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Bioremediation of Spent Bleaching Earth (SBE) Waste Using Lipolytic Fungi

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Abstract

The purpose of this research was to obtain the effectiveness level of bioremediation process of oil residue on SBE waste by using the isolates of lipolytic fungus *Aspergilus fumigatus*, *Cylindrocladium* sp and *Fumago* sp. This research was conducted by using completely randomized factorial design with 3 factors of treatment which consist of nutrient ratio (N:P:K) that were divided into three levels, namely n1=A(7:1.5:0.5), n2=B(14:3:1), n3=C(21:6:1.5). The humidity were divided into 3 levels treatment i.e. k1 (90%), k2 (80%), k3 (70%) and time that were divided into four levels, namely w1 (week 1), w2 (week 2), w3 (week 3), and w4 (week 4) with fixed pH i.e. pH 6, so that were obtained 36 combination of treatment and for each treatment combination repeated 3 times. Sampling of SBE waste was conducted in one cooking oil factory in South Sumatera using randomized sampling method as much as 24.000 g. The results showed that 80% humidity and nutrient A as the best treatment for lipolytic fungus consortium performance to degrade the SBE waste with the average percentage of oil degradation as much as 74.83%. 80% humidity and nutrient A was an effective interaction in affecting the percentage of oil degradation.

Keywords: SBE Waste, Bioremediation, Lipolytic Fungi

Abstrak (Indonesian)

Penelitian ini bertujuan untuk mendapatkan tingkat keefektivitasan bioremediasi residu minyak pada limbah SBE menggunakan isolate jamur lipolitik *Aspergilus fumigatus*, *Cylindrocladium* sp, dan *Fumago* sp. Penelitian ini dilakukan dengan menggunakan Rancangan Acak Lengkap (RAL) berpola faktorial dengan 3 faktor perlakuan yaitu faktor 1 berupa rasio nutrien (N:P:K) yang terdiri atas tiga taraf, yaitu n1=A (7:1,5:0,5), n2 = B (14:3:1), n3 = C (21:6:1,5). Faktor 2 berupa kelembaban yang terdiri dari 3 taraf yaitu k1 (kelembaban 90%), k2 (kelembaban 80%), k3 (kelembaban 70%) serta faktor 3 berupa waktu yang terdiri atas empat taraf yaitu w1 (minggu ke-1), w2 (minggu ke-2), w3 (minggu ke-3), dan w4 (minggu ke-4) dengan pH yang dikondisikan tetap, yaitu pH 6 sehingga didapatkan 36 kombinasi perlakuan dan masing-masing diulang sebanyak 3 kali. Pengambilan sampel limbah SBE dilakukan di salah satu pabrik minyak goreng di Sumatera Selatan dengan menggunakan metode *Random sampling* sebanyak 24.000 g. Hasil penelitian menunjukkan bahwa kelembaban 80% dan nutrien A sebagai perlakuan terbaik untuk kinerja konsorsium jamur lipolitik dalam mendegradasi limbah SBE dengan rata-rata persentase degradasi minyak sebesar 74,83%. Kelembaban 80% dan nutrien A merupakan interaksi yang efektif dalam mempengaruhi persentase degradasi minyak.

Kata Kunci: Limbah SBE, Bioremediasi, Jamur Lipolitik

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INTRODUCTION

The palm oil processing industry that processes fresh fruit bunches into CPO continues to increase along with increasing area and production. The clearing of CPO to be processed into cooking oil can be done chemically (neutralization, bleaching, and mild deodorization) or physically (degumming, bleaching, and stripping). CPO Purification by adding bleaching substances will produce cooking oil and residues that can contaminate the environment in the form of Spent Bleaching Earth (SBE) waste. This SBE residue has an acid pH. The percentage of CPO residues in SBE can reach 20-30% and they are difficult to be separated without special handling such as extraction [1]. Therefore, this SBE waste must be treated with caution before being discharged into the environment.

