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Potential Oil Diesel Biodegradation by Marine Yeast Isolated from East Java Sea.

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ABSTRACT

Oil waste waters increases along with industrial and transport activities that leave residue of oil in the area around the beach. This condition can be overcome using environmentally substances, which is using microorganisms. Marine yeast is one of marine microorganisms which can be potentially an agents that environmentally degrade diesel fuel. The purpose of this study was to determine the ability of diesel fuel degradation by marine yeast isolates which was isolated from two different locations, Probolinggo fishing port and KondangMerak beach. Result of biodegradation test with different concentration of diesel fuel as much as 10 ml , 15 ml and 20 ml showed that marine yeasts have the ability to degrade diesel fuel. Results of biodegradation test using gravimetric method following the Standard Method 21stEdition 2005 APHA - AWWA - WEF 5520 B #) showed that average degradation of marine yeast from Probolinggo fishing port is 71.66 % ± 0.109 and where as diesel fuel from KondangMerak beach is 66.1 % ± 0.085. Result of gravimetric assay indicated that themaximumdegradationabilityoftheyeastwasfoundat10mlconcentrationofdieselfuel.

Keywords: Biodegradation, Yeast Peptone Dextrose Agar, Gravimetric

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INTRODUCTION

Environmental pollution is increasing marine waters by hydrocarbons has given rise to problems for the organism and its environment. Petroleum processed would result from a product that can be used for diesel fuel is diesel. Diesel fuel composed of benzene, toluene, xylene, and other alkyl on polyaromatic hydrocarbons. Compounds contained in diesel will cause chronic effects on organism, especially mammals such as immunological disorders, reproductive, and the emergence fetotoxic effects and genotoxic [1]. Petroleum is the main energy source used for industrial activities and transport. Increased industrial activity and transport to be near the beach and offshore can improve marine pollution. According Ristiati [2], oil (diesel) marine waters spill could cause serious problems for the biotic communities and aquatic habitats. The oil spill will spread on the surface of the sea that will happen modifications and changes in the oil composition.

Environmental conditions polluted waters can be done by using the method of handling chemistry, physics and biology. One method that can be used to combat pollution of an environmentally friendly way is to bioremediation. Bioremediation agent remidiator requires that microorganisms can degrade chemical compounds so as not to pollute the environment.

Microorganisms which can be used in the process of biodegradation most predominantly found in areas contaminated hydrocarbons. Allegedly microorganisms in the region have the ability to degrade hydrocarbons for the purposes of metabolism, so having the ability to degrade better than in the less polluted hydrocarbons. One of microorganisms that can be used as an agent of the oil is degrading yeasts. Marine yeast can be categorized as an agent to degrade the oil if the growth of the yeast can use carbon from oil as the sole carbon source for growth.

The purpose of this study was to determine the potential of isolates of yeast marine isolated from two different places, namely fishing port Probolinggo and KondangMerak beach, the effectiveness of isolates yeast sea fishing port Probolinggo and KondangMerak beach in degrading solar, the best concentration in the addition of diesel oil in the degradation process. Selection of sampling sites is based on the quality of the environment. Fishery Port Probolinggo represent the north coast which has a lot of shipping activity, while the KondangMerak Beach represent the south coast is still maintained the quality of its environment.

RESEARCH METHOD

Time and Place Research

This study was conducted from March to June 2016. Samples previously been taken in the area of Probolinggo and Fishing Port Kondang Merak Beach in April 2015. Samples of existing marine yeast cultured in the laboratory of the Center of Biological Sciences (LSIH) Brawijaya University. The process of biodegradation test carried out in the laboratoryare Marine Sciences Faculty of Fisheries and Marine Sciences. Gravimetry test conducted in SUCOFINDO Analytical and Testing Laboratories, Surabaya.

Isolation and Culture of Marine Yeast

Marine yeasts were isolated into YPDA media (Yeast Peptone Dextrose Agar). Media created in 750 ml of seawater was added to the 15g Agar PA, 5g Yeast Extract, Dextrose PA10 gr, 10gr Peptone PA, and 0.25 ml Clhoramphenicolas an antibacterial. Isolation is done by pour plate method with the addition of sea water samples of 1 ml. Media containing sample was then incubated in the incubator for 10x24 hours using a temperature of34˚C.

Marine yeast isolates were incubated for 10 days subsequently cultured in liquid media. Marine yeast culture process by using Artificial Sea Water as much as 1 L with the addition of sugar 0.5 % (w/v) and foliar fertilizers Growhore 0.2 % (w/v). Marine yeast aerated for 3x24 hours.

