Ethanol and Sugar Production from Sorghum (Sorghum bicolor) Starch at Varying Acidity

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Abstract. Sorghum (Sorghum bicolor), in addition to producing foodstuffs, feed, industrial use, is also potential for ethanol production. Sorghum is also a potential developed because it has a complete adaptation power in marginal land and other non-productive lands. The use of sorghum starch for ethanol production is a renewable fuel alternative and friendly for the environment. The aim of the research was to study the fermentation of sorghum starch into simple sugars and ethanol at varying acidity (pH). The study is experimental research with two treatment factors: pH (4; 6; 7; 8; 9; 10) and sugar levels. Data were collected and analyzed with simple and multiple linear regression analyses. The result showed that the pH treatment applied to influence the enzyme fermentation of sorghum starch. The conclusion of the study is the optimum pH of amylase enzyme activity in fermenting sorghum starch into glucose is pH 8. The relationship between the amount of alcohol, sugar, and pH is very strong at 95.1%. Multiple regression equations to explain the relationship of the three variables that the amount of alcohol (Y) produced in sorghum starch fermentation can be estimated additive by the amount of sugar formed (X\textsubscript{1}) and pH (X\textsubscript{2}).

Keywords: Alcohol, Fermentation, Ph, Sorghum Starch, Sugar.

1. Introduction

Sorghum (Sorghum bicolor) is a food companion of rice that has a comparative advantage over other cereals such as corn, wheat, and rice. Excess sorghum as a food ingredient is the potential of functional food contained in its seeds. The element of functional food includes the variety of antioxidants, mainly Fe minerals, dietary fiber, oligosaccharides, β-agate including non-starch polysaccharide carbohydrate components (NSP), and others. Sorghum contains 3.1 g of fat, 10.4 g protein; Carbohydrate 70.7 g; Crude Fiber 2.0 g, and energy 329 kcal. Sorghum flour contains leucine amino acids (1.31-1.39%) higher than flour (0.88%). Sorghum lysine flour levels are only 0.16%, much lower than 0.38% flour [1]. Sorghum flour replaced 15-50% flour without reducing the flavor, texture, and aroma of the product. Flour of sorghum contains a higher nutritional than flour of rice, corn, and cassava. Sorghum seeds contain 83% starch, 3.60% fat content and 12.3% protein [2].

Sorghum, as a seasonal plant from the Gramineae family, is able to grow in the area of marginal land. Sorghum does not need much water as rice plants. One of the advantages of sorghum is tolerant of drought and waterlogging [3]. Sorghum plants have a dry potency developed in less fertile soil and the availability of water only depends on rainfall. Sorghum can be harvested repeatedly so that the effort lasts year-round without intensive soil treatment and reduce the risk of erosion [4].
Sorghum seeds contain 67-71 percent starch. Sorghum seed starch can be hydrolyzed into simple sugar. Simple sugar obtained from the seeds of sorghum can be fermented to become alcohol. Sorghum is a potential energy crop because it can produce 7000 bioethanol per hectare per year. Bioethanol, as an alternative to fossil fuels, is mainly produced by yeast fermentation from different feedstocks. It is a high octane number fuel, and its physicochemical features are considerably different compared to the gasoline [5]. Other types of biomass have potential as raw materials for bioethanol production. Because of their chemical composition, i.e., carbohydrate sources, they mostly form three groups: (i) sugar-containing raw materials: sugar beet, sugarcane, molasses, whey, sweet sorghum, (ii) starch-containing feedstocks: grains such as corn, wheat, root crops such as cassava, and (iii) lignocellulosic biomass: straw, agricultural waste, crop and wood residues [6]. Bioethanol was interpreted as a chemical produced from foodstuffs containing starch, such as cassava, sweet potatoes, corn, and sago [7].

Bioethanol was generally from sorghum seeds inferior quality or moldy. The sugar-containing sorghum can be fermented and produce double ethanol than corn [4]. Ethanol is obtained from sorghum as a renewable and environmentally friendly. Plant development as an energy source for the manufacture of bioethanol carried out on several crops, namely rubber cassava [8], leather banana kapok [9], corn waste [10].

Fermentation is the process of forming sugar into ethanol made by the yeast-containing microorganisms. Yeasts, which are the most common microorganisms in bioethanol production, play an essential function in fermenting sugars to ethanol. Yeast is a potential for the fermentation of ethanol is Saccharomyces cerevisiae because it has the conversion power to ethanol very high, metabolism is known, the primary metabolite of ethanol, carbon dioxide, water, and other metabolites [10]. Bioethanol fermentation is then separated from solids and water to obtain pure ethanol through distillation and dehydration processes [11].

