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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.

Keywords: Bioethanol, Cellulose, SSF Method, Qualitative and Quantitative Test.

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 (Risdianto et al. 2009). 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 (Ginting, 2013). 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 (Kadarmoidheen, 2012).

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 (Marques, 2006).

Materials and Method

Instrument

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

Materials

The materials used in this study include: Pine Leaves from Top selfie tourist spots. Aqua, NaOH, NH_3, Buffer pH6, Jones Reagents, Yeast, Ethanol, Ether.Water filtration experiment was conducted with a nitrate solution at varied initial concentration by a flow adsorption system using a coloumn filled with ceramic granule. At each adsorption simulation, flow rate was at around 3-5mL/min. The effluent samples were taken from certain volume sequentially and the nitrate concentration in the samples were determined by using colorimetric brucin method using a spectrophotometer.

Procedure

Preparation process of dipping of pine leaf sample.

Samples (*pine leaves*) blend and weighed as much as 10 grams. Samples are inserted into 100ml beaker, by adding 50ml NaOH 1% and 50ml NH_3OH 4%. Silence for 72 hours in closed condition. Filtered and taken the residue (*sediment*). Washed with aquades (*pure of water*). The process of preparation of enzymes from oyster mushroom stem waste. Stem the mushroom cut and in the blender until smooth. Added pH6 citrate buffer inside in blender. Filtered and taken filtrate (*cellulose enzyme in the fungus*)

Bioethanol production process by SSF method.

Samples from soaking results are taken 5 grams. Samples are included in the beaker and mixed with the enzyme Cellulose in mushrooms. Added cherry buffer pH6 and plus yeast. At fermentation with two treatments that is 48 hours and 72 hours

Quantitative analysis of ethanol.

Determining the obtained ethanol content used analysis using UV-Vis double-beam spectrophotometer using Jones reagent with the comparison of standard solution and heat treatment for 30 minutes.

Qualitative Analysis of Ethanol.

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

Results and Discussion

This research was conducted in laboratory FMIPA UII, with main material Is a pine leaf. The pine leaves are taken from the pine forest Magelang of (*Java Central*). In the preparation phase of dried pine leaf sample is smoothed up into a small powder, functioning so that the pores on the leaf surface open. Aim to increase the surface area on the leaves with a solvent will be used so that the cellulose compound is present in the pine obtained maximally.

After becoming fine powder then pine leaf performed immersion with NaOH (Sodium Hydroxide) and NH_4OH (*Ammonia Hydroxide*) for 72 hours in a closed state. Aims to remove the lignin that is contained in the pine leaf. Lignin itself Is a major constituent component of plant cell walls and some algae, lignin is also associated with cellulose and hemicellulose. The structure of lignin very complex results in lignin lignocellulose components that are difficult to broken down. Therefore, the use of both basic solutions is considered effective to reduce the lignin contained in pine leaves.

In this study also used the help of enzymes to help bioethanol production process. The enzyme is derived from oyster mushroom stems, besides the waste turns inside the stem of oyster mushroom contains a useful enzyme in maximizing the fermentation process. The use of oyster mushroom stem enzymes tailored to how many samples will be used in the process fermentation. The enzyme is obtained from a mixture of oyster mushroom sticks with Solution of citrate buffer pH 6 with ratio 1: 3, then smoothed then filtered and filtrate taken. The filtrate will be

blended into bioethanol production process by method SSF (*Simultaneous Scarification Fermentation*).

The subsequent preparation is the production of bioethanol by the method of SSF (Simultaneous Scarification Fermentation). Method of SSF (Simultaneous Scarification Fermentation) by converting polysaccharides into compounds monosaccharide compounds without turning back to polysaccharide compounds because passing through the fermentation stage to become ethanol. This method is effective in producing bioethanol. In bioethanol production process by using Method of SSF (Simultaneous Scarification Fermentation) by adding some materials such as enzymes derived from oyster mushroom filtrate, solution Buffer pH6 and commercial yeast.

In bioethanol production is done two different 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 and followed by an enzyme filtrate ratio of 5 mL, buffer citrate pH 6 with Banner 1: 4 (*citric acid solution: sodium citrate solution*) and commercial yeast 0,5 Gram, stir until blended until well blended, aiming for the process Fermentation goes max.

After the fermentation process is completed, some tests are performed proves that in the sample there is bioethanol. In the first test with using Jones Reagents is reagents from a mixture of potassium dichromate and sulfuric acid (H_2SO_4) 5 M, with the manufacturing process in a cold state and It 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 fermentation It's just that testing is done different day. By preparing five solutions standard (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) diluted ethanol Into several percent concentration variations. Samples for test Jones Reagent divided into two namely the original sample sample and sample of 10 times dilution. Each standard solution, the original sample and the sample yielded 10 times dilution added 5 ml of Jones Reagent, then heated for 30 Min until green turns indicates the presence of ethanol in the sample.

