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A Test on the Potential of Marine Yeasts to Degrade Diesel Oil Isolated from Kili-Kili Beach, Trenggalek, East Java.

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ABSTRACT

The main source of marine pollution comes from the oil spill in either of the ships, offshore drilling as well because of shipwreck. Oil spill at sea continuously increases in number and frequency, resulting in damage to the coastal and marine areas. Biodegradation is one attempt to reduce the pollutants with the help of organisms (bacteria and fungi) in the elimination process of hydrocarbons at sea. The purpose of this study was to determine the potential of marine yeast isolates isolated from Kili-Kili Beach, Trenggalek, East Java and to determine the percentage in the decrease in diesel oil and marine yeasts. The method used is the experimental with completely randomized design (CRD) of one factor and four treatments (2 ml, 5 ml, 10 ml and 15 ml) and 3 repetitions (n = 4). Test parameters observed were marine yeasts isolated using solid and liquid media to determine the growth curve by observing the optical density value. Furthermore, the main research was to examine oil biodegradation process by measuring the pH, salinity, temperature, and data on the percentage of decrease of diesel oil. The results show that there are two types of organisms playing role in the process, i.e. marine yeasts and fungi. Pure marine yeasts isolated have an optical density value of 1.433 (adaptation phase) on the first day 1, 2.849 (logarithmic phase) on the second day, 2.088 and 1.706 (stationary phase) on the third and fourth day, and 1.033 on the fifth day. The results of biodegradation of the most decrease happens to the treatment on the volume of 2 ml and 15 ml (0.31 and 0.23), and the least decrease happens to the treatment on the volume of 5 ml and 10 ml (0.50 and 0.46). The best decrease in diesel oil 15 happens to the treatment on the volume of 15 which is equal to 98.49% and the lowest happens to the treatment on the volume of 2 ml which is equal to 5 84.56%.

Keywords: marine yeasts, biodegradation, diesel oil

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INTRODUCTION

Background of the Study

Environmental pollution by hydrocarbons of oil continues to increase and this has caused significant impact to living organisms. Layer of oil on the water surface disrupts the lives of organisms in the water because it hinders the diffusion of oxygen from the air into the water and blocks the entry of sunlight into the water. In addition, water contaminated by oil cannot be consumed by humans because it often contains toxic substances such as compounds of benzene and toluene[1].

Handling of environment contaminated with oil can be done through physical, chemical, and biological methods. An effort commonly done to solve oil pollution is chemical method that can be implemented easily and quickly, but it can destroy the flora and fauna of the sea and can kill microbes that decompose oil[2]. It is necessary to look for oil pollution prevention and responses that are not harmful biologically (biodegradation) as an alternative to tackle oil pollution in an environmentally friendly way.

Biodegradation is defined as a process of oxidation of organic compounds by microorganisms, both in soil, water, or wastewater treatment plant[3]. In the ecosystem, there are many microorganisms (bacteria, yeasts) capable of degrading oil[4]. Hydrocarbon degrading microorganisms are often found in oil-polluted area. According to Ahearn and Meyer[5], marine yeast population density in the oil-polluted area is more than in the uncontaminated region.

Several studies have shown that marine yeast that comes from the ocean can be used as an agent to degrade diesel oil. In the study by Nurhayati[6], yeast isolates are taken from oil-polluted area in the Surabaya Harbor and nine isolates are collected, some of them are from the genus of *Rhodotorula* and *Candida*. Miranda *et al.*[7] have isolated marine yeasts from the charging stations with diesel oil from the Suape Pernambuco Harbor, Brazil and two isolates of *Candida emobii* UFPEDA 862 and *Rhodotorula aurantiaca* UFPEDA 845 are collected.

Studies on the response of microorganisms toward diesel oil are closely related to efforts to improve the quality of the environment, especially marine waters. The ocean has long become dumping grounds for wastes that contain high pollutant compounds. Therefore, this study is expected to obtain pure marine yeast isolates derived from Kili-Kili Beacah that can later be used to reduce the concentration of diesel oil, thereby reducing its abundance on the ocean due to shipping activities in the surrounding area.

Research Objectives

The purpose of this study was (1) to determine the potential of marine yeast isolates isolated from Kili-Kili Beach, Trenggalek, East Java and (2) to determine the percentage in the decrease in diesel oil and marine yeasts.

Research Potential

This study has the potential as a new method on the biodegradation of diesel oil by using a type of yeast microorganisms from the sea. In addition, the results from this study are expected to be complementary to the studies done previously.

The specific benefits of this research are (1) to understand, examine, and broaden knowledge about the ability of marine yeasts in reducing diesel oil (2) to provide information on biodegradation processes done by marine microorganisms.

