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INDIGEN BACTERIA FROM *SPENT BLEACHING EARTH* WASTE AS AN REMOVAL AGENT OF Fe And Cu

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Abstract

Abstract- The most pollution produced by oil palm factory is Spent Bleaching Earth, because the waste contains residue of oil and dangerous metal that enable bacteria to live hence the research was done to get bacteria of Fe and Cu metal as bioremoval agent. Waste sampling method is random sampling, then purification, selection and potency test. Bacteria that pass potency test on metal containing media are identified and characterized by their genus. The result of the research indicated that the indigenous bacteria genus for good accumulation of Fe metal is Salmonella sp (B7) and for Cu metal is Escherichia sp (B1).

Keywords: ***Bacteria, Metal, Spent Bleaching Earth***

Abstrak (Indonesian)

Abstrak- Pencemaran yang paling banyak dihasilkan oleh pabrik kelapa sawit adalah Spent Bleaching Earth karena didalam limbah ini terdapat residu minyak dan logam berbahaya yang memungkinkan bakteri untuk hidup maka dilakukan penelitian untuk mendapatkan bakteri logam Fe dan logam Cu sebagai agen bioremoval. Metode pengambilan sampel limbah diambil secara random sampling, kemudian dilakukan pemurnian, seleksi, uji potensi. Bakteri yang lulus uji potensi pada media yang mengandung logam di identifikasi dan karakterisasi genusnya. Hasil dari penelitian menunjukan bahwa bakteri indigen yang terdapat di dalam limbah SBE mampu menurunkan Logam Fe dan Cu yang terdapat di dalam limbah SBE. Genus bakteri indigen pengakumulasi logam Fe yang baik adalah Salmonella (B7) dan logam Cu adalah genus Escherichia (B1).

Kata Kunci: ***Bakteri, Logam, Spent Bleaching Earth***

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INTRODUCTION

Oil palm plantations are plantations managed by the private sector and communities that do not yet have their own management. The production of oil palm is getting attention from local governments, where the processing of oil palm itself must be done by large industries that process oil palm into vegetable oil. The industry of oil palm contains a lot of residue or waste from its processing. Wastewater treatment from these industries must be reduced by the impact of environmental pollution, at least to suppress high levels of pollutants in industrial areas. It is better for industries that produce waste to process waste before disposal into the environment so as not to become a waste that endanger the environment [1].

The handling of Spent Bleaching Earth (SBE) waste into landfills, resulting in contaminated land on organic compounds that can be done by ex-situ and in-situ, a way that can be used to overcome environmental pollution is to involve plants and microorganisms that can reduce the impact of damage to the environment caused by SBE waste. Decomposition of microorganisms in a polluted medium can be done by natural recovery process.

According to Aulia, many microorganisms are tolerant of dangerous heavy metals [2].

Microorganisms or bacteria that can be used to accumulate hazardous metals can be isolated from the waste site itself as one of the heavy metal bioremoval agents of the environment. To obtain the bacteria accumulating heavy metals is carried out several stages, namely isolation from where there is waste then selection, characterization and identification as a first step to determine the ability of the bacteria with a potential test, because the ability of isolated bacteria differs from its maximum ability level to accumulate metals. The bacterium is used because it has adapted to the polluted environment so that this activity can minimize organic pollutant compounds and turn them into non-hazardous materials. However, each bacterial treatment usually has a weakness because it is not effective in some contaminated land, due to high hazardous substances such as metals, organic compounds chlorinated and organic salts [3].

In this research, isolation of indigenous bacteria capable of degrading copper (Cu) and iron (Fe) metal, where the bacteria can be obtained from SBE waste, it is known that they can adapt to the environment.

MATERIALS AND METHODS

Tools and materials

The tools to be used in this research are: petri dish, autoclave, 250 ml Erlenmeyer flask, dropper dropper, eppendorf, AAS (Atomic Absorption Spectrophotometry), desiccator, plastic bag, cool box, scissors, paper label, aluminum foil, . Materials needed are 90-95% alcohol, distilled water, micro filter 0.22 µm, PGE medium (pepton glucose - yeast extract), Nutrient Agar (NA), Cu (NO3) solution 1000 ppm, FeSO4 solution .7H2O 1,000 ppm and SBE waste samples.

Sampling

Sampling of SBE waste in one of CPO oil factories in Banyuasin Regency is done by random sampling method. SBE waste samples were taken from 4 sampling points representing as much as 250 grams ago in composite, after which it was inserted into the plastics and labeled. The composited sample of 4 sampling points stored in a sealed plastic is then fed into the cool box to be brought to the laboratory and subsequent testing.

