediately reached an exponential phase. The lag phase that was reached by treatment B and C was a period of bacterial adjustment to the environment, ranging from one hour to several days. The period depends on the type of bacteria, the age of the culture, and the availability of nutrients in the medium. The bacteria in this phase are adapted to the environment and have not been able to breed. However, the cell metabolism and the size of the bacterial is increase. Whereas, the exponential phase in treatment A was a period of rapid breeding and there were characteristics of active cells. The cells in this phase were divided and increased by time. Some bacteria in this phase usually produce primary metabolites, such as carbohydrates and proteins. The phase on the curve was characterized by a straight line in the plot of the number of cells against time (Nugroho 2007). The highest peak of bacterial growth in each treatment was relatively varied where treatment A showed the highest growth on the 7th day, treatment B on the 14th day, and treatment C on the 21st day. The variation of maximum growth was caused by the ability of different bacteria to adapt to their new environment before being able to utilize the crude oil contained in seawater as a source of carbon (Puspitasari et al., 2020). The stationary phase and the death phase were not experienced by all treatments. The stationary phase occurs on treatment B from the 21st day to the 28th day where there was a balance between the growth rate and the mortality rate. Thus, the number of all living bacteria will remain. Moreover, the death phase occurred in treatment C where the rate of reduction in bacteria exceeded the rate of bacterial culture (Nugroho 2007).

Figure 3 shows treatment A that reached a significant decrease on the 14th day of the bioremediation process. This is due to the performance of bacterial consortium that works simultaneously where complex types of hydrocarbons are degraded in different ways. Thus, they will decrease in certain conditions. Atlas (1981) reported there are no individual microorganisms capable to completely degrade hydrocarbons. Thus, the bacterial consortium with various enzymatic capacities is needed to degrade complex hydrocarbon mixtures, such as petroleum. The use of the bacterial consortium will increase the degradation of petroleum. This phenomenon is due to the bacterial consortium which can degrade different components of petroleum. Thus, it has a more complete enzymatic ability in degrading petroleum (Patowary et al., 2016).

The immobilized bacterial consortium was relatively able to survive for 1 month (Figure 3). It is shown by the high number of each treatment on the 28th day. The total number of bacterial cells was 1 x 10^9 CFU/ml (A), 1 x 10^8 CFU/ml (B), and 5 x 10^8 CFU/ml (C). The viability of each treatment was due to the correlation between the viability of bacteria in maintaining the metabolic and enzymatic with the carriers (Obuekwe & Al-Muttawa 2001). The best viability performance of immobilized bacterial consortium was shown by treatment A that reached an exponential phase

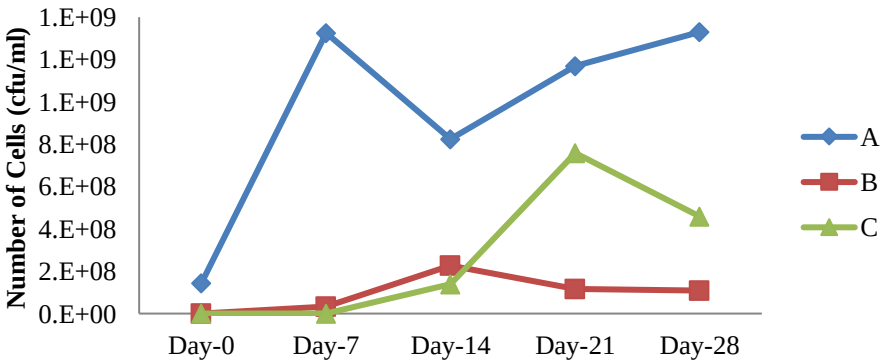

Figure 3. Growth of bacterial population for 1 month; (A) 0 day storage (B) 7 days storage (C) 41 days storage

of 1.33 x 10^9 CFU/ml on the 28th day, whereas treatment B and C had a significant decrease in bacterial cells. This is related to the statement by Obuekwe et al. (2001) which reported that immobilized bacteria at the beginning of the storage period can utilize carbon compounds and not affect metabolic and enzymatic performance in utilizing their nutrients.

