

The Effect of Nitrogen Fertilizer Addition on Bioethanol Production From Sweet Sorghum Bran

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Abstract. Bioethanol is a renewable energy source made by fermenting plants glucose or starch components. The previous study indicated that sweet sorghum could be used as a feedstock for ethanol production. The high carbohydrate content of the sweet sorghum bran can be hydrolyzed into glucose using α -amylase and glucoamylase. Besides carbohydrates, nitrogen is a critical component in ethanol production. The study investigated the effects of different concentrations of urea addition on sweet sorghum bran ethanol production. First, the sweet sorghum bran was analyzed for proximate and carbohydrate content. Then, the sweet sorghum bran was hydrolyzed using α -amylase and glucoamylase. In the end, ethanol production was optimized using *Saccharomyces cerevisiae* (2 g w/w) and different urea concentrations (1, 2, and 3 g). Parameters analyzed were pH, total dissolved solids, sugar content, and ethanol content. Ethanol content was analyzed using gas chromatography. The result showed that a combination of *S. cerevisiae* and 2 g of urea was the best. Ethanol reached 4,8% after 72 h of fermentation.

Keywords: amylase, bioethanol, nitrogen, sweet sorghum bran

1. Introduction

The need for energy sources is increasing along with population growth. Indonesia's oil dependence, especially in the Middle East, reaches around 35% (1) (DEN, 2019). This condition is not balanced with the availability or renewal of existing energy sources. The agricultural sector provides solutions to meet the community's needs. As a rich country of natural resources, especially in the agricultural sector, it is a great chance to produce renewable energy from plant-based materials (biofuels).

Bioethanol is a biofuel derived from the edible part of a plant made by fermentation (Richana, 2011, Laila et al., 2019). Bioethanol can be processed from natural sources containing glucose, carbohydrates, and cellulose, such as rice bran (Falaah and Kusumayanti, 2021), cassava & corn (Kusmiyati and Sitophyta, 2014), yam tubers (Hargono, 2020), cassava skin waste (Ariyanti et al., 2019), water hyacinth (Sari et al., 2017), and wood/lignocellulose (Mansur et al., 2016). Sorghum is a prospective source because all biomass components such as seeds, stems, and leaves can be used as bioethanol in addition to food and feed (M. Arsyad Biba 2011). As a food source, sweet sorghum contains high glucose and ranks fifth after wheat, rice, corn, and barley (Human, 2007). Therefore, it has a great potential to generate bioethanol (Richana, 2011). However, the fulfillment of energy needs using biofuels is often not in line with the procurement of food needs, so it is often considered one of the causes of the food crisis (Apriwinda, 2013). In addition, sorghum plants can grow on marginal land, whereas other plants cannot grow optimally (Kasmiyati et al., 2015). This indicates that sorghum is the potential to be used as a material in bioethanol production.

The milling sorghum processing produces waste in the form of bran. In addition, it will reduce the nutritional value of sorghum seeds due to epidermis erosion which contains nutritional components such as protein, fat, and carbohydrates (Widowati 2011). However, it properly makes sorghum bran as waste and has the benefit of being used as biomass which has the potential to produce bioethanol. The principle of bioethanol production from sorghum bran is based on the sugar conversion through

fermentation using the yeast *Saccharomyces cerevisiae*. Amylase is used to convert starch into monosaccharides through enzymatic processes. This process consists of liquefaction and saccharification, which are the initial stages in handling sorghum bran raw materials containing starch. Liquefaction requires α -amylase enzymes, and saccharification needs glucoamylase enzymes. Liquefaction is the starch changing process from thickened starch to liquid (Simanungkalit et al., 2016). When a mixture of starch and water (starch suspension) is heated until boiling, it becomes thick. With the addition of enzymes, the mixture will become watery. Next is the saccharification process, which is dextrins hydrolyze into glucose (Budyanto and Richana 2011). In addition, the addition of yeast and nitrogen source has been shown to increase the effectiveness of ethanol production (Hastuti et al., 2015). However, the addition of nitrogen fertilizer on bioethanol production from sorghum bran has not been studied. Therefore, this study aims to obtain the optimum urea concentration as a nitrogen source in the production of bioethanol from sorghum bran.

