Removal of Total Petroleum Hydrocarbon (TPH) crude oil by consortium bacteria acetobacter tropicalis and lactobacillus casei

F. Azzahra[1], *A.* Rinanti[1*], *Rositayanti* Hadisoebroto[1], *A.* Minarti[1], *S.* Aphirta[1], *L.* Rahmiyati[1], *S.M.P.* Mahrendra[1], and *T.* Sunaryo[1]

[1]Faculty of Landscape Architecture and Environmental Technology, Department of Environmental Engineering Universitas Trisakti, Jakarta, Indonesia

Abstract. The objective of this research is to remove Total Petroleum Hydrocarbon (TPH) contained in crude oil by utilizing Acetobacter tropicalis and Lactobacillus casei bacteria consortium through degradation process. Crude oil degradation was conducted on Stone Mineral Salt Solution (SMSS) liquid media and artificial sea water in a controlled environment with limited system. The implemented variations on SMSS liquid media are differentiated based on acidity (pH) of 3, 5, and 7, with contact time of 7, 14, and 21 days. On the other hand, the variation implemented on artificial sea water only applied on contact time of 7, 14, and 21 days. Samples were incubated on a shaker incubator with 30°C and 150 rpm. The sensitivity test revealed that consortiom bacteria are resistant against crude oil, which proven by the missing inhibiting zone formation around disc paper that contains crude oil. This research shows that the optimum condition to degreade TPH both on SMSS liquid media and artificial sea water is at pH level of 7 in 7 days with TPH removal efficiency of 94%. This research provides an important information that Acetobacter tropicalis and Lactobacillus casei bacteria consortium has the potential to degrade crude oil TPH in a controlled environment.

1 Introduction

Crude oil is one of the most important commodities because almost every country in this world utilizes crude oil as their main energy source [1]. Crude oil can be utilized to produce fuel for cars, motorbikes, airplanes, and other vehicles that require gas to run. Apart from that, crude oil can also be utilized as industrial raw material. Surely, crude oil utilization will also lead to waste production that has negative impacts on the environment. Numerous crude oil processing activities, starting from the refinery up to exploration, have the potential to produce oil sludge as waste. Oil spill is only discovered in land, but also in water ecosystem [2].

Oil pollution in water is caused by oil spill generated by shipping activities, motor-boat machine, and leakage on oil storage facilities [3]. The location polluted by crude oil can

* Corresponding author: astririnanti@trisakti.ac.id

generally be recovered through physics-chemical methods. The separation of crude oil component is started by distillation, which then creates oil vapors that will be further processed and perfected into a more benefitful products. However, physics-chemical treatments are quite expensive and require a long period of time [4,5].

Another alternative to process crude oil is known as bioremediation technique, which is a technique that utilizes microbes to degrade organic compounds [6]. Bioremediation possesses ecological advantage when faced with areas polluted by crude oil hydrocarbon because of its relatively low cost, positive effect, and minimum secondary pollution. On crude oil bioremediation process, the utilization bacteria consortium can influence crude oil degradation process because each bacterium require specific substrates to degrade crude oil components [7,8]. Bacteria utilized to degrade crude oil are able to survive in a contaminated area by using hydrocarbon compounds as nitrogen sources [9,10]. The objective of this research is to overcome crude oil pollution on water with biotechnological approach through bioremediation by bacteria consortium enzymatic activities. This research is also implemented to determine growth response of

Acetobacter tropicalis and Lactobacillus casei bacteria consortium on crude oil polluted environment based on the variation of acidity level (pH) and contact time (Td), determine the optimum condition for Acetobacter tropicalis and Lactobacillus casei bacteria consortium that generates the most efficient Total Petroleum Hydrocarbon (TPH) removal, and to determine TPH removal efficiency level by Acetobacter tropicalis and Lactobacillus casei bacteria consortium.

2 Materials and method

1.1. Crude Oil, Bacteria Consortium Growth Media, and Artificial Sea Water Preparations

This research utilized crude oil sample taken from the collection of Faculty of Earth and Energy Technology Laboratory of Universitas Trisakti obtained from Pertamina EP Asset 3 Jatibarang Field, with research parameter of initial Total Petroleum Hydrocarbon (TPH) concentration on crude oil sample. Characterization test was implemented at Lemigas ESDM Laboratory Jakarta, through gravimetric method. As Acetobacter tropicalis and Lactobacillus casei bacteria consortium growth media, we utilized Stone Mineral Salt Solution (SMSS) liquid solution with the following compositions 0.5g $CaCO_3$, 2.5g NH_4NO_3, 0.5g KH_2PO_4, 1g $Na_2HPO_4.7H_2O$¸ 0.5g $MgSO_4.7H_2O$, and 0.2g $MnCl_2.7H_2O$. These materials were dissolved on 1 litre of aquades and sterilized by using autoclave for 60 minutes on 121°C temperature. Powder artificial sea water media with compositions of $CaCl_2$, NaCl, NaF, KCl, $MgCl_2.6H_2O$, $SrCl_2.6H_2O$, KBr, Na_2SO_4, $NaHCO_3$, and H_3BO_3 were dissolved on 1 litre of aquades, and sterilized on an autoclave on 121°C temperature for an hour.

