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## Hydrolysis optimization of tobacco stems with ultrasonic-assisted hydrolysis method

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**Abstract.** The development of biofuel in Indonesia is rife to reduce the consumption of fuel oil. Bioethanol has advantages compared to fuel oil, such as lower carbon monoxide emissions making it environmentally friendly. Besides that, from being used as a fuel, bioethanol is used as raw material for alcohol, pharmaceutical, and cosmetics derivatives. Biomass is one of the bioethanol's raw materials available in Indonesia, one of which is tobacco stem. In this research, the tobacco stem hydrolysis process carried out using the ultrasonic-assisted hydrolysis method. The variable of the hydrolysis process is H<sub>2</sub>SO<sub>4</sub> solution, particle size, and time. The data analysis used Design Expert with a Central Composite Design method. The purpose of this research was to determine the optimum of particle size, H<sub>2</sub>SO<sub>4</sub> concentration, and the time in the hydrolysis process of tobacco stems with the ultrasonic-assisted hydrolysis method. The optimum resulting in reducing sugar of ultrasonic-assisted hydrolysis is 6.921 µg/µL.

**Keywords:** Bioethanol; H<sub>2</sub>SO<sub>4</sub> concentration; Particle size; Tobacco stem; Ultrasonic-assisted hydrolysis

### 1. Introduction

The development of Biofuel in Indonesia is rife to reduce the consumption of fuel oil. This is in line with the Republic of Indonesia Presidential Regulation No. 5 of 2006 that targets the use of biofuel at 5% of total primary energy by 2025. This policy is also supported by Minister of Energy and Mineral Resources Regulation No. 12 of 2015, which targets the use of bioethanol to 20% of the total oil demand diesel and gasoline in 2025. The use of bioethanol as a fuel has several advantages over to fuel oil, such as the combustion is cleaner and more environmentally friendly because carbon monoxide gas emissions are lower so it does not contribute to the accumulation of carbon dioxide in the atmosphere [1]. Besides being used as a fuel, bioethanol is also used as raw material for the alcohol, pharmaceutical, and cosmetic derivative industries [2].

Bioethanol can be made from biomass raw materials that contain sugar, starch, or cellulose. The main characteristics of raw materials that can be used as ingredients for bioethanol are high sugar and carbohydrate content such as sugar cane, cassava, sorghum, roomie, sweet potato, arrowroot, corn, straw, corncobs, and wood [3]. Biomass is a raw material that is readily available in Indonesia because it is endowed with fertile soil and abundant biological resources. One of the abundant natural



resources in Indonesia is tobacco. Based on the Indonesian Plantation Statistics data, the area of tobacco plantations of smallholders and state plantations reached a total of 189,657 ha in 2018. East Java Province is a province that has the largest tobacco plantation area in Indonesia, which is 94,887 ha. Jember Regency itself has 6,078 ha of tobacco plantations. At present, the use of tobacco is still focused on the leaves, while the stems are only left as waste. Even after the harvest season, tobacco stems are only left and stacked on the edge of the rice fields. However, this tobacco stem is one of the biomass that can be used as raw material for making bioethanol. With a population range of 22,000 trees per hectare of land and an estimated weight of 0.5 kg of tobacco stems, 66,858 tons of tobacco stem waste will be available in Jember Regency.

Tobacco stems have high cellulose content so that they have the potential to become biofuel raw material for bioethanol. The cellulose content in tobacco stems can be up to 40% for dry tobacco stems [4]. Tobacco stems contained 56.10% cellulose components, lignin 15.11%, and nicotine 0.26% [5]. The content of lignin, cellulose, and hemicellulose in tobacco stems were 25.2%, 44.6%, and 30.2%, respectively [6]. Lignocellulose in tobacco stems has a density of about 260-350 kg/m<sup>3</sup> with a chemical structure and composition similar to wood from broadleaf wood species [7]. Naturally, cellulose is bound by hemicellulose and protected by lignin [8].

Bioethanol has the same molecular formula as ethanol or ethyl alcohol, C<sub>2</sub>H<sub>5</sub>OH, which is a derivative of hydroxyl compounds or OH groups [9]. Ethanol is volatile, soluble in water, colorless, has a molecular weight of 46.1, has a density of 0.789 at a temperature of 20°C, a boiling point of 78.3°C, a freezing point of -117.3°C, latent heat of evaporation 204 cal/g, octane number 91-105, and calorific value of 7077 cal/g. Based on alcohol content, bioethanol is divided into three grades, namely (1) industrial-grade with 90-94% alcohol content, (2) neutral with 96-99.5% alcohol content which is usually used for liquor or pharmaceutical raw materials, (3) fuel grade with 99.5-100% alcohol content [10].