2. Research Methods

The research consisted of 3 stages, namely 1). characterization of raw materials, 2) optimization of enzymatic hydrolysis in the production of simple sugars from sweet sorghum bran, 3). Optimization of the effect of adding urea to the bioethanol production process from sweet sorghum bran.

Characterization of sorghum bran. The sorghum bran, a by-product of grinding sorghum seeds, was firstly sieved to remove impurities and then baked for 2-4 hours at 60°C. Sugar production was carried out through an enzymatic process. First, as many as 1200 g of sorghum bran was added with 6000 mL of water. Next, the sorghum bran mixture was added with 1 mL/kg α -amylase enzyme and heated to 100°C while stirring. The mixture was cooled to a temperature of 60°C, added the enzyme glucoamylase 1ml/kg, and stirred evenly. The mixture was then allowed for one night to optimize the saccharification and liquefaction process. Next, the mixture was filtered and divided into two parts, with a total of 6 buckets. Each bucket was added 2 g of yeast and urea (1 g, 2 g, and 3 g, respectively) with two replicates and stirred until evenly distributed. The buckets were tightly closed during fermentation and observed for pH, total dissolved solids, total sugar, and alcohol content.

3. Results and Discussion

Bioethanol production from sorghum uses liquid sugar (juice) from the stalk as its raw material. However, sorghum seeds with relatively high starch content can also be used as raw material for making bioethanol (Rahmi. E et al., and Tri P, 2009). The utilization of agricultural waste such as sorghum bran as raw material for bioethanol production requires preliminary tests, namely proximate and starch content. The results of the analysis of the sorghum bran can be seen in Table 1.

The moisture indicates the amount of water, and the ash content indicates the value of inorganic compounds contained in the sorghum bran. The carbohydrate content of sorghum bran was 84.59%, and the starch content was 55.93%, with a moisture value of 7.47%. Endang et al. (2012) stated that the starch content in sorghum flour is 79.37%, with a moisture value of 10.21%, and M. Arsyad Biba stated that the carbohydrate content of sorghum flour was 73%. The value of starch content indicates the amount of glucose in the sorghum bran, which can be converted into bioethanol through fermentation. Ash content in the sample affects the alcohol yield. Natural resources can be used as a raw material for bioethanol production if ash content is less than 10 %. A high percentage of ash content can inhibit the fermentation process and cause scale on the equipment during distillation (Wardani AK, Pertiwi FNE; Arif). The results showed that the ash content of the sorghum bran was 2.52% and fulfilled the requirements as raw material for bioethanol production.

Table 1. The proximate analysis of sorghum bran

Sample	Percentage (%)					
	moisture	ash	fat	Protein	carbohydrate	starch
Sorghum bran	7.47	2.52	4.50	0.92	84.59	55.93

Based on the proximate analysis, sorghum bran has a high percentage of starch. Therefore, it can be used as a raw material in bioethanol production. Bioethanol production uses starch sources through three steps liquefaction, saccharification, and fermentation (Richana, 2011). In the liquefaction process, α -amylase hydrolyzes the -1,4 glycosidic bonds of amylose and amylopectin in starch (Siswanti et al., 2014). According to Purbawani (2006) in Sefriana (2010), the breakdown process by α -amylase occurs randomly from the middle or interior of the starch molecule. As a result, it produces branched or straight oligosaccharides, namely glucose maltose and various types of -limit dextrins. Furthermore, in the saccharification process, glucoamylase plays a role in hydrolyzing oligosaccharides with α -1,6 glycosidic bonds and maltose into unbroken glucose.

According to previous research conducted by Budiyanto and Richana (2011), α -amylase and glucoamylase enzymes used were 1 mL/kg. Enzyme activities are influenced by pH and temperature conditions. The addition of α -amylase was carried out at the beginning of heating the sorghum bran until boiling while continuously stirring to avoid burning. It was due to the α -amylase enzyme working optimally at a temperature of 90-100 °C and a pH of 5-6.5 (Richana 2011). Glucoamylase works optimally at 60 °C, pH 4.5-5.0 (Richana 2011), so glucoamylase was added after the bran mixture was cooled to 55-60 °C.