According to Loh [1], SBE is a solid waste that were produced in the process of purifying oil in the vegetable oil industry. If SBE waste accumulates in the terrestrial environment, the penetration of O_2 in the soil will be disrupted which causes the nutrients cannot be absorbed completely into the body of the plants, so that the plants become damaged or even died that culminate in the destruction of the balance of the ecosystems. One way to deal with the dangers of SBE waste is by bioremediation techniques to decompose the oil that still contained in SBE with the help of soil microorganisms that are lipolytic. However, most of the SBE (Spent Bleaching Earth) wastes generated from the palm oil clearance process by industry are still largely abandoned and dumped on the ground resulting in the accumulation of waste and cause environmental pollution.

SBE waste can be degraded in nature by physical, chemical, and biological techniques. Biological degradation of SBE waste can be done by bioremediation technique using microorganisms such as bacteria or fungi. According to Wang [2], microorganisms have an important role in overhauling organic pollutants in the soil through its metabolism process.

According to Juwarkar [3], bioremediation is an alternative method by utilizing conventional methods to reduce contaminants in waste compounds and media by utilizing natural microbial activity in the form of different microbial consortiums and can be enhanced by fertilizing (adding nutrients such as carbon, nitrogen, and phosphorus) and add the appropriate microbial population. Piotrowska [4] stated that among microorganisms responsible for biological corrosion, bacteria and fungi tolerate low temperature and humidity, as well as a wide range of pH levels, and they also easily adjust to unfavorable environmental conditions. Humidity and degree of substrate acidity (pH) are very important factors for the growth of fungi. Fungi are more tolerant organisms in the environment with acidic condition. Mukharomah[5] mentioned that there are types of lipolytic fungi that are able to degrade the oil contained in the SBE waste such as *Aspergilus fumigatus*, *Cylindrocladium* sp, and *Fumago* sp. The three isolates of this fungus work synergistically in lowering the oil content in SBE waste.

Based on the above information, the researchers need to conduct a research by utilizing a consortium of fungus *Aspergilus fumigatus*, *Cylindrocladium* sp, and *Fumago* sp. in degrading the contaminants in the form of oil residues that were contained in the SBE waste with due regard to environmental factors such as humidity, nutrients, and pH. Therefore, researcher interested in conducting research entitled "Bioremediation Spent Bleaching Earth (SBE) Waste Using Lipolytic Fungi".

MATERIALS AND METHODS

Materials

The materials used were aluminum foil, autoclave, beaker glass, note book, Bunsen, petri dish, measuring cup, Erlenmeyer, hot plate, incubator, ose needle, camera, cotton, magnetic stirrer, paper disk, paper label, pH meter, spatula, soil tester, and tissue roll. The tools used in this study were sterile distilled, contaminated soil Spent Bleaching Earth (SBE) waste and lipolytic fungus isolates, 70% alcohol, n-hexane, PDA and PDB medium, nutrient source (N, P, and K) in the form of urea fertilizer, TSP fertilizer, and KCl fertilizer.

Sterilization Tools and Media

The tools, materials and media that will be used were sterilized using autoclave at 1 atm pressure and 121 °C for 15 minutes.

Medium Preparation for Fungi Rejuvenation

Nutrient agar as much as 2.34 grams were put into Erlenmeyer, then dissolved in 60 mL distilled water. Then heated over hot plate and homogenized using a magnetic stirrer until boiling. The prepared media was then sterilized using an autoclave at 121 ° C for 15 minutes. As much as 10 mL of nutrient agar was inserted into the test tube, allowed standing at room temperature until the medium freezes in a sloping position, and then the nutrient agar was ready to be used for the rejuvenation of the fungus [6].

Medium Preparation for the Making of Inoculum of Fungi Consortium

Medium were being used for the manufacture of inoculum fungus consortium was the mineral medium. The mineral medium was homogenized by using a magnetic stirrer above the hot plate. After homogeneous, the media was sterilized using an autoclave at 121 ° C with 1 atm pressure for 15 minutes [6].

Research Procedures

Lipolytic Fungi Rejuvenation

Three isolates of lipolytic fungi as much as 1 ose each were inoculated into a tilted PDA medium by placing an ose needle which were contained cultured on the bottom of the tilt agar and drawn with a zigzag movement. The stock culture was incubated at room temperature for 48 hours [7].