The process of fermentation of alcohol is influenced by several factors, namely the concentration of sugar, temperature, pH, nutrient material, and the time required for the fermentation process. The sugar concentration used in the fermentation process is 10-18%. Sugar concentrations that are too high cause yeast growth to decrease so that the fermentation time becomes long [10]. Temperature is very influential in the activity of yeast enzymes and alcohol results due to evaporation. The fermentation reaction rate will increase according to the optimum temperature of 27-32°C. On the other hand, the optimum temperature of bread yeast has a maximum temperature of about 19-32°C [12]. The extreme acidity can cause inactive enzymes. Enzymes usually have maximal activity at the optimum pH. Most enzymes have an optimum pH value of around 4.8 – 8.0. The optimum pH value affects the maximum reaction speed, the increased affinity of enzymes with the substrate, and the stability of the enzyme [13].

Annual bioethanol production is constantly increasing, and the prediction of worldwide bioethanol production and its consumption is an increase to nearly 134.5 billion lt by 2024 [5]. Research on the fermentation of starch sorghum becomes sugars, and alcohol should be done in order to increase the value-added of sorghum plants, the diversity of raw material sources of the industry, and the development of the product of fermentation. The encouraging factor for this research is the potential of sorghum plants as a source of cheap, simple, fast, and plenty of carbohydrate biomass. The research aims to study the fermentation of sorghum starch into simple sugars and ethanol at varying acidity (pH).
2. Research Methods
The research was conducted in June 2018 to November 2018 at the chemistry laboratory of the Faculty of Agriculture. This research is experimental research, with factors, namely pH value and reducer sugar (milligram/100 ml samples) as independent variables. The treatment pH value is 4; 6; 7; 8; 9 and 10. The dependent variable is the percentage of ethanol.

The pH treatment is repeated 2 times, so there are 12 experimental units. The sorghum seed is required as much as 60 kg (5 kg each). From 5 kg, sorghum seed would have obtained ± 2-liter sediment protein separated by filtering. One liter of free protein fermentation liquid was sampled to measure reducing sugar using refractometers and other one liter for measuring alcohol with alcoholmeters.

Fermentation of sorghum starch into ethanol using bio-process technique based IREC (Indonesia Renewable Energy Corporation) method. IREC method includes 4 stages, namely: hydrolysis process, fermentation process, distillation process, and dehydration process. This research only performs the first 3 processes to obtain ethanol yield 95% (technical quality) and reduction of sugar levels.

Sorghum seeds are reported might be able to produce a ratio of biomass and ethanol as much as 2.5:1, meaning that every 2.5 kg of sorghum seeds might be able to make 1 liter of low-quality ethanol, or approximately 40% ethanol. The alcohol yield is measured for each substrate's pH treatment above. The observation data used were simple and multiple linear regression analysis. The relationship between pH and sugar; pH and alcohol using a simple regression analysis while the relation between variables of pH, sugar levels, and ethanol using multiple linear regression analysis.

3. Result and Discussion
The pH treatment applied to influence the enzyme fermentation of sorghum starch (Table 1). pH 8 produces the highest amount of sugar and alcohol of 9.65% and 18.50%, respectively. The pH below or above pH 8 produces fewer sugars and alcohol. pH 10 produces the lowest sugar, 4.10% with alcohol 13.75%. pH 4 produces 4.75% sugars but the lowest alcohol 10.25%.

Some factors that affect the fermentation process include a substrate, temperature, pH, and oxygen microbial used [14]. pH is one of the factors that should be observed during the fermentation process. pH affects the growth of *Saccharomyces cerevisiae*. The results of the experiment showed that the fermentation process was optimal at pH 8 by producing the highest sugar and alcohol. Below or above the pH 8, the activity of the α-degrading and β amylase enzyme decreases so that the sugar and alcohol produced are less. pH affects the effectiveness of enzymes produced by microorganisms in forming substrate enzyme complexes. The pH change causes the denaturation process to decrease enzyme activity [15].

| pH | % Gula (Brix) | % Alcohol |
|----|--------------|-----------|
| 4  | 4.75         | 10.25     |
| 6  | 5.30         | 14.25     |
| 7  | 5.50         | 14.25     |
| 8  | 9.65         | 18.50     |
| 9  | 4.25         | 14.50     |
| 10 | 4.10         | 13.75     |
| Total | 33.55   | 85.50     |

The relationship between pH variables and the amount of sugar and pH with the amount of alcohol show changes in its pH followed the changes the sugar and alcohol (Figure1). The activity of
an enzyme amylase most active, so production sugar from decomposition starch sorghum at pH 8. The yeast that contains *Saccharomyces cerevisiae* change sugar as a source of carbon to become ethanol. The use of *Saccharomyces cerevisiae* in this study because microbes *Saccharomyces cerevisiae* has some advantages compared to other microbes, *Saccharomyces cerevisiae* can produce alcohol up to 2% in 72 hours [16]. Microbes *Saccharomyces cerevisiae* produces the enzyme invertase and zymase enzyme in both enzymes the microbes *Saccharomyces cerevisiae* can correlate the sugar into ethanol. The sugars of the Disaccharide group will hydrolyze invertase enzyme into subsequent monosaccharides of zymase enzyme will convert monosaccharides into alcohol and carbon dioxide [17]. The value of ethanol levels tends to increase as the fermentation temperature increases.