Hypothesis

The hypotheses are: (1) there is a potential of marine yeast isolates isolated from Kili-Kili Beach, Trenggalek, East Java to degrade diesel oil and (2) there are differences in the percentage in the decrease of diesel oil and marine yeasts.

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Time and Place

This study was conducted from April to July, taking water samples taken from Kili-Kili Beach, Trenggalek, East Java. Furthermore, water samples were processed at the Central Laboratory of Microbiology Laboratory of Biological Sciences (LSIH) and the Laboratory of Marine Sciences of University of Brawijaya.

MATERIAL AND METHOD

Research Material

Material

The materials used in this study are YPDA (Yeast Peptone Dextrose Agar), sea water, distilled water, alcohol 70%, methylene blue, cotton, aluminum foil, label paper, tissue, paper, rope, plastics, methylated spirits, immersion oil, HCl, and n-hexane.

Equipment

The equipment used in this study is a global positioning system (GPS), a polyethilen bottle, coolbox, autoclave, digital scales, microscopes, erlenmeyer, petri dish, glass beaker, measuring cups, micropipette, spatula, Bunsen, ose needle, object glass, cover glass, sprayer, shelf staining, crushable tank, triangle, spoons, thermometer, pH meter, DO meter, salinometer, pumpkin separation, distillation flask, funnel, and desiccator.

Research Method

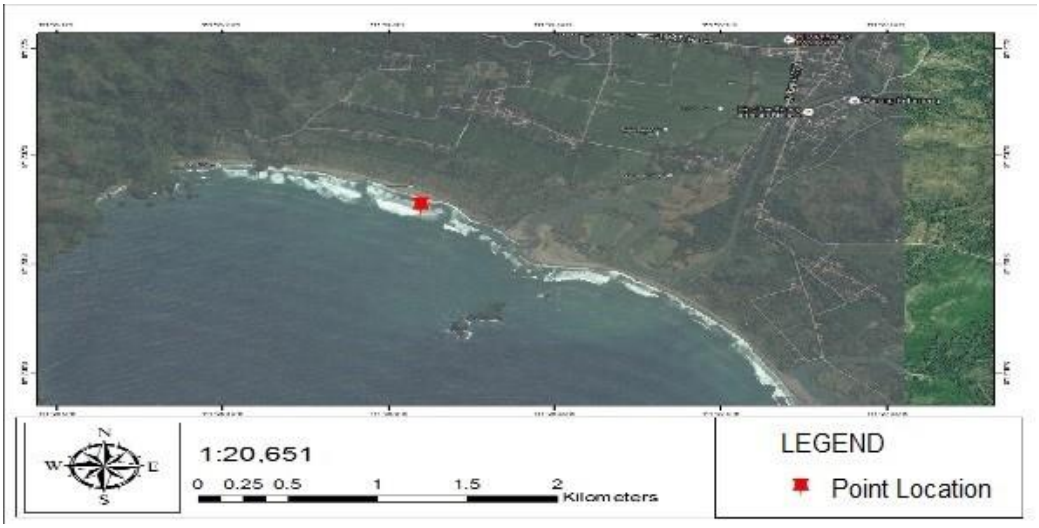
This study used experimental method of completely randomized design (CRD) with four treatments and one factor (2 ml, 5 ml, 10 ml, and 15 ml) and 3 repetitions (n = 4). Test parameters observed were marine yeasts isolated using solid and liquid media to determine the growth curve by observing the optical density value. Furthermore, the main research was to examine oil biodegradation process by measuring the pH, salinity, temperature, and data on the percentage of decrease of diesel oil.

Analysis Procedures

Sea Water Sampling

Sea water sampling locations to get the microorganisms were in Kili-Kili Beach, Trenggalek, East Java, with the value of the environmental parameters as follow: temperature of 30.9°C, pH 7.6, salinity 35%, and DO 17.6 mg/L. Sea water samples were placed in the aseptic PVC bottle. Samples of sea water were taken 3 times.

Figure 1. Locations of Sea water Sampling



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Isolation of Marine Yeasts

Sea water sample of 1 ml was added to the solid medium (yeast extract 10 g/l, glucose 20 g/l, peptone 20 g/l, 150 ml of distilled sea water and chloramphenicol) that had been sterilized for 15 minutes. Then, it was incubated in an incubator for 10 days. Marine yeast cells were isolated using inoculant needle and incubated for 3 days.

