PRODUCTION OF BIOETHANOL FROM BANANA PEELS ASSISTED BY HYDROLYSIS OF TERMITE GUT MICROBES USING SIMULTANEOUS SACHARIFICATION AND FERMENTATION (SSF) METHOD

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Abstract

Study of bioethanol production from banana peels assisted by hidrolysis of termite gut microbes using Simultaneous Sacharification and Fermentation (SSF) has been conducted. Banana peels are waste that has not been widely utilized by the public, therefore in the long period, this waste can be one of land contamination issues. Banana peels contain quite high lignocellulose which can be decomposed into glucose. It is the main source of bioethanol formation. Microbes in the gut of termites contain cellulolytic bacteria that can help degrade lignocellulosic to form glucose. Therefore this research was done without prior delignosellulosa process using an acid. Furthermore glucose is formed through fermentation with the help of *Saccharomyces cerevisiae* to form ethanol. Termite gut microbes were isolated using Nutrient Agar, then the resulting microbial colonies were added to the fermentation process as much as 0 mL, 1 mL, 3 mL, 5 mL, 7 mL, and 9 mL. The analysis was conducted qualitatively by GC and quantitatively using UV-Vis spectrophotometer. The result shows that the termite gut microbes increase the production of bioethanol and optimum volume termite gut microbes were added about 3 mL with the resulting yield of 17.784%.

Key words: *bioethanol, banana peels, termite gut microbes, fermentation*.

Introduction

The more advanced the times affects on the increasing of fuel compsumpsion yet it is not balanced with the availability of the petroleum resources. Therefore, it is necessary to search for alternative materials that can be used as a substitute for petroleum. Bioethanol is one of the alternative fuels that have many strenght compare from foil. Bioethanol contains lower CO gas emission when compared to oil of about 19-25% (Syam et al, 2009). Bioethanol can be produced from materials that contain lots of cellulose. Cellulose exists in agricultural waste or plantations that have not been utilized optimally.

Banana peel is a waste material (waste of banana) which is quite a lot everywhere. It contains one of the largest sources of lignocellulose and has not been utilized optimally in Indonesia. People usually threw banana peels away and it has become a serious problem because it will increase the acidity of the soil and

pollute the environment. Banana peel composition based on cell wall analysis (% dry weight) i.e. 37,52% hemicellulose; 12,06% cellulose; and 7,04% lignin (Robetson, 1993). So that the content of cellulose in banana peel is potentially developed as substrate in bioethanol production.

The termite gut contains cellulolytic bacterial microorganisms that can help digest cellulose. Cellulolytic bacteria are found in the termite hindgut or termit gut. Lignocellulose is degraded optimally in termite hindgut due to the role of lignocellulose degrading bacteria (Papoola and Opayele, 2012). Digestive activity in termite involves not only bacteria in the digestive tract but also by activity of cellullose enzymes produced by the body of termites. Microbes in the termite gut are microbial symbionts interconnected with other organism in the lignocellulose degradation process (Shelton and Grace, 2003; Mackenzie, 2007; Berlanga et al., 2011).

The aim of this research is to know the effect of microbial addition and optimum addition volume of termite gut on bioethanol production from banana peels by SSF method.

Material and Method

Materials

The instrumentation used in this research were laboratory glassware, microwave autoclave, centrifugator, mixer, buchner funnel vacum filtration, Gas Chromatography-Mass Spectroscopy (GC-MS Buck Scientific 910), and UV-Vis double beam Spectrophotometer (Hitachi U-2010). Materials used in research were banana peels, termite gut, buffer solution pH 7, ethanol 70%, nutrient agar (NA), aquadest, aluminium foil, cotton, ethanol p.a (Merck), reagent of sulfuric acid and potassium dichromate, *Saccaromyces cerevisiae.*

Procedure

Preparation of extract termite gut

Fifty termites were surface sterilized with 70% ethanol and washed with three times with water. The gut was pressed out with sterile tweezers and crushed until smooth. The mixture was added 10 mL buffer solution at pH 7 and

centrifuged at 2000 rpm for 15 minutes. After that, transfer supernatant to a new tube.