1.2. Acetobacter tropicalis and Lactobacillus casei Bacteria Consortium Cultivation on SMSS Media without Crude Oil Addition

Acetobacter tropicalis and Lactobacillus casei bacteria consortium cultivation was conducted by implementing batch system that utilizes 80% SMSS liquid meia of the total capacity of erlenmeyer (250 ml) the remaining space was considered as free board. Bacteria consortium was cultivated on a shaker incubator with 150 rpm rotation speed on 30°C temperature for 120 hours. The utilized composition in bacteria cultivation without crude oil consists of 85% SMSS media (v/v), 10% of bacteria consortium (v/v), and 10% NPK liquid fertilizer, meanwhile bacteria cultivation with additional crude oil consists of 85% of SMSS (v/v), 10% of bacteria consortium (v/v), 10% o NPK liquid fertilizer (v/v), and 10% crude oil (v/v). Bacteria consortium growth was calculated by using Total Plate Count (TPC) method, with the following formula:

Colony per mL or per gr (CFU/mL or CFU/gr) =

$$\frac{\text{Number of Colony per Flask}}{\text{Dilution Factor}} \quad (1)$$

1.3. Bacteria Consortium Sensitivity Test on Crude Oil

The sensitivity test was carried out on a petri dish containing a solid medium of Gelatine Nutrient (GN). Acetobacter tropicalis and Lactobacillus casei bacteria consortium was mixed into GN media and then allowed to solidify first. Discs that had been smeared with petroleum were placed in GN containing a solid consortium of bacteria, then the petri dishes were incubated at 37°C for 48 hours. This sensitivity test is carried out to determine the sensitivity or sensitivity of the bacterial consortium to petroleum.

1.4. Crude Oil Degradation Test on SMSS Liquid Media

Crude oil degradation test by a consortium of bacteria was carried out on SMSS liquid media with the aim of obtaining optimum conditions for all variables used. The variables used were acidity (pH) with variations of 3, 5, 7, and contact time (days) with variations of 7, 14, and 21. Each variation was carried out in duplicate. The compositions used for optimization of pH and contact time are SMSS 70% (v/v), 10% bacterial consortium (v/v), 10% crude oil (v/v), and 10% NPK liquid fertilizer (v/v).). Incubation was carried out using a shaker incubator with a rotating speed of 150 rpm at 30°C [11]. The degradation test was carried out in a 250 ml Erlemeyer using 80% of the total capacity. Each sample will be taken to the Lemigas ESDM Laboratory, Jakarta to be analyzed for levels of Total Petroleum Hydrocarbon (TPH). The lowest final TPH level from one of the results of optimization of pH and contact time will be the optimum conditions for Acetobacter tropicalis and Lactobacillus casei bacteria in removing the TPH content of crude oil. The formula for calculating the efficiency of TPH removal is as follows:

$$\text{TPH Removal Efficiency} = \frac{B-A}{B} \text{ x } 100\% \quad (2)$$

Where:

A : Concentration after degradation process (%)

B : Concentration before degradation process (%)

1.5. Crude Oil Degradation Test on Artifical Sea Water Media

250 ml Erlemeyer using 80% of the total capacity and conditioned at the optimum pH of the crude oil degradation test stage in SMSS media, mixed culture bacteria 10% (v/v), and 10% crude oil concentration(v/v). Incubation was carried out using a shaker incubator with a rotating speed of 150 rpm at 30°C with each contact time of 7, 14, and 21 days. Each variation of contact time is made up of two samples or in duplicate, and then each sample will be taken to the Lemigas ESDM Laboratory, Jakarta for analysis of Total Petroleum Hydrocarbon (TPH) levels. The lowest final TPH level from the variation of contact time will be the optimum contact time for Acetobacter tropicalis and Lactobacillus casei bacteria in removing the TPH content on crude oil.

1.6. Analysis of pH Changes and Bacteria Growth Kinetics

Changes in pH during the crude oil degradation test in SMSS liquid media and artificial sea water media by a consortium of bacteria will be measured using a pH meter. Measurement of pH will be carried out at each initial and final condition in each stage of the independent variable petroleum degradation test. The calculation of bacterial growth by connecting the concentration of nutrients with the growth rate of bacteria can be carried out using the Monod equation with the following equation:

$$\frac{dx}{dt} = ?\,?\,x \tag{3}$$

Where:
X = Cellular Concentration (g/L)
T = Time (hour)
? ? = Specific Growth Rate (Hour^{-1})
q parameter which is the rate of specific substrate utilization value can be calculated with the following formula:

$$\Delta S = qx\ \Delta t \tag{4}$$

$$\frac{dS}{dt} = qx \tag{5}$$

$$q = \frac{(dS/dt)\,u}{x} \tag{6}$$

The specific growth rate, in relation with specific substrate utilization will generate slope = 1/YT from intercept b. The total growth can be stated with the following formula.

$$q = \frac{1}{Y_T}\,\mu + b \tag{7}$$

Where:
q = Specific substrate utilization rate
YT = total growth
μ = specific growth rate (hour-1)
b = intercept
According to [12] the growth yield always comes with a constant value if the biomass composition and environmental condition are stable. Because of that, the initial biomass and substrate contribution represented by Xo, So and S are concentration during growth process. The relationship between growth process and substrate utilization can be measured with the following formula:

$$x - x_o = Y\,(S_o - S) \tag{8}$$

Where:
x – xo = Biomass concentration
so – s = Substrat Concentration
1.7. Pilot Scale Implementation
The results of the crude oil degradation test at each stage starting from the pH optimization stage to the contact time will be implemented on a larger scale, namely the pilot scale. It is planned that the container for the petroleum degradation reactor will be filled with the composition of 10% (v/v) Acetobacter tropicalis and Lactobacillus casei bacteria consortium, 80% (v/v) oil polluted water, and NPK fertilizer as an additional 10% (v/7v) nutrient. The reactor will also be conditioned to a temperature of 30°C, the optimum pH for the degradation test stage in SMSS media, and the optimum contact time for the degradation test stage in artificial seawater media.