Marine Yeast Culture on Liquid Media

Marine yeast culture procedure was performed according to the method of Sukoso[8]. Materials for culture media i.e. sugar, fertilizer, and sea water were prepared. Then sea water was sterilized and was put in the bottle. Sugar of 0.5% (w/v) and fertilizer of 0.2% (w/v) was added and it was aerated for 5 days. Then, it was observed with a microscope every day.

Determining the Growth Phase of Marine Yeasts

Marine yeasts were taken using 5 ml pipette and were put into a test tube. Then, the sample was homogenized and turbidity values were measured with a spectrophotometer.

Biodegradation of Diesel Oil

Marine yeasts on growth media were taken using 5 ml pipette and were aerated. Then, diesel oil 2 ml, 5 ml, 10 ml, 15 ml was added. Salinity, pH, and temperature were measured. Then, they were put in a shaker with a speed of 70 rpm for 7 days.

Analysis of Diesel Oil Levels in Gravimetry

Levels of diesel oil at each concentration are then analyzed by gravimeter. Gravimetric in chemistry is one of the analytical methods to determine the quantity of a substance or component that has been known by measuring the weight of the component in a pure state after a separation process. Gravimetric analysis involves the isolation and measurement of the weight of a specific element or compound.

Design of Treatment

Table 1. Design of Treatment

Volume / Sample	2 ml (1)			5 ml (2)			10 ml (3)			15 ml (4)			Control (5)
Kili-Kili Beach, Trenggalek (T)	T1 a	T1 b	$T1_c$	T2 a	T2 b	T2 c	T3 a	T3 b	T3 c	T4 a	T4 b	T4 c	Control

Data Analyisis

Data from the analysis of the content of diesel oil in different volumes in 3 repetitions were analyzed using SPSS version 16.

RESULTS AND DISCUSSION

Isolation of Marine Yeasts

Isolation on Solid Medium

Two microorganisms were found, i.e. marine yeasts and fungi, as shown in Figure 2.

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Figure 2. Initial Isolation Results of Sea Water

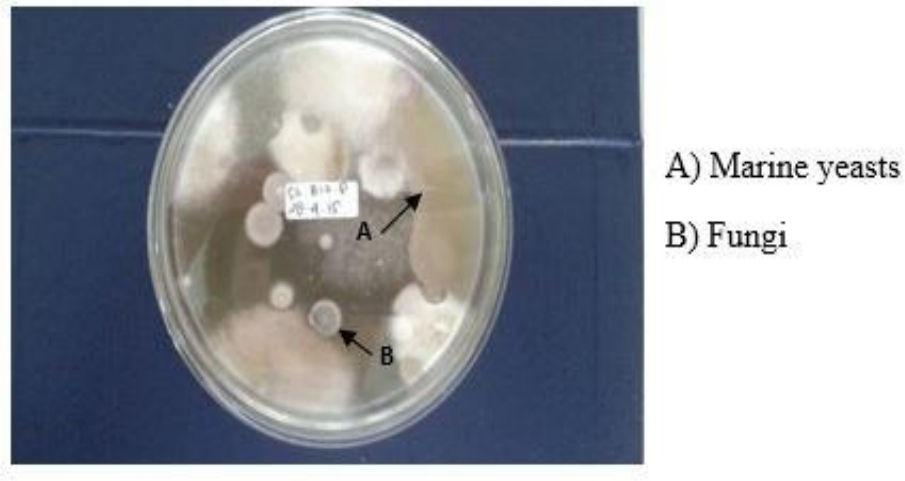

Figure 2 shows the results of the initial isolation on the growth medium using YPDA media, and the medium has been proven to be able to foster yeasts and molds. Marine yeasts are marked with arrows (A) and fungi are marked with arrows (B). At this initial isolation, the growth of yeasts marine could not be analyzed properly because marine yeasts were mixed with the other type of microorganism, i.e. fungi. Fungus is a class of multicellular microorganism having filaments and it growth in the food can be easily seen because of the appearance that looks like cotton. Its growth will be initially white, but if the spores have emerged, it will form different colors depending on the type of fungi.

