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Production of Pachouli Oil by Fermentation Methode Using *Phanerochaete Chrysosporium* With Kieserite as Sustitution $MgSO_4$

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Abstract: One of the factors that affect the fermentation process on the production of patchouli oil by *Phanerochaete chrysosporium* is the composition of media and fermentation time. In general, the type of media used to growth of *Phanerocheate chrysosporium* is the NLM that it contains pure $MgSO_4$. On the other hand, the kieserite has high concentration of $MgSO_4$ which is expected to replace pure $MgSO_4$ as a growth medium for *Phanerochaete chrysosporium* and the price is cheaper than $MgSO_4$. The aims of this research was to determine the effect of kieserite addition and fermentation time to yield, refractive index, and patchouli alcohol content of patchouli oil production. Research done by the fermentation of patchouli leaf powder by *Phanerochaete chrysosporium* using media containing kieserite. Fermented was dissolved in n-hexan that subsequently separated between the oil in n-hexan and the residue. Product of patchouli oil was adsorbed by bentonit, and patchouli oil was separated by filtration. The final product of patchouli oil was analyzed yield, refractive index, fat content, and % patchouli alcohol to get of the best yield.The variables used in this research are fermentation time (7, 9, 11, 13, 15 days) and kieserite addition (0.5, 1, 1.5 g / L media). The results showed that the decrease kieserite added and increasing fermentation time causes increased yield. The best of the product on this research was result of variable fermentation time 15 days and kieserite addition 0,5 g/L media which the best result was yield (5.32%), refractive index (1.509), fat (negative), and patchouli alcohol (34.3%).

Keywords: *Phanerochaete crysosporium*, patchouli oil, kieserite, fermentation time, yield

I. INTRODUCTION

Patchouli oil is one of the essential oils that are most needed by the international parfume market. This is because patchouli oil is asa binder (fixative) to other fragrance ingredients that work to prevent evaporation of the fragrance substances and lead to longer lasting fragrance odor [20]. Indonesian patchouli oil are produced from some of the region including are as in Aceh, Sulawesi, Maluku, and the southern part of Java Island.
The quality of patchouli oil produced in Indonesia the average is still below the standard that has been set SNI. Standard Test for export which contains at least 30 % patchouli alcohol [19]. On the other hand, patchouli oil quality standards are influenced by concentration of patchouli alcohol contained in patchouli oil.
Patchouli oil in Indonesia are generally produced using distillation process, which through this process the resulting yield about 2-3 % with patchouli alcohol content 28 % [5]. Farmers can only produce patchouli oil with patchouli alcohol content of 26-28 % [8].

Patchouli leaves distillation process with direct drying is not perfect because patchouli oil is still attached to the leaf tissue [1]. Therefore, new methods are needed to destroy the patchouli leaf tissue so that the amount of patchouli oil that remove more. Fermentation method is one alternative to break down lignin and cellulose tissue present in patchouli leaves. Fermentation using *P. chrysosporium* is to break down cellulose because these fungi produce cellulose enzyme [18]. While the principles of fermentation using *P.chrysosporium* is to break the lignin (biodelignification) and break down cellulose [18]. The outbreak of lignin (biodelignification) and cellulose from patchouli leaves causing patchouli oil separated from the leaves so that it can be iso lated more easily [1].

Nasruddin, et.al [9] said that the process of delignification in patchouli leaves using NaOH solution at a temperature of 55 ^{o}C and fermented for 6 days using *Trichoderma viride* to remove cellulose can produce yield of 2.35 %. Diana (2011) [1] shown that the patchouli leaf fermentation using variables (leaf, stem, leaf and stem mixture) as a pretreatment before refining process and wrap patchouli leaves wet for 24 hours and followed by distillation can produce the highest yield of 2.97 %.