3 Result and discussion

The crude oil characterization conducted in Lemigas ESDM Laboratory in Jakarta generated the results that can be viewed and categorized based on their specific gravity or APIravity. The crude oil sample characteristics can be viewed on Table 1.

Table 1. Crude Oil Sample Characteristics.

Parameter	Unit	Results	Method
Mass	-	0.8814	ASTM D 4052
°API	-	29.1	ASTM D 4052
TPH	%	81.94	Gravimetry

The acquired API mass and gravity values based on the characteristic test are 0.8814 and 29.1. Based on the results obtained according to [13] the crude oil samples in this study included as heavy crude oil with an API gravity range of 22° - 30°. The Total Petroleum Hydrocarbon (TPH) was also tested in crude oil characterization, and the test results showed a TPH value of 81.94%. The results of this crude oil characteristic are used as a reference for the initial TPH concentration before the TPH crude oil removal by Acetobacter tropicalis and Lactobacillus casei bacteria consortium.

Cultivation of Acetobacter tropicalis and Lactobacillus casei bacteria consortium was carried out in a 250 mL erlenmeyer with two different conditions, namely condition of Stone Mineral Salt Solution (SMSS) media without crude oil and the condition of the SMSS media containing crude oil. Pollutant-free cultivation was carried out to obtain an exponential phase of Acetobacter tropicalis and Lactobacillus casei bacteria consortium. The growth of the bacterial consortium was obtained by counting the number of living bacteria using the Total Plate Count (TPC) method. The growth phase of the bacterial consortium Acetobacter tropicalis and Lactobacillus casei can be seen in Figure 1.

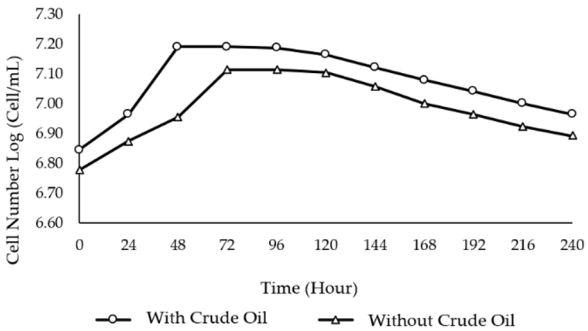


Fig. 1. Mixed Culture Bacteria Growth Curve on SMSS Media with and Without Crude Oil.

As shown in Figure 1. The Acetobacter tropicalis and Lactobacillus casei bacteria consortium cultivation occurred for 240 hours or 10 days. During the cultivation stage, there are 3 growth phases namely exponential, stationary, and death phases. Cultivation without crude oil in an SMSS media reached exponential phase between 0 to 72 hours. In this phase, bacteria consortium started to adapt with the growth media and experience and rapid increase of cell numbers. Bacteria consortium is starting to enter stationary phase after the 72nd hour until 144th hour, marked with constant growth of bacteria cells. After the 144th hour, the bacteria enter death phase marked with lowered number of bacteria due to lack of nutrition on growth media, which led to lower cell metabolism.

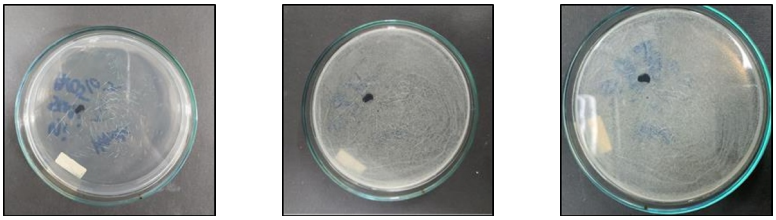
Cultivation of the bacterial consortium in SMSS media containing crude oil showed an exponential phase that occurred faster at 0 hours to 48 hours. The stationary phase occurs

after the 48th hour to the 120th hour, this is indicated by the constant growth of the number of bacterial cells. The bacterial consortium began to enter the death phase with a decrease in the number of bacterial cells after passing the 120 hours.

Based on Figure 1 and the explanation of the growth curve above, the cultivation of bacteria containing crude oil experienced a faster lag and exponential phases and also a longer stationary phase compared to the cultivation of bacteria without crude oil. This indicates that Acetobacter tropicalis and Lactobacillus casei bacteria consortium is able to utilize the carbon (C) element in crude oil as a source of nutrition for cell metabolic activity, so that the number of bacterial cells is faster to divide.

The availability of nutrients, both macronutrients and micronutrients that are complete, suitable, and in the right ratio, is very important for bacterial growth. The main constituents of bacterial cells are the elements carbon, oxygen, hydrogen and nitrogen. These elements, especially carbon, are very important substrates for energy production in bacteria so that bacterial metabolism can properly take place [14,15].

The sensitivity test Acetobacter tropicalis and Lactobacillus casei bacteria consortium was carried out in petri dish containing Gelatine Nutrient (GN) media with disc paper that had been exposed to petroleum added. Petri dishes were incubated for 48 hours at 37°C. This sensitivity test was conducted to determine Acetobacter tropicalis and Lactobacillus casei bacteria level of susceptibility to compounds or substances that can inhibit bacterial growth. The results of the sensitivity test of mixed culture bacteria Acetobacter tropicalis and Lactobacillus casei to crude oil can be seen in Figure 2.