Figure 3. Pure Isolates of Marine Yeasts

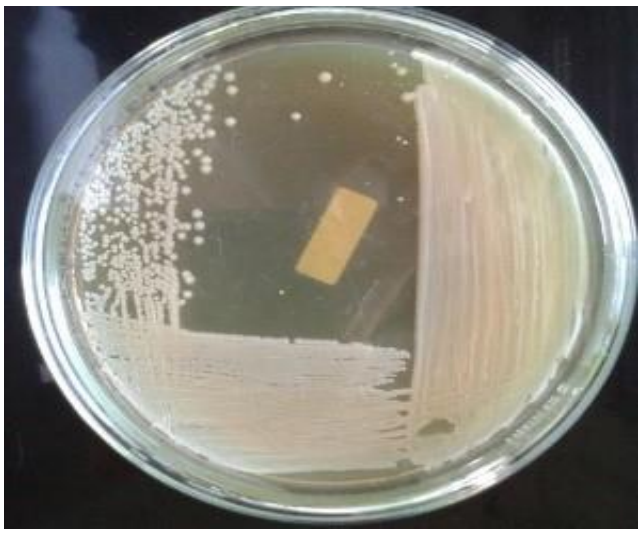

Figure 3 is the result of purification of the initial isolation of which microorganisms were marine yeasts. Based on visual observation, marine yeasts looked a little slimy, bright in color, white cream in color, and is has slightly pungent odor. Fungi, on the other hand, have a variety of colors, have mycelium, and have spores. Yeast is a class of eukaryotic only forms blastopores (round, oval, cylindrical and spherical oval influenced by the strain).

Isolation on Liquid Medium

Figure 4 shows the results of the culture on the liquid medium.

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Figure 4. Marine yeast culture on the liquid medium (A) Marine yeast cells (1) Cell division (2) Parent cells (B) The growing phase of marine yeasts

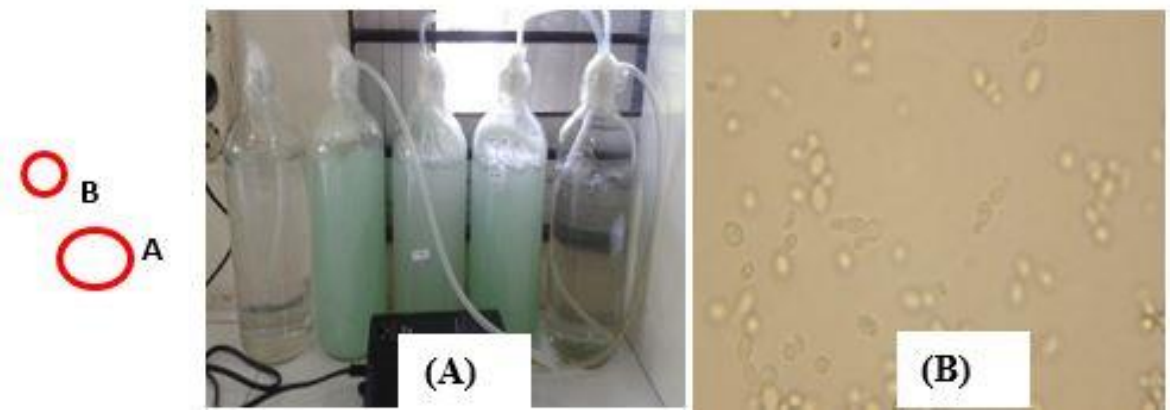

Figure 4 (A) shows that the marine yeast cells are circle and some of these cells are small circles stuck together; a small circle indicates that the yeast cells are undergoing cell division. Research by Sukoso[8], shows marine yeasts can reproduce sexually and asexually, in which sexual reproduction occurs with sporulation. Ascosporous of marine yeast are more resistant to heat in the dry environment compared to vegetative cells of marine yeasts. Sporulation is affected by internal and external factors of marine yeasts. Internal factors include cell health and life, while external factors include temperature, oxygen, nutrients, and pH[8,9].

Figure 4 (B) shows marine yeast culture process which is cultivated in a glass bottle using a starter on a solid medium which has been purified from the first isolation. Starter of the marine yeast used is about 5 ml using a pipette volume and then the glass bottle is aerated to provide oxygen evenly to the yeast cultures.

