JURNAL ILMIAH BERKALA SAINS DAN TERAPAN KIMIA

Jurnal Ilmiah Berkala: Sains dan Terapan Kimia
Vol. 16 No. 1, Januari 2022

The Effect of Hydrolysis Time Using Microwave on Bioethanol Production from Sorghum Waste (*Sorghum Bicolor L.*)

Pengaruh Waktu Hidrolisis Menggunakan *Microwave* Terhadap Produksi Bioetanol Dari Ampas Sorgum (*Sorghum Bicolor* L.)

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ABSTRACT

In this study, the production of bioethanol from sorghum bagasse was carried out to understand the surface morphology of sorghum bagasse before and after hydrolysis, the effect of hydrolysis time using a microwave, and the concentration of inoculum on the ethanol content produced. A total of 10 grams of sorghum bagasse was suspended with 250 mL of 2% H2SO4 solution and then heated using a microwave at a temperature of 150ºC with variations in heating time of 20, 30,40, and 50 minutes. The heated hydrolyzate was analyzed for reducing sugar content using UV-Vis. The morphology of sorghum bagasse before and after hydrolysis was analyzed using SEM and quantitative analysis of fermented ethanol using a pycnometer and GC. The results showed that there were differences in the surface of sorghum bagasse before and after hydrolysis, the highest reducing sugar content was obtained at 30 minutes of hydrolysis, which was 30.4 g/L, and the highest concentration of Saccharomyces cerevisiae was 8% which resulted in 5.325% ethanol content using a pycnometer and 9.05% using GC.

***Keywords**:* Sorghum bagasse, Hydrolysis, Microwave, Bioethanol

ABSTRAK

Pada penelitian ini dilakukan produksi bioetanol dari ampas sorgum dengan tujuan untuk mengetahui morfologi permukaan ampas sorgum sebelum dan sesudah hidrolisis, pengaruh waktu hidrolisis menggunakan microwave dan konsentrasi inokulum terhadap kadar etanol yang dihasilkan. Sebanyak 10 gr ampas sorgum disuspensi dengan 250 mL larutan H_2SO_4 2% lalu dipanaskan menggunakan microwave pada suhu 150 ºC dengan variasi waktu pemanasan 20, 30, 40 dan 50 menit. Hidrolisat hasil pemanasan dianalisis kadar gula pereduksi menggunakan UV-Vis, morfologi ampas sorgum sebelum dan sesudah hidrolisis dianalisis menggunakan SEM dan analisis kuantitatif etanol hasil fermentasi menggunakan piknometer dan GC. Hasil penelitian menunjukkan adanya perbedaan pada permukaan ampas sorgum sebelum dan sesudah hidrolisis, kadar gula pereduksi tertinggi diperoleh pada waktu hidrolisis 30 menit yaitu sebesar 30,4 g/L dan konsentrasi tertinggi saccharomyces cerevisiae adalah 8% yang menghasilkan kadar etanol sebesar 5,325% menggunakan piknometer dan 9,05% menggunakan GC.

Kata Kunci: *Ampas sorgum, Hidrolisis, Microwave, Bioetanol*

Submitted: August 23, 2021; **Accepted**: February 18, 2022: **Available online**: March 8, 2022

P-ISSN: 1411-1616, E-ISSN: 2549-8215

1. INTRODUCTION

The current energy crisis is caused by dependence on fossil fuels which are non-renewable sources. Therefore, it is critical to developing new alternative energy, namely bioethanol. Bioethanol can be produced from starch- or carbohydrate-containing plants such as corn (Zea mays L.), sweet sorghum (Sorghum bicolor L.), sugarcane (Saccharum officinarum L.), cassava (Manihot esculenta C.), and sugar beet (Beta vulgaris) (Ray et al., 2019). However, the current bioethanol production is from starch derived from food. It may have a detrimental effect on food supply due to the conflict between food and energy. To address this issue, it is required to perform research using non-food raw materials, including sorghum bagasse. Sorghum pulp has 72.05 % carbohydrates, making it suitable for bioethanol production (Arif et al., 2018). The redevelopment of the sorghum plant is deemed necessary because apart from being used as food, this plant can also be used as an energy source.