On the other hand, *Phanerochaete chrysosporium* is a white rot fungus that has the ability to degrade lignin and cellulose [18]. White rot fungi produce enzymes Lignin Peroxidase (LiP), Manganese Peroxidase

(MnP), and cellulase. The enzyme has plays a role in degrading lignin and cellulose. *Phanerochaete chrysosporium* have optimum grow that 37 °C, pH 4 - 7, and aerobic which *Phanerochaete chrysosporium* is able to carry out delignification corn stalks with a loss rate of 81.4 % lignin and cellulose of 22.3 % [3].Sri Rulianah [17] said that the isolation of patchouli oil with fermentation method using a fungi *Phanerochaete chrysosporium* and NLM media can produce yield of 10 %. This research uses the substitution medium for the growth of fungus *Phanerochaete chrysosporium* is kieserite fertilizers. The use of kieserite fertilizers aims to replace MgSO4 in NLM media that has been used in previous research that using pure $MgSO_4$ media [16]. On the other hand, the price of pure $MgSO_4$ much more expensive than the price of kieserite fertilizer, so that the research is expected to produce patchouli oil with good quality and operational costs of production lower.

The purpose of research was to determine the effect the use of kieserite on the growth of fungi *Phanerochaete chrysosporium* and to determine the effect of the kieserite addition and fermentation time on the yield and patchouli alcohol concentration in patchouli oil.

The scope of the research includes there generation of fungi *Phanerochaete chrysosporium* starter, spore suspension production, production of starter in a liquid medium, drying and size reduction of patchouli leaf, patchouli leaf fermentation, separation of oil from the mixture, adsorption by bentonite for purification, and analysis of the quality of patchouli oil.

II. METHODOLOGY

Materials used in research, among others: media potato dextrose agar, nitrogen limited (NLM) media modified ($MgSO_4$ replaced with kieserite), patchouli leaf powder, distilled, *Phanerochaete chrysosporium* starter, molasses, N-hexan, bentonite, pH paper, patchouli oil standards, alcohol, and spirtus.

Equipments used, among others: autoclave, incubator shaker, incubator oven, a needle ose, glass beaker, fatty cotton, screen, spatula, measuring cups, analytical balance, erlenmeyer, flask, refractometer, test tubes, vacuum erlemeyer, a set of distillation equipment, Gas chromatography, bunsen, and oven.

Variables consisted of depending and independing variables. Variable independing, among others: type of fungi *Phanerochaete chrysosporium*, patchouli leaf powder with a water content of ± 12 %, fermentation pH 5-6, and the fermentation temperature is room temperature. Meanwhile, as the variables depending are: fermentation time (7, 9, 11, 13, and 15 days), and the addition of kieserite (0.5;1; 1.5g/L media).

Stages in the research are as follows: 1). Regeneration of microorganisms (*Phanerochaete chrysosporium*) on PDA (potato dextrose agar), 2). Drying and size reduction of patchouli leaf, 3). Making the growth curve, 4). Making starter *P.Chrisosporium* in liquid media and scale up, 5). Fermentation patchouli leaf powder using *Phanerochaete chrysosporium* where fermentation process aims to degradation of lignin and cellulose by using a modified NLM media ($MgSO_4$ replaced with kieserite) as variables, 6). The purification of results include: soaking the results withn - hexan, filtration, agitation, separation with a separating funnel, distillation to separate n – hexan of patchouli oil, adsorption by bentonite, bentonite filtering of patchouli oil, and analysis of the results include yield, refractive index, fat content, and the patchouli alcohol content.

III. RESULTS AND DISCUSSION

The results of fungus *Phanerochaete chrysosporium* regeneration in the media indicate that *Phanerochaete chrysosporium* can grow and develop properly on PDA and NLM media modified ($MgSO_4$ replaced with kieserite) as shown in Figure1.

(a) **(b)**

Figure.1: The Breeding of *P. chrysosporium*

a. PDA media, (b). *Phanerochaete chrysosporium* scale up results in a liquid medium that has been adapted with patchouli leaves and with the addition of kieserite

Further more, the growth of fungi *Phanerochaete chrysosporium* made a growth curve as such as in the Figure 2. The results showed that *Phanerochaete chrysosporium* growth curve has fairly long stationary phase. Thus it can be expressed that *Phanerochaete chrysosporium* have the ability to degrade lignocellulose

compounds with high molecular weight through the mechanism of the enzymatic system produced by the reaction of secondary metabolites which the reaction of secondary metabolites occurs when stationary fase [15].