Logarithmic Phase of Marine Yeast

Figure 5. Logarithmic Phase of Marine Yeast

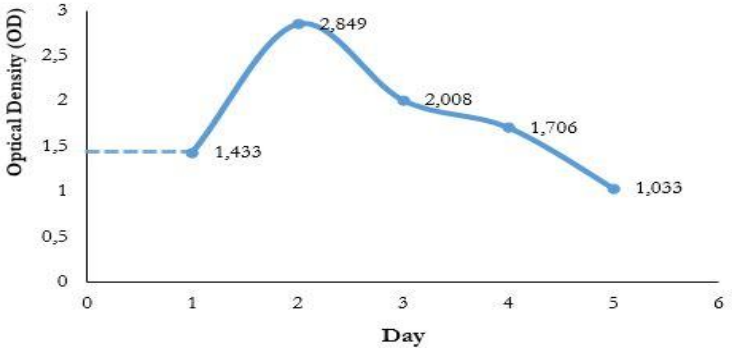

Figure 5 shows that on day 0-1 was a phase of adaptation or adjustment to the growth media with optical density value of 1.433. Growth of yeast on the second day produced optical density values of 2.849 characterized by rapid growth of the yeast. This growth indicated that the yeast cultured was going through a log or exponential phase. According to Pelczar and Chan[10], the multiplication phase (logarithmic or exponential) is a phase in which cells will divide at a constant rate meaning a mass of cells are to be doubled at the same rate, the constant activity of metabolites and balanced growth situation. On the third day, pattern of growth of yeast decreased to 2.008 and on the fourth day, it decreased to 1.706. This might have been due to the decreased nutrients in the culture bottle as for the previous log phase microbes required more energy and slowed cell division process. The third and fourth day was the stationary phase. On the fifth day, the optical density decreased to 1.003. This indicated that the yeast has experienced death phase.

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Biodegradation Test

Measurement of Environmental Parameters on the Yeast Culture on Biodegradation Tests.

pH

Figure 6. Degree of Acidity

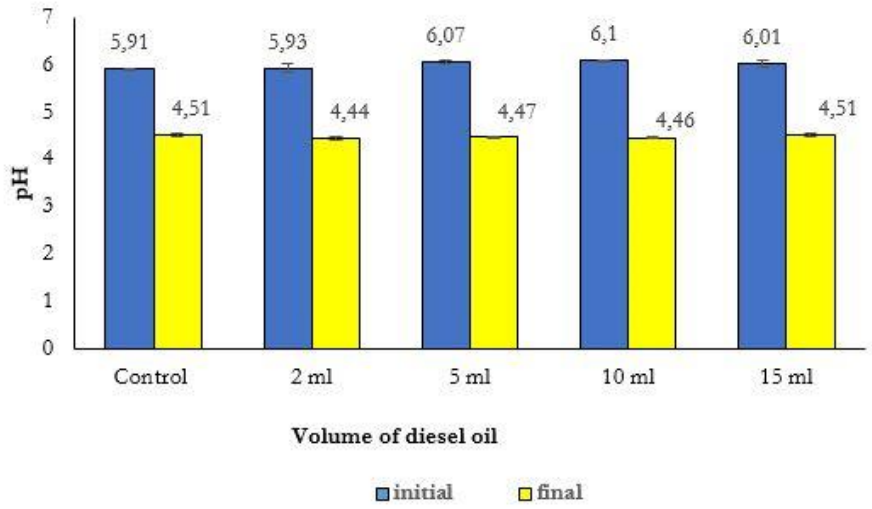

Figure 6 shows the degree of acidity or pH value was 6.10, the highest before biodegradation at the 10 ml volume of diesel oil, and the lowest was 5.93 of 2 ml volume. The highest pH value after biodegradation was 4.51 at the 15 ml volume of diesel oil, and the lowest was 4.44 at the volume of 2 ml. This is due to the early stages of biodegradation occurs bio surfactant formation; increased bio surfactant causes an increase in pH. The more bio surfactant formed, the higher the pH will increase. After the oil is almost completely emulsified, the pH will continue to decrease due to the activity of bacteria that form acid metabolites, mainly the result of hydrocarbon degradation metabolites[11]. Biodegradation of oil will be faster with increasing pH and optimum speed at alkaline pH[12]. According to Nghia[13], the optimum pH for biodegradation is in the range between 6 and 8.

