Bioethanol Production from Sweet Sorghum Bagasse Through Enzymatic Process

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Abstract. Bioethanol is one of renewable energy considered as fuel petroleum substitute. Generally, bioethanol can be obtained from raw material which contain of sugar, starch or lignocelluloses, i.e sorghum. The aim of this study was to obtain the optimum condition of NaOH concentration and cellulase:xylanase enzymes for bioethanol production from sweet sorghum bagasse. Raw material used was sweet sorghum bagasse. The experiment was conducted four stages: 1) characteristics of raw materials; 2) optimization effect of NaOH solution on delignification process, experimental design at this stage was completely randomized design (CRD) with 1 factor; 3) optimization effect of cellulase and xylanase enzymes (1:1% and (2:2%) to bioethanol production on scale of 500 g raw materials, statistical analysis that used in this stage was t-student test; 4) optimization process of bioethanol production on scale of 50 kg raw material. The results showed that the optimum condition to produced bioethanol from sweet sorghum bagasse was addition of 10% NaOH solution at a temperature of 120-130 °C for 20 minutes. Furthermore, the resulted material from delignification was proceeded to saccharification and hydrolysis using xylanase: cellulase with ratio of 1:1. The bioethanol produced was 13.7% from total sweet sorghum bagasse powder used with alcohol content of 82.8%.

1. Introduction
Bioethanol is one of the new and renewable energies which is widely considered as fuel for substituting petroleum [1]. The use of ethanol as a substituent fuel will reduce harmful gas emissions (CO, NO, and SO₂) and produce very low greenhouse gases when compared to burning petroleum. Bustaman [2] stated that the use of ethanol has several advantages, i.e. high oxygen content reaches 35% so that if burned is very clean, environmentally friendly because it does not contribute to the accumulation of carbon dioxide in the atmosphere and it is renewable.

Generally, the raw material of bioethanol can be obtained which contain sugar, starch and lignocelluloses [3] [4]. Lignocelluloses biomass is a promising new and renewable energy source. The use of lignocelluloses materials for bioethanol production has received special attention in addition to encouraging the development of renewable energy businesses and also to reduce production costs because of the low price [5][6][7]. The conversion of lignocelluloses materials to bioethanol has received important attention because it can be used to substitute the needs of fuel oil in Indonesia. Bioethanol is known to be a mixture of gasoline to fuel motorized vehicles with the advantage of octane number values and evaporation heat [8].
In general, lignocelluloses material is waste from harvesting or processing of agricultural products that are left behind materials and not yet widely used in Indonesia. The potential of lignocelluloses material in Indonesia is quite abundant, including sorghum bagasse. Sorghum bagasse content based on dry weight is cellulose (17-18%), hemicelluloses (18-21%) and lignin (22-23%) [9].

The availability of abundant sorghum bagasse has the opportunity to bring benefits to bioconversion into ethanol. However, the development of the use of lignocellulosic material is still constrained by the problems of their economic and technological feasibility. This is due to the very tight structure and natural characteristics of lignocelluloses and requires pretreatment to break down the sugar components [10][11]. Factors that must be considered to reduce ethanol production costs are efficient processing of raw materials to produce high ethanol yields and high ethanol concentrations [11]. Bioethanol yield from lignocelluloses material is strongly influenced by the pre-treatment and saccharification process to produce glucose and also the fermentation process of glucose to ethanol by \textit{Saccharomyces} sp microbes. Thus, research is needed to improve the efficiency of the delignification process [12], saccharification, and fermentation of glucose produced for bioethanol production. The aim of this study was to obtain the optimum condition of NaOH concentration and cellulase:xylanase enzymes for bioethanol production from sweet sorghum bagasse.

2. Materials and methods
Research was conducted at the Microbiology and Chemistry Laboratory of Indonesian Center for Agricultural Postharvest Research and Development (ICAPRD) and Chemical Research Center LIPI. The raw material used in this research was sweet sorghum bagasse. Chemicals used were aquadest and NaOH (technical grade). Isolate used in this research was the culture of \textit{Saccharomyces cereviceae}. While enzymes used were xylanase and cellulase enzymes (Novozon).

2.1. Raw materials characteristics
At this stage, sweet sorghum bagasse powder was analyzed for proximate analysis (water, fat, protein, ash) and fiber content (lignin, hemicelluloses and celluloses).

2.2. Optimization effects of addition 10% NaOH solution and enzymes (cellulase and xylanase) for bioethanol production on scale of 500 g raw materials
At this stage, sweet sorghum bagasse powder with a water content of <10% was pretreated. The pretreatment process was carried out by soaking sorghum bagasse powder with 10% NaOH solution at a temperature of 120-130°C and pressure of 1 Bar. There were two treatments of different concentration ratio of cellulose: xylanase enzyme namely 1%: 1% and 2%: 2%. Each treatment was repeated 6 times. Observations carried out on the alcohol content using Gas Chromatography (GC) tool. Data of alcohol content obtained were carried out by t-student analysis to determine the best enzyme concentration addition treatment.