This success was supported by the statistical results which showed the effect of the addition of crude oil and seawater to the growth phase at each immobilized bacterial consortium. It was indicated by the number of F count> F table. Further test results showed the immobilized bacterial consortium on 0 day storage (fresh) had a significant effect on using crude oil during the breeding period. The ability of bacteria to degrade petroleum is due to its ability to produce enzymes that can break down complex organic compounds into simpler compounds. Monooxygenase enzymes and dioxygenase enzymes produced by bacteria can open carbon bonds in aromatic rings and produce a primary alcohol. The dioxygenase enzyme produced by these bacteria then forms cis-dihydrodiol where this compound will be dehydrogenated to form dihydroxy-PAHs which are the substrate for ring-opening enzymes. Through the provision of one oxygen molecule, the monooxygenase enzyme can also degrade Polycyclic Aromatic Hydrocarbons (PAHs) and form arene oxides, then these molecules will be used by microbes as a source of nutrition for growth and energy (Hasyimuddin et al., 2016).

The Results of Environmental Parameter Measurement

Power of Hydrogen (pH)

The biodegradation process was supported by the increase of bacterial population and decrease of pH on each treatment. The pH measurement results during the experiment; day-0 and day-28 are presented in Figure 4.

Figure 4 shows the degression of pH that is affected by the carbon compound in crude oil which is modified by the bacterial consortium to produce CO_2 and reacts with water to form acidic H_2CO_3 or Volatile Organic Acid (VOA). The increase of organic acids accumulation was the result of the metabolic process along with the incubation (Hassanshahian & Cappello 2013). This is related to Krachler et al., (2009) which reported the decrease of pH during biodegradation is the formation of acidic such as acetic acid and propionic acid as well as gluconic, pyruvic, citric, and succinic acids (Gosalam *et al.* 2008). Thus, it reduced the value of pH in the medium.

Bacteria in general able to live at 5 to 8 of pH and 7 as the optimum scale (Purnawan et al., 2015). It shows the decrease of pH during the biodegradation process was normal. Moreover, Fernández-Calviño & Bååth (2010) reported that bacteria can grow and thrive in neutral pH conditions. At neutral pH, the viability of enzymes produced by the bacteria is maximum in degrading oil.

Temperature

Temperature is directly related to the chemistry of pollutants and affects the physiology of microbes in the environment. Thus, the temperature has an important role in the biodegradation of hydrocarbons. The results of temperature measurement during the experiment; day-0 and day-28 are presented in Figure 5.

Figure 5 shows the temperature was relatively homogeneous. It was due to the sample was placed at room temperature (25°C-28°C) where this range is good enough for bioremediation. Pande et al., (2020) reported that the optimal temperature in the bioremediation process is approximately 25°C-40°C and most hydrocarbonoclastic microorganisms are generally active at 20°C - 35°C. The diagram shows an increase in

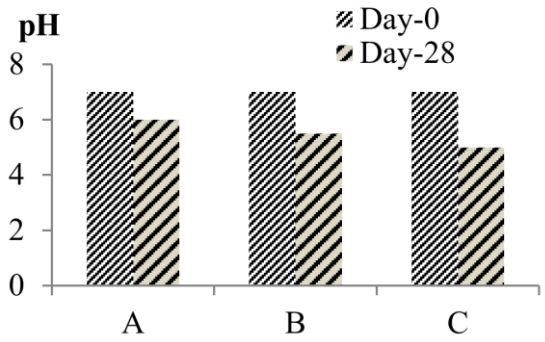

Figure 4. Diagram of pH; (A) 0 day storage (B) 7 days storage (C) 41 day storage.

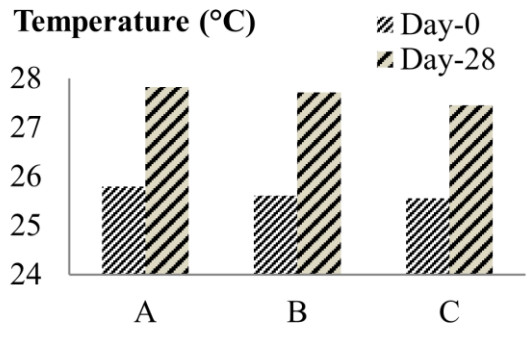

Figure 5. Diagram of temperature; (A) 0 day storage (B) 7 days storage (C) 41 days storage.

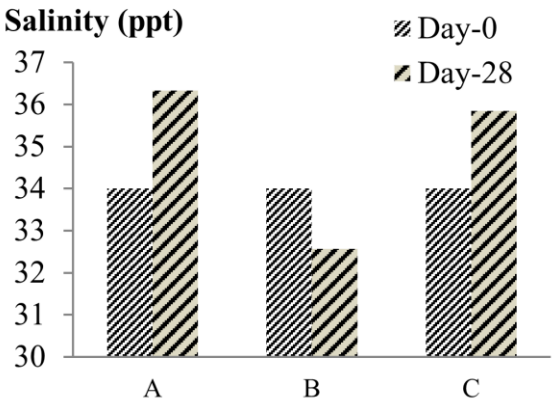

Figure 6. Diagram of salinity (A) 0 day storage (B) 7 days storage (C) 41 days storage.