Isolation of termite gut microbes

Nutrient Agar medium was produced by 7 gram Nutrient Agent and 250 mL aquadest. The mixture was heated in Microwave until homogeneous for 5 minutes. Medium was removed to erlenmeyer flask and cover with cotton and alumunium foil. Medium was sterilized in autoclave for 120 minutes.

In the next methods, termite gut microbes were allowed to grow in sterile petri dish. Nutrient Agar medium was placed inside half volume of petri dish until solid. Extract of termite was taked one ose and lubricated to the medium. The petri dish was incubated for 48 hours at 37°C. Termite gut microbes were pressed out from nutrient agar medium with use ose. Then, added to 100 mL cool NaCl solution 1% at pH 7 and centrifuged at 4200 rpm for 60 minutes. After that, transfer supernatant to a new tube.

Production of bioethanol using SFF method

Banana peels were sun dried and ground to fine powder. 10 gram powderbanana peels was carried out in 250 mL bottle of sample. Added 100 mL aquadest and termite gut microbes to the bottles with different volume such as 0 mL, 1 mL, 3 mL, 5 mL, 7 mL, and 9 mL. Added 4 gram *Saccaromyces cerevisiae* and mixed until homogeneous. All bottles were carried out at room temperatures for 24 hours. Product of fermentation was filtered using funnel buchner that contains of water and alcohol. Alcohol was characterizad using Gas Chromatography-Mass Spectroscopy and UV-Vis Spectrophotometer

Result and Discusussion

Quantitative analysis of ethanol using UV-Vis Spectrophotometer

Ethanol product was analyzed by UV-Vis Spectrophotometer at 585 nm wavelenght. The resulted show that absorbantion of each sample then calculated to adjust the ethanol standard calibration curve obtained.

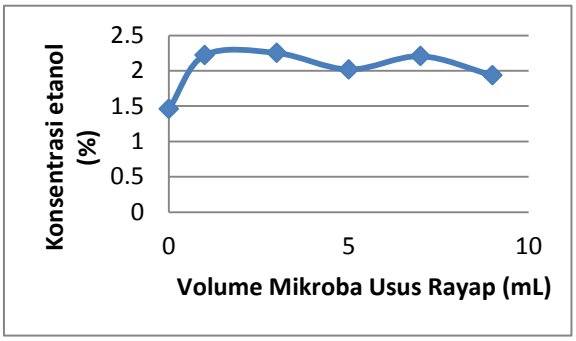

Fig 1. The curve relation between volume of temites gut microbes and ethanol product

The results showed that fermented banana peels without temite gut microbes produced the lowest ethanol, so it can be seen that the addition of termite gut microbes affects the ethanol production process from banana peels. The isolated termite gut microbes in this reseach can help this is degrade lignocellulosic in banana peels this is likely due to the presence of lignocellulose degrading bacteria in these microbes. Lignocellulose is degraded optimally in termite hindgut due to the role of lignocellulose degrading bacteria (Papoola and Opayele, 2012). Microbes in the termite gut are microbial symbionts interconnected with other organism in the lignocellulose degradation process (Shelton and Grace, 2003; Mackenzie, 2007; Berlanga et al., 2011).

The optimum addition volume of termite gut microbial was found to be 3 mL. From the various volumes that were added, i.e. 0; 1; 3; 5; 7; and 9 mL, the ethanol produced was increased and reached its optimum at 3 mL and gradually decreased at a higher volume. The result showed that after the optiumum fermentation condition was archieved, as the volume of microbes was increasing the amount of fermented ethanol was decreasing. This condition could be explained by the fact that besides the substrate was converted into product through the metabolic process, microorganisms also need some substrate and nutrient for growth, as well as the reproduction of new cells and also enlarging of the cellular size. This means that not all of the substrates were converted into products. As for after the optimum fermentation condition were achieved, the increase of microbial volume needs a higher amount of substrates for cellular growth, resulted

in the least amount of the products produced. It has been know that bioethanol can be toxic to microorganisms, thus with the formation of the fermentation products in the form of bioethanol, it will decrease the productivity of microorganisms (Yuni, 2013).