In general, there are three stages in producing bioethanol from starch, namely hydrolysis, fermentation, and distillation. Hydrolysis is one of the critical processes in bioethanol production. This process uses acids or enzymes catalyst (Rani et al., 2019). Various studies on bioethanol using the acid hydrolysis method have been carried out. One of them was by Kolo et al. (2020), who reported that bioethanol concentration from elephant grass with microwave irradiation at the optimum concentration of 2% sulfuric acid (H2SO4) with a hydrolysis time of 30 minutes and a temperature of 90°C was 10.79 g/L. One factor affecting the bioethanol concentration is the amount of reducing sugar produced from the hydrolysis process. Kolo et al. (2021) found that under the optimum conditions of dilute acid hydrolysis using microwave irradiation was 150°C, 50 minutes, the concentration of reducing sugar was 33.41 g/L, and the hydrolysis efficiency reached 83.52% (v/v). Research by Ahmed El-Imam et al. (2019) found that the glucose level from the hydrolysis of sorghum pulp using a 3% H_2SO_4 concentration was 34.53 g/L.

In this study, sorghum bagasse taken from North Central Timor Regency (TTU) was converted into bioethanol through a hydrolysis process using dilute acid. Before and after the hydrolysis process, the carbohydrate texture was analyzed by SEM (Scanning Electron Microscopy). Furthermore, the reducing sugar content was analyzed using UV-Vis Spectro, and the bioethanol content was measured using a pycnometer and GC (Gas Chromatography). This study aimed to study the surface morphology of sorghum bagasse before and after hydrolysis to determine the optimum hydrolysis time and the highest bioethanol content of sorghum bagasse based on variations in inoculum concentration.

2. MATERIALS AND METHODS

2.1. Materials

The materials used were sorghum taken from North Central Timor (TTU) Regency, yeast, pure isolate of *Saccharomyces cerevisiae* taken from Gadjah Mada University Yogyakarta, NaOH (Merck), H_2SO_4 (Merck), aluminum foil, wrapping, tissue, cotton, glucose (pro analysis), distilled water, alcohol, PDA media, inoculum medium (Glucose 10 g/L (pro analysis), yeast extract 0.1 g/L (pro analysis), KH_2PO_4 0.1 g/L (pro analysis), $MgSO_4.7H_2O$ 0.1 g/L (pro analysis), $(NH_4)_2SO_4$, 0.1 g/L (pro analysis), and fermentation media. The tools used were petri dish, beaker, Erlenmeyer flask, graduated cylinder, funnel, stirrer, test tube, and dropper, pH meter analytical balance, oven, GC instrument, UV-Vis, microwave, SEM, magnetic stirrer, cuvette, wire loop, hot plate, autoclave, thermometer, and distillation apparatus.

2.2. Sample preparation

The sorghum seeds were dried and ground. Then the pulp was taken and sieved using a 100 mesh sieve to obtain uniform size. Milling and sifting aimed to expand the surface of sorghum to facilitate the hydrolysis process.

2.3. Hydrolysis

A total of 10 grams of sorghum powder with a size of 100 mesh was suspended with 250 ml of 2% H_2SO_4 solution and then heated using a microwave at 150°C with variations in time (20, 30, 40, and 50 minutes). The heating results were filtered and neutralized using a 2% NaOH solution, then analyzed for glucose content using UV-Vis at a wavelength of 540 nm. The surface of the hydrolyzed powder was analyzed using SEM (Scanning Electron Microscopy).

2.4. Stock breeding and rejuvenation of Saccharomyces cerevisiae

S. cerevisiae breeding stock and rejuvenation were carried out by inoculating *Saccharomyces cerevisiae* in 250 ml of PDA media (9.75 g of PDA) and incubated for 48 hours. Furthermore, the rejuvenated *S. cerevisiae* was inoculated in 50 mL of inoculum media (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 in an Erlenmeyer, then incubated for 48 hours using an orbital shaker at 120 rpm (Samsuri et al., 2007).