Biodegrdation Thests and Test Gravimetry

The experimental design in this study using factorial completely randomized design (RAL Factorial) consisting of three (3) different treatment of solar concentration using three (3) repetitions in each

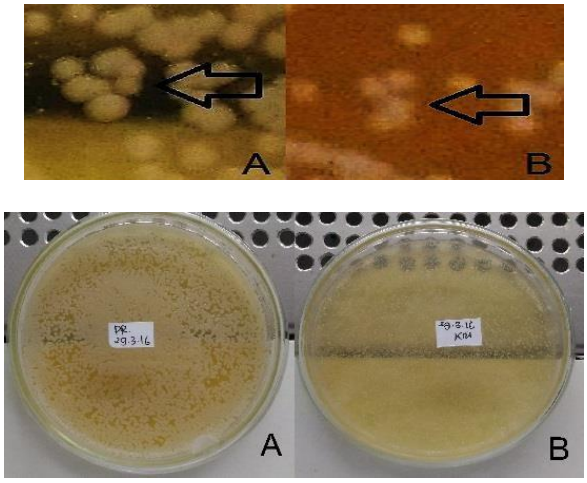
comparison. Design of experiments is shown in Table 1. Each experiment is added with nutrients ie 5 grams of sugar as a source of carbon and 2g Growhore foliar fertilizers as nitrogen source. Samples were aerated and dishaker with a speed of 80 rpm for 7 days (168 hours). Samples are already in the shaker for 7 days and then analyzed in order to determine the gravimetric residual fuel oil after seacoupled with yeast. Analysis of the gravimetric method was performed according to the Standard Method 21st Edition 2005 APHA-AWWA-WEF. This standard describes the procedure for checking various kinds of quality water, sewage, fresh water and high salinity water. In this study, using 5520 B#), the standard used to analyze the Oil and Grease by gravimetric method using the solventn-hexan.

RESULTS ANDDISCUSSION

Isolation and Culture of MarineYeast

Isolation sea yeasts done using two (2) different medium that is denser medium YPDA (Yeast Peptone Dextrose Agar) with chloramphenicol addition of 0.25 ml of liquid media and Sea water. Isolation intended to obtain a pure culture yeast sea. Results obtained insulation marine yeast colonies are round white. Marine yeast colony size is almost uniform in Probolinggo Port Fishing or Kondang Merak Beach. Marine yeast colony isolation results can be seen in Figure1.

Figure 1: Result of Isolation



(A)Probolinggo Port Fishing (B) KondangMerak Beach

Table 1: The Design of The Research Treatment

Location	Treatment									Control
	10 ml (X)			15 ml (Y)			20 ml (Z)			
	1	2	3	1	2	3	1	2	3	
Factor 1	XA1	XA2	XA3	YA1	YA2	YA3	ZA1	ZA2	ZA3	KA
Factor 2	XB1	XB2	XB3	YB1	YB2	YB3	ZB1	ZB2	ZB3	KB

Marine yeast colonies isolated and then cultured in media sea water with the addition of fertilizer and sugar as a nutrient for the growth of yeasts sea. Marine yeast in liquid culture given treatment aeration serves as a supplier of oxygen required for the metabolism process. Aeration the marine yeast cultures performed for 3 x 24 hours. Marine yeast culture processes can be seen in Figure 2. In the process of yeast culture sea from Fishery Port Probolinggo and Kondang Merak Beach no difference in treatment. On the first day the sea yeast culture (1x24 hours) resulted in the media is still the clear blue color, whereas on the third day (3x24 hours) media have changed color to bluecloudy.

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Figure 2: Culture Marine Yeast (A) 1x24 hours, (B) 3x24 hours

The success of marine yeast culture is indicated by a change in the brightness of the water. The more turbid culture media, the growth of yeasts sea is increasing.

Biodegradation Test

Biodegradation test was performed by using sterile sea water media is added sugar and foliar fertilizers then added different concentrations of diesel oil (10 ml, 15 ml, 20 ml) and added marine yeast cultures of 7 ml. Sterile sea water that had been treated later in the shaker with the objective to condition biodegradation media make it more dynamic and to homogenize the yeast in order to get the nutrients evenly , shaker speed was 80 rpm and aeration are given as the source of oxygen for 24 hours. Biodegradation process can be seen in Figure3.

Figure 3: The Process of Biodegradation in Shaker

Their aeration is essential to the process of degradation, due to marine yeasts need oxygen for the metabolic process. Given sufficient oxygen during the degradation process, the yeast will work optimally degrade diesel oil. Besides necessary for metabolism, oxygen is also required for the marine yeast oxidation process chemical compounds contained in diesel oil. In the process of degradation can be seen by a color change in the test medium.

Medium test which was originally colored clear and there is a layer of oil on the surface turns into a murky. Before and after the process of degradation can be seen in Figure 4.

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Figure 4: Before And After The Process of Degradation

The color change in the media can be assumed that the degradation of marine yeast uses sugar and fertilizer to supply energy required to break the bond chain diesel oil, so that the diesel oil can also be used as food for the marine yeast growth process. According Ristiati [2], diesel oil which originally formed a separate layer on the surface of the media will be split into small granules. The formation of small granules in the media caused by the presence of biosurfactant. Biosurfactant is a complex compound consisting of hydrophilic and hydrophobic groups that are soluble in water and oil.

Calculation of Marine Yeast Growth Phase

The growth of microorganisms starts from the beginning of growth until the end of growth activity can be described in the form of curves. In the present study the growth of yeasts sea is measured by Optical Density values with a wavelength of600 nm using a spectrophotometer. Principles calculations using a spectrophotometer was leavened by measuring the amount of light transmitted by the marine yeast cells are suspended. Probolinggo marine yeast growth curve can be seen in Figure 5 to Figure 8.