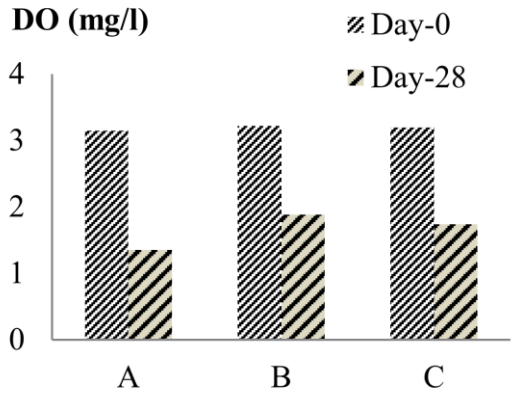

Figure 7. Diagram of dissolved oxygen (A) 0 day storage (B) 7 days storage (C) 41 days

temperature during the biodegradation process for 28 days of treatment. This is related to Sihag Pathak & Jaroli (2014) that the high activity of microorganisms in degrading oil was supported by an optimal temperature, and the biological processes in bacteria generally increase during the increasing temperature. Furthermore, bacterial cells were possible to be inhibited and killed by the denaturation of maximum temperature (Pande et al., 2020).

Salinity

The occurrence of the biodegradation process was indicated by the fluctuation of salinity in each treatment through the fluctuation of bacterial population and metabolism are presented in Figure 6.

Bacteria require an osmotic balance between the intracellular environment and the intercellular environment (Qin et al., 2012). Figure 6 shows the ranges of salinity were optimum (32-36 ppt). Qin et al., (2012) also reported that the normal range of salinity approximately 30-35 ppt. However, according to Darmayati et al., (2015) biodegradation process of oil is still running well even though the salinity in the environment reaches 56 ppt. The diagram shows a fluctuation on each treatment on the 28th day. The fluctuation was due to the high concentrations of soluble salts. Yan et al., (2015) reported that High-concentrations of soluble salts affect microbes via two primary mechanisms: osmotic effect and specific ion effects. Moreover, many studies showed that salinity reduces microbial activity, microbial biomass and changes microbial community structure. Salinity reduces microbial biomass mainly because the osmotic stress results in drying and lysis of cells.

Dissolved Oxygen (DO)

Bacterial growth is generally affected by the availability of dissolved oxygen. The lack of dissolved oxygen will be the inhibiting factor for degradation. The dissolved oxygen measurement results during the experiment; day-0 and day-28 are presented in Figure 7.

Figure 7 shows the significant degression of dissolved oxygen 1.35-3.15 mg / L which was due to the equilibrium between bacterial growth and oxygen demand. Graves et al., (1991) reported that the increase of oxygen demand is following the decrease of dissolved oxygen. The optimum dissolved oxygen for marine biota and marine microorganisms growth is more than 5 mg / L (Spietz et al., 2015). The availability of oxygen in bioremediation will increase the degradation process. Oxygen is required to be inserted into the hydrocarbons by the enzyme oxygenase, and also required for hydrocarbon oxidation.

4. Conclusion

The immobilized bacterial consortium in all storage periods were viable on sawdust carriers which related to the high number of bacterial populations on the 28th day where the bacterial population on 0-day storage was 1.33 x 10^9 CFU/ml, the 7 days storage was 1.10 x 10^8 CFU/ml, and the 41 days storage was 4.60 x 10^8 CFU/ml. The most viable of immobilized bacterial consortium on sawdust was the 0-day storage (9.59 x 10^8 CFU/ml) which was indicated by the increases phase at the end of the day. It was proved that the bacteria are still productive and had the ability to degrade petroleum hydrocarbons.

Acknowledgment

This paper was supported by the Indonesian Institute of Science and Faculty of Marine Sciences and Fisheries of Raja Ali Haji Maritime University.