The pH value of the mixture decreased with the length of fermentation time (Figure 1). The fermentation time affects the pH of the mixture (Arif et al., 2016). The pH of the mixture ranged 3.43-5.30 that the optimum pH for fermentation using *S. cerevisiae*. The pH value was decreasing due to the metabolisms process of *S. cerevisiae*, followed by bioethanol production, acetaldehyde, CO₂, and the breakdown of pyruvate as a final product. Cahyani et al. reported that the pH mixture was getting lower due to acetaldehyde and CO₂ production during bioethanol production. The addition of 2 g of urea for 72 hours of fermentation showed the smallest pH of 3.14 (Figure 1). This value indicates that *S. cerevisiae* has grown optimally. The urea addition of 1-3 % increased the biohydrogen production, in contrary decreased by 4 and 5% of urea addition (Maghfiroh and Agustini, 2013)

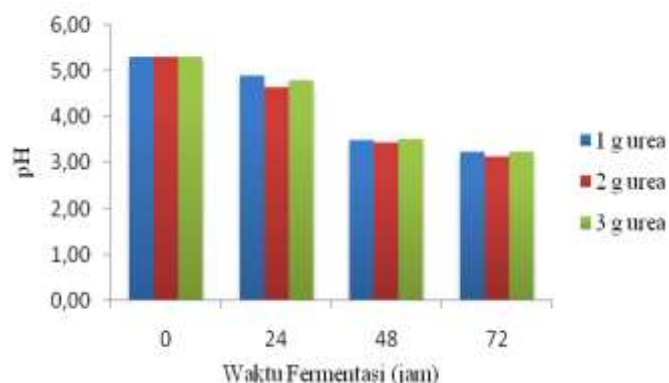


Figure 1. pH values during fermentation time on several nitrogen fertilizer concentrations

Figure 1 showed that the highest pH value decreasing in SSC occurred after 48 hours of fermentation. It was assumed that pH conditions had reached the optimum pH for yeast metabolisms during fermentation. At 72 hours of fermentation, yeast metabolism entered the stationary phase, and the pH value did not significantly change. Besides pH and temperature, the presence of the nutrient is also an essential factor in yeast activities or growth. Nitrogen fertilizer or urea is commonly used as a nitrogen source for *S. cerevisiae* growth. Therefore, the amount of nitrogen source should fulfill *S. cerevisiae* needs. Less nitrogen source will reduce yeast activity and affect the fermentation in bioethanol production. The availability of nitrogen sources correlated with yeast activity. SSC value is a value that indirectly indicates the amount of glucose contained in the sorghum bran mixture. The urea

concentration did not affect the SSC value at 24 and 72 hours of fermentation time. However, at 48 hours of fermentation, the SSC value decreased in 2 g of urea application compared to 1 and 3 g of urea.

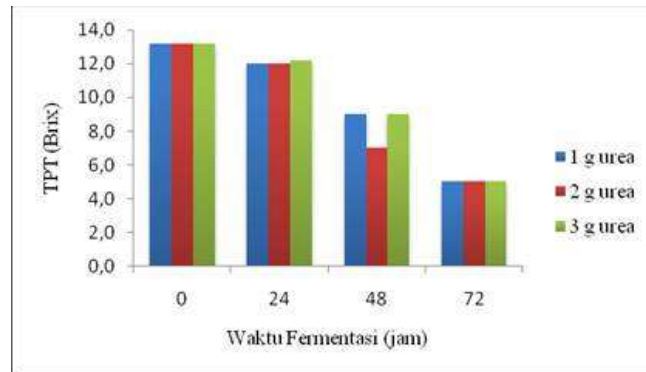


Figure 2. SSC values during fermentation time on several nitrogen fertilizer concentrations

The initial sugar concentration significantly affects ethanol yield and sugar conversion in fermentation (Asli, 2009). The initial total sugar content of the sorghum bran mixture was 7.07% (Figure 3) and decreased during fermentation. Total sugar decreased daily during 72 hours of fermentation and significantly decreased at 48 h of fermentation. It was assumed that the optimum growth of *S. cerevisiae* occurs at 24-48 hours of fermentation. The lowest total sugar obtained from fermentation using 2 g of urea was 2.58% and 1.71% at 48 and 72 hours, respectively. While total sugar from 1 g and 3 g of urea. 3.40% and 3.30% and continued decrease to 1.97% and 1.91%, respectively. Sugar content decreasing in bioethanol production could be assumed that *S. cerevisiae* was able to convert sugar into alcohol (Arif et al., 2016). The ability to produce bioethanol could be seen from the amount of sugar consumption and yeast growth during fermentation. Wardani et al. (2013) reported that there were correlations between sugar content, cell concentration, and length of fermentation. Our result study was in line with Wardani et al. (2013) that length of fermentation decrease sugar content and increases cell concentration.