Figure 5: Marine Yeast Probolinggo Growth Curve With Concentration Diesel Oil 10 ml

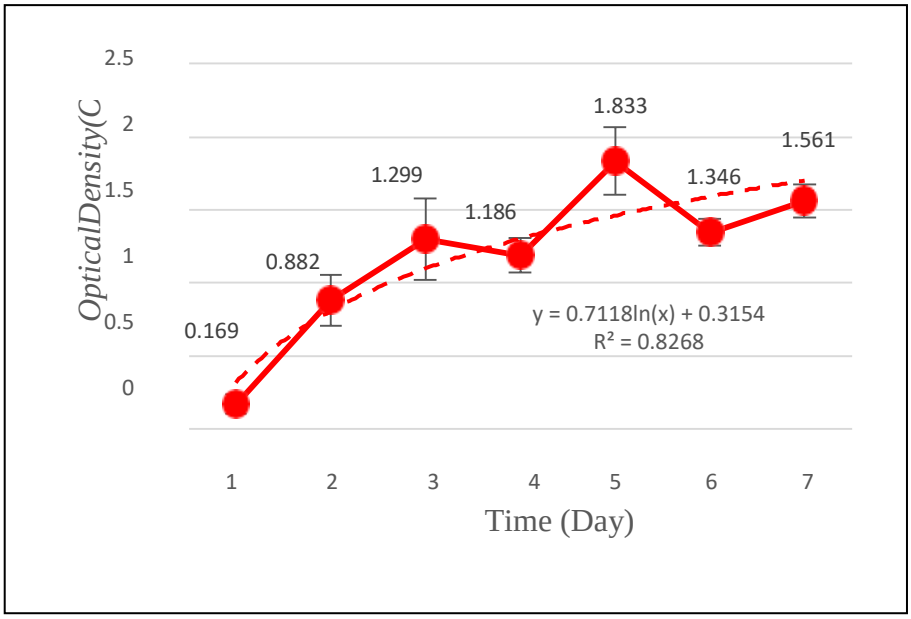

Figure 6: Marine Yeast Probolinggo Growth Curve WithConcentrtion Diesel Oil 15 ml

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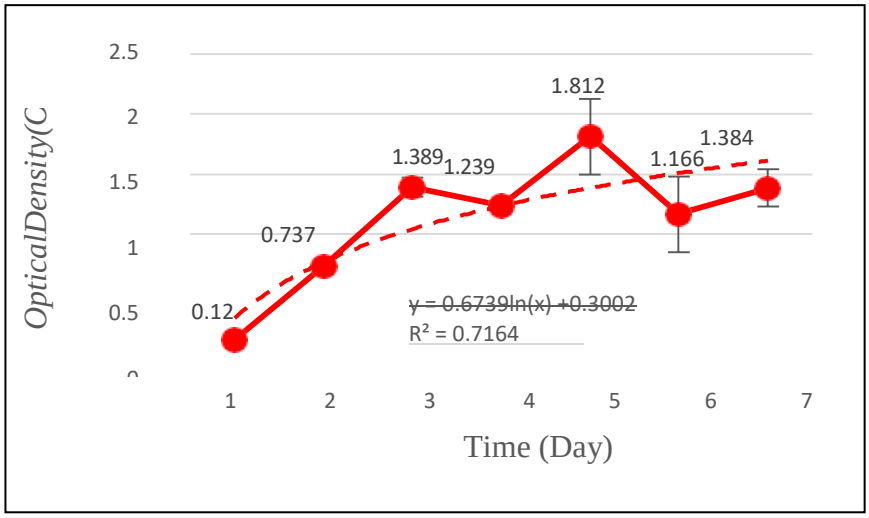


Figure 7: Marine Yeast Probolinggo Growth Curve With Concentration Diesel Oil 20 ml