Salinity

Figure 7. Salinity Before and After Degradation of Diesel Oil

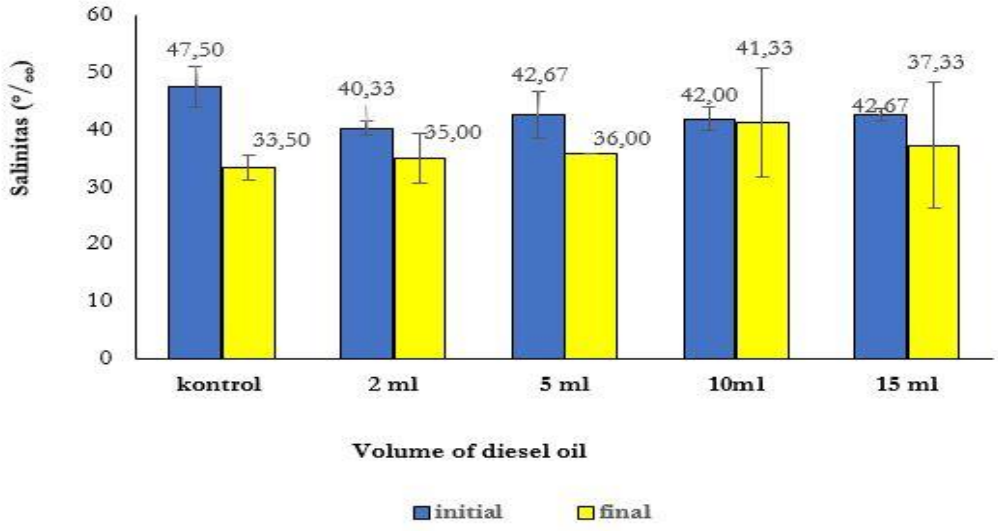

Figure 6 shows the highest salinity value before biodegradation was 42.67% at the 5 ml volume and 15 ml volume of diesel oil, and the lowest was 40.33% at the volume of 2 ml of diesel oil. The highest salinity value after biodegradation was 41.33% at the 10 ml volume of diesel oil, and the lowest was 35% at the volume of 2 ml of diesel oil. According to Dewilda *et al.*[14] the salt concentration continues to decline due to the reduced volume of water. The concentration of salt in sea water is 3% of the weight of the water entirely. In addition to experiencing decrease, the salt concentration can be increased by evaporation.

Temperature

Figure 8. Temperature Before and After Degradation of Diesel Oil

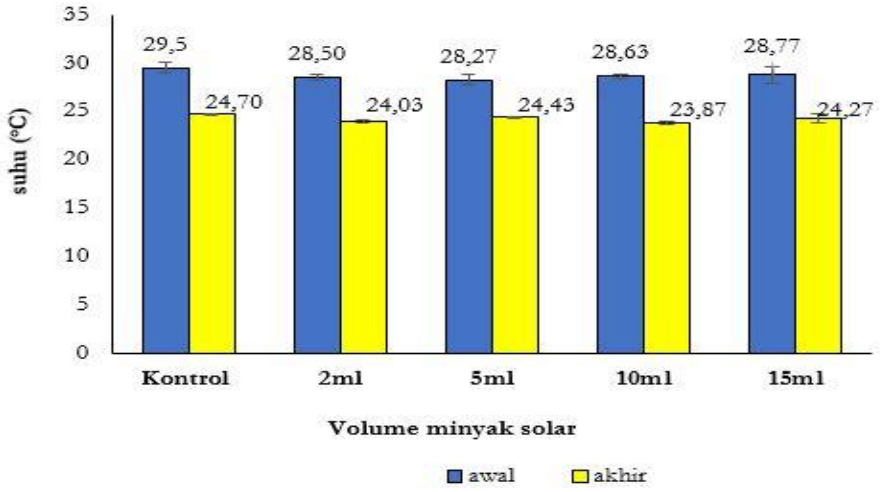

Figure 8 shows the highest temperature value before biodegradation was 28.77^{0}C at the 15 ml volume of diesel oil, and the lowest was 28.27^{0}C at the volume of 5 ml of diesel oil. The highest temperature value after biodegradation was 24.43^{0}C at the 5 ml volume of diesel oil, and the lowest was 23.87^{0}C at the volume of 10 ml of diesel oil. According to Leahy and Colwell[15], temperature also affects the physical condition of the hydrocarbons in petroleum slurry waste and microorganisms that consume it. At low temperature, viscosity of the oil increased, and evaporation of short-chain alkanes becomes more toxic than other hydrocarbons, thus delaying the process of biodegradation. Generally, the speed of oil degradation by aerobic bacteria is optimum at temperatures ranging from 15 - 30^{0}C[16].

The Biodegradation Results by Marine Yeasts

The results of biodegradation test for 7 days using diesel oil volume of 2 ml, 5 ml, 10 ml, and 15 ml show yeast isolates have the ability to degrade the oil, seen from the decline in oil content after tests, and to break down complex organic compounds into simpler compounds. According to Mauersberger *et al.*[17] yeasts degrade petroleum by means of oxidizing hydrocarbons substrates by the enzyme of P450 monooxygenases which oxidize n-alkanes to alcohols. Furthermore, alcohol is oxidized to aldehydes. Aldehyde is then hydroxylated to fatty acids.

Figure 9 shows that the degradation ability of each isolate of yeasts with the addition of diesel oil volume of 2 ml, 5 ml, 10 ml, and 15 ml is very high. Decreased levels of diesel oil are caused by the very high supply of oxygen in the volume of 15 ml and 12 ml of diesel oil so yeasts in the biodegradation process can play their role properly. Oil volume 5 ml and 10 ml experiences slightly lower decrease than 2 ml and 15 ml oil volume. In the aeration process, oxygen flowing in the bottle treatment decreases slightly as a result of not optimal treatment process of biodegradation in the bottle. According to Hess *et al.*[18] to degrade hydrocarbons quickly, extra oxygen, nitrogen, and phosphorus inputs are needed. Another thing that leads to the decrease in oil levels is the group of alkanes. Alkanes are the largest component in the diesel oil by 76%. Alkanes are straight chain of hydrocarbons that have a single chemical bond between the carbon atoms. Alkanes may be straight chain, branched chain, or ring structure. One of the most important properties of alkanes is volatile and may be biodegraded by microbes. N-alkanes are degraded faster than the branched chain of alkanes; n-alkanes easily and quickly degraded are those containing C10-C18[7].