(a) **(b)** **(c)**

Fig. 2. The Sensitivity Results of Acetobacter tropicalis and Lactobacillus casei Bacteria on Crude (a) 0 hour; (b) 24 hour; (c) 48 hour.

In Figure 2 it can be seen that the inhibition zone was not formed during the 48 hour incubation period. This proves that Acetobacter tropicalis and Lactobacillus casei bacteria consortium is resistant and able to grow in an environment polluted with crude oil. The natural resistance of Acetobacter tropicalis bacteria against crude oil is supported by the finding of Majolagbe et al. (2019) who stated that Acetobacter sp. bacteria can be utilized to degrade crude oil sub products on growth culture media [16]. On the other hand, the natural resistance of Lactobacillus casei bacteria against crude oil is supported by a finding that Lactobacillus sp. bacteria is able to bind and detoxify xenobiotic compounds [17-21]. We also managed to conduct crude oil degradation test on SMSS liquid media. The pH optimation was implented with TPH concentration and the removal efficiency n be seen in Table 2.

Table 2. Crude Oil TPH Removal with pH Variations on SMS Liquid Media.

pH	Innitial TPH Concentration (%; w/w)	Final TPH Concentration (%; w/w)	Removal Efficiency (%)
5.0	81.94	5.06	93.82
6.0	81.94	4.75	94.20

7.0	81.94	4.54	94.46

The degradation process for 7 days with variations in pH 3, 5, and 7 showed an increasing efficiency of removal. Based on Figure 3, it can be seen that the lowest removal efficiency occurred in the treatment of samples with pH 3 which showed the final TPH concentration decreased to 5.06% (w/w), so that the removal efficiency was 93.82%. Sample treatment with pH 5 showed an increase in removal efficiency of 94.20% with the final concentration of TPH being 4.75% (w/w). The highest removal efficiency occurred in the treatment of samples with pH 7 which showed the final TPH concentration decreased to 4.54% (w/w), so that the removal efficiency was 94.46%. Based on the results of the analysis above, it can be seen that the optimum variation in acidity (pH) for the degradation process of crude oil TPH by Acetobacter tropicalis and Lactobacillus casei bacteria consortium is a concentration of pH 7.

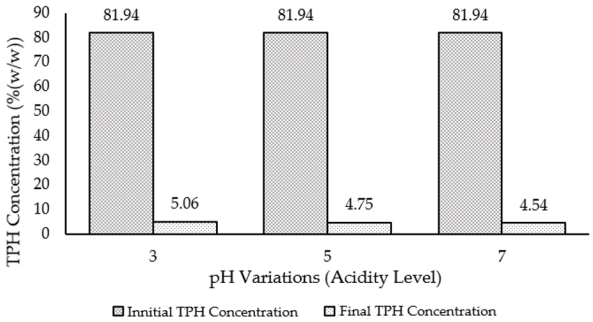


Fig. 3. Crude Oil TPH Removal with pH Variations in SMSS Liquid Media.

Research finding by Wang et al., (2019) who utilized Bacillus subtilis bacteria isolate that included in the hydrocarbonoclastic bacteria group, was able to degrade crude oil with removal efficiency of 65% on pH 7 condition [22]. The pH condition on the research is similar to the optimum pH condition in this research. That is why, the results of that previous research can be compared with the results obtained in this research. The TPH degradation by Acetobacter tropicalis and Lactobacillus casei bacteria consortium shows removal efficiency of 29.46%, higher than removal efficiency generated by using hydrocarbonoclastic bacteria isolate. After obtaining opimum pH level to degrade crude oil on SMSS media, we also obtained the optimum contact time to degrade crude oil on SMSS media. The TPH concentration results and removal efficiency with contact time variations can be seen in Table 3.

Degradation process occurred at 7, 14, and 21 days show relatively similar removal efficiency level. Based on Figure 4, we can see that the TPH of 7 days contact time variation finally reached 4.54% with removal efficiency of 94.46%. 14 days of contact time shows final TPH concentration of 4.96% the removal efficiency of 93.95%. Variation with 21 days contact time shows final TPH concentration of 4.695 with removal efficiency of 94.28%. Based on this results, we can see that the removal efficiency of day 7 to day 14 experienced decrease, but from day 14 to day 21, the removal efficiency is increased.

Table 3. Crude Oil TPH Removal with Contact Time Variations in SMSS Liquid Media.

Contact Time (Day)	**Initial TPH Concentration (%; w/w)**	**Final TPH Concentration (%; w/w)**	**Removal Efficiency (%)**
7	81.94	4.54	94.46

14	81.94	4.96	93.95
21	81.94	4.69	94.28

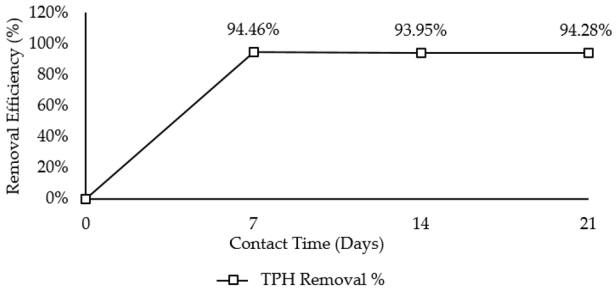


Fig. 4. Crude Oil TPH Removal with Contact Time Variations in SMSS Liquid Media.