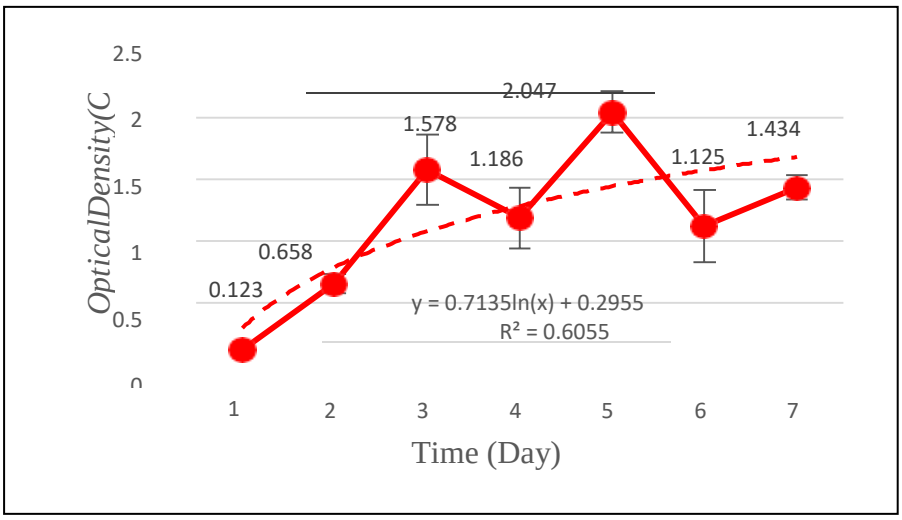
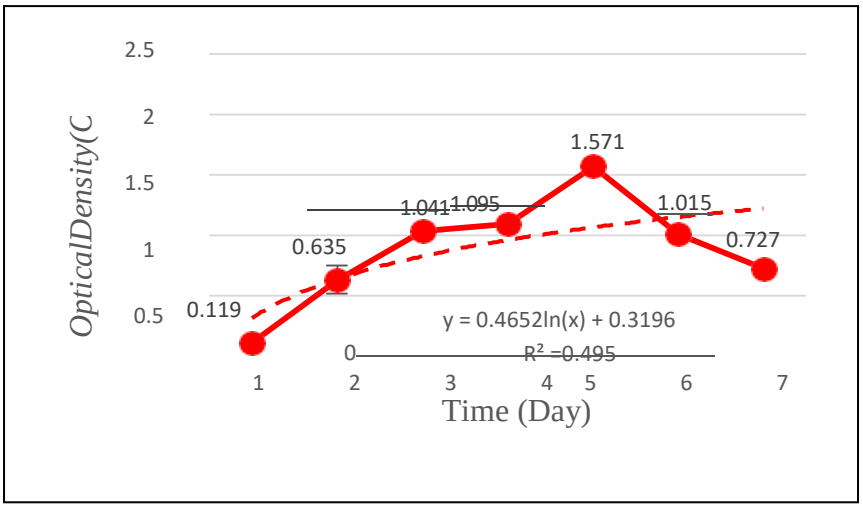


Figure 8: Marine Yeast Probolinggo Growth



In general microorganisms especially bacteria through a phase lag, log, stationary, and death within 24 hours.On the chart the growth of marine yeasts Proboling growth the addition of solar 10 ml,15 ml and 20

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ml did not show the growth phase in a matter of days, it can be seen from the Optical Density on the first day of the third to the growth of yeasts sea has increased dramatically (logarithmic), but on the fourth day of marine yeast growth decreasing, and back up on the fifth day. This in an indication that the yeast growth phase with the addition of marine diesel oil was at 24 hours. In contrast to the growth of marine yeasts without the addition of diesel fuel, the growth phase can be seen in a matter of days, on the first day of leavened marine entered a phase of adaptation, on the second day to the fifth day marine yeasts entered logarithmic phase and stationary phase, on the day of the sixth and seventh yeasts sea entered a phase dead.

The growth curve of marine yeast Kondang Merak Beach can be seen in Figure 9 through Figure 12 as follows:

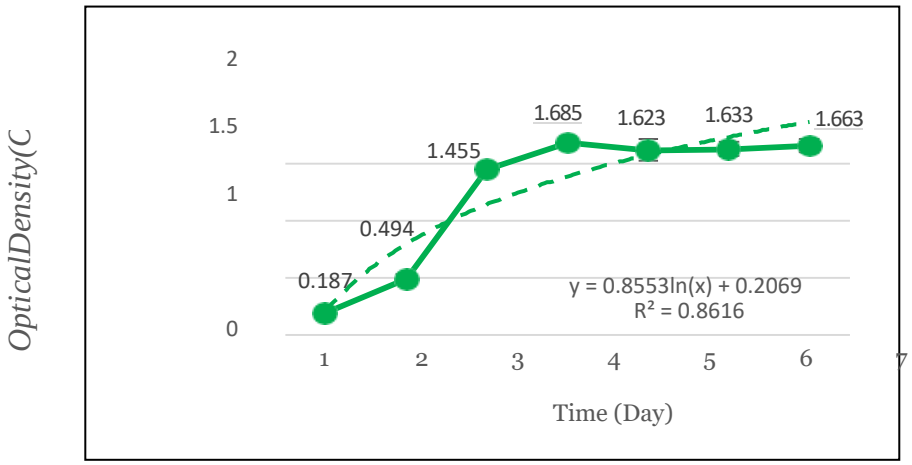

Figure 9: Marine Yeast KondangMerak Growth Curve With Concentration Diesel Oil 10 ml