2.5. Preparation of fermentation media

The fermentation medium was prepared by mixing 10 g/L glucose, 0.1 g/L yeast extract, 0.1 g/L KH_2PO_4, 0.1 g/L $MgSO_4.7H_2O$, and 0.1 g/L $(NH_4)_2SO_4$ in 50 mL aquadest. It was then sterilized using an autoclave at 121°C for 30 minutes.

2.6. Bioethanol production

Fermentation with yeast *Saccharomyces cerevisiae* used variations in inoculum concentrations of 4, 6, and 8%. A total of 100 ml of the hydrolyzate was put into an Erlenmeyer and neutralized using 2% NaOH. The fermentation medium was added and sterilized at 121°C for 30 minutes. Then it was cooled, and the inoculum medium was added, then covered with cotton and aluminum foil. Fermentation was carried out anaerobically for 7 days.

2.7. Destilation

The sample to be distilled was put into a distillation flask and heated. Observations were made on the time and temperature of the product's boiling point that dripped from the first to the end. The volume of the distillate obtained was measured using a graduated cylinder. Density was measured using a pycnometer and ethanol content using GC.

2.8. Characterization and analysis

A. Texture analysis of powder surface

The surface texture of sorghum powder was analyzed using SEM before and after hydrolysis. The sorghum bagasse before and after hydrolysis were put into the SEM chamber for position setting and image recording. SEM images were taken at 800x magnification.

B. Analysis of reducing sugar

The reducing sugar content of the hydrolyzed sorghum pulp was analyzed using the DNS (Dinitrosalicylate) method. A total of 1.5 ml of standard glucose solution with concentrations of 1000, 2000, 3000, 4000, 5000 ppm was put into a test tube, and then 1.75 ml of DNS reagent was added. The standard solution and the sample were put in a 100°C water bath for 20 minutes and allowed to stand until room temperature. The absorbance was measured at a wavelength of 540 nm. The hydrolysis efficiency (EH) is calculated using the following equation:

$$\text{EH (\%)} = \frac{[Glucose]gr/L}{[Biomass]gr/L} \times 100\%$$

C. Analysis of ethanol concentration

1. Qualitative analysis of ethanol

Distilled ethanol was added with 2 mL of 2% $K_2Cr_2O_7$ and 5 drops of concentrated H_2SO_4. Then the solution was homogenized and added with 1 mL of distillate until the color changed from orange to greenish.

b. Quantitative Analysis of Ethanol

Quantitative analysis was carried out in two ways, namely:

a. The pycnometer with known volume was activated in the oven for 10 minutes at 105°C and then cooled. Then the empty pycnometer was weighed, and the weight was recorded. Water was added to the pycnometer until it was filled and then weighed. The same procedure was carried out by replacing the distilled water with the sample (Saleh et al., 2016). The following equation calculates the density of ethanol:

$$\rho_1 = \frac{W1}{W2} \times \rho 2$$

description:

W_1=weight of ethanol

W_2=weight of aquades

ρ_1 = density of the sample

ρ_2 = density of the sample

Density obtained from the measurement using a pycnometer was then used to calculate the ethanol concentration, yield (Y), fermentation efficiency (EF), and conversion efficiency (EK) using the following equation:

$$Y\ (\%) = \frac{ethanol\ concentration\ (g/L)}{sugar\ concentration\ (g/L)} x100$$

$$EF\ (\%) = \frac{ethanol\ concentration\ (g/L)}{0{,}51\ x\ sugar\ concentration (g/L)} x100$$

$$EK\ (\%) = \frac{ethanol\ concentration\ (g/L)}{biomassa\ (g/L)} x100$$

b. Analysis of Ethanol Concentration Using Gas Chromatography (GC)

The concentration of fermented ethanol was analyzed using GC. A total of 1 µL of sample solution was injected into the column. The area of the ethanol peak from the chromatogram was then calculated. The ethanol concentration was determined using the standard curve equation.

3. RESULTS AND DISCUSSION

3.1. Surface Texture Analysis Using Scanning Electron Microscopy (SEM)

Before and after hydrolysis, sorghum starch was characterized using Scanning Electron Microscopy (SEM). SEM analysis aims to determine the morphological structure of sorghum starch before and after the hydrolysis. The results of the SEM analysis are depicted in Fig. 1.