The process of making bioethanol from lignocellulosic raw materials is done through 4 main stages, namely pretreatment, hydrolysis, fermentation, and distillation. The existence of lignin in the lignocellulosic raw material is one of the factors of the difficulty of hydrolysis of lignocellulosic raw material. This has become one of the important factors in the pretreatment process, which aims to break the bonds of lignin, cellulose, and hemicellulose. This process is also often referred to as the lignin degradation process (delignification). The delignification process is carried out to condition the lignocellulosic material both in structure and size by breaking up the lignin and hemicellulose content, damaging the cellulose crystals, and increasing the porosity of the material [11].

In this study, the hydrolysis process was carried out using low concentrations of strong acids. The acid solution used in the hydrolysis process is H<sub>2</sub>SO<sub>4</sub>. The hydrolysis process is carried out using strong acids with low concentrations due to avoid the reduction in the amount of reducing sugar if done at high concentrations. This is inseparable from the nature of glucose, which is easily decomposed. Hydrolysis with low concentrations makes the process last longer than high concentration but can reduce the breakdown of glucose by acids [12]. The hydrolysis process at high temperatures can be carried out in the temperature range of 160°C-240°C, while hydrolysis at low temperatures can be carried out at a temperature range of 80°C-140°C [13]. Chemical hydrolysis can break the glycoside bonds randomly. Termination of this bond will affect the glucose levels obtained from the hydrolysis process. The hydrolysis process of tobacco stems was carried out by the ultrasonic-assisted hydrolysis method using variable H<sub>2</sub>SO<sub>4</sub> solution concentration, time, and particle size. The ultrasonic-assisted hydrolysis method is a hydrolysis method by applying sound energy or vibration to move particles in a sample. The use of ultrasonic-assisted hydrolysis methods requires lower energy and is considered a green technology that has the potential to reduce reaction times and chemical loading during the hydrolysis process [14]. The ultrasonic frequency that is usually used is ≥ 20 kHz. The time spent on hydrolysis using ultrasound ranges from 10 - 70 minutes [15]. Ultrasonic-assisted hydrolysis has a significant impact on the hydrolysis process because it has mechanical effects in the form of vibrations and rotations that can destroy the hard lignocellulosic structure of biomass [16]. The use of ultrasonic can reduce biomass particle size. The purpose of this study was to

determine the optimum particle size, time, and H<sub>2</sub>SO<sub>4</sub> concentration in the hydrolysis process using ultrasound.

## 2. Materials and methods

### 2.1. Materials

The materials used include tobacco stems (from Tamansari, Jember Regency, Indonesia), distilled water, H<sub>2</sub>SO<sub>4</sub>, Nelson's A, Nelson's B, arcnomolybdate, aluminum foil, and filter paper. Lignin, cellulose, and hemicellulose content in raw material can be seen in table 1.

**Table 1.** Cellulose, hemicellulose and lignin composition in tobacco stems

Raw Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reducing Sugar (µg/µL)
Tobacco stems	50.320	6.495	32.005	3.387

### 2.2. Methods

The method used in this research includes pretreatment with chemical methods and hydrolysis using the ultrasonic-assisted hydrolysis method.

*2.2.1. Sample preparation.* The tobacco stems are dried beforehand in the sun using sunlight. After drying, the tobacco stems are crushed with a chopper machine. Next, the tobacco stems were sieved with 60 mesh sieves so that a uniform size was obtained. The raw material is weighed and dried in the oven for one hour at a temperature of 60-70°C.

*2.2.2. Pretreatment.* One gram of tobacco stem sample was put into a beaker glass and soaked with 10 mL 4% H<sub>2</sub>SO<sub>4</sub>, then heat it at 100°C for 60 minutes [17]. Samples are filtered and washed with distilled water. Then dry the sample in the oven. Finally, cool the sample at room temperature.

*2.2.3. Hydrolysis.* There are three types of variables used, time, particle size, and H<sub>2</sub>SO<sub>4</sub> concentration, in accordance with the Design Expert's result with Response Surface Method (RSM) of the Central Composite Design (CCD) type in table 2. The process of data retrieval with an ultrasonicator. First, the pretreatment tobacco stems are taken as much as 3 g. Then add 75 mL of H<sub>2</sub>SO<sub>4</sub>. Then the sample is processed by an ultrasound device. Repeat for the 2<sup>nd</sup> to 20<sup>th</sup> sample. Then, analysis of reducing sugar levels.