Isolation

Isolation was done by dilution method SBE waste samples in weigh as much as 1 gram then dissolved in 9 ml distilled water, then homogenized using vortex, so obtained level of dilution 10-1. The above working procedure will be repeated continuously to dilution level 10-6. Sample SBE wastes have been diluted each 1 ml of dilution of 10-4, 10-5 and 10-6 dilution then incorporated into the medium to prepare PGE cup (peptone glucose - yeast extract) by spreading cup method then incubated at 370C, then put in a dark room for 2-3 days and observed growing colonies.

Purification

Colonies that grow with different characteristics, based on morphological differences (such as color, colony shape and colony surface). Each was purified by scratching on a sterile NA medium in a petri dish, and then incubated for 2 x 24 hours at 37°C. This technique is repeated until the colony grows apart as an early indication of a pure colony [4].

Selection

After the colony grows and has a diameter of 2-4 mm, it is transferred to a PGE medium containing Fe and Cu metal, after being overgrown with microbial accumulating heavy metals in culture agar in a cup containing Fe and Cu with a concentration of 5 ppm. The culture was incubated by manually shaking by hand every 5 hours at room temperature for 3 days. Colonies that are able to accumulate heavy metals in isolation from medium agar to newly diagnosed PGE are darker than bacteria whose medium does not use metals [5].

Potential Test

Selection results obtained are the four bacteria that have the ability to reduce Fe and Cu metals. This test was performed on Erlenmeyer containing 50 mL of PGE medium, Fe metal and Cu metal as much as 15 mL, and bacterial isolate. The culture is incubated by means of a 24 Hour shaker for 5 days. Furthermore, the calculation of metal residues was done after the test.

Measurement of percentage of metal decline by bacteria using the formula:

$$R = \frac{C0 - Ceq}{C0} \times 100\ \%$$

Characterization and Identification

The results of bacterial isolate which has been tested in subsequent degrading of metals in characterization to simplify identification. Characteristics were performed on colony morphology on various forms of agar media, cell morphology, biochemical testing, sugar fermentation, starch hydrolysis, gelatin hydrolysis, indole test, red metal test, vogos-preskuer test, citrate test, citrate test, H2S test, urea hydrolysis test , citrate test, H2s test,

urea hydrolysis test, catalase test, and motility test. Bacteria capable of degrading metals were identified using Bergey's manual [6].

RESULTS AND DISCUSSION

Bacterial Isolates

Bacteria are isolated from SBE wastes which contain a lot of waste from the process of refining the cooking oil, which is contained in the waste containing Fe and Cu metals [7]. Bacteria are found in nature in mixed populations, only by purification so in pure bacteria. Purification of bacteria taken from samples of SBE separated from bacteria one with other bacteria in the media according to morphological characteristics. The bacteria isolated into the PGE medium (Pepton Glucose Yeast Extract) were carried out to a dilution level of 10-6, after which the bacteria were incubated at room temperature for 5 days to obtain 8 bacterial isolates from each dilution as listed inTable 1.

Tabel 1. Result of insulation and purification of metal bacteria on medical waste (Spent Bleaching Earth).

Types of Sample	Number of Isolates	Bacterial Isolate Code	Selection Results
SBE Waste *(Spent Bleaching Earth)*	8 Isolates	B1, B2, B3, B4, B5, B6, B7, B8	B1, B3, B6, B7

Based on Table 1, it is known that the result of isolation obtained from several dilutions there are 8 different colonies, it can be concluded that the existence of the diversity contained in homogenized samples first and able to live in some places containing SBE waste which indicated that the bacteria could reduce Fe and Cu metals contained in the SBE waste. The isolate codes obtained from the 10-6 dilutions are, B1, B2, B3, B4, B5, B6, B7 and B8. Based on the result of Selection of Fe and Cu metals, it is found that from 8 isolates capable of adapting and living in medium containing Fe and Cu only 4 bacterial isolates can grow on selective medium condition B1, B3, B6 and B7.

Bacteria that can accumulate Fe and Cu metals have a slightly darker colony characteristic than other colonies. This selection test aims to look at the ability of bacteria that can degrade metals. The presence of bacteria in a habitat is generally greatly influenced by the availability of nutrients and the physical and chemical factors of the environment.

The isolated bacterium obtained was purified based on the morphological characteristics of each isolate. The isolates were viewed based on the morphology of each colony having different shapes, elevation, surface, color and diameter of colonies. The result of characteristic and physiological observation of bacteria of biochemical test obtained then sought genus having the same characteristics using Bergey's Manual of Determinative Bacteriology 8th edition and Bergey's Manual of Determinative Bacteriology 9th edition, obtained 4 different indigenous bacteria genus namely Isolate B1 is genus Escherichia, isolate B5 is genus Pseudomonas, isolate B6 is Enterobacter genus, isolate B7 is genus of Salmonella bacteria.