Based on Figure 1, there are differences between Figures 1a and Figure 1b, where Figure 1a shows sorghum starch which is still tightly arranged, regular, and rigid. It is due to the presence of wax, hemicellulose, lignin, and other binder components that bind to each other in sorghum starch that has not been hydrolyzed (Umagiliyage et al., 2015). While in Figure 1b, there is a change in the structure of the sorghum starch fiber, which looks more crushed and makes a uniform structure and small size. Microwave hydrolysis accelerates the reduction and degradation of cellulose, resulting in a solid structure with reduced size (Riazi et al., 2015). The low contact time and energy consumption of the microwave reaction will reduce the formation of unwanted secondary products. Microwave heating can rapidly convert starch to sugar. Compared with conventional heating, the reaction rate of starch hydrolysis into glucose increases 100 times with the use of microwave irradiation (Nikolic et al., 2010)

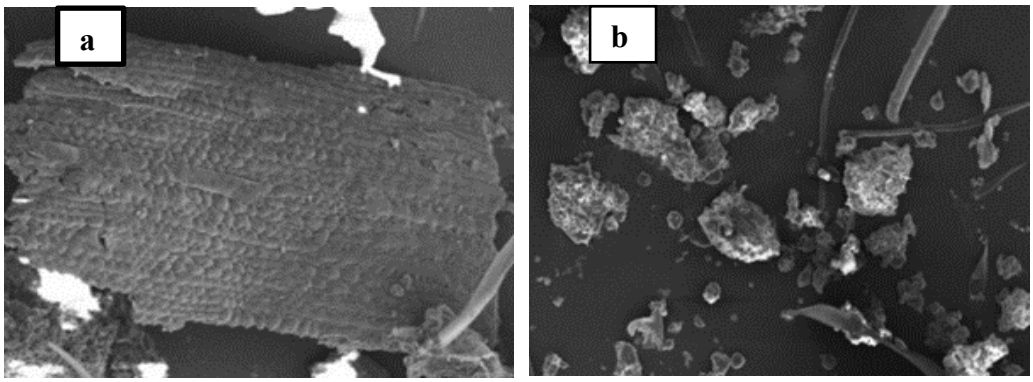


Figure 1. Results of SEM Analysis

Notes: (a) Before Hydrolysis, (b) After Hydrolysis (Source: ITS Energy Laboratory Surabaya)

3.2. Analysis of reducing sugar

Sorghum bagasse was hydrolyzed by microwave at 150°C with variations in heating time of 20, 30, 40, and 50 minutes and using 2% sulfuric acid as a catalyst. Based on the time variation, it was seen that there was a color change with increasing heating time on the sorghum starch hydrolyzate (Fig. 2). The longer the heating time, the darker the color of the hydrolyzate produced. It indicates that there has been a complete degradation of hemicellulose and cellulose into glucose (Widyastuti, 2019), but if the hydrolysis process is continued, charcoal will form on the Erlenmeyer wall. It implies that the glucose produced is damaged or burned. The microwave method is more profitable than the standard reflux method because of the shorter reaction time (in minutes), fewer solvent, and higher yield (Chen et al., 2012).

Figure 2. Hydrolysis results from sorghum bagasse (a) 20 minutes, (b) 30 minutes, (c) 40 minutes, (d) 50 minutes

The reducing sugar content of the hydrolyzed filtrate was analyzed using the DNS (Dinitrosalicylic Acid) method with 3,5-dinitrosalicylic acid reagent. The reducing sugar concentration was determined based on the formation of a red-brown reduced product when the sugar in the sample reduced 3,5-dinitrosalicylic to 3-amino-5-nitrosalicylic acid during heating. Glucose testing was carried out at a wavelength of 540 nm because the brownish-red color was absorbed maximally at that wavelength. Glucose can react with DNS so that by processing absorbance values, its levels can be measured spectrophotometrically (Galung, 2021). The measurement results of reducing sugar content are presented in Table 1.