**Table 2.** Sample variations based on design expert

Run	Time (minutes)	Particle Size (mesh)	H <sub>2</sub> SO <sub>4</sub> Concentration (%)
1	30	60	1
2	84	100	2
3	50	100	2
4	70	140	1
5	50	100	3.7
6	30	140	3
7	50	100	2
8	50	100	2
9	70	140	3
10	50	100	2
11	16	100	2
12	30	60	3

13	50	100	0,3
14	50	33	2
15	50	100	2
16	70	60	3
17	50	167	2
18	50	100	2
19	30	140	1
20	70	60	1

2.2.4. *Analysis of reducing sugar.* Hydrolyzed tobacco stems are then analyzed for reducing sugar levels. The reducing sugar test was carried out by the Somogyi-Nelson method [18]. The testing step can be done by weighing a sample of 5 g and diluting with distilled water to 50 mL. Shake for 1 hour, after which it is filtered, and the filtrate is taken. The filtrate is diluted with distilled water to 100 mL, then shaken. The result of dilution is taken 1 mL, then it is carried out in series. 1 ml is taken from the dilution series, then put into a test tube and added 1 mL of Nelson's A and Nelson's B. reagents. The sample is heated with a bath for 20 minutes, and then cooled with running water. Samples added 1 mL of arsenomolybdate, then added 7 mL of distilled water. The absorbance samples were measured with a UV-Vis spectrophotometer at a wavelength of 540 nm.

### 3. Results and discussion

Tobacco stems are crushed with a chopper and crushed using a blender. Sample preparation begins with the sieving process in the Basic Laboratium and Chemical Engineering Process. The sieve results can be seen in figure 1.



Figure 1. The sieve results are 60 mesh, 80 mesh, 100 mesh, 120 mesh, and 140 mesh

The pretreatment samples are then hydrolyzed using an ultrasonicator at the Center for Development of Advanced Science and Technology (CDAST) Laboratory of Jember University. Sample variations were prepared according to the results of the Design Experts in Table 2. The samples that have been cultivated can be seen in figure 2.



Figure 2. Samples that have been hydrolyzed by ultrasonicator

#### 3.1.1. Reducing sugar level test

Samples that have been hydrolyzed with an ultrasound are then analyzed for reducing sugar levels by the Somogyi-Nelson method. The results of the reducing sugar test can be seen in table 3.

**Table 3.** Reducing sugar test results

Run	Time (minutes)	Particle Size (mesh)	H <sub>2</sub> SO <sub>4</sub> Concentration (%)	Reducing Sugar Levels (µg/µL)
1	30	60	1	5.371
2	84	100	2	3.751
3	50	100	2	3.639
4	70	140	1	6.921
5	50	100	3.7	2.996
6	30	140	3	5.524
7	50	100	2	3.723
8	50	100	2	3.164
9	70	140	3	4.924
10	50	100	2	3.220
11	16	100	2	3.192
12	30	60	3	3.178
13	50	100	0.3	3.066
14	50	33	2	2.996
15	50	100	2	3.234
16	70	60	3	2.843
17	50	167	2	4.197
18	50	100	2	3.778
19	30	140	1	6.670
20	70	60	1	3.052

### 3.1.2. Effects of time, particle size, and concentration on the hydrolysis process

Based on table 3, it can be seen that the highest reducing sugar yield is 6.921 µg/µL. These results were obtained at 70 minutes time variation, 140 mesh particle size, and 1% H<sub>2</sub>SO<sub>4</sub> concentration. The results from table 3 were then analyzed using a Design Expert with the RSM method. From the analysis method, ANOVA with Linear Model can be seen in table 4.

**Table 4.** ANOVA with the linear model

Source	Sum of Squares	df	Mean square	F-value	p-value	
<b>Model</b>	12.54	3	4.18	3.96	0.0274	significant
A-time	0.3116	1	0.3116	0.2955	0.5942	
B-concentration	2.35	1	2.35	2.23	0.1551	
C-particle size	9.88	1	9.88	9.37	0.0075	
<b>Residual</b>	16.87	16	1.05			
Lack of fit	16.47	11	1.50	18.78		
Pure error	0.3986	5	0.0797		0.0023	significant
<b>Cor Total</b>	29.41	19				

