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EFFECT OF PHP PREPROCESSING AND OPTIMIZATION OF ENZYMATIC SACCHARIFICATION ON BIOETHANOL PRODUCTION FROM TEKI GRASS WITH ITS BENEFITS AS SHIP FUEL IN SUPPORTING ENERGY AVAILABILITY FOR SHIP FUEL IN COASTAL AREAS

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Abstract: *The availability of environmentally friendly energy as ship fuel in coastal areas is one of the challenges faced today. One alternative that can be used is bioethanol. This study analyzed the potential of Cyperus rotundus (teki grass) as the main ingredient of bioethanol G2 through preprocess-PHP and optimization of enzymatic saccharification. Preprocess was carried out at two temperatures (40°C and 50°C) with time variations, followed by saccharification and fermentation using Aspergillus niger and Saccharomyces cerevisiae. The results showed that optimal saccharification in preprocessing at 50° C produced 13.66% cellulose content. The best bioethanol content was obtained at a fermentation time of 7 days with a temperature of 38°C. This research contributes to the development of an effective method for producing bioethanol from teki grass, supporting the sustainability of renewable energy while considering environmental aspects.*

Keywords: *Cyperus rotundus, Bioethanol, Preprocess-PHP, Enzymatic Saccharification*

Introduction

Marine transportation is one of the rapidly growing technological sectors. This is demonstrated by the increasing number of passengers and cargo transported by ships each year. The evolution of demand must align with the development and improvement of marine transportation facilities to meet demand and provide optimal service [1]. The availability of environmentally friendly energy as ship fuel in coastal areas is one of the challenges faced today. One solution to overcome the availability of renewable fuels is to find alternative fuels such as bioethanol.

Bioethanol is a plant processing product that can be used as a renewable alternative fuel [2], an important component in the manufacture of bioethanol is glucose, for example, sugar that has glucose content. Producing bioethanol requires tubers, corn, or other plants that contain sugars, carbohydrates, and cellulose [3]. The utilization of biomass for bioethanol production typically involves high cellulose content. In plants, cellulose is bound to lignin, forming lignocellulose, which can inhibit the processing of cellulose. Therefore, preprocessing is necessary to degrade lignin from the cellulose structure by employing chemical processes, such as NaOH treatment.

The research and manufacture of bioethanol by various countries across the developing world has only generally taken place in the last ten years. There is a tendency to choose the use of non-food feedstocks, such as lignocellulosic waste as the main feedstock. This choice is based on the abundance of availability, but not only that, this choice can also avoid competition in the supply of food in a country [4].

Bioethanol production is divided into three generations that distinguish the use of basic materials. The first generation uses raw materials derived from plants rich in glucose, the second generation utilizing the use of plants that have lignocellulose content, and the third generation utilizes microalgae [5]. One of the plants that have lignocellulose content that can be used as a basic material for making bioethanol is Teki grass (*Cyperus rotundus*). Based on research conducted by [6], teki grass contains 20.76% cellulose, 12.93% hemicellulose, and 11.88% lignin. The teki grass content changes after preprocessing, to 30.07% cellulose, 15.87% hemicellulose, and 4.03% lignin. The cellulose content contained in teki grass can be converted into second-generation bioethanol by using cellulose as raw material.

Bioethanol production from lignocellulosic materials involves several serious stages, including preprocessing, hydrolysis, fermentation, and distillation for product separation. Up to currently, there are many evolving preprocessing methods, which are tailored to the type of lignocellulosic primary materials.

Therefore, the objectives of this study were to identify efficient preprocess conditions with *phosphoric acid* and *hydrogen peroxide*, optimize the Enzymatic saccharification process on *Cyperus rotundus*, and improve the efficiency of bioethanol products.

Methodology

1. Teki Grass Substrate Preparation

The collected teki grass is dried under the sun, to reduce or eliminate the water content in the grass. It is crushed and sieved using a 60-mesh sieve to obtain a fine powder. The aim is to simplify the biomass so that it is easy to preprocess using PHP.

2. PHP *Preprocess*

The PHP preprocess in the study utilizes *phosphoric acid* (H_3PO_4) and *hydrogen peroxide* (H_2O_2), removing hemicellulose and lignin efficiently whereas increasing temperature and time can accelerate hemicellulose and lignin removal [7]. According to Qiu, et al (2017), the most optimal conditions for preprocessing are at a temperature of 40°C with a duration of 2 hours and a proportion of H_3PO_4 of 70.2%, and H_2O_2 of 5,2%. PHP solution was put into a 250 ml Erlenmeyer sealed using aluminum foil was stirred for 2 hours with the specified temperature variation. The solid substrate sediment from the *preprocess* was filtered with 96% ethanol 3 times. The substrate that

had been washed with ethanol was stored in a freezer at a temperature of -20°C.

3. *Simultaneous Saccharification Fermentation (SSF)*

A sample weighing 13 g was put into a 250 ml Erlenmeyer flask. The sample was dissolved in 130 ml of distilled water and added Aspergillus Niger 20% of the dry weight of the sample and 15% of the weight of the Saccharomyces cerevisiae sample. Aspergillus niger is a fungus that is used for the production of cellulase enzymes to break down cellulose into glucose. While Saccharomyces cerevisiae converts glucose into bioethanol [8]. Mixed then covered using aluminum foil with a hole the size of a needle. Erlenmeyer is placed on a magnetic stirrer set at a temperature variation of 32° C, 35° C, and 38° C. The fermentation process is carried out by conditioning the environment to suit the conditions of the temperature variables. The fermentation process lasts for 7 days.