Tabel 1. Measurement Results of Reducing Sugar Levels

Time (minute)	Reducing Sugar Level (g/L)	Hydrolysis Efficiency (%)
20	29.2	73
30	30.4	76
40	24.3	60
50	22.2	55

Measurement of reducing sugar content using the DNS method in Table 1 shows an increase in reducing sugar content at a hydrolysis time of 20-30 minutes, from 29.2-30.4 g/L, and hydrolysis efficiency from 73-76%. The increase in reducing sugar levels is caused by the hydrolysis process, which takes longer to cause more H^+ ions to break the cellulose polymer chain, resulting in high free radicals that will bind to OH^- ions to form glucose monomers (Melwita et al., 2014). Suppose the hydrolysis process is allowed to continue continuously. In that case, it will cause reducing sugar content, as shown in Table 1, where the hydrolysis time of 40 and 50 minutes had reduced sugar content from 24.3-22.2 g/L. Susansti et al. (2013) reported that the decrease in reducing sugar content was due to a too long hydrolysis time, causing the converted glucose to be damaged (sugar caramelization) due to excessive heating (Figure 3). Another factor causing the decrease in reducing sugar levels is the hydrolysis of glucose into hydroxymethylfurfural (HMF) and further reaction to form formic acid (Sukowati et al., 2014). The maximum production of reducing sugar was obtained at 30 minutes of heating, which was 30.4 g/L with a hydrolysis efficiency of 76%. This result is higher than Kolo & Sine (2019) on the hydrolysis of sorghum pulp which produces a reducing sugar content of 34.3 mg/L at a hydrolysis time of 40 minutes.

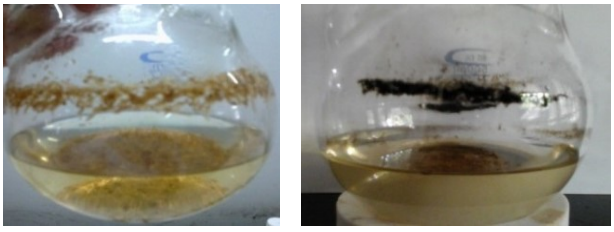
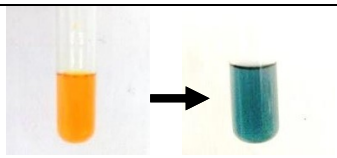
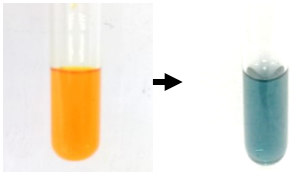


Figure 3. The appearance of Microwave Hydrolyzed Solution

3.3. Qualitative analysis of ethanol

Fermentation of glucose hydrolyzate in 2% sulfuric acid treatment was carried out at 150°C for 30 minutes. Fermentation in this study used sorghum bagasse hydrolyzate as a substrate fermented using *Saccharomyces cerevisiae*. The yeast *Saccharomyces cerevisiae* was chosen because it possesses various advantages, including a high rate of survival and the ability to produce an adequate amount of alcohol (Jayus et al., 2016). Before proceeding with Gas Chromatography analysis, a qualitative analysis using potassium dichromate (K_2CrO_7) was performed to verify that the sample from the stepwise distillation produced ethanol. The results of the analysis are presented in Table 2.

Table 2. Qualitative results of ethanol analysis

Sample	Results	Image
Pure ethanol	Positif (+)	
Sorghum ethanol	Positif (+)	

The images in Table 2 show the color change from orange to blue. It proves the presence of ethanol in the distillate (Saleh et al., 2016). Color change due to oxidized alcohol. Cr^{6+}, which is yellow, is reduced to Cr^{3+}, which is blue, according to the following reaction (Sania et al., 2015):

$$3CH_3CH_2OH + Cr_2O_7^{2-} + 8H^+ \longrightarrow 3CH_3COH + 2\ Cr^{3+} + 7H_2O$$

3.4. Quantitative analysis of ethanol

a. Analysis results of ethanol concentration using pycnometer

The fermentation process in this study was carried out with various inoculum concentration treatments, namely 4, 6, and 8%. Analysis using a pycnometer was carried out to determine the density of fermented ethanol. The ethanol density results obtained were matched with the specific gravity conversion table to determine the ethanol concentration. Conversion of specific gravity of ethanol concentration for each treatment (inoculum concentration 4, 6, and 8%) is depicted in Table 3.