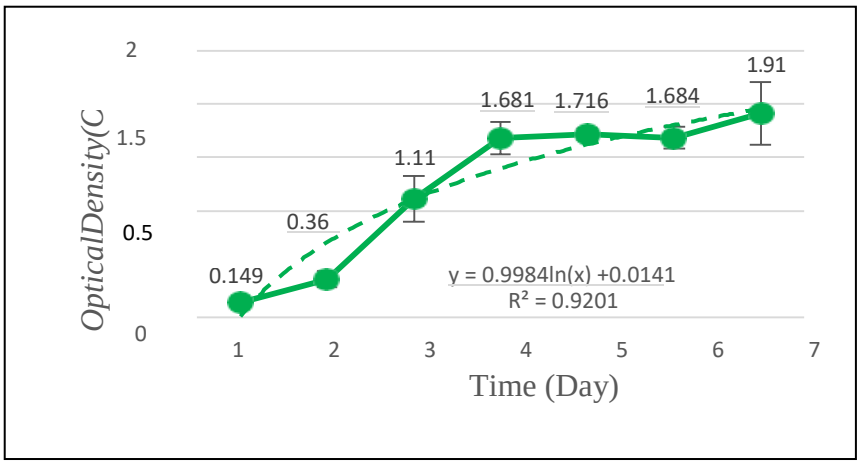

Figure 10: Marine Yeast KondangMerak Growth Curve With Concentration Diesel Oil 15 ml

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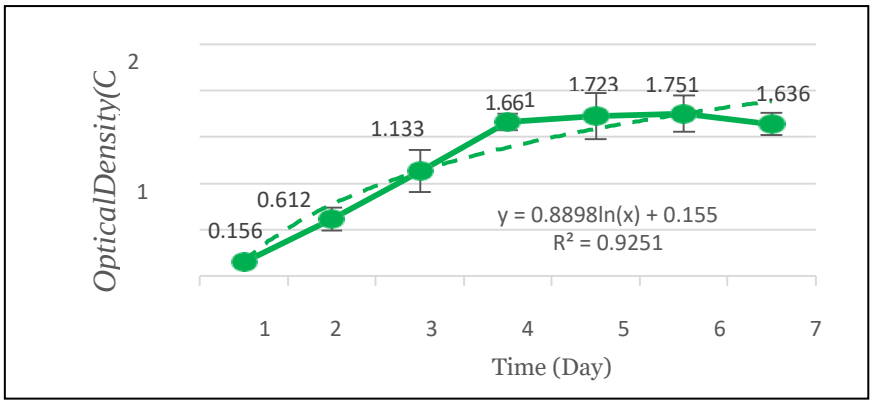

Figure 11: Marine Yeast KondangMerak Growth Curve With Concentration Diesel Oil 20 ml

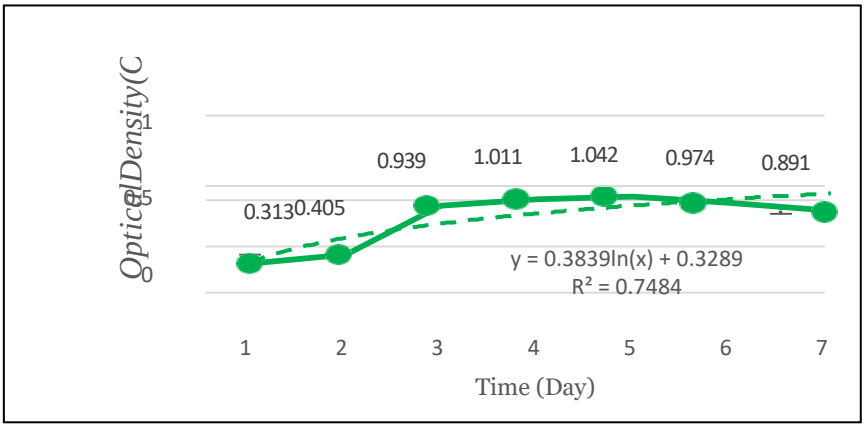

Figure 12: Marine Yeast KondangMerak Growth Curve Without Addition Diesel Oil

On the chart the growth of marine yeasts Kondang Merak Beach with the addition of diesel by 10 ml, 15 ml and 20 ml showed that on the first day of leavened sea entered a phase of adaptation, on the second day until the fourth day showed yeasts sea entered logarithmic phase, the fifth to sixth day marine yeast shows a stationary phase, on the seventh day marine yeast enters the death phase.

The difference between the yeast growth phase of Probolinggo Fishing Port and KondangMerak Beach can diakaitkan with water quality yeast origin. In yeasts from Probolinggo waters accustomed exposed to the solar waste, no adaptation when given additional treatment diesel oil causing growth time is getting shorter because it was directly familiar with the presence of hydrocarbons. But the marine yeast in waters Probolinggo can regenerate fast time, it can be seen from the Optical Density values were decreased and increased. In yeasts of the waters of the KondangMerak, the quality of the waters is still awake, yeasts adapt when treated the addition of diesel, so the time of growth can be seen in a matter of days. Marine yeast waters of the KondangMerak also proved to be able to use hydrocarbons from diesel oil for nutritional growth. It can be seen from the increasing value of Optical Density from day to day, generally have the growth and metabolism of microorganisms capable of rapid adaptation to various environmental conditions. Inconditions permit, the presence of carbon and nutrients are sufficient, microbes can increase this number by dividing themselves. The time required for dividing microbe called generation time (genetationtime).

Measurement of Environmental Parameters

1. Temperature

Temperature is one parameter that is essential for the growth of marine yeasts. Marine yeast can grow on a very wide range of temperatures, ranging from 22˚C to 30˚C [3]. Temperaturecurve of medium degradation by the addition of marine yeast Probolinggo Fishing Port and KondangMerak Beach can be seen in Figure 13 and Figure14.