To know the variable relationship of sugar amounts with alcohol if the pH variable is ignored, then use multiple partial regression analyses. The mathematical relationship of the amount of sugar and alcohol where pH is ignored is \( Y = 8.729 + 0.987X_1 \), with a coefficient of determination of 0.603 (Figure 2). The equation of the regression showed that without the lack of sugar, then alcohol produced at 8.729, and with the addition of sugar 1 unit, the resulting alcohol rose by 0.987. The value of the coefficient of determination is high, indicating that the influence of sugar to alcohol variables is 60.3%.

Multiple regression analyses produce mathematical equations namely: \( Y = 3.799 + 0.996X_1 + 0.666X_2 \) with correlation coefficient and coefficient of determination of 95.1% and 90.5% respectively. The equation shows that when the sugar and pH variables do not exist, then the amount of alcohol is 3.799. The addition of 1 unit sugar will increase the number of alcohol0.996, and the addition of a pH of 1 unit increases the amount of alcohol by 0.666. The mathematical equation of those three variables can be used to predict how large the amount of alcohol is associated with the number of sugars formed in the fermentation process and its relation to the pH value. The correlation coefficient value of 95.1% indicates that the correlation between the three variables is very strong, at 95.1%. The value of the coefficient of determination 90.5% is strongly indicated that the effect of sugar and pH to alcohol is 90.5%.

The optimum temperature for the growth of *Saccharomyces cerevisiae* and its activities is at a temperature of 25–35°C. The weather plays an important role because it can directly affect the activity
of *Saccharomyces cerevisiae* and indirectly affects the level of ethanol produced. The ability of yeast to produce ethanol depends on glucose, pH, oxygen levels, and other environmental factors [18].

Microbial growth is influenced by temperature environment fermentation. Microbes have different growth criteria. *Saccharomyces cerevisiae* has a growth temperature range of between 20-30°C [15]. *Saccharomyces cerevisiae* will grow optimally in the temperature range of 30-35°C, and peak alcohol production is achieved at 33°C temperature. If the weather is too low, then the fermentation will take place slowly, and vice versa, if the temperature is too high, then *Saccharomyces cerevisiae* will die so that the fermentation process will not take place [19].

Distillation technique used to separate alcohol (filtrate fermentation) which have different boiling point [20]. The treatment showed some types of alcohol as filtrate the result of fermentation of sorghum starch, namely: methanol, ethanol, isopropyl alcohol (Table 2). The first distillation out is methanol, where methanol has a boiling point range 68-70°C. Other distillation produced is ethanol and isopropyl alcohol, with a boiling point of 78°C and 80°C.

**Table 2.** Component of alcohol constituent in 42 liters filtrate from fermented of 20% sorghum starch

| Time  | Temperature | % Alcohol | Volume | Description         |
|-------|-------------|-----------|--------|---------------------|
| 13:18 | 70          | 92        | 200    | Methanol            |
| 13:42 | 78          | 89        | 1500   | Ethanol             |
| 13:58 | 78          | 89        | 1500   | Ethanol             |
| 14:38 | 78          | 86        | 1500   | Ethanol             |
| 15:58 | 78          | 80        | 1500   | Ethanol             |
| 16:03 | 82          | 75        | 750    | Ethanol+Isopropyl Alcohol |
| Total |             |           | 6950   |                     |

Description: Ethanol Ratio: filtrate = 6/42 or every 7 liters filtrate obtained 1 liter of ethanol 86%.

The distillation technique is purification to separate 3 types of alcohol (methanol, ethanol, isopropyl alcohol) fermented results. The principle of distillation technique is the difference in the boiling point of liquids at specific pressures. In this experiment, the highest ethanol content (89%) is generated at a boiling point of 78°C. Ethanol levels decrease along with the length of distillation time because the higher the boiling and the length of time, the distillation causes many ethanol to evaporate, thereby lowering the ethanol levels. The treatment fermented of sorghum starch can be estimated that each 100 kg of sorghum starch obtained 100/20 x 6 liters of ethanol = 30 liters of ethanol, sufficient economic value means.