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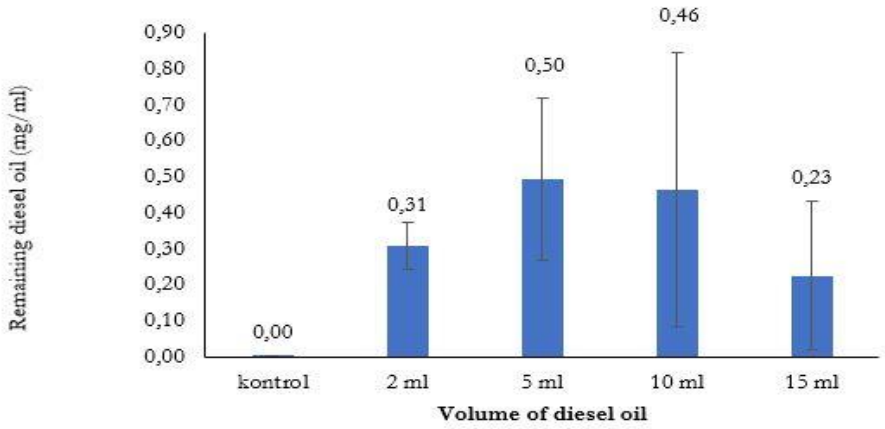
Figure 9. The Biodegradation Results of Oil Diesel by Marine Yeasts



Final Percentage of Diesel Oil

The decrease in oil content is expressed as a percentage (%) and the percentage after 7 days of decreased levels of oil can be seen in Table 2.

Table 2. Decreased Percentage of Diesel Oil

No	Initial Concentration (ml/l)	Final Concentration (ml/l)	Percentage of Remaining Diesel Oil (%)	Percentage of Decrease of Diesel Oil (%)
1	Control	0.00	0	100
2	2 ml	0.31	15.44	84.56
3	5 ml	0.49	9.91	90.09
4	10 ml	0.46	4.64	95.35
5	15 ml	0.23	1.51	98.49

In the above table, biodegradation test results for 7 days with 2 ml volume is capable of degrading oil up to 15.44%, 5 ml volume is capable of degrading oil up to 9.91%, 10 ml volume is capable of degrading oil up to 4.64%, and 15 ml volume is capable of degrading oil up to 1.51%. The decrease is due to even oxygen supply on each bottle treatment. This decrease in the percentage of oil content is also influenced by synergism between isolates of yeast to produce enzymes that break down the hydrocarbon structure. According to Siregar (2009), biodegradation test results after 14 days of test show that at a concentration of 1% of oil, consortium isolates are capable to degrade oil as much as 68% and at a concentration of 3% of oil, consortium isolates are capable to degrade oil as much as 65%. This is because at oil concentration of 1%, composition of aromatic hydrocarbons is lower than composition of aromatic hydrocarbons at oil concentration of 3%; thus, 1% concentration is friable and is easily utilized by yeast as a source of energy. Nababan[19] adds that on the seventh day, consortium isolates are able to degrade oil at 1.5 ml volume with remnant of 0.55 ml (36.7%), oil at 3 ml volume with remnant of 1.11 ml (37%), and oil at 4.5 ml volume with remnant of 3.05 ml (67.8%).

CONCLUSIONS AND SUGGESTIONS

Conclusions

Based on the study, the conclusions are:

- Marine yeasts isolated from Kili-Kili Beach, Trenggalek, Jawa Timur are able to degrade diesel oil at a volume of 2 ml, 5 ml, 10 ml, and 15 ml.
- Marine yeasts with a volume of 2 ml of diesel oil can reduce diesel oil of 84.56%, a volume of 5 ml of diesel oil can reduce diesel oil of 90.09%, a volume of 10 ml of diesel oil can reduce diesel oil of 95.35%, nd a volume of 2 ml of diesel oil can reduce diesel oil of 98.49%.