Based on the ethanol results and the parameters of the three variations of inoculum concentration, it can be concluded that the highest ethanol concentration was obtained from fermentation with 8% inoculum, namely 5.325%. These results indicated that the ethanol concentration increased with increasing inoculum concentration. It is because the percentage concentration of inoculum is inversely proportional to the lag phase, where the higher the inoculum concentration, the shorter the lag phase so that it quickly reaches the exponential phase. The exponential phase occurs when the yeast fully grows and adapts, allowing for maximum sugar conversion and the formation of a product (ethanol). The purpose of inoculum preparation is to shorten the lag phase (adaptation) of fermentation, resulting in a shorter fermentation time and a higher alcohol content produced (Pramita et al., 2014). The highest values obtained from a pycnometer examination of ethanol content were used for GC analysis, namely fermentation with 8% inoculum (Table 4). The quantitative test of ethanol using a pycnometer and GC showed that the ethanol concentration increased with the increase in the inoculum concentration.

Table 3. Substrate Parameters and Experimental Results

Yeast Concentration %	Ethanol Concentration (%)	Y (%)	EF%	EK%
4	0.465	1.52	2.98	1.15
6	5.034	16.33	32.03	12.41
8	5.325	17.25	33.86	13.12

*Description: Y: Yield, EF: Fermentation Efficiency, EK: Conversion Efficiency

b. Analysis of ethanol concentration using GC

Standard chromatograms and sample analysis using GC are presented in Fig. 4 and Fig. 5.