4. Separation

The fermentation results from each sample variation are filtered to separate the fermentation process [9].

Results and Discussions

1. Analysis of preprocessing temperature variation results

This research uses PHP preprocess with temperature variation. The higher the cellulose content obtained from the preprocess determines the level of bioethanol produced. PHP preprocess produces cellulose, hemicellulose, and lignin content, as seen in Table 1.

Table 1. Analysis of preprocessing temperature variation results

Content (%)	Temperature 40° C	Temperature 50° C
Cellulose	4.09	13.66
Hemicellulose	9.58	5.32
Lignin	6.74	11.45

Table 1 states that the cellulose content at 40° C was 4.09% and 50° C was 13.66%. The hemicellulose content decomposes a lot at a temperature variation of 50° C, leaving a hemicellulose content of 5.32%. Increasing the preprocess temperature is useful for removing hemicellulose and cellulose is not too affected by the preprocess temperature because along with the increase in preprocess temperature the cellulose content also increases. Less hemicellulose content results in more efficient enzymatic hydrolysis [7]. Low lignin content was obtained at a preprocess temperature of 40° C with a content of 6.74%. At a temperature of 50° C, a high lignin content of 11.45% was still obtained. High lignin content is usually caused by binding with cellulose and hemicellulose so it is difficult to decompose in the rumen [10].

2. Results of Simultaneous Saccharification Fermentation (SSF) Temperature Analysis of Teki Grass Bioethanol

In testing the analysis of bioethanol content with temperature variations displayed in

qualitative and quantitative data. In qualitative testing using FTIR and quantitative using GC.

The FTIR test is shown in Figure 1 and the GC test in Figure 2.

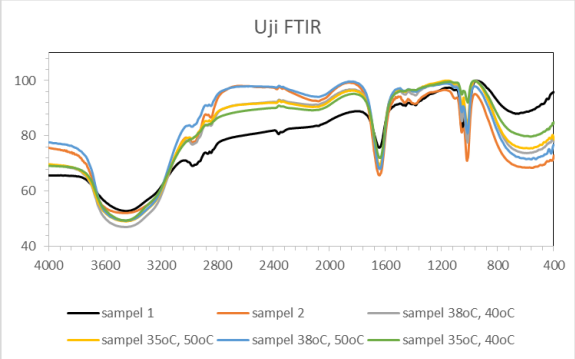

Figure 1. Graph of FTIR analysis results

Figure 1, Fermented samples that have been distilled are analyzed using FTIR (Fourier Transform Infrared Spectroscopy). The analysis result on all 6 samples showed the presence of absorption at specific wave numbers for ethanol. Samples 1-6 have wavelengths in the range of 3448.72–3452.58 cm. According to the statement [11], the O-H structure bond absorbs at a wavelength between 3230-3550 cm^{-1} In research [12], ethanol spectra, the absorption of the fall - OH is found at a wavelength of 3622 cm^{-1} From the data and statements above, it can be concluded that each variation contains ethanol content, and it can be observed that the highest OH group absorption value is obtained in the sample fermentation variation of 38°C, with 40°C preprocess.

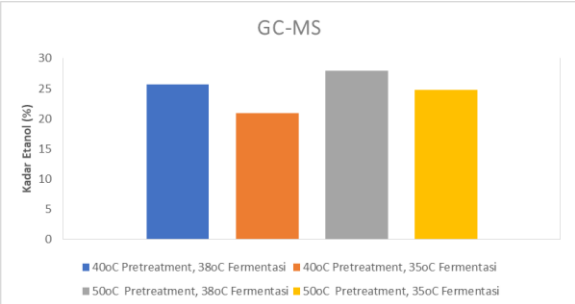

Figure 2. Graph of GC analysis results

Figure 5 shows the best ethanol results obtained in the GC test, temperature variation 38°C fermentation of 50°C preprocess substrate, which produces ethanol content of 27.92093%. The higher the cellulose content of the substrate used, the higher the level of bioethanol produced [13]. The longer the fermentation time, the higher the ethanol content produced [14]

The GC (Gas Chromatography) test performed only presented data temperature variations 35°C and 38°C This is because the sample temperature variation of 32°C produced little bioethanol so it can only be used for the FTIR test. In addition, other statements support that the temperature of 32°C produces low levels of bioethanol. A temperature of 32°C is a temperature that is less than optimal for the fungus *Aspergillus niger* can grow and develop. *Aspergillus niger* has an optimum temperature range of growth between 35 - 37°C [15]. From this statement, it can be concluded that a temperature of 32°C is less optimal for bioethanol production.

Conclusion

Based on research on preprocess-PHP and SSF of teki grass (Cyperus rotundus) for bioethanol production, it can be concluded that preprocessing affects increasing cellulose content. The higher the preprocess temperature, the higher the cellulose content in the teki grass substrate. Preprocess 40° C has a content of 4.09% cellulose, 9.58% hemicellulose, and 6.74% lignin. At a preprocess temperature of 50° C teki grass, 13.66% cellulose, 5.32% hemicellulose, and

11.45% lignin were obtained. The preprocess temperature variation of 50° C resulted in the best increase in cellulose content. The temperature of simultaneous saccharification and fermentation (SSF) affects the production of bioethanol from teki grass. The higher the fermentation temperature, the higher the bioethanol content produced. Among the 3 temperature variations, the best bioethanol content was obtained at a temperature variation of 38° C with a preprocess of 50° C giving the highest bioethanol content, with a bioethanol content of 27.92093%.

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