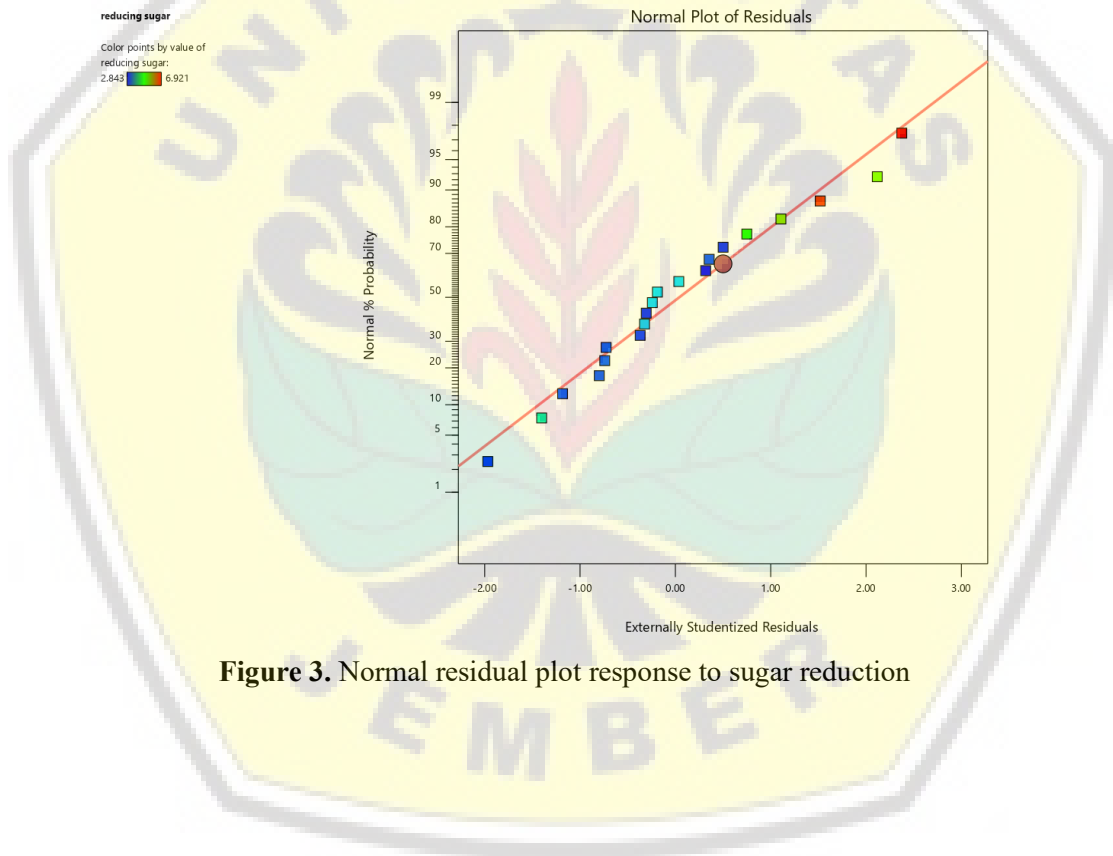
This linear model is significant for this hydrolysis process. It can be seen the F-value and p-value of this model from table 4. The F-value of 3.96 indicates that this model is significant, while the p-value of 0.0274 indicates that there is only interference of 2.74% of this model. The p-value of each

variable less than 0.05 indicates that the variable has a significant influence during the hydrolysis process. Table 4 shows that the particle size factor has a p-value of 0.0075 so that it greatly influences the hydrolysis process. The F-value for the lack of fit of 18.78 and p-value of 0.0023 shows that this model is significant because the disturbance that may occur in this model is only 2.3%. The linear equation for the hydrolysis process can be written in equation 1. From equation 1, it can be seen that reducing sugar will increase with the smaller particle size of the sample.

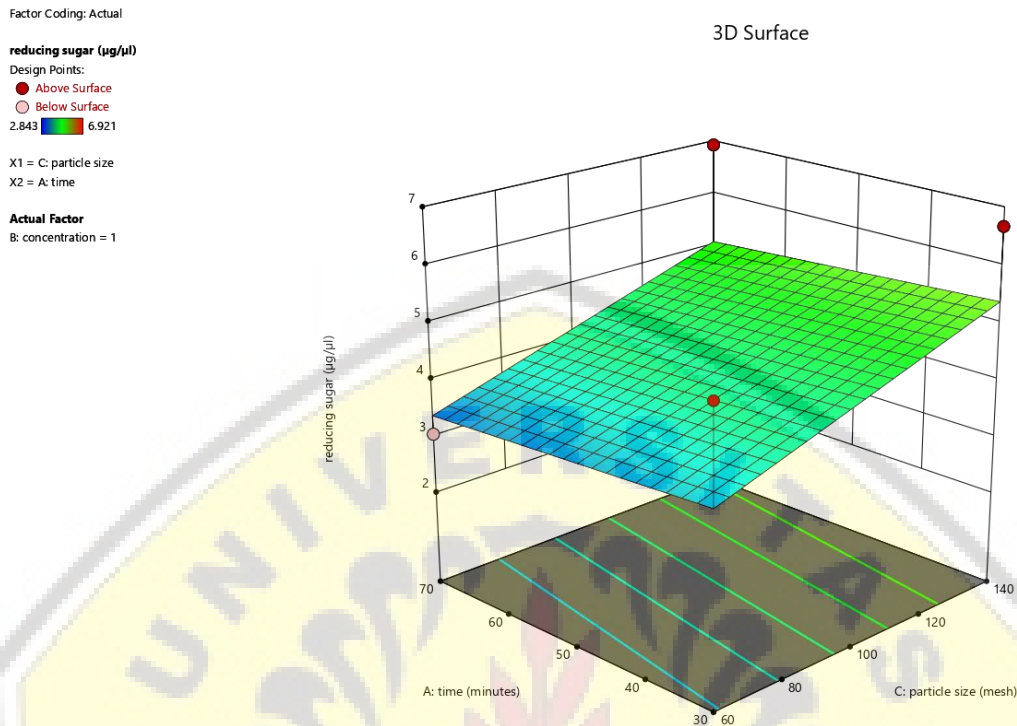
$$\text{Reducing sugar} = 3,97 - 0,1511 A - 0,4146 B + 0,8505 C \tag{1}$$