References

Al-wasify, R.S. & Hamed, S.R. 2016. Retracted: Bacterial biodegradation of crude oil using local isolates. In International Journal of Bacteriology. 2016:1–1. https://doi.org/10.1155/2016/6013871

Andilas, D.D. & Yanggana, L.A. 2017. Pelaksanaan program tol laut PT Pelayaran Nasional Indonesia. Jurnal Manajemen Transportasi dan Logistik. 4(1):1. https://doi.org/10.25292/j.mtl.v4i1.36

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiological Reviews. 45(1):180–209. https://doi.org/10.1128/mr.45.1.180-209.1981

Bayat, Z., Hassanshahian, M. & Cappello, S. 2015. Immobilization of microbes for bioremediation of crude oil polluted environments: A mini review. The Open Microbiology Journal. 9:48–54. https://doi.org/10.2174/1874285801509010048

Darmayati, Y., Sanusi, H.S., Prartono, T., Santosa, D.A. & Ruyitno, R. 2015. The efficacy of bioaugmentation on remediating oil contaminated sandy beach using mesocosm approach (Efikasi tehnik bioaugmentasi dalam memulihkan pantai berpasir tercemar minyak menggunakan pendekatan mesokosm). Ilmu Kelautan: Indonesian Journal of Marine Sciences. 20(3):143. https://doi.org/10.14710/ik.ijms.20.3.143-152

Dzionek, A., Wojcieszyńska, D. & Guzik, U. 2016. Natural carriers in bioremediation: A review. Electronic Journal of Biotechnology. 23(28–36). https://doi.org/10.1016/j.ejbt.2016.07.003

Fernández-Calviño, D. & Bååth, E. 2010. Growth response of the bacterial community to pH in soils differing in pH. FEMS Microbiology Ecology. *73*(1):149–156. https://doi.org/10.1111/j.1574-6941.2010.00873.x

Graves, D.A., Lang, C.A. & Leavitt, M.E. 1991. Respirometric analysis of the biodegradation of organic contaminants in soil and water. Applied Biochemistry and Biotechnology. 28–29(1):813–826. https://doi.org/10.1007/BF02922652

Hassanshahian, M. & Cappello, S. 2013. Crude oil biodegradation in the marine environments. Biodegradation-Engineering and Technology. https://doi.org/10.5772/55554

Hasyimuddin, H., Natsir Djide, M. & Farid Samawi, M. 2016. Isolasi bakteri pendegradasi minyak solar dari perairan Teluk Pare-Pare. Biogenesis: Jurnal Ilmiah Biologi. 4(1):41–46. https://doi.org/10.24252/bio.v4i1.1119

Hazaimeh, M., Abd Mutalib, S., Abdullah, P.S., Kok Kee, W. & Surif, S. 2014. Enhanced crude oil hydrocarbon degradation by self-immobilized bacterial consortium culture on sawdust and oil palm empty fruit bunch. Annals of Microbiology. 64(4):1769–1777. https://doi.org/10.1007/s13213-014-0821-3

Komarawidjaja, W. 2016. Karakteristik dan pertumbuhan konsorsium mikroba lokal dalam media mengandung minyak bumi. Jurnal Teknologi Lingkungan. 10(1):114. https://doi.org/10.29122/jtl.v10i1.1510

Krachler, R.F., Krachler, R., Stojanovic, A., Wielander, B. & Herzig, A. 2009. Effects of pH on aquatic biodegradation processes. Biogeosciences Discussions. 6(1):491–514. https://doi.org/10.5194/bgd-6-491-2009

Nugroho, A. 2007. Dinamika populasi konsorsium bakteri hidrokarbonoklastik : Studi kasus biodegradasi hidrokarbon minyak bumi skala laboratorium (The dynamic population of the bacterial hydrocarbonoclastic concorsium in the crude oil sludge degradation). Ilmu Dasar. 8(1):13–23.

Nugroho, A. 2010. Biodegradasi sludge minyak bumi dalam skala mikrokosmos: simulasi sederhana sebagai kajian awal bioremediasi land treatment. Makara of Technology Series. 10(2):82–89. https://doi.org/10.7454/mst.v10i2.427

Obuekwe, C.O. & Al-Muttawa, E.M. 2001. Self-immobilized bacterial cultures with potential for application as ready-to-use seeds for petroleum bioremediation. Biotechnology Letters. 23(13):1025–1032. https://doi.org/10.1023/A:1010544320118

Okoro, C.C. 2010. Enhanced bioremediation of hydrocarbon contaminated mangrove swamp in the nigerian oil rich niger delta using seawater microbial inocula amended with crude biosurfactants and micronutrients. Nature and Science. 8(8):195–206.

