BIOETHANOL PRODUCTION OF PINE MERKUSII LEAF WITH SELECT ENZYME USING METHOD (SSF)

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Abstract - Increased fuel demand encourages the search for material sources raw material that can be used to produce fuel as a source renewable. Therefore, it needs a new innovation to create as well developing renewable energy from waste, its potential very fulfilling or easy to get around. Bioethanol is a source of energy renewable environmentally friendly from waste raw materials. Bioethanol (C_2H_5OH) is one of the fuels that comes from a biological source that can replace petroleum. Bioethanol can be produced from the fermentation of sugar from source of carbohydrates (starch) using the help of microorganisms that can obtained from materials containing cellulose. This study was conducted for utilizing the waste of pine leaf which is very abundant to reduce environmental pollution in this innovation using stems from oyster mushrooms which is useful as enzyme activation in bioethanol making by method SSF (Simultaneous Scarification and Fermentation) by converting the polysaccharide compound becomes a monosaccharide compound without turning back to polysaccharide compounds. This research uses qualitative analysis with GC instruments that show that pine leaves can produce bioethanol as a substitute for petroleum, as well as quantitative tests using UV-Vis spectrophotometer instrument with optimum yield on 72-hour time variation.

Index Terms - Bioethanol, Cellulose, SSF Method, Qualitative and Quantitative Test.

I. INTRODUCTION

The development of the era makes the use of fuel continues to increase while the fuel oil used is increasingly depleted. Indonesia is one of the countries that supply the most energy due to the increasingly populated population of Indonesia. The need for fuel or energy today is still widely supplied from fossil fuels. Increased fuel demand encourages searching for sources of raw materials that can be used to produce fuel as a renewable source. Therefore, a new innovation is needed to create and develop renewable energy from waste, whose potential is very fulfilling or easily obtainable. Various kinds of alternative energy such as biodiesel, biogas and one of them that can replace the fossil energy source is bioethanol. Bioethanol is a liquid produced from the fermentation of sugar from carbohydrate sources (starch) using the help of microorganisms. Bioethanol can also be obtained from materials containing cellulose. The cellulose is a polysaccharide which, when further processed, can produce ethanol [8]. One of the ingredients used is pine leaf, wandering the number of pine forests making new innovations from the pine leaves are very abundant to be a source of renewable energy. The need for bioethanol very high encourages innovation in producing bioethanol to run effectively and efficiently, so as to meet the needs and to minimize the energy from fossils. Pine Plant is a reforestation plant that can reduce the occurrence of landslides because it has a deep root and heavy, reducing erosion because the canopy can inhibit the kinetic power of falling rain water, and pine leaf has a high evapotranspiration value, so it will quickly reduce the moisture content in the soil [3]. During this time many pine leaves used as pine-scented essential oil products only while pine leaf is an organic waste containing cellulose that can be used as the basic ingredients of bioethanol manufacture. One handling the abundance of pine leaf waste by converting it into a more environmentally friendly renewable energy. An innovation that has a high selling value of environmentally friendly energy that can be used by the community.

In addition to the pine leaves in this innovation using stems from oyster mushrooms are useful as enzyme activation in the manufacture of bioethanol. Fungus is an organic decomposition agent, especially containing cellulose. Oyster mushroom with a Latin name (Pleuritic stratus) has the ability to decompose cellulose in dead plant tissue [4]. The method used to produce bioethanol is by the method of SSF (Simultaneous Scarification and Fermentation) by converting the polysaccharide compound into monosaccharide compound without changing back to the polysaccharide compound as it passes through the fermentation stage to become ethanol. SSF method becomes very important to be developed because it can shorten the process of making bioethanol [5].

II. FORMULATION OF THE PROBLEM

1. How to use pine leaf waste to convert tobioethanol?
2. What is the fermentation time required to obtain theconditionwhich is optimum with the method of SSF (Simultaneous Scarification andFermentation)?

III. DESTINATION OF THE PROBLEM

1. To know the potential of cellulose contained in

pine leaves.

2. To find out the fermentation time required to obtain Optimum condition with method SSF(Simultaneous Scarification and Fermentation)

IV. BENEFITS OF THE RESEACH

A. For the government

Helping the government to overcome the problem of depletionPetroleum energy resources by creating production innovationsBioethanol with the latest method which is cheaper and affordable by ccommunity.

B. For the community

The community is able to process pine waste (CupressusLusitanica) are abundant by making bioethanol.