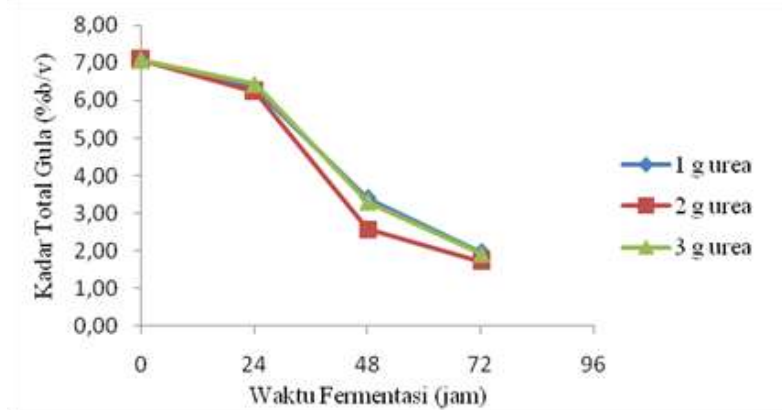


Figure 3. Total sugar values during fermentation time on several nitrogen fertilizer concentrations

Total sugar content decreased during fermentation contradicts with an increase in ethanol content. Based on Figure 4, the result showed that ethanol content increased in each treatment during 72 hours of fermentation. Bioethanol production was in line with fermentation duration (Surati 2015). However, fermentation duration exceeding the limit will reduce the ethanol production yield. It might be caused by the nutrients available for yeast have been exhausted. The highest ethanol yield was obtained in the addition of 2 g of urea. The difference in ethanol yield increased up to 72 hours of fermentation duration in all treatments, and this was due to yeast differentiation in stationary phase achievement. The

stationary state is the condition the yeast reaches its maximum growth and is powerful to produce alcohol (Andari et al., 2015). The ethanol yield with 2 g of urea at 48 hours was 3.50%, while the addition of 1 g and 3 g of urea produced 1.30% and 2.10% of ethanol. It is known that the amount of urea is proportional to the amount of yeast. Urea provides suitable nutrition for the development and activity of yeast in fermentation. During fermentation, nitrogen compound decreases due to the precipitation of some protein (Winarno 2007). The addition of 1, 2, and 3 g of urea increased ethanol yield during 72 hours of fermentation by 3.34%, 4.78%, and 4.20%, respectively (Figure 4).

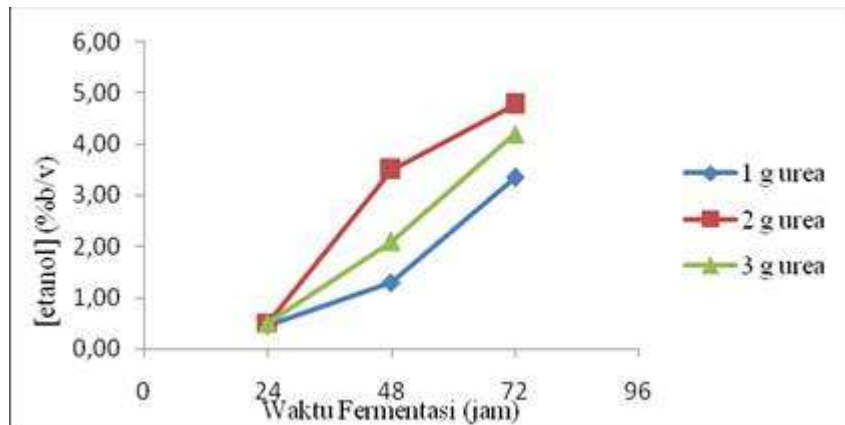


Figure 4. Ethanol values during fermentation time on several nitrogen fertilizer concentrations

4. Conclusion

Ethanol production was optimized using *Saccharomyces cerevisiae* (2 g w/w) and different urea concentrations (1, 2, and 3 g). Parameters analyzed were pH, total dissolved solids, sugar content, and ethanol content. Ethanol content was analyzed using gas chromatography. The result showed that a combination of *S. cerevisiae* and 2 g of urea was the best. Ethanol reached 4.8% after 72 h of fermentation.

5. References

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