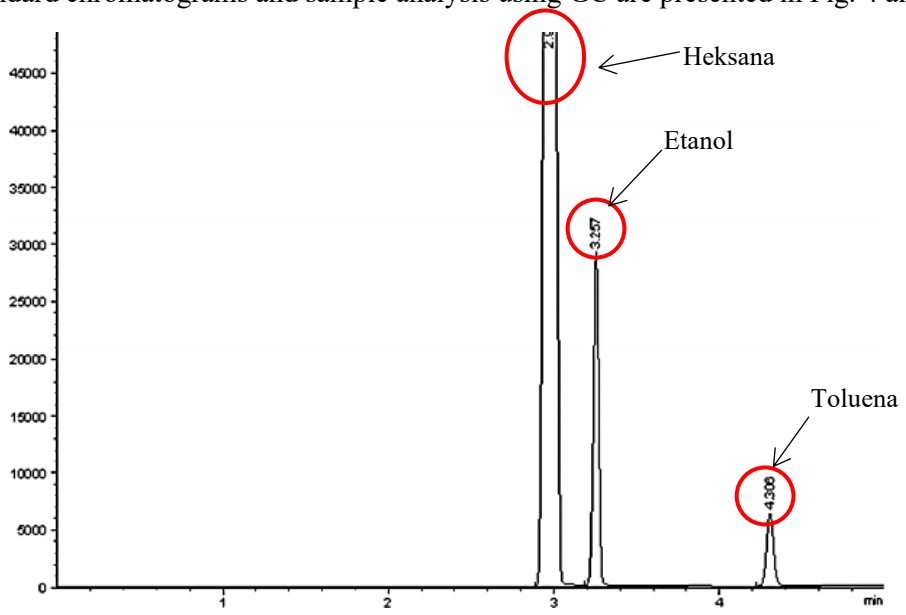


Figure 4. Ethanol standard chromatogram

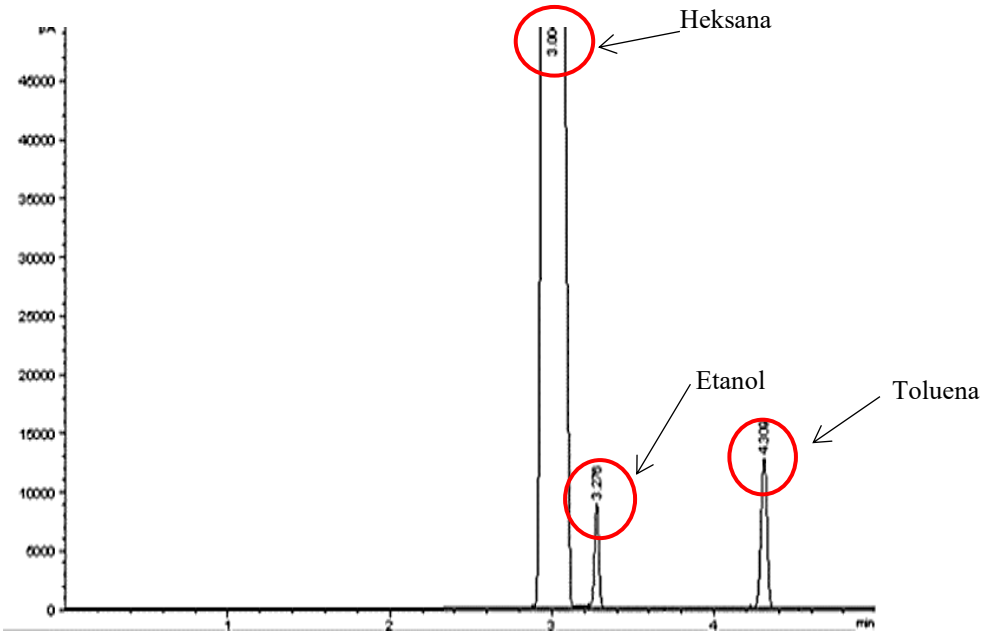


Figure 5. Bioethanol Chromatogram of Sorghum Bagasse

The presence of ethanol in the fermentation product was determined by comparing the sample's retention time to the standard, as seen in Figures 4 and 5. The chromatogram results showed no significant difference between the standard solution and the sample. Retention time on standard ethanol is 3.257 minutes and on the sample is 3.276 minutes. It indicates that the analysis results contain pure ethanol. At the same time, the toluene that comes out at a retention time of 4.306 minutes on the standard and 4.309 minutes on the sample is the internal standard used in the analysis using GC. This study chose toluene as the internal standard because toluene has a molecular formula similar to ethanol. Due to the similarities of the two structures, it is simple to determine the solubility of the two solutions (like dissolves like) (Solikha, 2017). Quantitatively, the measurement of ethanol content using GC was performed by comparing the area of the ethanol peak with the area of the standard and multiplied by the standard concentration. The calculation results of bioethanol concentration, yield, fermentation efficiency, and conversion efficiency are presented in Table 4.

Table 4. Substrate Parameters of GC Analysis

Ethanol Concentration %	Y (%)	EF%	EK%
9,05	29,35	57,56	22,31

***Description:** Y: Yield, EF: Fermentation Efficiency, EK: Conversion Efficiency

The highest ethanol yield was obtained at an inoculum concentration of 8%, namely 9.05 % with a yield of 29.35 %, fermentation efficiency of 57.56 %, and conversion efficiency of 22.31 %. The ethanol yield value indicates the amount of ethanol produced from the substrate. The efficiency of fermentation is used to determine the success of the fermentation. The higher the fermentation efficiency, the higher product is produced, and the conversion efficiency reflects how many substrates are converted to ethanol products. According to the GC analysis results presented in the table, the fermented sorghum bagasse contained 9.05 % ethanol. This result is lower than that of Sarongallo et al. (2017), who purified bioethanol from sweet sorghum juice at a concentration of 43%. This difference could be explained by the fact that prior investigations used sorghum juice, but this study used sorghum bagasse. Other possible explanations for the low ethanol content in this investigation include the absence of an ethanol purification step. The distillation results still contain water and inhibitor chemicals that prevent the creation of ethanol. It is supported by Roni (2015), who states that inhibitor compounds formed in the hydrolysis process using dilute acid can inhibit the fermentation process.

4. CONCLUSION

Based on the findings of the investigation, the following conclusion could be drawn:

1. SEM analysis revealed that the sample's surface was flat, rough, and rigid prior to hydrolysis, but after hydrolysis, the surface was damaged, appearing crushed with a smaller size.
2. The optimum hydrolysis time using a microwave at 150°C was 30 minutes, resulting in the highest reducing sugar content of 30.4 g/L.
3. The highest ethanol content obtained from sorghum bagasse fermentation using an inoculum concentration of 8% was 5.325% (pycnometer) and 9.05% (GC).

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