C. For academics

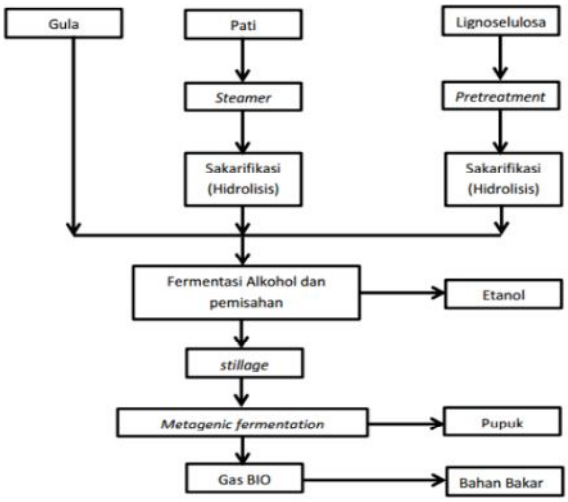
From this research, academics can do researchfurther to look for other alternatives of pine leaf waste treatment(cupressus lusitanica) to get bioethanol.

D. For the author

Being the first step of the author to develop works thatOther than the results of this study are expected to be useful forDevelopment of chemistry related to alternative energy sourcesand increase the use value of pine waste (Cupressus lusitanica).

V. LITERATURE REVIEW

Bioethanol is one of the biofuel that comes as fuelan environmentally friendly and renewable alternative. Bioethanol (C_2H_5OH)is a biochemical fluid from the fermentation process of sugar from carbohydrate sourcesusing the help of microorganisms. Ethanol is widely used as a solvent,germicides, beverages, anti-frozen ingredients, fuels, and intermediates forsynthesis of other organic compounds. Ethanol as a lot of solventused in the pharmaceutical, cosmetics, and resin and laboratory industries.At room temperature ethanol is a clear, volatile liquid, and a distinctive smell [2]. There are two types of bioethanol generation based on its raw materialsfirst generation bioethanol with raw materials used comes from the materialfood-based starch [7] and bioethanol the second generation whose raw materials come from biomass waste [9]. First generation bioethanol has started to be developed in iIndonesia, but this generation bioethanol uses food and feedthe price is still relatively high so that the material-based raw materialsfood will lead to competition between energy needs withfood needs, and extensive land use for food crops the. Therefore, the second generation bioethanol that usesbiomass waste materials. Biomass waste has a considerable amount inIndonesia so that more effective biomass waste should be developed is to be moneyed into second generation bioethanol. At the stagesecond generation bioethanol production consists of smoothing process, treatmentearly (delignification), hydrolysis (scarification), fermentation, and continued processdistillation (purification). Differences in the production process of first generation bioethanol andsecond generation bioethanol can be seen Picture 1.



Picture 1. Stages of bioethanol process based on the raw material [1].

Pine leaves (cupressus lusitanica) are used because they contain substratescellulose is potential for bioethanol production. The carbohydrate is broken downfirst through the process of hydrolysis then in fermentation into alcohol.Yeast or yeast is a mushroom consisting of a single cell, and it is notforming hyphae. Including fungus class (Ascomycotina). Reproduction withform budding (budding). The yeast used in this studyis commercial yeast. These microorganisms are chosen because the commercial yeast issaccharomyces crevice which can produce large amounts of alcoholand has a tolerance to high alcohol levels. Alcohol levels aregenerated at 8-20% under optimum conditions. Yeast tape that is stable,harmless or toxic, easy to obtain and easier insidesugar starch lignocellulosesteamerscarification(Hydrolysis)alcohol fermentation andseparationstillagemutagenic fermentationpretreatmentscarification(Hydrolysis)ethanolfertilizergas bio fuel maintenance. Bacteria are not widely used to produce alcoholscommercial, because the bacteria cannot tolerate high levels of alcohol[10].

Fermentation is the process of energy production in cells in an anaerobic state(Without Oxygen). In general, fermentation is one form of respirationanaerobic however, there is a clearer definition that definesfermentation as respiration in an anaerobic environment with no acceptorexternal electrons [11]. Fermentation of bioethanol canhave defined as the process of decomposition of sugars into bioethanol andcarbon dioxide-induced enzymes

produced by microbial cell masses.The changes that occur during the fermentation process are glucose becomingbioethanol by yeastcells of tape and bread yeast [6].

VI. METHODELOGY

A. Tools Used

The tools used in this research include: A set of glasswarelaboratory, blender, analytical balance, desilator device, 3ml pickonometer,Buchner Vacuum pump. Instruments used include: SpectrophotometerUV-Visible double beam, GC (Gas Chromatography).