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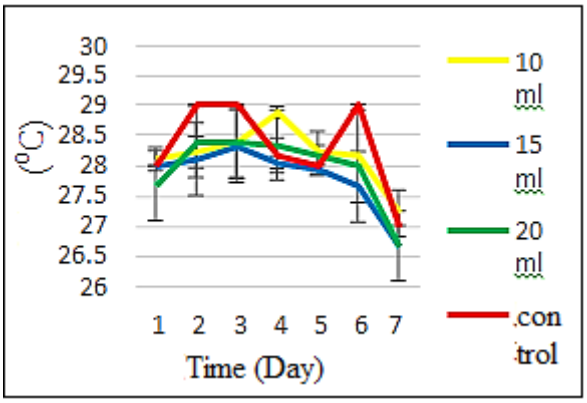

Figure 13: Temperature Curve Medium Degradation Yeast Addition Probolinggo

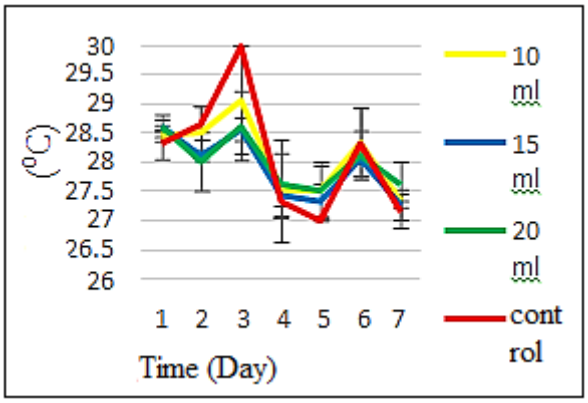

Figure 14: Temperature Curve Medium Degradation Yeast Addition KondangMerak

In this study, obtained temperature values are still within the range of 26˚C to 30˚C. The average value of the temperature medium degradation of Fishery Port Probolinggo yeasts that 28,05˚C±0.304, while the average value of the temperature for medium degradation on Kondang Merak Beachis 28,03˚C±0.335.

Temperature is an environmental factor that greatly affects the process of biodegradation of hydrocarbons. In general, an increase in temperature greatly affect metabolic processes, growth rate, and enzyme activity in microorganisms. Beyond the optimum temperature, the growth of microorganisms will be slow[4].

2. Salinity

Salinity in the test medium degradation is very high, ranging from 35‰ to 43‰. This is because the degradation test media are growhere fertilizer which contains Mg and Ca. During the degradation process in the test medium salinity decreased, and then increased due to evaporation. The average value of salinity medium degradation yeast of Fishery Port Probolinggo is 38.82‰±1.806, while the average value of salinity for medium degradation yeast on KondangMerak Beach is 41.12‰±1.31. Salinity curve on medium degradation can be seen in Figure 15 and Figure16.

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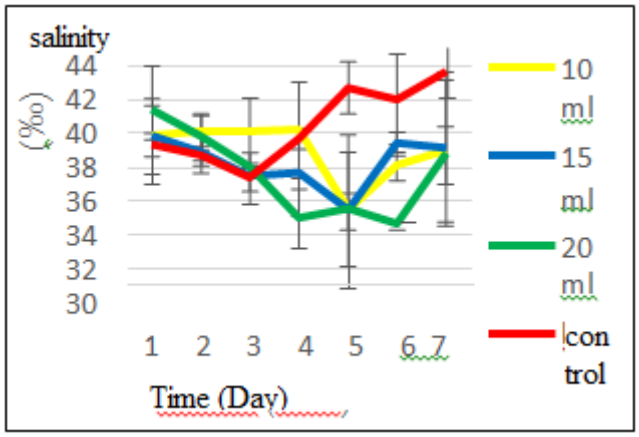

Figure 15: Salinity Curve Medium Degradation Yeast Probolinggo

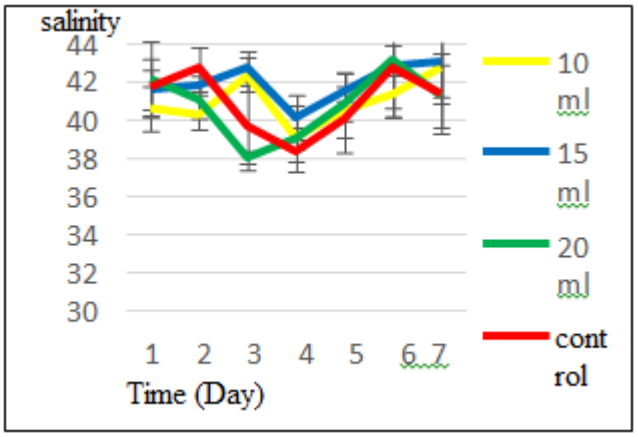

Figure 16: Salinity Curve Medium Degradation Yeast KondangMerak

According Mujab [5], the optimum salt concentration for a review of the growth of microorganisms is 3% of the overall weight. Oil degrading microorganisms are only able to grow under conditions of low salinity. However, the marine yeast is one of the microorganism capable of living at high salinity, is evident from the increasing value of Optical Density as an indicator of the growth process.

