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The effect of time and temperature variations on the hydrolysis of *sargassum muticum* using microwave irradiation in the synthesis of bioethanol as renewable energy

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Abstract

Consumption of fuel oil and the increasing population are increasing daily. The high level of energy consumption results in the oil supply reserves in the earth's bowels being depleted and will run out for the next few years. Bioethanol is ideal for replacing fossil energy because it has renewable, environmentally friendly, and renewable properties. Sargassum muticum can be converted into bioethanol because it contains monosaccharide carbohydrates such as glucose, galactose, and mannose and polysaccharides such as xylan, galactan, and mannan. The carbohydrate content of Sargassum muticum can be converted to bioethanol through a chemical hydrolysis process using a 3 % sulfuric acid catalyst and fermented with the help of Saccharomyces cerevisiae yeast with a 10 % inoculum for 6 days. The reducing sugar obtained from the hydrolysis process was analyzed by the DNS method using a UV-Vis spectrophotometer. Ethanol levels were analyzed qualitatively using potassium dichromate and quantitatively using a hand refractometer. The conversion of Sargassum muticum resulted in a reducing sugar content of 92.90 g/L at a temperature of 250 °C and a hydrolysis time of 60 minutes. The bioethanol content obtained from the fermentation of the hydrolyzed glucose was 42.32 %.

Keywords: bioethanol, fermentation, hydrolysis, renewable energy, sargassum muticum.

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1. INTRODUCTION

In recent years, concerns over energy scarcity have become a hot topic of discussion. The root of the problem lies in the depletion of oil reserves, which cannot keep up with human consumption. This is due to the energy-intensive industrial sector, which consumes 3.691.993 terajoules (53.4 %), and the increasing population, with Indonesia reaching 255,461,686 million people. Households consume 1.554.160 terajoules (22.5 %) of energy, while the transportation sector consumes 1.263.435

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terajoules (18.3 %) (BPS Statistic Indonesia, 2023). The data rapidly indicates the emergence of environmental problems and energy resource scarcity (Zhang *et al*., 2023). The continuous utilization of fossil fuels hurts climate change, such as increasing greenhouse gas emissions and CO_2 emissions (Polikovsky *et al*., 2020). To address the issue of energy scarcity and the negative impacts caused by the use of fossil fuels, we need to shift towards renewable energy sources that are environmentally friendly and have abundant raw materials, such as bioethanol.

Bioethanol is a potential alternative fuel to replace fossil fuels. Bioethanol can be produced from various renewable sources, such as carbohydrate-containing plants (Zabed *et al*., 2017). Bioethanol is an alternative fuel with a high-octane number (108). This high octane number can increase the compression ratio and combustion, which can contribute to reducing CO_2 emissions in engines (Manmai *et al*., 2020).. Bioethanol is generally produced from biomass such as crop residues, wood, grass, and wheat (Nahak et al., 2024). However, these raw materials are still competing with the food and feed sectors. One biomass that is considered not to compete with food and feed is marine biomass such as seaweed.

Seaweed has garnered significant attention in recent years. Its intrinsic properties, such as rapid growth, high biomass productivity, and ease of cultivation without requiring land, have made it an attractive commodity. The abundant carbohydrate content in seaweed makes it highly suitable as a raw material in the production of biofuels, including ethanol, hydrogen, and butanol (Río et al., 2019). Global data shows that the volume of seaweed and aquatic plants reached 30.4 million tons in 2015. Seaweed growth in Indonesia in 2014 reached 10.2 million tons. Seaweed is a rich source of carbohydrates, with carbohydrate content ranging from 20-72 %. The carbohydrate content in seaweed consists of various types, including hydrocolloids, hemicellulose, and cellulose. The carbohydrate content varies depending on the type of seaweed (Hakim *et al*., 2017). This high carbohydrate content has the potential to be converted into bioethanol. One potential feedstock for bioethanol conversion is *Sargassum muticum.*

Sargassum muticum is highly suitable for bioethanol conversion due to its carbohydrate content, which includes monosaccharides such as glucose, galactose, and mannose, as well as polysaccharides like xylan, galactan, and mannan(Hakim *et al*., 2017). According to Pandey et al. (2020), brown seaweed Sargassum muticum contains 17.06 % carbohydrates. The high carbohydrate content of Sargassum muticum can be converted into bioethanol through hydrolysis, fermentation, and purification (distillation) processes. The use of microwaves in the hydrolysis process is an efficient method. Microwaves heat evenly, making it more energy-efficient than conventional heating, which only heats the surface. This results in shorter heating times, better performance, and greater flexibility in starting and stopping the process (Mikulski *and* Kłosowski, 2020).