The Making of Fungi Consortium Inoculum

Mixed cultures were made from fungi *Aspergilus fumigatus*, *Cylindrocladium* sp, and *Fumago* sp. The mixed culture inoculum was prepared by inoculating each of *Aspergillus fumigatus*, *Cylindrocladium* sp, and *Fumago* sp into 50 mL of PDB and agitated at 120 rpm. If the number of fungus cells has reached ± 10^7CFU/mL, then it can be used to create fungi mixed cultures. Fungi-mixed cultures were prepared by mixing all single cultures into the Erlenmeyer and then incubated for 6 hours at 37 ° C. After finished being incubated for 6 hours, then the fungi consortium inoculum was ready for the next process.

Preparation of Incubator

SBE waste 24,000 grams was prepared, then separated into 3 parts that contained 8000 grams of each parts, and each part was loaded by the sources of N, P, and K with the ratio of n1 (7: 1,5: 0,5) which was symbolized by the letter A, n2 (14: 3: 1) which was symbolized by the letter B, and n3 (21: 6: 1,5) which was symbolized by the letter C. Humidity of k1 (70%), k2 (80%), and k3 (90%), and time that were divided into four levels, namely w1 (week 1), w2 (week 2), w3 (week 3), and w4 (week 4) with fixed pH i.e. pH 6,so that were obtained 36 combination of treatment and for each treatment combination repeated 3 times. Sampling of SBE waste was conducted in one cooking oil factory in South Sumatera using randomized sampling method as much as 24.000g.

Analysis and Measurement of Observation Variables

Analysis of Gravimetric Oil Levels

The oil content analysis was performed on each treatment; the oil content was analyzed once a week during the bioremediation process by Gravimetric [8].

pH and Humidity Measurements

The pH and humidity measurements were performed at the beginning and the end of the bioremediation process trial in each treatment. The pH measurements were obtained by placing a soil tester on an incubator. The needle on the soil tester will move according to the pH and moisture in the incubator.

The Calculation of the Number of Fungus Colonies

The calculation of the number of fungus colonies was performed once a week during the bioremediation process for each treatment. The calculation of the number of fungi cells was performed by using TPC method[9]

Variable of Observation

The observation variables in this research were: the number of fungi cells in each treatment sample that was calculated in CFU/mL once a week, the pH value in each treatment sample at the beginning and the end of the experiment, and the percentage of Oil Degradation every week.

Analysis and Presentation of Data

Data in the number of fungus colonies and percentage of oil content degradation were analyzed using ANAVA at a level of 0.05. If ANAVA results showed a significant effect, it will be continued with Duncan's New Multiple Range Test (DNMRT). Data processing was performed by Software Statistic 8

RESULT AND DISCUSSION

Number of Fungus Colonies

Based on data of research result and result of Analysis of Variance (ANAVA), it was found that humidity factor greatly influences the number of fungus colonies that grow with p = 0.0039. P value of treatment level was smaller than 0.05 indicates that the humidity factor significantly affected to the number of fungus colonies production. The result of further test of DNMRT 5% showed that the largest number of fungus colonies was found at 80% humidity. This can be seen in Table 1.

Table 1. Average Number of Fungus Colonies in Various Humidity

Humidity (%)	Nutrient	Average Number of Fungus Colonies (CFU/mL)
70	A	8.67×10^{4a}
70	B	11.33×10^{4abc}
70	C	14.59×10^{4bcd}
80	A	20.17×10^{4e}
80	B	13.22×10^{4abc}
80	C	15.27×10^{4cde}
90	A	18.84×10^{4de}
90	B	13.84×10^{4abcd}
90	C	9.60×10^{4ab}

Based on Table 1, the consortium of lipolytic fungi that was used can grow very well at 80% humidity. Humidity has a very big influence on the growth of fungi, because if the temperature and humidity is not achieved to the growth's requirement then the substrate will dry out and the growth of the fungus will be hampered. Piotrowska [4] stated that temperature and humidity can determine the success of soil bioremediation using fungi.