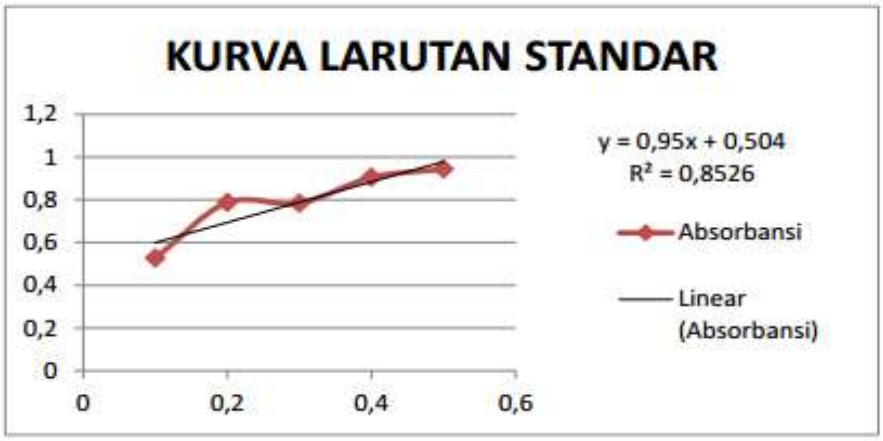

Fig. 1. The curve of standard solution

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

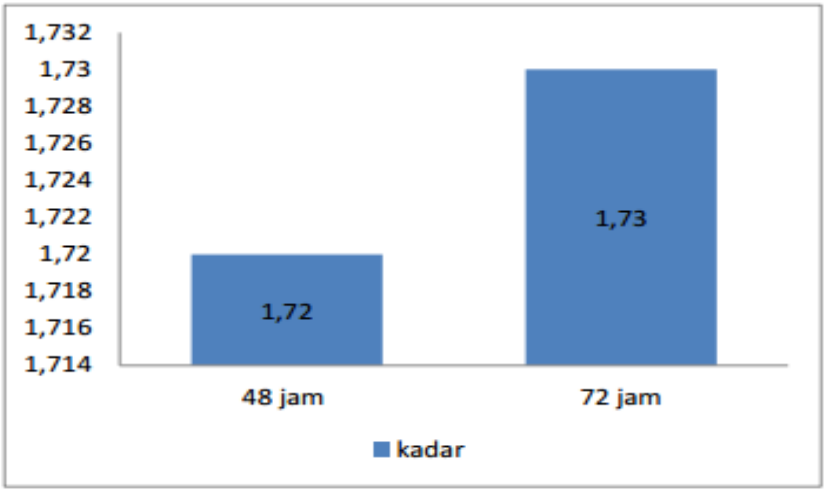

Fig. 2. Diagram of ethanol content in pine leaves

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

level at most in the second sample (72 hour fermentation) which is seen In the following diagram.

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) and The second sample (fermentation 72 hours) there is ethanol as in the following figure:

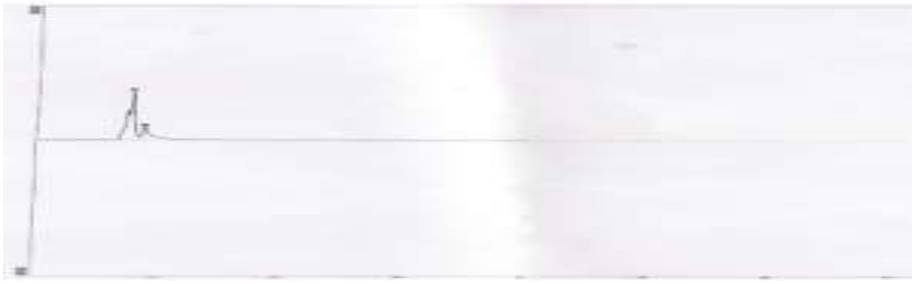

Fig. 3. Graph of standard solution of 0.5% ethanol

Fig. 4. Graph the second 48-hour fermaentation sample

Fig. 5. Graph The third fermentation sample is 72 hours

Conclusion

That in pine leaves there is a content of cellulose compounds Can be used as bioethanol, so potentially as a substitute Fossil energy. Results show on qualitative test of jonne's reagent and GC Indicating that in both samples there is ethanol, whereas In the quantitative test shows the best levels in the sample Fermented 72 hours with a content of 1.73 g/mL.

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