B. Materials Used

The materials used in this study include: Pine Leavesfrom Top selfie tourist spots. Aqua, NaOH, NH_3, Buffer pH6, Jones Reagents,Yeast, Ethanol, Ether.

C. Ways of working

1. Preparation process of dipping of pine leaf sample.
   a. Samples (pine leaves) blend and weighed as much as 10 grams.
   b. Samples are inserted into 100ml beaker, by adding50ml NaOH 1% and 50ml NH_3OH 4%
   c. Silence for 72 hours in closed condition
   d. Filtered and taken the residue (sediment)
   e. Washed with aquades (pure of water)

2. The process of preparation of enzymes from oyster mushroom stem waste.
   a. Stem the mushroom cut and in the blender until smooth
   b. Added pH6 citrate buffer inside in blender
   c. Filtered and taken filtrate (cellulose enzyme in the fungus)

3. Bioethanol production process by SSF method.
   a. Samples from soaking results are taken 5 grams
   b. Samples are included in the beaker and mixed with the enzymeCellulose in mushrooms
   c. Added cherry buffer pH6 and plus yeast
   d. Atfermentation with two treatments that is 48 hours and 72 hours

4. Quantitative analysis of ethanol.

Determining the obtained ethanol content used analysis usingUV-Vis double-beam spectrophotometer using Jones reagentwith the comparison of standard solution and heat treatmentfor 30 minutes.

- **Preparation of Jones reagent**
  1. 1.5 gram of $K_2Cr_2O_7$ dissolved in 50 mL $H_2SO_4$5M
  2. The dissolution of $K_2Cr_2O_7$ powder by H_2SO_4 is carried out in ice waterin a waterbath container.
- **Preparation of standard solution**

Made a solution of alcohol and alcohol with aquades with a measureas follows:

Volume larutan induk alkohol 96 % (mL)	Volume akuades (mL)	Konsentrasi larutan standar (%)
0,02	24,98	0,1
0,05	24,95	0,2
0,07	24,93	0,3
0,1	24,9	0,4
0,13	24,87	0,5

5. Qualitative Analysis of Ethanol.

The result of distillation obtained then analyzed the contentThe compound uses GC(Gas Chromatography)with appropriate columns and standard solutionsUsed with a concentration of 0,5%.

VII. DISCUSSION

This research was conducted in laboratory FMIPA UII, with main materialIs a pine leaf. The pine leaves are taken from the pine forest Magelangof (JavaCentral). In the preparation phase of dried pine leaf sample is smoothed upinto a small powder, functioning so that the pores on the leaf surfaceopen. Aim to increase the surface area on the leaves with a solventwill be used so that the cellulose compound is present in the pine obtained maximally. After becoming fine powder then pine leafperformed immersion with NaOH (Sodium Hydroxide)and NH4OH (AmmoniaHydroxide) for 72 hours in a closed state. Aims toremove the lignin that is contained in the pine leaf. Lignin itselfIs a major constituent component of plant cell walls and somealgae, lignin is also associated with cellulose and hemicellulose. The structure of ligninvery complex results in lignin lignocellulose components that are difficult tobroken down. Therefore, the use of both basic solutions is considered effectiveto reduce the lignin contained in pine leaves.



Picture 2. Pine powder soaking result for 72 hours

In this study also used the help of enzymes to helpbioethanol production process. The enzyme is derived from oyster mushroom stems, besidesthe waste turns inside the stem of oyster mushroom

contains a useful enzymein maximizing the fermentation process. The use of oyster mushroom stem enzymestailored to how many samples will be used in the processfermentation. The enzyme is obtained from a mixture of oyster mushroom sticks withSolution of citrate buffer pH 6 with ratio 1: 3, then smoothed thenfiltered and filtrate taken. The filtrate will be blended intobioethanol production process by method SSF (Simultaneous ScarificationFermentation).

The subsequent preparation is the production of bioethanol by the methodof SSF (Simultaneous Scarification Fermentation). Method of SSF (SimultaneousScarification Fermentation) by converting polysaccharides into compoundsmonosaccharide compounds without turning back to polysaccharide compounds becausepassing through the fermentation stage to become ethanol. This method is effective inproducing bioethanol. In bioethanol production process by usingMethod of SSF (Simultaneous Scarification Fermentation) by addingsome materials such as enzymes derived from oyster mushroom filtrate, solutionBuffer pH6 and commercial yeast. In bioethanol production is done twodifferent treatments on the variation of fermentation time is on the fermentation of two-days(48 hours) and three-day fermentation (72 hours) with a sample weight of 5.5 grams andfollowed by an enzyme filtrate ratio of 5 mL, buffer citrate pH 6 withBanner 1: 4 (citric acid solution: sodium citrate solution) and commercial yeast 0,5Gram, stir until blended until well blended, aiming for the processFermentation goes max.