3. Power of Hidrogen (pH)

pH (Power of Hydrogen) is a scale used to measure the acidity or alkalinity of a solution. Marine yeast is one of the microorganisms that can survive in an unfavorable environment. In this study, the average pH value of medium degradation of Fishery Port Probolinggo yeasts which 5±0.667, while the average pH value to medium degradation of marine yeast in KondangMerak Beach is 6±0. Graph medium pH degradation of the marine yeast Probolinggo Fishing Port and KondangMerak Beach can be seen in Figure 17 and Figure18.

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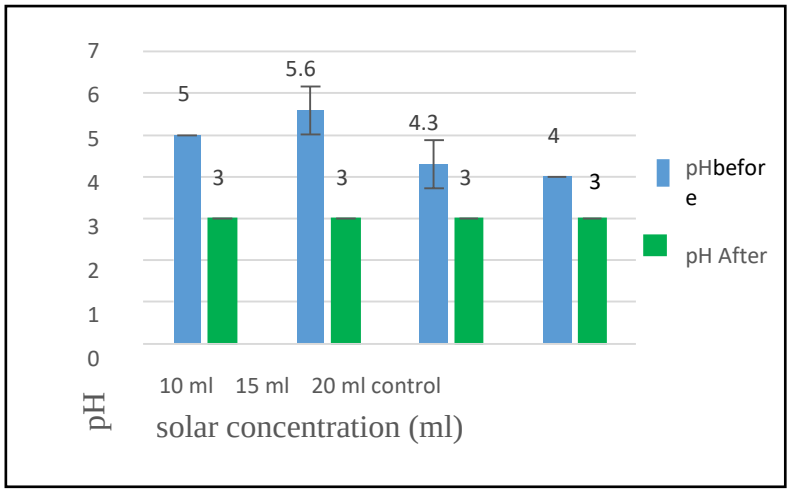

Figure 17: pH Medium Degradation Marine Yeast Probolinggo

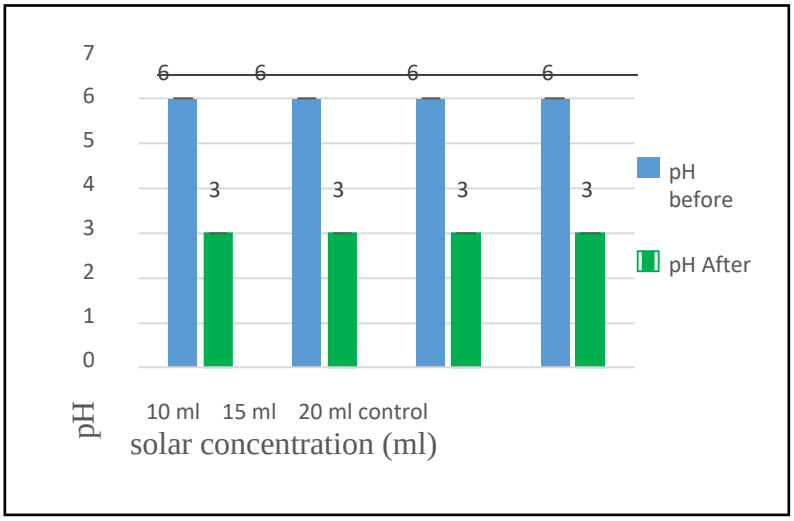

Figure 18: pH Medium Degradation Marine Yeast KondangMerak

In this study, the pH was measured at the beginning and end of the degradation with the aim to determine the presence of metabolites of degradation. If the yeast can degrade diesel oil, it will produce acid metabolites which resulted in a decrease in the pH value. According Ristiati [2], at the beginning of the process of degradation will occurbio surfactant formation which will cause an increase in pH. During the process of degradation of the pH decrease, and this is because yeast produces acid metabolites, the result of the process of degradation of hydrocarbons found in diesel.

Analysis of Gravimetry

Gravimetric method is the method used to determine the levels of oil and grease in water medium. This method is used to handle the emulsion a substance that cannot evaporate. The test results in the addition of solar degradation as much as 10 ml, 15 ml and 20 ml showed marine yeasts have the ability to degrade diesel oil. The value of the magnitude of the degradation of diesel fuel can be seen in Table 2, which shows that the addition of diesel fuel as much as 10 ml and 20 ml ability to isolate marine yeast of Fishery Port Probolinggo able to degrade diesel oil is greater than isolates marine yeast of KondangMerak Beach while the addition of diesel oil by 15 ml marine yeast isolates the ability of KondangMerak Beach greater than the degrading yeast isolates of Fishery Port Probolinggo. This may be due to aeration in the test medium with the addition of yeast from KondangMerak Beach greater than aeration in the test medium with the addition of yeast from Probolinggo Fishery Port. The results showed that the marine yeast of the Fishing Port Probolinggo can degradean average of 71.66%±0.109 for diesel oil, while the yeast of KondangMerak Beach can degrade an

average of 66.1%±0.085 for diesel oil. From the test results gravimetric known to decrease the maximum found in concentrations of diesel oil 10 ml, this could be caused due to the amount of hydrocarbon fuel oil fewer and more easily decomposed or degraded compared to the amount of hydrocarbon fuel oil at a concentration of 15 ml and 20 ml is too concentrated and need time longer to be decomposed or degraded.