The highest yield of bioethanol production from banana peels with termite gut microbes was 17,784% ang 2,254% for ethanol concentration. In this research, the converted bioethanol was high relatively. Muhammad et al (2016) have researched bioethanol from Azadiractan leaves as main source which hydrolyzed with acid and used *S. Cerevisiae* for fermentation process, yield in 6,25%. The other research from Mishra (2013) study of bioethanol production from *Lantana camara* with cellulose bacteria from termite gut was resulted ethanol concentration in 11,66%. In Seftian (2012) research, bioethanol could obtain from banana peels with enzymatic hydrolisis from *Aspergilus niger* cellulase with ethanol concentration in 13,1154% but from the last two of research did the deligncellulose with acid before fermentation.

Qualitative analysis of ethanol using GC- Mass Spectroscopy

In this research, gas chromatography was used as qualitative analysis. With compared between retention time of sample fermentation product and ethanol 10% as standard in the same condition of analysis. The spectrum of the product can be seen in the following picture (Fig 2).

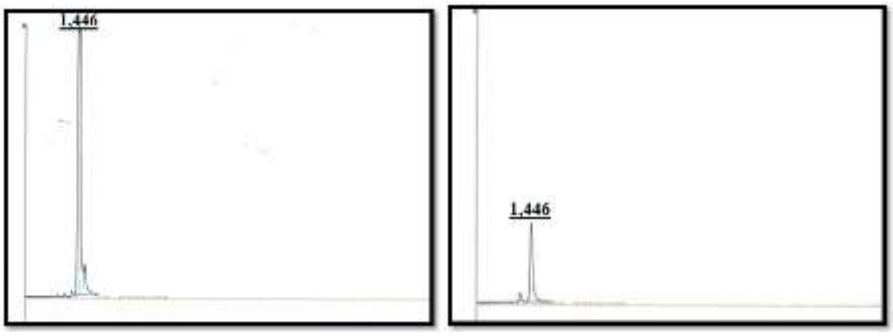

Fig 2. Chromatogram-spectrum of ethanol, (A) ethanol standard 10%; (B) added termite gut 3 mL

Fig 2 shows optimum percentages of ethanol from fermentation product with previous quantitative analysis. An analysis carried out only on the optimum sample as a corroborative evidence that the quantitative results of the previouse analysis of the presence of ethanol qualitatively with GC is true. From the result of GC analysis performed on the 10% ethanol standard dhowed the formation of one peak in the retention time (Rt) area 1,446. Then on the analysis of the sample with the highest ethanol content that is on the addition of 3 mL termite gut microbial obtained the same that is 1.446 min. The retention time is showed as qualitative analysis that the sample contains the same compound to the standard (containing the ethanol compound). The peak produced between ethanol standard 10% and the sample is higher so the area is larger than the peak on chromatogram in the sample. Based on quantitative analysis of this sample yield ethanol concentration about 2,254%. This is consistent with the theory that quantitative analysis using GC can be done by comparing the area in the chromatogram. So it can be seen that ethanol analysis method using UV- spectrophotometer with the addition of dichromate reagent can be done.

Conclusion

Bioethanol was produced from banana peels assisted by hydrolisis of termite gut microbes with the help of *Saccharomyces cerevisiae* to form ethanol using simultaneous sacharification and fermentation (SSF) method. With added termite gut at this process can increase percentage of ethanol product. The result show that the optimum volume termite gut microbes were added to production bioethanol about 3 mL. The consentration ethanol from banana peels was 2,254% with 17,784% yield.

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