The fluctuated removal efficiency occurred around 94% during 7 to 21 days is predicted to occur due to accelerated exponential phase of Acetobacter tropicalis and Lactobacillus casei bacteria than SMSS media with crude oil. This occurred because the available nutrition is complete and suitable. Research by Munawar & Zaidan (2013) that utilized Pseudomonas sp. and Bacillus sp. bacteria consortium shows TPH removal efficiency of 91.04% in just 42 days [23]. Other research conducted by Afianti & Febrian (2020) produced TPH removal efficiency of 52.9% in 42 days by utilizing mangrove sediment mixed culture bacteria of Xylocarpus granatum from Lagoi, Riau Islands [24]. Compared to this research, the utilization of Acetobacter tropicalis and Lactobacillus casei bacteria consortium produced better TPH removal; 94.46% of TPH removal in just 7 days of time.

We also conducted crude oil degradation test on artificial sea water. The TPH concentration and removal efficiency obtained in the degradation process can be seen in Table 4.

Table 4. Crude Oil TPH Removal with Contact Time Variations on Artificial Sea Water.

Contact Time (Day)	**Initial TPH Concentration (%; w/w)**	**Final TPH Concentration (%; w/w)**	**Removal Efficiency (%)**
7	81.94	7.27	91.13
14	81.94	5.00	93.90
21	81.94	4.66	94.32

7 days degradation process produced final TPH concentration of 7.27% w/w) with removal efficiency of 91.13%. The 14 days degradation process shows improvement by obtaining final TPH concentration of 5% (w/w) and removal efficiency of 91.13%. 21 days degradation process shows the highest efficiency level which is at 94.32% with final TPH concentration of 4.66% (w/w).

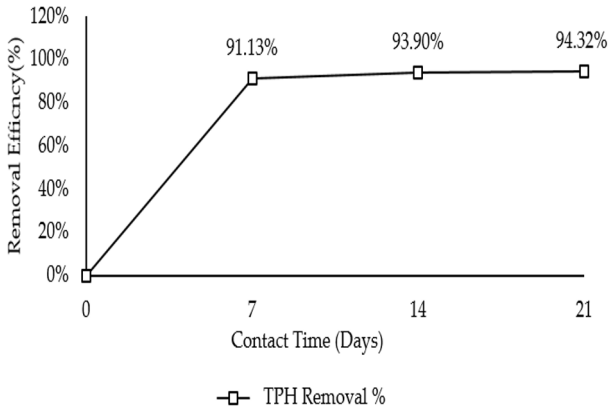


Fig. 5. Crude Oil TPH Removal with Contact Time Variation in Artificial Sea Water.

Based on Figure 5, it can be seen that the consortium of bacteria Acetobacter tropicalis and Lactobacillus casei can degrade the TPH content of petroleum with the highest efficiency within 21 days using artificial seawater media. The allowance at this stage reached 94% within 21 days due to the exponential phase that occurred longer than in SMSS media, although the bacterial consortium was still able to set aside crude oil TPH for longer. This is supported by statements from several researchers that Acetobacter tropicalis and Lactobacillus casei are a group of thermotolerant bacteria that have the ability to grow in conditions of high salinity, making them useful and possible for sea water bioremediation and also crude oil degradation [25-27]. Another research by Chekroud et al., (2021) that utilizes Acinetobacter sp., Aerobacter sp., and Bacillus sp. mixed culture bacteria was able to degrade crude oil on artificial sea water media with removal efficiency of ±95% in 42 days [28]. Compared to this research, Acetobacter tropicalis and Lactobacillus casei bacteria consortium can degrade crude oil TPH of 94% in a relatively shorter period of time, which is 21 days. During crude oil TPH degradation by utilizing Acetobacter tropicalis and Lactobacillus casei bacteria both on SMSS liquid media and artificial sea water media, the pH change occurred both on the initial and the final conditions are measured by pH meter. The pH change occurred during degradation process can be seen in Table 5.

Table 5. The pH Change during Crude Oil TPH Degradation Process.

Sampel Type		Innitial pH	Final pH
pH Variation Stage on SMSS Liquid Media	pH 3	3	3.82
	pH 5	5	5.75
	pH 7	7	5.38
Contact Time Variation Stage on SMSS Liquid Media	Td 14	7	7.18
	Td 21	7	7.47
Contact Time Variation Stage on Artificial Sea Water Media	Td 7	7	6.89
	Td 14	7	6.47
	Td 21	7	5.53

Based on the results obtained in Table 5, pH changes that occur in SMSS liquid media and artificial seawater media show different results. In the SMSS liquid medium in the pH variation stage, the variation in pH 3 showed an increase in pH to 3.82. Variations in pH 5 also showed an increase in pH to 5.75, but variations in pH 7 showed a decrease in pH to 5.38. In the SMSS liquid medium, the contact time variation of 14 and 21 days also showed an increase in pH to 7.18 and 7.47. The increase in pH that occurs is suspected because the pH of the environment is too acidic, causing the bacterial consortium to carry out a reaction

to reduce the level of acidity in its environment. According to Nugroho, (2010), increased pH can be caused by the effort of bacteria to survive on acidic environment by switching ion H+ in the environment with ion K+ in bacteria cell to lower the environmental acidity [29].