According to Siregar [6],the results of biodegradation test known yeast isolates the treated oil 1 % had a reduced oil content higher than 3 % oil concentration. This is due to the oil concentration of 1% of the total hydrocarbon concentration lower than the 3% oil, so oil at a concentration of 1 % is more easily broken down and utilized by yeast as a source of energy. This means that the composition of the hydrocarbons in the oil effect on the process of biodegradation.

Analysis of Data

Analysis of the data used is the analysis ANOVA (One Way Analysis of Variance). According to Sugiharto [7], the analysis ANOVA was used to test the null hypothesis of the amount of data that is expected to have a value, compute the average of the same. Variable used in this analysis in the form of quantitative variables. This analysis is used to determine whether there are differences in the ability of marine degradation yeastisolates from Probolinggo Fishing Port and KondangMerak Beach based on the results of a gravimetric test of the increasing concentration of diesel oil by 10 ml, 15 ml, 20ml.

ANOVA results of calculations known that the value F.HitungF. Tabel greater than 5%, which means that reject H0.Based on calculations using statistics show that the difference in ability between the degradation of marine yeast isolates Probolinggo Fishing Port and Kondang Merak Beach.

ANOVA test only gives an indication of whether or not the difference between the average of the overall treatment that the addition of yeast isolates of Probolinggo fishing port and beach KondangMerak, necessitating the holding of further tests. Further tests (Post Hoc Test) is performed to determine whether or not its difference each addition of solar concentration. Further tests using the LSD (Least Significant Difference) or so-called LSD (Least Significance Different). This method makes the value of LSD or LSD value as a reference in determining whether an average of two treatments statistically significantly different ornot.

Based on the LSD (Least Significant Difference) showed that the addition of solar concentration of each treatment on the addition of yeast and Coastal Fishing Port ProbolinggoMerakKondang differ significantly. Significant differences can be seen from the presence of an asterisk (*).From the above table are all marked with an asterisk (*) which means that all treatments have significant differences. In addition to do LSD (Least Significant Difference), Duncan test was also done to determine the value of the difference between each treatment regardless of the number of treatments. From the test results can be known Duncan each treatment and the addition of diesel 10 ml, 15 ml and 20 ml differ significantly. Based on the average value of the smallest diesel oil are the largest decrease in the concentrations of diesel oil adding 10ml.

CONCLUSION

Based on the results of this study concluded that :

1) Marine Yeast isolated from the Fishing Port Probolinggo and KondangMerak Beach shown to degrade diesel oil at a concentration of 10 ml , 15 ml and 20ml.
2) Isolate marine yeast of Probolinggo Fishing Port and KondangMerak Beach have differing abilities to degrade diesel oil. Gravimetric test results indicate that the yeasts of the Probolinggo Fishing Port can degrade an average of 71.66%±0.109 for diesel oil, while the marine yeast of KondangMerak Beach can degrade an average of 66.1%± 0.085 for diesel oil.
3) From the gravimetric assay results are known to decrease the maximum at a concentration of 10 ml of diesel oil, it indicates that the concentration of the best diesel oil can be degraded by the yeast to the maximum sea is at a concentration of 10 ml of diesel oil.

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REFERENCES

[1] Nashikin, R., M. Shovitri. *Isolasidan Karakterisasi Bakteri Pendegradasi Solar dan Bensindari PerairanPelabuhan Gresik*. 2013. JurusanBiologi. FMIPA. InstitutTeknologiSepuluhNopember. Surabaya.

[2] Ristiati,N.P. UjiKemampuanIsolat Bakteri Pendegradasi Minyak Solar TerhadapLimbahOli Dari PerairanPelabuhanCelukanBawang. 2013. *Seminar Nasional FMIPA UNDIKSHA III Tahun 2013*.

[3] Pelczar, M.J., E.C.S. Chan. *Microbiology.* 1989. Payne Publishers.New York.

[4] Dewilda, Y., R.Afrianita, F.F.Iman.DegradasiSenyawaFenolOlehMikroorganismeLaut. *JurnalTeknikLingkungan UNAND*.2012,9(1): 59-73, ISSN 1829-6084.

[5] Mujab, A.S. Penggunaanbiokomposdalambioremediasilahantercemarlimbahlumpurminyakbumi. 2011.*Unpublished Thesis*.Universitas Islam NegeriSyarifHidayatullah. Jakarta.

[6] Siregar, S.R. PotensiKhamirPendegradasiMinyak Solar Dari Air LautBalawan.2009. *Unpublished Thesis*. SekolahPascaSarcajana. Universitas Sumatera Utara.

[7] Sugiharto,T.*AnalisisVarians*. 2009.BumiPustaka. Semarang.