4. **Conclusion**

The optimum pH of amylase enzyme activity in describing sorghum starch into glucose is pH 8. The relationship between the amount of alcohol, sugar, and pH is very strong at 95.1%. Multiple regression equations illustrate the third relationship of the variable that the amount of alcohol (Y) produced in sorghum starch fermentation can be estimated additive by the amount of sugar formed (X1) and pH (X2).

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References

[1] Suarni, “Potential of Sorghum as a Functional Foodstuff,” Food Crop Sci., 2012.

[2] M. P. Sirappa, “The prospect of developing sorghum in Indonesia as an alternative commodity for food, feed, and industry,” J. Agric., 2003.

[3] Anas, “Development of White Sorghum as a Base for the Development of Flour-Based Food Products,” in Proceedings of the National Symposium on Food Integrative, 2011.

[4] B. Irawan and N. Sutrisna, “Sorghum Development Prospects in West Java Support Food Diversification,” Agro Econ. Res. forum, 2016, doi: 10.21082/iae.v29n2.2011.99-113.

[5] A. Bušić et al., “Bioethanol production from renewable raw materials and its separation and purification: A review,” Food Technology and Biotechnology. 2018.

[6] S. I. Mussatto et al., “Technological trends, global market, and challenges of bio-ethanol production,” Biotechnology Advances. 2010.

[7] N. Sarkar, S. K. Ghosh, S. Bannerjee, and K. Aikat, “Bioethanol production from agricultural wastes: An overview,” Renewable Energy. 2012.

[8] M. A. Hapsari and A. Pramashinta, “Manufacture of bioethanol from rubber cassava (Manihot glaziovii) for household stove fuel in accelerating the conversion of kerosene to vegetable fuels,” Chem. Ind. Technol., 2013.

[9] S. Bahri, A. Aji, and F. Yani, “Making Bioethanol from Banana Kepok Skin by Fermenting using Bread Yeast,” J. Unimal Chem. Technol., 2019, doi: 10.29103/jtku.v7i2.1252.

[10] A. R. Fachry, P. Astuti, and T. G. Puspitasari, “Manufacture of Bioethanol from Corn Cob Waste with Variations in Hydrochloric Acid Concentration and Fermentation Time,” J. Chem. Eng., 2013.

[11] H. Zabed, J. N. Sahu, A. Suely, A. N. Boyce, and G. Faruq, “Bioethanol production from renewable sources: Current perspectives and technological progress,” Renewable and Sustainable Energy Reviews. 2017.

[12] N. Nurkholis, N. R. Afifah, and S. Nealma, “Synthesis of Bioethanol From Berenuk Fruit (Crescencia cujete L.) With Alcoholic Hydrolysis And Fermentation Methods,” J. Technol., 2019.

[13] S. Octavia, T. H. Soerawidjaja, R. Purwadi, and I. D. G. A. Putrawan, “Early Processing of Lignoselulosa Using Ammoniac to Increase Fermentation Sugar Acquisition,” Proc. Natl. Semin. Chem. Eng., 2011.

[14] U. Kunaepah, “The Effect of Long Fermentation And Glucose Concentration on Antibacterial Activity, Total Polyphenols, And Chemical Quality of Kefir Milk Red Beans Semarang Graduate Program,” Diponegoro Univ., 2008.

[15] L. Wang, J. Littlewood, and R. J. Murphy, “Environmental sustainability of bioethanol production from wheat straw in the UK,” Renewable and Sustainable Energy Reviews. 2013.

[16] V. S. O’Leary, R. Green, B. C. Sullivan, and V. H. Holsinger, “Alcohol production by selected yeast strains in lactase-hydrolyzed acid whey,” Biotechnol. Bioeng., 1977, doi: 10.1002/bit.260190706.

[17] M. G. Judoamidjojo, M., Darwis, A.A., Sa’id, Fermentation Technology. Jakarta: Rajawali Pers, 1992.

[18] E. R. Dyartanti and M. Margono, “CONTINUOUS PRODUCTION OF BIOETHANOL FROM SORGHUM BICOLOR FROM FLUIDIZED BED BIOREACTORS,” EKUILIBIUM, 2015, doi: 10.20961/ekuilibrum.v14i2.2040.

[19] S. H. Mohd Azhar et al., “Yeasts in sustainable bioethanol production: A review,” Biochemistry and Biophysics Reports. 2017.

[20] H. Dalimunthe, L. A. Harahap, and A. P. Munir, “Effect of Distillation Temperature on Quality and Yield of Patchouli Oil of Direct Steam Destillation Type,” J. Food Agric. Eng., 2015.