The stages of variation in contact time on artificial seawater media all showed a decrease in pH. The variation of the 7-day contact time showed a decrease in pH to 6.89, the pH for the 14-day contact time decreased to 6.47, and the pH for the 21-day contact time decreased to 5.53. The decrease in pH occurs because the metabolic activity of bacteria that produce organic acids can lower the pH value during the degradation process [30,31]. The pH value is one of the important parameters that characterize the life of bacteria, and fluctuating pH values indicate that bacteria carry out a metabolic condition to be able to grow in their environment [32]. After obtaining the optimum environmental conditions at each stage of the petroleum degradation test, it is necessary to calculate the kinetics of petroleum removal. The consortium of bacteria Acetobacter tropicalis and Lactobacillus casei had specific growth rate values (μ) ranging from 0.0440 – 0.0952 hours-1 and specific substrate utilization rates (q) ranging from 0.000028 – 0.000102 hours- 1. The relationship between the values of and q will get a total growth value (YT) of 714 hours-1 and a Kd value of 0.05 hours-1. The graph between the specific growth rate and the specific substrate utilization rate can be seen in Figure 6.

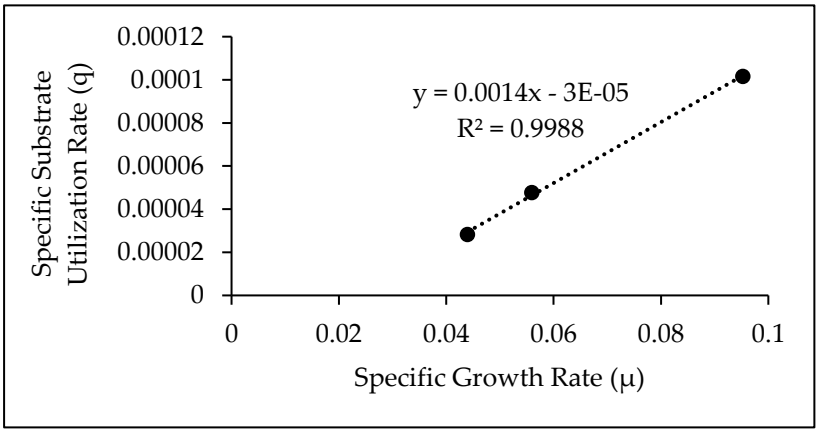


Fig. 6. The Relationship between Specific Growth Rate (μ) and Specific Substrate Utilization Rate (q).

The limited utilization of specific substrates for bacterial growth can be defined using the Growth Yield equation. The graph of the relationship between the utilization of specific substrates (S) and the amount of bacterial growth in limited culture (Xm) will produce a linear line which then forms a Yobs slope (slope/S) with a value of 15405 hours-1. The relationship between specific substrate utilization (S) and the amount of bacterial growth in limited culture (Xm) can be seen in Figure 7.

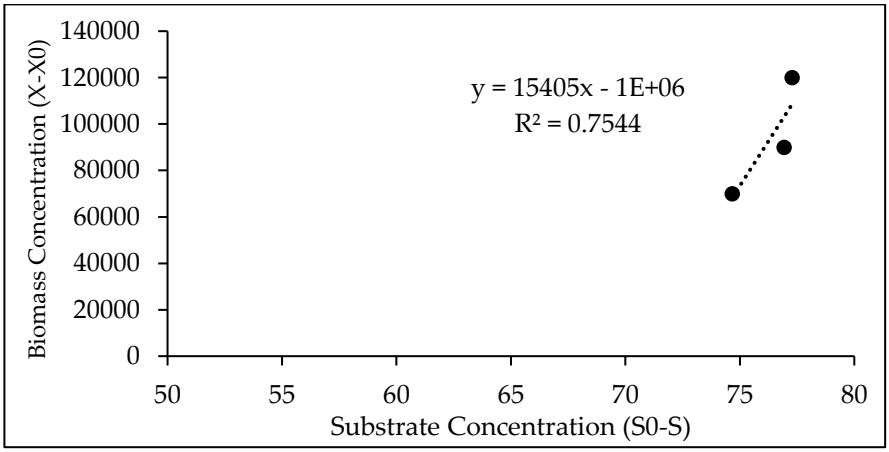


Fig. 7. Relationship between TPH Limit (S0-S) with Bacteria Growth (X-X0).

Furthermore, there is a relationship between the specific growth rate and the concentration of TPH which is shown in Figure 8. The maximum specific growth rate (μ) value is 0.0952 and half of the maximum specific growth rate value is 0.0476, based on the two values, it can be obtained the value of saturated concentration (KS) is 4.8%.

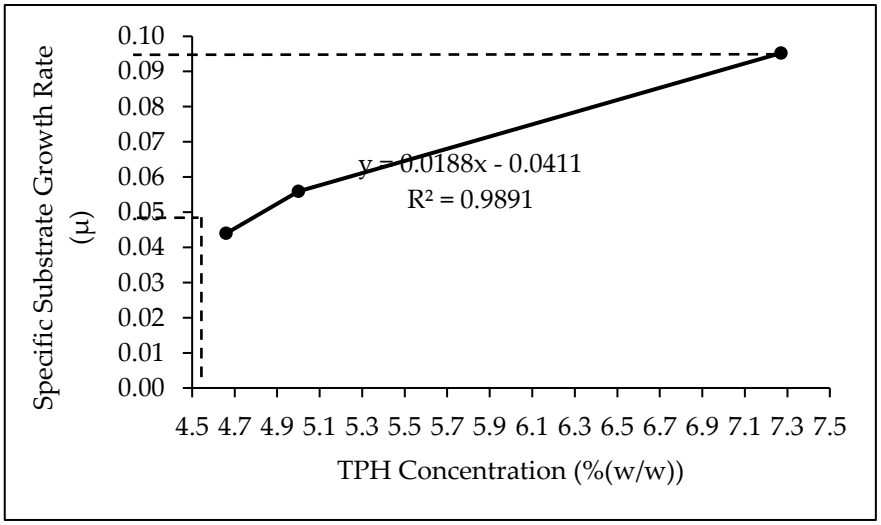
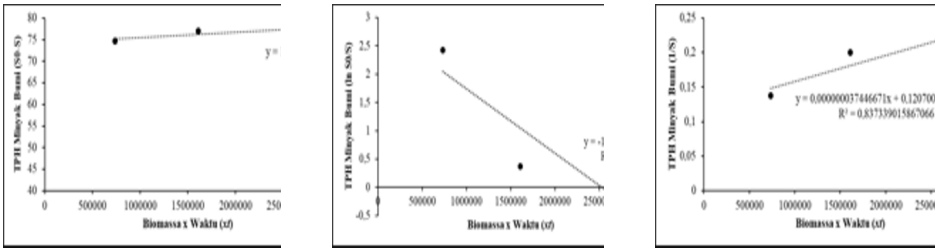