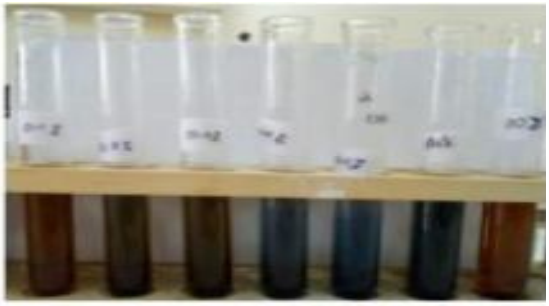


Picture 3. Fermented samples using SSF method (SimultaneousScarification Fermentation)

After the fermentation process is completed, some tests are performedproves that in the sample there is bioethanol. In the first test withusing Jones Reagents is reagents from a mixture of potassium dichromate andsulfuric acid (H_2SO_4) 5 M, with the manufacturing process in a cold state andIt is necessary to apply on a mixture of both solutions. In the jones reagent test, the sample used is the second fermentation sample and the third fermentationIt's just that testing is done different day. By preparing five solutionsstandard (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) diluted ethanolInto several percent concentration variations. Samples for test Jones Reagent divided into two namely the original sample sample and sample of 10 timesdilution. Each standard solution, the original sample and the sample yielded 10 timesdilution added 5 ml of Jones Reagent, then heated for 30Min until green turns indicates the presence of ethanol in the sample.

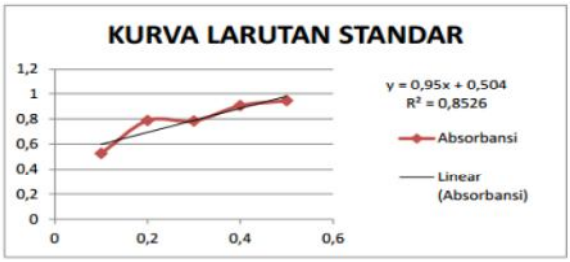


Picture 4. Heating for 30 minutes under boiling water conditions



Picture 5. Samples with jones reagent test

The UV-Visible test is then performed by looking at the second time variationSample, how much bioethanol is obtained by comparingwith a standard solution (0.1%, 0.2%, 0.3%, 0.4%, 0.5%) ethanol solution, it will visible levels of ethanol in the sample.



Graph 1. The standard solution curves of five different concentrations

Test results on UV-Visible showed that the levels obtained at the first bioethanol sample (48 hours fermentation) with absorbance atoriginal sample (1.750 Abs) with a content of 1.72 g/ml. While in the second sample(Fermentation 72 hours) absorbance value of the original sample (2,149Abs) with the concentration of 1.73 g/ml. The curve yield in the standard solution shows the equation y = 0.95X + 0,504With R^2 = 0.8526. Based on the result of UV-Visible sempel showing the levelat most in the

second sample (72 hour fermentation) which is seenIn the following diagram (figure 8)

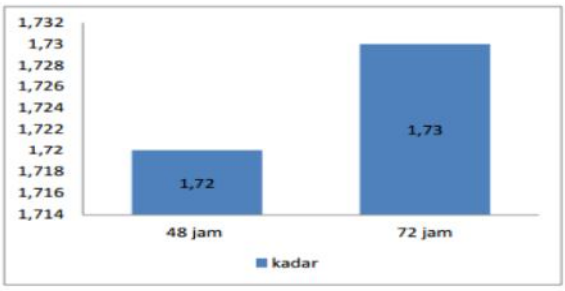


Diagram 8. Ethanol content in pine leaves

The result of the test on GC (Gas Cromathography) shows the qualitative result that in the sample There is an ethanol content. In the first sample (fermentation 48 hours) andThe second sample (fermentation 72 hours) there is ethanol as in the following figure:



Analisys 1. Graph of standard solution of 0.5% ethanol.



Analisys 2. Graph The second 48-hour fermaentation sample.



Analisys 3. Graph The third fermentation sample is 72 hours.

CONCLUSION

That in pine leaves there is a content of cellulose compoundsCan be used as bioethanol, so potentially as a substituteFossil energy. Results show on qualitative test of jonne's reagent and GCIndicating that in both samples there is ethanol, whereasIn the quantitative test shows the best levels in the sampleFermented 72 hours with a content of 1.73 g/ml.

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