The interaction between humidity factor and nutrient also has a big influences to the number of growth of fungus colonies with p = 0.00024 which means that the interaction between humidity and nutrients significantly affects to the number of fungus colonies productions. The result of further test of DNMRT 5% on the humidity and nutrient interaction are presented in Tablel 2.

Table 2. Average Number of Fungus Colonies inVarious Humidity and Nutrient Combinations

Humidity	Average Number of Fungus Colonies (CFU/mL)
70	11.53×10^{4a}
80	16.22×10^{4b}
90	14.09×10^{4ab}

Description: The numbers followed by the same small letters show no significant difference according to DNMRT α 0.05

Based on Table 2 above, there are nine combinations between moisture and nutrients that produce a high average number of fungus colonies. From the data that was obtained, found that 80% of humidity and nutrient A (with the ratio N: P: K = 7: 1.5:0.5) gave the best results for the number of fungus colonies production. This means that 80% humidity and nutrient A are highly effective for the growth of colonies of lipolytic fungi in the bioremediation process. At 80% of humidity, the growth of the consortium of lipolytic fungi is very effective because at 80% of humidity the oxygen demand for growth of lipolytic fungi is sufficient so that the lipolytic fungi is able to grow well to to decompose the oil contained in SBE waste by going through the process of bioremediation. The consortium of lipolytic fungi is an aerobic fungus that needs oxygen for its growth. The oxygens were obtained from aerobic respiration is used to break down the organic materials in SBE waste as a source of nutrients for the lipolytic fungus consortium.

Additional nutrients such as nitrogen, phosphorus and potassium are utilized by the lipolytic fungus consortium to carry out metabolic processes[10]. Nutrients and oxygen that enter the body of microorganisms are used to stimulate the biodegradation of contaminants. Energy metabolism results are used as a source of energy to perform fungi consortium activities, such as for growth and fungus cell division. The substrate is the main nutrient source for fungus growth. New nutrients can be exploited after the fungus expresses extracellular enzymes that can reduce the complex compounds from the substrate into simpler compounds.

pH level

The pH that was used at the beginning of this study is pH 6. Neto [11] suggested that pH 5.5-6.5 is the best pH for fungi growth. The highest pH degradation was in SBE waste with 80% of humidity factor and nutrient A. Effective lipolytic fungi consortium growth was chosen at pH 6 because at pH 6, the fungus was able to grow optimally. It is known that pH 6 is an ideal pH for the growth of lipolytic fungus consortium because of the absorption of CPO as a source of energy and enzymatic reaction is good. At pH 6, the lipase enzyme works optimally and a consortium of fungi consisting of *Aspergillus fumigatus*, *Cylindrocladium* sp, and *Fumago* sp. experienced good on growth, thus resulting in a large decrease of pH. The fungus are more at acidic pH, whereas the bacteria favor in a neutral conditions. Souza [12] stated that at the optimum pH the side chains of amino acid is in an appropriate state so that the enzyme is very efficient in accelerating a very specific reaction. The effect of interaction between moisture and nutrient on the percentage of pH decrease are presented in Table 3. Based on Table 3 above, there are nine moisture and nutrient interactions that affect the percentage of pH decrease. The interaction between moisture and nutrient factors shows various percentage of pH decrease. The presence of a minus number in the percentage of pH

decrease that is presented in Table 3 shows that there is an increase in the final pH with pH higher than six.

Table 3. Interaction between Moisture and Nutrient to the Percentage of pH Decrease

Interaction of Humidity and Nutrient	Percentage of pH Decrease
70% A	-2.75
70% B	7.11
70% C	-4.13
80% A	18.47
80% B	-4.82
80% C	-14.29
90% A	18.36
90% B	-6.43
90% C	-16.12

This can occur because the addition of urea fertilizer to meet the nutritional needs of the nitrogen element in lipolytic fungi and the addition of TSP fertilizer to meet the needs of the P element on the lipolytic fungus can raise the pH of SBE waste. The more fertilizer added to the SBE waste, the higher SBE waste pH. However, along with the bioremediation process by lipolytic fungi, acidic substances are generated from the decomposition process of the oil contained in the SBE waste so that the pH of waste decreased. In general, mushrooms require a medium with a pH around 6. However, there are some fungi that also prefer the sour media condition. Rousk [13] adds that fungi generally exhibit a wider pH range than bacteria for optimal growth. Therefore, the fungus *Aspergillus fumigatus*, *Cylindrocladium* sp, and *Fumago* sp can still grow optimally despite being exposed to pH higher than six.