Fig. 8. Relationship between Specific Substrate Growth Rate and TPH Concentration.

Determination of the order of the reaction is done by graphing the growth rate of order 0, order 1, and order 2. Then the R^2 value in each graph is chosen which is closest to number 1. Each order kinetics graph can be seen in Figure 9.



(a) (b) (c)

Fig. 9. Kinetic Order (a) 1; (b) 2; (c) 3.

Based on Figure 9, it can be seen that the R2 value closest to 1 is the reaction order 2 with a value of 0.837. The graph rate on the order of reaction 2 is also in accordance with this study, namely the greater the value of biomass x time (xt), the higher the TPH value of the oil that is set aside is also greater. Reaction order 0 and reaction order 1 were not used in this study because both graphs show that biomass x time (xt) has no effect on TPH removal, and is not in accordance with this study. The modeling of the relationship between the substrate and the concentration of bacteria obtained from this kinetic calculation can be used to estimate the degradation process periodically on a pilot scale [33,34]. The results of this laboratory-scale research are planned on a larger scale or pilot scale to treat 1000 L of oil-contaminated water by utilizing Acetobacter tropicalis and Lactobacillus casei bacteria consortium. It is planned that a tubular reactor with a volume of 1250 liters has dimensions of 1.8 m high, 1 m diameter, and 1.4 m high volume of wastewater. To treat 1000 L of waste water, a consortium of 111.11 liters of bacteria is needed. The composition of the bacterial consortium used in the pilot scale is the same as the laboratory scale, which is 10% of the reactor volume.

The results of the calculation of the second order TPH removal kinetics rate and the bacterial consortium cultivation curve equation were used to obtain the residence time required by the bacterial consortium to eliminate TPH in the pilot scale calculation. The residence time required to set aside TPH with an initial concentration of 81.94% is 1686 hours or 70 days. The quality standard of the Decree of the Minister of the Environment Number 128 of 2003 concerning Requirements for Biologically Treated Crude Oil Waste states that the initial concentration of TPH before going through the biological processing process is a maximum of 15% and the final concentration of TPH after processing is 1%. Based on these quality standards, the results of the removal of TPH in this study have not yet reached the quality standard because the initial concentration of TPH has exceeded the quality standard. To achieve the final quality standard, it is necessary to conduct further research to increase the removal efficiency higher than 94%, namely by varying the temperature and TPH of petroleum as independent variables.

4 Conclusion

The growth response of Acetobacter tropicalis and Lactobacillus casei bacteria consortium showed that these bacterial cultures could grow well on SMSS liquid media and artificial seawater containing petroleum pollutants with controlled environmental conditions, namely in the pH range of 3 to 7, temperature range 27 -30°C, within 7 to 21 days. The optimum environmental conditions for the growth of Acetobacter tropicalis and Lactobacillus casei bacterial consortium in SMSS liquid media and artificial seawater containing crude oil pollutant were at a temperature of 30°C and pH 7, in a contact time of 7 days. Total Petroleum Hydrocarbon (TPH) which can be degraded by Acetobacter tropicalis and Lactobacillus casei

bacterial consortium resulted in a removal efficiency of 94% under controlled conditions at 30°C and pH 7, in a contact time of only 7 days.