Figure 2 shows that the stationary phase started on day 5. This means that the secondary metabolites by *Phanerochaete chrysosporium* started on day 5. Thus, on day 5 *Phnerochaete chrysosporium* began to produce lignocellulosic enzymes. Lignocellulosic enzyme consists of Lignin Peroxidase enzymes (LiP) and Manganese Peroxidase enzyme (MnP) and cellulase enzymes which lignocellulosic enzymes have play a role in the degradation of lignin and cellulose in patchouli leaf tissue. Thus, on day 7 of Lignin Peroxidase enzyme (LiP) and Manganese Peroxidase enzyme (MnP) and cellulose enzyme produced is stills lightly. This causes degradation of lignin and cellulose in patchouli leaf tissue is stills lightly so that the amount of patchouli oil obtained slightly. By changing the operating conditions of fermentation include the fermentation time(7, 9, 11, 13 and 15 days) and the addition of kieserite of 0.5; 1; and 1.5g/L media so that obtained of patchouli oil yield as shown in Figure 3.

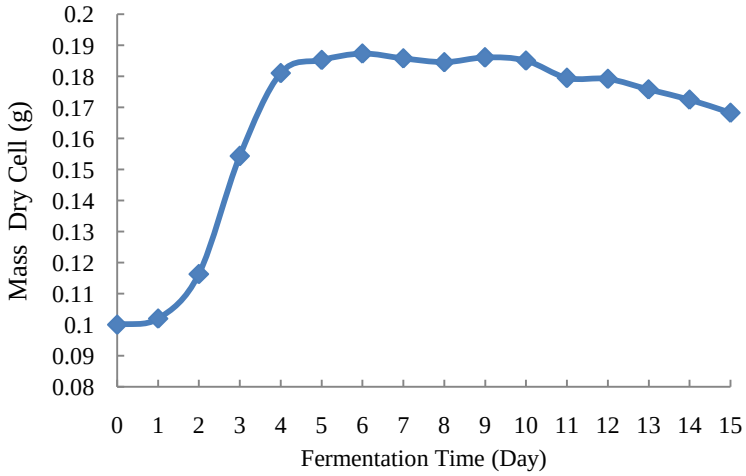

Figure.2: *Phanerochaete chrysosporium* growth curve with NLM media that has been modified with kieserite

Figure 3 shows that the smallest yield was obtained at 7 days (the shortest time) for the addition kieserite of 0.5; 1; and 1.5g/L media. Meanwhile, the highest yield was obtained at day15 for the addition kieserite of 0.5; 1; and 1.5g/L media. This indicates that the fermentation time greatly affects the yield. The longer the fermentation time caused the higher yield. The highest yield of 5.201 % was obtained at 15 days of fermentation time and the addition of kieserite 0.5 g/L media. While the low yield of 2.606 % was obtained at the time of fermentation 7 days and the addition of kieserite 1.5 g/L media. This indicates that the activity of the lignoselulotic enzyme produced by *Phanerochaete chrysosporium* on the 15th day more than a day to 7, to 9, and to 11, and to13.

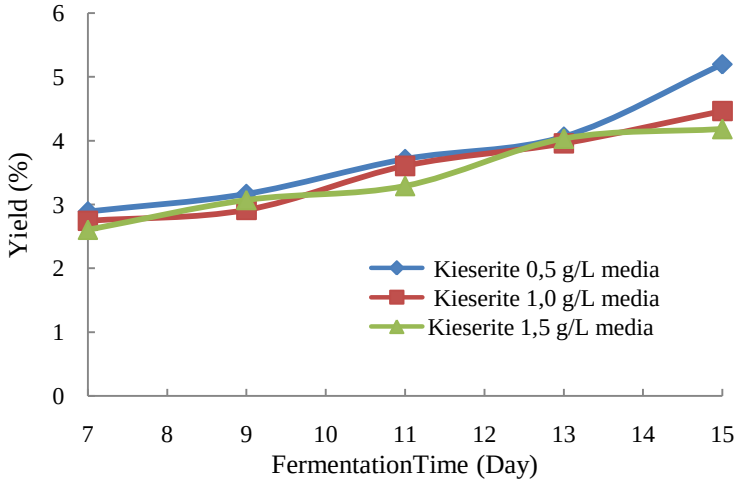

Figure. 3: The relationship between yield with fermentation time for various additions of kieserite ($MgSO_4$)