Isolate B1 belongs to the genus Escherichia, where the characters are similar to the genus, short rod, gram negative, facultative anaerobic, motile and have no endospores, do not produce H_2S, test PoscousproskeurVoges and use citrate as energy. B3 isolates belonging to the genus Pseudomonas have similar characters to the genus is straight or curved, gram negative, aerobic, motile and has no endospores, does not produce H_2S, Negative ProskeurVoges test, Methyl Red Negative Test. Pseudomonas is able to grow in an environment containing oil and other fuel oil.

Enterobacter genus is isolate B6 Where cell morphology results obtained rod-shaped bacterial cells, gram-negative gram and did not form endospora, for the results of biochemical tests obtained the bacteria are motile with positive catalase test. According to Buchanan & Gibon [5], the Enterobacter genus is motile; a bacterial cell is stem and gram negative.

For B7 isolates where the characteristic results have similarities with the genus Salmonella which exhibit characteristics of bacilli-shaped bacteria or stems, gram negative, motile, have no facultative anaerobic endospores, where the Methyl red and positive catalase test, negative prouskauer voges test, glucose fermentation produce acid and gas, and produce H_2S gas.

Test of Decreasing Metals Potential using ANOVA

The use of ANOVA tools has become common in research. ANOVA has the advantage of being able to see the influence of several factors at the same time so that the results obtained become more accurate [8]. Based on the results of the Analysis of Variance (Anova) shows that the significance value of treatment of Fe metal reduction is 0.313 where the value is> 0.05 which means that the effect of treatment is not significant (effect is not real). The results of metal residues for Fe metal are 0.004 <0.005, meaning that the effect of the treatment is

significant (significant effect). For the significance value of treatment on Cu metal results from the Analysis of Variance (Anova) is 0,000 <0,005 which means that the treatment results of reduction of Cu Metal and Cu Metal residue both have significant treatment effects (significant effect).

So it can be concluded that overall there are significant differences between treatment averages, but not necessarily the average treatment without bacterial control is different from the average treatment contained in bacteria.

Table.1. Fe Metal Residue and Decreased Fe Metal by indigenic bacteria

Treatment	Fe (ppm) metal residues	Decreases in Fe (%)
Without Bacteria (B0)	3.18 b	-*
Escherichia (B1)	1.13 a	64.25
Pseudomonas (B3)	1.95 a	38.46
Enterobacter (B6)	1.56 a	50.73
Salmonella (B7)	1.81 a	42.87

Information:
The numbers followed by the same lowercase letters indicate a non-significant difference according to DNMRT 5%
* Not tested

In Table 1 the results of Duncan's further test for Metal Fe treatment values of bacteria B1, B3, B6 and B7 were both followed by letters meaning that the treatment of the four bacteria was not significantly different in effect. For the best treatment, the results of Fe Metal reduction were Escherichia bacteria (B1) of 64.25% and the most abundant Fe Metal residues were Pseudomonas (B3) of 1.95 ppm, so it can be ascertained that Pseudomonas (B3) obtained from SBE waste not effective for lowering Fe metal. Microorganisms have different adaptive abilities and sensitivity and this difference is related to the mechanism of bacterial response to some metals.

Based on the reduction of Cu Metal in Table 2, it was found that the decrease of Cu metal by 5 ppm by indigent bacteria showed that Salmonella (B7) bacteria were able to reduce Cu Metal by 74.67%. The results of statistical analysis through Duncan's follow-up test at a significance level of 0.05 showed significant differences in all treatments used. Based on these results it can be seen that Salmonella (B7) bacteria is the most effective treatment for reducing Cu Metal at a concentration of 5 ppm.

Table 2. Cu Metal Residues and Decreased Cu Metal by indigenic bacteria

Treatment	Cu (ppm) metal residues	Decreases in Cu (%)
Without bacteriaB0)	4,37 c	-*
Escherichia (B1)	1,69 b	61,33
Pseudomonas (B3)	3,40 c	22,20
Enterobacter (B6)	2,85 b	34,62
Salmonella (B7)	1,10 a	74,67

Information:
The numbers followed by the same lowercase letters indicate a non-significant difference according to DNMRT 5%
* Not tested

Microbial activity as an energy source, carbon source or acceptor electron for its metabolism. The entry of certain amounts of bacteria, especially bacteria that are additive and resistant to polluted media, can bind heavy metals because microbes produce extracellular compounds or enzymatic syntheses that are able to bind heavy metals through the process of adsorption [9].

CONCLUSION

Based on the results of the study of Indigen bacteria from SBE waste which can reduce Fe and Cu metals, it was concluded that 4 isolates from SBE waste have the ability to reduce Fe and Cu i.e. B1, B3 , B6 and B7. The highest genus of indigenizing metal Fe bacteria is Escherichia (B1) of 64.25% and Cu is Salmonella (B7) of 74.67%.

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