To the best of the authors' knowledge, the first study on bioethanol production from *Sargassum muticum* was conducted by Rio *et al*., (2019), using enzymatic hydrolysis (CellicCTec2, Viscozyme) and simultaneous fermentation with Saccharomyces

cerevisiae. The study yielded 8.35 g/L of sugar and 68 % ethanol. Chemical production of bioethanol using acid catalysts has not been previously investigated. Therefore, this study will produce bioethanol from *Sargassum muticum* chemically using 3 % sulfuric acid (H_2SO_4) catalyst with variations in time and temperature of microwave irradiation during the hydrolysis process. The objective of this research is to determine the optimal time and temperature for the hydrolysis of *Sargassum muticum* and to determine the resulting bioethanol content, which will be analyzed using a hand refractometer and GC-FID

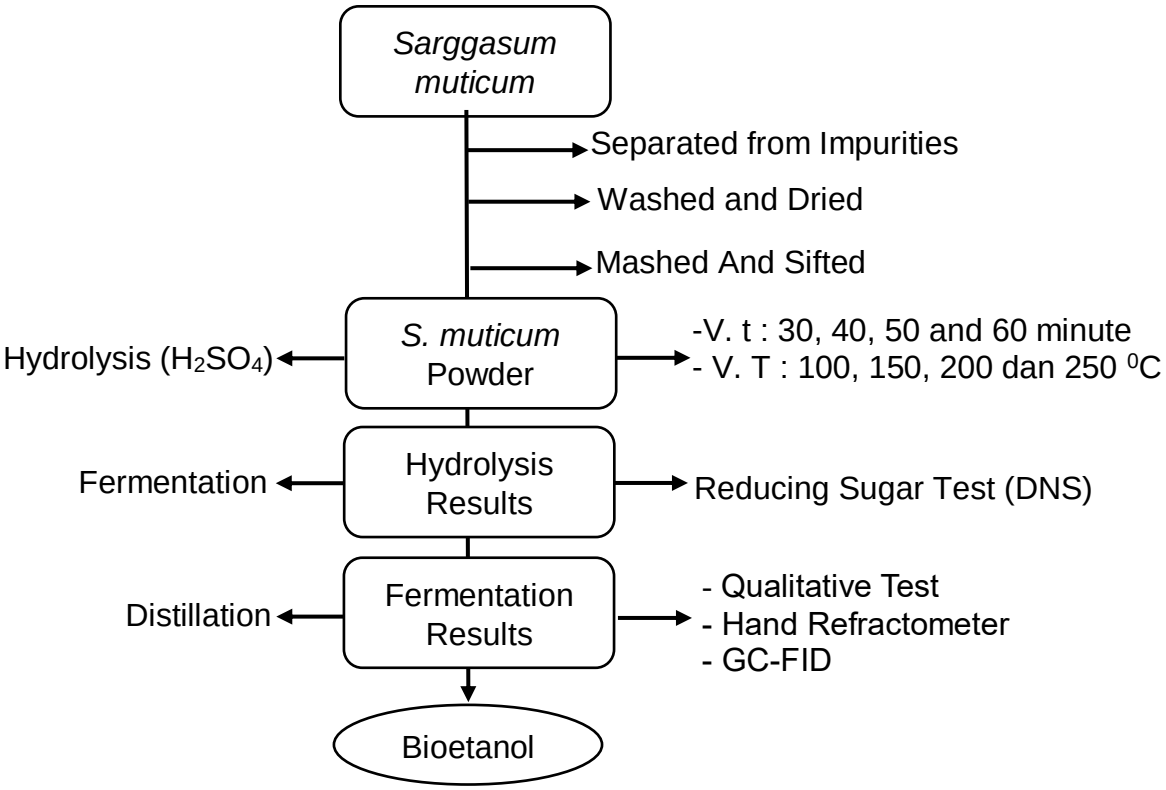
2. MATERIALS AND METHODS

Bioethanol production from *Sargassum muticum* includes preparing raw materials, hydrolysis, fermentation, and distillation. Reducing sugar analysis was also carried out using a UV-Vis spectrophotometer, bioethanol content analysis using a hand refractometer, and gas chromatography.