where A is the reaction time (minutes), B is the concentration of H<sub>2</sub>SO<sub>4</sub> (%), and C is the particle size (mesh).

From this modeling, the normal plot can be seen in figure 3. Figure 3 shows that the predicted distribution of sample points approaches the normal line so that the distribution is categorized as normal. The 3-dimensional graph showing the correlation of each variable with the resulting reducing sugar can be seen in figure 4.



**Figure 3.** Normal residual plot response to sugar reduction



**Figure 4.** The 3-dimensions graph of the relationship between variables with reducing sugar

From figure 4, the smaller the particle size, the greater the surface area so that the contact surface area during the hydrolysis reaction is even greater. This makes the hydrolysis reaction get optimal results on reducing sugar. Effect of particle size is a complex phenomenon associated with a significant change in physical and chemical properties of substance due to direct reduction of the particles, the contribution of the interface to system properties, and due to particle size being commensurate with the physical parameters of the diameter dimension [19]. The longer the hydrolysis time, the more cellulose which is converted into simple glucose [20]. In this study, with a hydrolysis time of 16-70 minutes, the optimal reducing sugar results were obtained at 70 minutes. The concentration of sulfuric acid used in this study was 0.3-3.7%, with the optimal result of reducing sugar obtained at a concentration of 1%. Sulfuric acid is a dehydration agent that can absorb water content in carbohydrate-containing materials [21].

A comparison of hydrolysis results with the Ultrasonic-Assisted Hydrolysis method, when compared with a previous study, can be seen in table 5.

**Table 5.** Comparison of reduction sugar results with another study

	This Research	Another Study [22]
Raw Material	Tobacco stems	Tobacco stems
Hydrolysis Method	<i>Ultrasonic-Assisted Hydrolysis</i>	Conventional
Type of Solvent	H <sub>2</sub> SO <sub>4</sub>	HCl
Reducing Sugar ( $\mu\text{g}/\mu\text{L}$ )	6.921	13.66

Based on table 5, the reducing sugar produced in this study is smaller than the reducing sugar from another study [22]. This is likely due to the tobacco stems used in this study were tobacco stems from Jember, while the tobacco stems used by another study [22] originated from Mataram, so they have different characteristics. Although the results of reducing sugars were not as great as in previous study



[22], the ultrasonic-assisted hydrolysis method has advantages that include minimization of flavor loss, greater homogeneity of treatment, and significant energy savings [23]. The reducing sugar in Jember tobacco stems before hydrolysis was  $3.387 \mu\text{g}/\mu\text{L}$ , which can be seen in table 1. So, with the hydrolysis process and obtained reducing sugar  $6.921 \mu\text{g}/\mu\text{L}$ , there has been an increase.

#### 4. Conclusion

The conclusion of this research is the hydrolysis process with the Ultrasonic-Assisted Hydrolysis method to obtain optimal results with a variation of time of 70 minutes, 140 mesh particle size, and 1%  $\text{H}_2\text{SO}_4$  concentration with the resulting reducing sugar  $6.921 \mu\text{g}/\mu\text{L}$ . Mathematical modeling for this hydrolysis process is reducing sugar =  $3.97 - 0.1511 A - 0.4146 B + 0.8505 C$ , where A is the reaction time (minutes), B is the concentration of  $\text{H}_2\text{SO}_4$  (%), and C is a measured particle (mesh).

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