This phenomen on indicates that the lignoselulotic enzyme can catalyze/degrade the substrate (lignin and cellulose) more so the more patchouli oil that can be produced from the tissue of leaf patchouli. Lignin and cellulose degradation process occurs through the process of destruction of lignin and cellulose in tissue of patchouli leaf, making it easier to isolate patchouli oil. Sri Rulianah [16] said that by using NLM media containing pure $MgSO_4$, the longer the fermentation time up to15 days will produce patchouli oil yield higher. With the addition of kieserite, showed that the more kieserite are added so the smaller the yield. This is seen in the kieserite addition of 0.5; 1; and 1.5 % in the fermentation time of 15 days where the highest yield was obtained at kieserite addition of 0.5%, and the lowest yield was obtained on addition of 1.5 %.

The quality and quantity of fungi growth effect on the amount of the yield where kieserite addition of 0.5 grams per liter NLM has more number of spores in spore sizes equally. The number of spores that many will be able to degrade lignin and cellulose in patchouli leaves better than the large number of spores but slightly. This is consistent with the results obtained which yield result is highest in the kieserite addition of 0.5 grams per liter NLM.

Patchouli alcohol concentration in this research showed at Figure 4 which the highest concentration patchouli alcohol obtained in the fermentation time of 15 days and the addition of kieserite as much as 0.5 g/L media. While the lowest concentration of patchouli alcohol obtained at 7 days of fermentation time and the addition of kieserite 1.5g/L media.

Figure 4 shows that the longer the fermentation time caused higher concentration of patchouli alcohol. The fermentation time period 7 days to 15 days, the highest concentration of patchouli alcohol of 35.22 % is achieved in the fermentation time of 15 days. This indicates that more and more degraded lignin and cellulose resulted in more and more patchouli oil are removed and the higher the concentration of patchouli alcohol. Fadilah [3] said that the longer the fermentation time so the higher concentration lignin and cellulose degraded. Mahyati [7] in lignin biodegradation research on corn cob using *Phanerochaete chrysosporium* mixture also mentioned that the longer the fermentation time so the greater the reduction of lignin and cellulose.

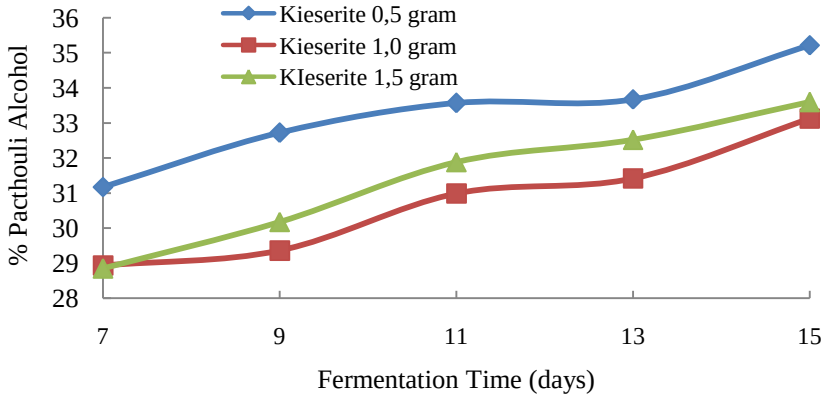

Figure. 4: The relationship between % Patchouli alcohol with fermentation time for various additions kieserite