Percentage of Oil Degradation

Based on Analysis of Variance of humidity, nutrient, and time factor and interaction between humidity and nutrient have significant effect on oil degradation percentage, with p = <0,05. The interaction between humidity factor and nutrient indicates that humidity and nutrients affect each other against the percentage of oil degradation, and because of the interaction between humidity and nutrient then the bioremediation process is more effective than only a single factor affecting the percentage of oil degradation. Appropriate moisture and nutrients will affect the percentage of degradation and the duration of biodegradation process. The results of further test of DNMRT 5% on percentage of oil degradation with various humidity levels are presented in Table 4 below.

Table 4. Effect of Humidity on Average Percentage of Oil Degradation

Humidity	Average Percentage of Oil Degradation
70	52.03
80	62.39
90	60.49

Based on Table 4 above, the results showed that the largest percentage of oil degradation is found in the treatment with 80% of humidity. Treatment with 80% of humidity is able to provide optimal results in degrading the oil content. This is because the lipolytic fungus can grow optimally at 80% of humidity so that the fungus can utilize the nutrients present in the substrate for metabolic processes and can utilize the oil as one of its food sources by overhauling the oil into fatty acids and glycerol which also causes the pH of the substrate to be more acidic because of the presence of fatty acids.

The effect of humidity on degradation percentage is a requirement for SBE waste biodegradation activity. Humidity helps the supply of oxygen that can keep environmental conditions in aerobic state to stimulate microbial oxidation and hydrolysis of hydrocarbon compounds.

The average percentage of oil degradation is inseparable from the role of nutrients for the growth of lipolytic fungi which causes the fungus to grow optimally and can break the oil into simpler compounds through its metabolic processes. The results of further test of DNMRT 5% on oil degradation percentage with various interactions of humidity and nutrient are presented in Table 5.

Based on the Table 5 above the highest percentage of oil degradation was found in the interaction between 80% of humidity and nutrient A. This means that at 80% of humidity and nutrient A, oil degradation is very effective, so that the time required for oil degradation is faster because this study used the fungi in the form of a consortium derived from SBE waste. The process of degradation of oil by using fungi in the form of a consortium is more optimal than by using single fungi.

The 80% of humidity is good humidity to improve the performance of a lipolytic fungus consortium in the SBE waste bioremediation process.

Table 5. Average Percentage of Oil Degradation in Various Combinations of Humidity and Nutrient

Humidity (%)	Nutrient	Average Percentage of Oil Degradation **(%)**
70	A	45.46[a]
70	B	53.51[a]
70	C	57.12[a]
80	A	74.83[b]
80	B	54.33[a]
80	C	57.99[a]
90	A	73.40[b]
90	B	55.99[a]
90	C	52.07[a]

Description : The numbers followed by the same small letters show no significant difference according to DNMRT α 0.05

Presumably at 80% of humidity interacting with nutrient A causes microorganisms easily soluble in water and optimize the work of lipase enzyme of microorganisms in degrading oil in SBE wastes. Based on the number of fungus colonies, pH levels, and degradation percentage it can be seen that initial pH 6 is the best pH for bioremediation by using lipolytic fungi. This is because at the pH is produced a large percentage of oil degradation. General lipase has an optimum PH and a little bit alkaline[14].

CONCLUSION

Based on the results of the research and the data analysis that has been done, it can be concluded that 80% of humidity as an effective humidity for the performance of a lipolytic fungus consortium in SBE waste bioremediation and interaction between 80% of humidity and nutrient A are capable to produce the largest percentage of oil degradation as much as 74.83%.

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