Pande, V., Pandey, S.C., Sati, D., Pande, V. & Samant, M. 2020. Bioremediation: an emerging effective approach towards environment restoration. Environmental Sustainability. 3(1):91–103. https://doi.org/10.1007/s42398-020-00099-w

Patowary, K., Patowary, R., Kalita, M.C. & Deka,

S. 2016. Development of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. Frontiers in Microbiology. 7:1–14. https://doi.org/10.3389/fmicb.2016.01092

Podorozhko, E.A., Lozinsky, V.I., Ivshina, I.B., Kuyukina, M.S., Krivorutchko, A.B., Philp, J.C. & Cunningham, C.J. 2008. Hydrophobised sawdust as a carrier for immobilisation of the hydrocarbon-oxidizing bacterium *Rhodococcus ruber*. Bioresource Technology. 99(6):2001–2008. https://doi.org/10.1016/j.biortech.2007.03.024

Purnawan, A., Capriyanti, Y., Kurniatin, P. & Rahmani, N. 2015. Optimasi produksi enzim amilase dari bakteri laut Jakarta (*Arthrobacter arilaitensis*). Indonesian Journal of Biology. 11(2).

Puspitasari, I., Trianto, A. & Suprijanto, J. 2020. Eksplorasi bakteri pendegradasi minyak dari perairan pelabuhan Tanjung Mas, Semarang. Journal of Marine Research. 9(3):281–288.

Qin, X., Tang, J.C., Li, D.S. & Zhang, Q.M. 2012. Effect of salinity on the bioremediation of petroleum hydrocarbons in a saline-alkaline soil. Letters in Applied Microbiology. 55(3):210–217. https://doi.org/10.1111/j.1472-765X.2012.03280.x

Safitriani., Thontowi, A., Yetti, E., Suryani. & Yopi. 2017. Pertumbuhan optimal bakteri laut *Pseudomonas aeruginosa* LBF-1-0132 dalam senyawa Piren. Jurnal Biologi Indonesia. 13(1):107–116. https://doi.org/10.47349/jbi/13012017/107

Setyonugroho, A. 2019. Kajian risiko penangulangan tumpahan minyak: Studi kasus di laut Jawa bagian Barat. Jurnal Pengelolaan Sumberdaya Alam Dan Lingkungan (Journal of Natural Resources and Environmental Management). 9(3):826–839. https://doi.org/10.29244/jpsl.9.1.826-839

Sihag Pathak, H. & Jaroli, D.S. 2014. Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. International Journal of Pure & Applied Bioscience. 2(3):185–202.

Sopiah, N., Oktaviani, A., Sulistia, S., Suciati, F. & Aviantara, D. 2016. Isolasi dan identifikasi bakteri pendegradasi hidrokarbon yang berasal dari tanah tercemar minyak bumi. Jurnal Teknologi Lingkungan. 12(3):291. https://doi.org/10.29122/jtl.v12i3.1238

Spietz, R.L., Williams, C.M., Rocap, G. & Horner-Devine, M.C. 2015. A dissolved oxygen threshold for shifts in bacterial community structure in a seasonally hypoxic estuary. PLoS ONE. 10(8):1–18. https://doi.org/10.1371/journal.pone.0135731

Suwedi, N. 2017. Model hubungan waktu terjadinya tumpahan minyak di suatu lokasi dengan bentuk sebarannya pada daerah terdampak. Jurnal Teknologi Lingkungan. 18(1):104–111.

Syakti, A.D., Yani, M., Hidayati, N.V., Siregar, A.S., Doumenq, P. & Made Sudiana, I.M. 2013. The bioremediation potential of hydrocarbonoclastic bacteria isolated from a mangrove contaminated by petroleum hydrocarbons on the cilacap coast, Indonesia. Bioremediation Journal. 17(1):11–20. https://doi.org/10.1080/10889868.2012.731446

Thamer, M., Al-Kubaisi, A.R., Zahraw, Z., Abdullah, H.A., Hindy, I. & Khadium, A.A. 2013. Biodegradation of kirkuk light crude oil by *Bacillus thuringiensis*, Northern of Iraq. Natural Science. 05(07):865–873. https://doi.org/10.4236/ns.2013.57104

Thapa, B., Ghimire, A. & Kumar, A. 2012. A review on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu University Journal Of Science. 8(I):164–170.

Triana, E., Yulianto, E. & Nurhidayat, N. 2006. Viability of encapsulated *Lactobacillus sp.* Mar 8. Biodiversitas Journal of Biological Diversity, 7(2):114–117. https://doi.org/10.13057/biodiv/d070204

Yan, N., Marschner, P., Cao, W., Zuo, C. & Qin, W. 2015. Influence of salinity and water content on soil microorganisms. International Soil and Water Conservation Research. 3(4):316–323. https://doi.org/10.1016/j.iswcr.2015.11.003