References

1. Mustika, Haryadi, Siti Hodijah, Jurnal Perspektif Pembiayaan Dan Pembangunan Daerah **2(3)**, 107–118 (2015) DOI: http://onlineJournal.unja.ac.id/index.php/JES/article/view/2267
2. D. Nuryana, Journal of Earth Energy Engineering **6(2)**, 9–13 (2017) DOI: https://doi.org/10.22549/jeee.v6i2.941
3. G.A.E. Mahmoud, M.M.K. Bagy, Microbial Action on Hydrocarbons, 299–320 (2019) DOI: https://doi.org/10.1007/978-981-13-1840-5_12
4. N. Mahmood Aljamali, Petroleum Engineering & Technology **11(2)** (2021) DOI: https://doi.org/10.37591/JoPET
5. S. Mehrdad, K. Saeb, L. Taghavi, M. Ghane, Iranian Journal of Toxicology **13(3)**, 21–26 (2021) DOI: https://doi.org/10.32598/IJT.13.3.594.1
6. T. Sunaryo, H. Widyatmoko, A. Rinanti, MATEC Web of Conferences **197**, 13007 (2018) DOI: https://doi.org/10.1051/matecconf/201819713012
7. F. Marsandi, S.P. Estuningsih, Proceeding Biology Education Conference: Biology, Science, Enviromental, and Learning **13(1)**, 807–813 (2016)
8. P. Dvořák, , P.I. Nikel, J. Damborský, V. de Lorenzo, Biotechnology Advances **35(7)**, 845–866 (2017) DOI: https://doi.org/10.1016/j.biotechadv.2017.08.001
9. S. Sihag, H. Pathak, D.P. Jaroli, International Journal of Pure & Applied Bioscience **2(3)**, 185–202 (2014)
10. A. Rinanti, I.J. Nainggolan, IOP Conference Series: Earth and Environmental Science **106**, 012100 (2018) DOI: http://iopscience.iop.org/article/10.1088/1755-1315/106/1/012100/meta
11. T. Wilan, N.A. Lieswito, A. Suwardi, et al., International Journal of Scientific and Technology Research **9(1)**, 3533-3536 (2020) DOI: http://www.ijstr.org/final-print/jan2020/The-Biosorption-Of-Copper-Metal-Ion-By-Tropical-Microalgae-Beads-Biosorbent.pdf
12. J. Monod, Annual Reviews in M **3, Xl**, 371–394 (1949)
13. V. Nandakumar, J.L. Jayanthi, Example from Mumbai Offshore, India. Energy and Fuels **30(5)**, 3776–3782 (2016) DOI: https://doi.org/10.1021/acs.energyfuels.5b02952
14. Gottschalk, Gerhard, Clinical Microbiology and Parasitology (For DMLT Students), 14–14) (2011) DOI: https://doi.org/10.5005/jp/books/12721_5
15. W.A. Spinosa, V. dos Santos Júnior, D. Galvan, J.L. Fiorio, R.J.H.C. Gomez, Vinegar rice (Oryza sativa L.) produced by a submerged fermentation process from alcoholic fermented rice. Food Science and Technology (Brazil) **35(1)**, 196–201 (2015) DOI: https://doi.org/10.1590/1678-457X.6605
16. O.N. Majolagbe, M.O. Olabemiwo, A. Ayandele, et al., International Journal of Current Microbiology and Applied Sciences **8(12)**, 2612–2622 (2019) DOI: https://doi.org/10.20546/ijcmas.2019.812.305
17. J.N. Bhakta, K. Ohnishi, Y. Munekage, K. Iwasaki, M.Q. Wei, Journal of Applied Microbiology **112(6)**, 1193–1206 (2012) DOI: https://doi.org/10.1111/j.1365-2672.2012.05284.x

18. R. Mishra, V. Sinha, A. Kannan, R.K. Upreti, Toxicology International **19(1)**, 25–30. (2012) DOI: https://doi.org/10.4103/0971-6580.94512
19. M. Monachese, J.P. Burton, G. Reid, Applied and Environmental Microbiology **78(18)**, 6397–6404 (2012) DOI: https://doi.org/10.1128/AEM.01665-12
20. S. Syukur, S. Yolanda, E. Fachrial, A. Jamsari, Journal of Chemical and Pharmaceutical Research **7(9)**, 235–241 (2015)
21. S.P. FHOLIClaus, H. Guillou, S. Ellero-Simatos, Npj Biofilms and Microbiomes **2**, 1–12 (2016) DOI: https://doi.org/10.1038/npjbiofilms.2016.3
22. D. Wang, J. Lin, J. Lin, W. Wang, S. Li, Molecules **24(17)** (2019) DOI: https://doi.org/10.3390/molecules24173021
23. Munawar, Zaidan, Sains & Matematika **1(2)**, 41–46 (2013)
24. N.F. Afianti, D. Febrian, Prosiding The 11th Industrial Research Workshop and National Seminar, 725–729 (2020)
25. I.M.A. Al-Maghrabi, A.O. Bin Aqil, M.R. Islam, O. Chaalal, Energy Sources **21(1–2)**, 17–29 (2010) DOI: https://doi.org/10.1080/00908319950014920
26. W. Soemphol, A. Deeraksa, M. Matsutani, et al., Bioscience, Biotechnology and Biochemistry 75(10), 1921–1928 (2011) DOI: https://doi.org/10.1271/bbb.110310
27. H.N.P. Trinh, B.H.D. Long, N.N. Thanh, et al., International Food Research Journal **25(2)**, 523–526 (2018)
28. Z. Chekroud, M.K. Gouda, M. Houhamdi, Current Advances in Chemistry and Biochemistry **6**, 67–79 (2021) DOI: https://doi.org/10.9734/bpi/cacb/v6/1976f
29. A. Nugroho, Bioremediasi Hidrokarbon Minyak (Universitas Trisakti, 2010)
30. Ristiati, N. P., M, S, Putra, I. M. G. P, *Uji Kemampuan Degradasi Minyak Solar Oleh Konsorsium Bakteri Hasil Preservasi Dengan Kombinasi Metode Liofilisasi Dan Metode Gliserol* (Prosiding Seminar Nasional MIPA, 2016)
31. E. Sari, R. Novianty, A. Awaluddin, Saryono, N. Pratiwi, Riau. Acta Biochimica Indonesiana **153**, 15–22 (2019)
32. M. Sarah, E. Misran, S. Maulina, I. Pertiwi, et al., Jurnal Teknik Kimia USU **10(1)**, 13–18 (2021)
33. Y. Wang, C. Qin, F. Witarsa, Waste Management **77**, 22–29 (2018) DOI: https://doi.org/10.1016/j.wasman.2018.04.040
34. M.F. Fachrul, A. Rinanti, T. Tazkiaturrizki, et al., Indonesian Journal of Urban and Environmental Technology **5**, 86-103 (2021) DOI: https://doi.org/10.1016/j.wasman.2018.04.040