The materials used in this research are Sargassum muticum samples, H_2SO_4, NaOH, distilled water, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4.7H_2O$, yeast extract, glucose, Saccharomyces cerevisiae yeast, tissue, wrapping seal, label paper, Erlenmeyer, spatula, beaker, stir bar, measuring flask, funnel, blender, falcon bottle, analytical balance, hot plate, shaker, microwave, vacuum pump, blender, 35 mesh sieve, autoclave, vacuum pump, UV spectrophotometer -Vis, a series of distillation equipment, hand refractometers and gas chromatographs.

2.1 General Research Methods

The synthesis of bioethanol from *Sargassum muticum* involves several stages, as illustrated in the following flowchart:



2.2 Sample Preparation

Sargassum muticum is collected from the white sand beach of Atapupu Regency of Belu, washed to remove impurities such as sand, and dried for ± 3 days. The dried Sargassum muticum is then ground using a blender and sieved using a 35-mesh sieve to obtain *Sargassum muticum* powder (Bria & Kolo, 2024).

2.3 Hydrolysis of *Sargassum muticum*

10 grams of *Sargassum muticum* powder is suspended in a 3 % sulfuric acid solution. The mixture is then heated in a microwave oven at a temperature of 150 °C for 30, 40, 50, or 60 minutes. The optimum time is then used for variations in heating temperature of 100, 150, 200, or 250 °C. The heated mixture is then filtered to obtain the hydrolysate filtrate (Kolo et al., 2021).

2.4 Fermentation of *Sargassum muticum*

The *Sargassum muticum* hydrolysate is adjusted to a pH of 4.5 and then measured to 300 mL. Fermentation media is then added, consisting of (glucose 10 g/L, yeast extract 0.1 g/L, KH_2PO_4 0.1 g/L, $MgSO_4.7H_2O$ 0.1 g/L, and $(NH_4)_2SO_4$ 0.1 g/L). The solution is then sterilized using an autoclave for 15 minutes at a temperature of 121 °C. It is then cooled and yeast Saccharomyces cerevisiae is added with an inoculum concentration of 10 %. The mixture is then incubated for 6 days (Kolo et al., 2022).

2.5 Distillation

In this study, the distillation apparatus was developed from the research of Bria and Kolo, (2023) which is a multi-stage distillation. After the fermentation process is complete, the next step is the distillation process to obtain bioethanol that is contained in Sargassum muticum (Rahmawati, 2018).

2.6 Reducing Sugar Content Test (UV-Vis)

1.5 mL of *Sargassum muticum* hydrolysis is taken, added with 1.5 mL of DNS reagent, and then heated in a water bath for 10 minutes. The heating results were then tested for reducing sugar content using a UV-Vis spectrophotometer at a wavelength of 450 nm (Agustini and Febrian, 2019).

2.7 Qualitative Test of Biotanol ($K_2Cr_2O_7$)

Two test tubes were prepared and 1 mL of 2 % potassium dichromate was added to each test tube. Each test tube was then added with 5 drops of concentrated sulfuric acid and shaken. 1 mL of standard ethanol (96 % ethanol) and bioethanol from distillation was added to each test tube and vortexed until a color change occurred. The presence of bioethanol was indicated by a color change from orange to greenish-blue (Bria & Kolo, 2023).

2.8 Semi-Quantitative Bioethanol Test (Hand Refractometer)

± 3 drops of distilled bioethanol are taken, then dropped onto the prism of a hand refractometer, and then directed towards a light to measure the concentration of bioethanol (Febriyanti et al., 2022).

2.9 Quantitative Analysis of Bioethanol

Analysis of bioethanol content using GC-FID, samples made with unknown concentrations, and standard solutions with known concentrations. The equipment is run under maximum conditions of 200 ^{0}C and the detector type is FID (Flame Ionization Detector). The nanometer pressure in the tube was measured at 3.5 kg/m. The velocity of the gas carrier (Helium) to the right or the left was measured at 300 mL/minute. Inject a standard solution of at least 1 µL of ethanol and make a chromatogram. The ethanol peak appears on the chromatogram (recording device) (Nggai et al., 2022).