Suggestions

In research on biodegradation of diesel oil by using marine yeast needs optimize culturing conditions, in this case the aeration process; this is necessary to improve the ability of marine yeasts to degrade diesel oil. In addition, sampling of sea water is better taken in an aquatic environment that has suffered pollution of diesel oil. For the treatment of diesel oil, it is better to use final concentration and labeling needs to be done carefully.

REFERENCES

[1] Nurhariyati, Ni'matuzahroh, T. Surtiningsih. Biodegradasi Minyak Oleh *Rhodotorula* dan *Candida* Hasil Isolasi Dari Pelabuhan Tanjung Perak Surabaya. *Jurnal Penelitian Hayati*. 2006, 12 (27-31).

[2] McConnaughey, R. Zottoli. *Introduction To Marine Biology*. 1983. The C.V Mosby Company. St. Louis, Toronto. London.

[3] Paramitha, P., M. Shovitro, N.D. Kuswytasari. Biodegradasi Limbah Organik Pasar dengan Menggunakan Mikroorganisme Alami Tangki Septik. *Jurnal Sains dan Seni ITS*. 2012, Vol.1, ISSN : 2301-928X.

[4] Jumiyati, S.H. Bintari, I. Mubarok. *Isolasi dan Identifikasi Khamir Secara Morfologi di Tanah Kebun Wisata Pendidikan Universitas Negeri Semarang*. 2012. Universitas Negeri Semarang.

[5] Ahearn, D.G., S.P. Meyers, P.G. Standard. The Role of Yeast in the Decomposition of Oils in Marine Environment. *Dev.Indust.Microbial.* 1972, 12: 126-134.

[6] Nurhayati, T. Biodegradasi Minyak oleh Yeast Hasil Isolasi dari Pelabuhan Tanjung Perak Surabaya. 2001. *Unpublished Thesis*. Universitas Airlangga. Surabaya.

[7] Siregar, R. Siregar. Potensi Khamir Pendegradasi Minyak Solar Dari Air Laut Balawan. 2009. *Unpublished Thesis*. Sekolah Pasca Sarcajana. Universitas Sumatera Utara.

[8] Sukoso. *Eksplorasi Potensi Khamir Laut*. 2012. Program Pascasarjana. Universitas Brawijaya. Malang.

[9] Judoamidjojo. *Teknologi Fermentasi*. 1992. 1st Edition. Rajawali Press. Jakarta.

[10] Pelczar, M.J., E.C.S. Chan. *Microbiology.* 1989. G. Wiliamson-W.J.A. Payne Publishers. New York.

[11] Ristiati, N.P. Uji Kemampuan Isolat Bakteri Pendegradasi Minyak Solar Terhadap Limbah Oli dari Perairan Pelabuhan Celukan Bawang. 2013. *Seminar Nasional FMIPA UNDIKSHA III Tahun 2013*.

[12] Foght, J.M., D.W.S. Westlake. *Bioremediation of hydrocarbons in freshwater.* 1987. In: Vandermeulen & Hrudey (Ed). *Oil in Freshwater:* Chemistry, Biology, Countermeasure Technology. Pergamon Press. New York.

[13] Nghia, N.K. Degradation of Aged Creosote and Diesel Contaminated Soils by Phytoremediation or Biostimulation (nutrients). 2007. *Unpublished Thesis*. Sveriges Lantbruksu Niversitet.

[14] Dewilda, Y., R. Afrianita, F.F. Iman. Degradasi Senyawa Fenol Oleh Mikroorganisme Laut. *Jurnal Teknik Lingkungan UNAND*. 2012, 9(1) : 59-73, ISSN 1829-6084.

[15] Leahy, J.G., R. Colwell. Microbial Degradation of Hydrocarbon. *Environmental Microbiologycal Review*. 1990. Volume 54.

[16] Englert, C.J. *Bioremediation of Petroleum Product in Soil*. 1993. Principles and Practices for Petroleum Contaminated Soil. Lewis. Michigan.

[17] Mauersberg, S., M. Ohkuma, W.H. Schunck, M. Takagi. *Candida Maltose*. 1996. In K. Wolf (ed.), Non-Conventional Yeast in Biotechnology. Springer-Verlag. Berlin. Germany.

[18] Hess, S.J., R.F. Roberts, G.R. Ziegler. Rheology Properties of Nonfat Yogurt Stabilized Using L. Delbruechii ssp. Bulgaricus Producing Exopolisaccharide or Using Commercial Stabilizer Systems. *J. Dairy Science*, 1997. 80: 252- 263.

[19] Nababan, B. 2008. Isolasi dan Uji Potensi Bakteri Pendegaradasi Minyak Solar Dari Laut Belawan. *Unpublished Thesis*. Sekolah Pascasarjana Universitas Sumatera Utara. Medan.