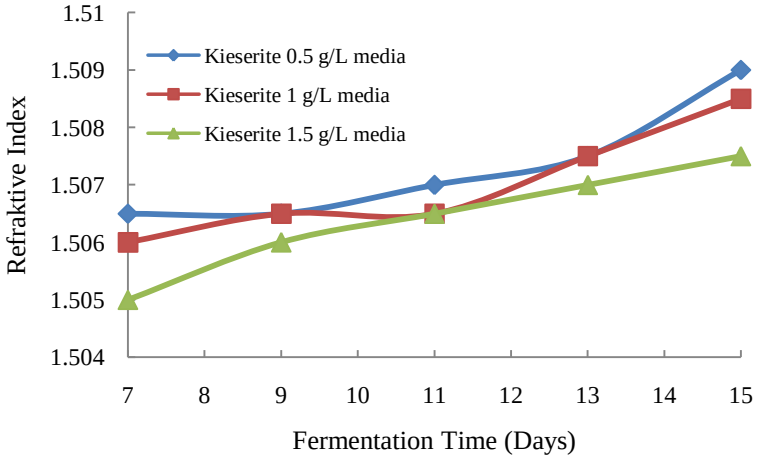

Figure.5: The relationship between the refractive index with fermentation time for various additions kieserite ($MgSO_4$)

Fungus *Phanerochaete chrysosporium* produces MnP, LiP, and cellulose enzymes which plays a role in the degradation of lignin and cellulose in the stationary phase so that the longer the fermentation time, the higher the % decrease lignin and cellulose concentration.

Sri Rulianah [18] in his research about the production of crude cellulase enzyme with bagasse as substrate using fungus *Phanerochaete chrysosporium* which to change the fermentation time can produce activity of crude cellulase enzyme increasing with the length of time of fermentation. With his research showed that the fungus *Phanerochaete chrysosporium* has the ability to degrade cellulose. While the relationship between yield, patchouli alcohol concentration, degradation of lignin and cellulose showed that the higher the degradation of lignin and cellulose causes the higher yield obtained and the higher concentration of patchouli alcohol in patchouli oil.

Effect of refractive index of the fermentation time and the addition of kieserite shown in Figure 5 which the highest refractive index of 1.509 was obtained at the fermentation time for 15 days, and adding kieserite 0.5 g/L media. While the low refractive index of 1,505 was obtained on day 7 with the addition kieserite of 1.5g/L media. Requirements of patchouli oil refractive index according to SNI 2006 (National Standardization Agency) are 1.507 - 1.517. At the time of fermentation 7 and 9 days have refractive index values below the standard, while for fermentation time 11, 13, and 15 days have met the SNI standard. The refractive index is one of the important optical properties of the medium which a high refractive index indicates the amount of turbidity and concentration substrate [14].

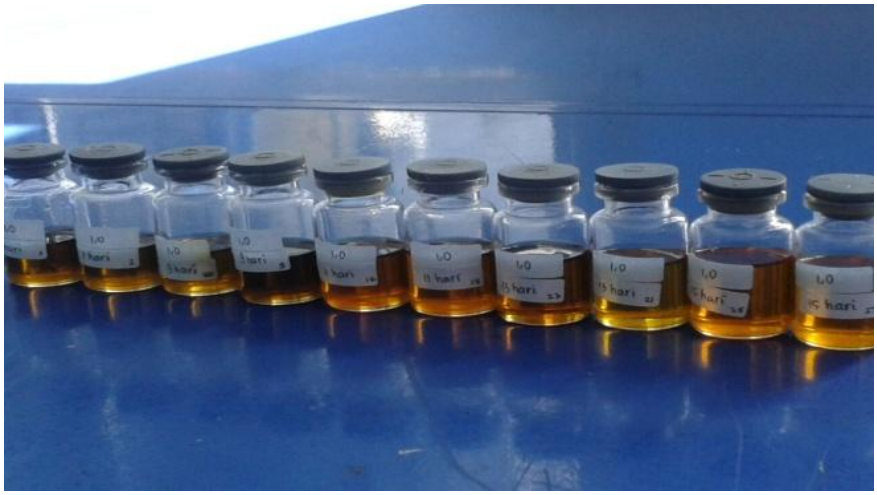

Figure.6: Patchouli oil of research results

IV. CONCLUSIONS

Result of the research can be concluded that *P.chrysosporium* can grow well on NLM media modified with kieserite as a substitute $MgSO_4$ and grow this best reached at kieserite addition of 0.5g/L media. The highest patchouli oil yield of 5.201 % was obtained in the fermentation time of 15 days with the addition of kieserite 0.5 g/L media. While the highest concentration of patchouli alcoholof 35.22 % and refractive index of 1.509 was obtained at 15 days of fermentation time and the kieserite addition of 0.5g/L.

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