3. RESULTS AND DISCUSSION

3.1 Hydrolysis and Analysis of Reducing Sugar Content (UV-Vis)

Sargassum muticum powder was hydrolyzed chemically using a 3 % sulfuric acid catalyst. The use of sulfuric acid catalyst aims to accelerate the process of cleavage of glycosidic bonds in cellulose into simpler sugar monomers. The use of low-acid catalysts aims to minimize environmental pollution. The result of hydrolysis was then analyzed for reducing sugar content using a DNS reagent. According to Kolo et al., (2023), the presence of reducing sugar in hydrolysis is marked by a change in the color of the hydrolysis solution from yellow to orange-red. The color change occurs due to the change of compounds from 3,5-dinitrosalisilic acid 3-amino in DNS reagent to 3-amino, 5-nitrosalisilic acid (**Figure 1**) (Agustini and Febrian, 2019). The more 3-amino, 5-nitrosalisilic acid compounds, the higher the absorbance value obtained from the UV-Vis spectrophotometer. The higher the absorbance, the higher the reduced sugar content produced.

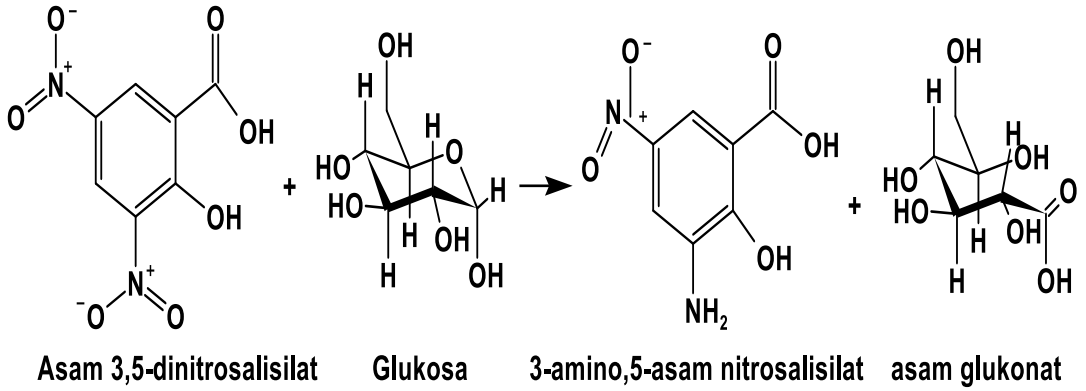


Figure 1. Reaction Mechanism Between DNS And Reducing Sugars (Kolo et al., 2022).

The results of measuring the reducing sugar content in the hydrolysis of Sargassum muticum with variations in Time and Hydrolysis Temperature can be seen in **Table 1**.

Table 1. Results Of Analysis Of Reducing Sugar Content (Time And Temperature Variations).

Time (Minutes)	Sugar level (g/L)	Temperature (^{0}C)	Sugar level (g/L)
30	63,63	100	44,50
40	68,50	150	46,90
50	72,10	200	47,50
60	**72,63**	250	**92,90**

From **Table 1** above, it can be seen that the longer the hydrolysis time and the higher the temperature used in the hydrolysis process, the higher the sugar content produced. This is because the longer and the higher the temperature used in the hydrolysis process, the more glycosidic bonds will be broken by H^+ ions, resulting in a higher sugar content (Herdini et al., 2020). This is supported by the change in the color of the hydrolyzate added with the DNS reagent, where the longer the time and the higher the temperature, the darker the color produced **(Figure 2).**

Figure 2. Hydrolyzate After Adding DNS Reagent.

3.2 Bioethanol Analysis

Bioethanol obtained from the fermentation process is first purified using a fractional distillation method to separate bioethanol from other impurities that are still mixed with ethanol such as water, yeast sediment, and acid catalyst. In this study, bioethanol obtained from the distillation process was then analysis qualitatively using $K_2Cr_2O_7$, semi-quantitative analysis using a hand refractometer, and quantitative analysis using gas chromatography.

3.3 Qualitative Analysis of Ethanol

The purpose of the qualitative analysis of ethanol is to determine the presence of ethanol from the fermentation results based on the color change. The color change can be seen in **Figure 3**.

Figure 3. Qualitative Test Results Of Biotanol

According to Nggai et al., (2022), the change in color from orange to blue is due to the oxidation of primary alcohol to aldehyde by potassium dichromate ($K_2Cr_2O_7$) solution. With this color change, it can be concluded that the ethanol obtained from the *Sargassum muticum* fermentation process contains bioethanol. According to Kolo et al., (2021), the change in color from orange to blue is obtained from the decrease in the bilogs value of Cr^{6+} to Cr^{3+}. The reaction to the decrease in blog value according to Nggai et al., (2022) is as follows:

$$3CH_3CH_2OH + Cr_2O_7^{2-} + 8H^+ \rightarrow 3CH_3CHO + 2Cr^{3+} + 7H_2O$$

Orange Blue

3.4 Semi-Quantitative Bioethanol Analysis Results (Hand Refractometer)

Bioethanol obtained from fermentation was purified using the distillation method and then analyzed using a hand refractometer to determine the ethanol concentration. The results of the ethanol analysis are shown in **Figure 4.**

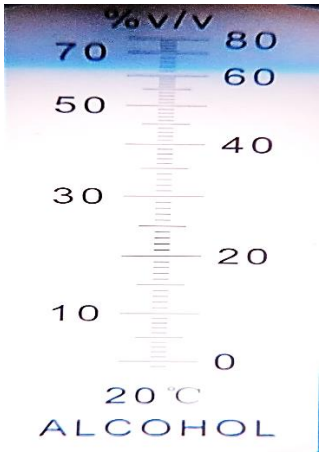


Figure 4. Results of Semi-Quantitative Analysis of Bioethanol Using a Hand Refractometer

Based on Figure 5 above, shows that the bioethanol concentration from the fermentation of *Sargassum muticum* reached 62 % (v/v) as seen from the color boundary of the light in the figure. The refractometer works by using white light, where the white light will be scattered when passing through the optics and the alcohol concentration will be automatically read. The bioethanol obtained from this study is lower than the study conducted by Rio et al., (2019) which was conducted simultaneously (SSF) and obtained an ethanol yield of 91.9 %. The bioethanol from this study is higher than the study conducted by Sari et al., (2020) which analyzed bioethanol using a hand refractometer. In this study, the ethanol obtained from the fermentation of cherry and pineapple peel was 13 %. In addition, the ethanol from this study is higher than the study conducted by Ramadhani et al., (2020) which produced bioethanol from red dragon fruit, which obtained an ethanol content of 26 %.

3.5 Results of Quantitative Analysis of Biotanol (GC)

In addition to semi-quantitative analysis using a hand refractometer, the distilled bioethanol was also analyzed quantitatively using gas chromatography (GC) to determine the concentration of bioethanol from the fermentation of Sargassum muticum that was purified using a distillation apparatus. Bioethanol concentration data analyzed using GC is presented in **Table 2**.

Table 2. Results Of Quantitative Analysis Of Bioethanol (GC)

Incubation Time (Days)	Inoculum Concentration (%)	Bioethanol Concentration (%)
6	10	42,32

Based on the data presented in Table 2, it can be seen that the concentration of bioethanol obtained is 42.32 %. The concentration of bioethanol obtained from the GC analysis is lower than the concentration of bioethanol obtained from the semi-quantitative analysis using a hand refractometer. This is because the two instruments

used in the analysis have different working principles. In addition, the analysis time using GC is longer after the distillation process because it must be sent to the laboratory that performs the analysis, so the concentration of bioethanol decreases. This is due to the volatile nature of bioethanol, which can evaporate if stored for a long time after distillation, thus reducing its concentration. In addition, it is suspected that the bioethanol obtained was further converted into acetic acid. However, the concentration of bioethanol obtained in this study is still relatively high compared to previous studies on bioethanol that were analyzed using GC, such as the studies conducted by Kolo et al., (2023).

4. CONCLUSION

From the results of the research carried out, the following conclusions can be drawn: The conversion of Sargassum muticum resulted in a reducing sugar content of 92.90 g/L at a temperature of 250 °C and a hydrolysis time of 60 minutes. The bioethanol content obtained from the fermentation of the hydrolyzed glucose was 42.32 %. It is expected that the research results can be developed continuously to address the energy crisis in Indonesia

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