2.3. Optimization of the bioethanol production process on a scale of 50 kg raw materials
At this stage, an optimization process of bioethanol production has been carried out using 50 kg of raw material per test. At this stage only two replications were carried out to see the stability of the best treatments obtained from previous stages in producing bioethanol. Observations carried out on content of lignin, cellulose, hemicelluloses, xylose, glucose, and alcohol content. Observation also carried out on the volume and yield of alcohol produced.

3. Results and discussion

3.1. Raw materials characteristics
Ash content is an important factor that can affect the yield of alcohol produced. The requirement for ash content of raw material for bioethanol production is not more than 10% [13]. High ash content can inhibit the fermentation process and cause crust on the tool during the distillation process [13]. The
results of the analysis showed that the ash content of sorghum bagasse was 0.112% (Table 1). The ash content of sorghum bagasse powder was still very suitable to be used as raw material for bioethanol. Cellulose is naturally bound by hemicelluloses and protected by lignin. Lignin compounds are the cause of lignocelluloses materials which are difficult to hydrolyze [14]. Bagasse sorghum powder has fiber content of 36.21% and lignin content of 17.98% (Table 1). High fiber content is expected to increase the yield of cellulose and xylan produced. However, the lignin content of sorghum bagasse powder was very high, so it needed to be pretreatment process both the reduction of raw materials and the use of chemicals. The process of pretreatment and hydrolysis is a very important process step that can affect the yield of ethanol.

| Parameter          | Content (%) |
|--------------------|-------------|
| 1. Moisture        | 9.923       |
| 2. Fat             | 0.899       |
| 3. Protein         | 2.048       |
| 4. Ash             | 0.112       |
| 5. Fiber content:  |             |
| Cellulose          | 36.210      |
| Hemicellulose      | 9.740       |
| Lignin             | 17.980      |

3.2. Optimization effects of addition 10% NaOH solution and enzymes (cellulase and xylanase) for bioethanol production on scale of 500 g raw materials

In this study, the pretreatment process was carried out using the addition of NaOH solution. The purpose of pretreatment is to open the lignocelluloses structure so that cellulose becomes more accessible to enzymes that break down the saccharides polymer into sugar monomers. In addition, pretreatment aims to reduce the crystallinity of cellulose, increase biomass porosity and achieve the desired fractionation [15]. With the increasing amount of lignin degraded, the hydrolysis process will be more perfect so that the fermentation process to convert into ethanol will be optimal [16]. The process of pretreatment (delignification) could reduce lignin contents and increase cellulas contents (Table 2). The degradation process of the lignin structure and hemicelluloses bonds could result in an increase in the amount of free cellulose present in biomass (Table 2).

| Parameter          | Delignification | t-value | t-table |
|--------------------|-----------------|---------|---------|
| Before             | After           |         |         |
| Lignin (%)         | 17.98           | 5.62    | -12.71* | 1.833   |
| Cellulose (%)      | 36.21           | 72.66   | 15.37*  | 1.833   |
| Hemicellulose (%)  | 9.74            | 7.50    | -3.49*  | 1.833   |

Remarks: * = Significant based on t-student test

The treatment dose of 10% NaOH and doses of xylanase: cellulase enzyme by 1%: 1% can produce alcohol content which tends to be higher than the doses treatment of xylanase: cellulase enzyme by 2%: 2% (Table 3). The addition doses of xylanase:cellulose enzyme by 2%: 2% did not increase the ability of cellulose hydrolysis. This is because of the higher the enzyme dose is given, the active side of the enzyme that contact with the substrate (cellulose and xylan) is also increasing so that more cellulose is hydrolyzed to glucose, but too much glucose content causes inhibition of glucose products. The glucose will be attached to the enzyme active side so that the surface areas contact to the substrate are restricted. This is in line with the results obtained by Retnoningtyas [17], namely the use of crude cellulase enzyme 10 ml/liter (1%) produces the highest alcohol content.
Table 3. Ethanol content after fermentation of sweet sorghum bagasse powder on two enzyme doses comparison and addition of *Sacharomycescerevisiae*

| Doses of xylanase:cellulase enzymes | Alcohol content (%) |
|-------------------------------------|---------------------|
| 1% : 1%                             | 5.038               |
| 2% : 2%                             | 3.098               |
| T-value                             | 4.657 *             |
| T-table                             | 2.228               |

Remarks: * = Significant based on t-student test

3.3. **Optimization of the bioethanol production process on a scale of 50 kg raw materials**

Lignin content from sorghum bagasse powder after the pretreatment process was very low, ranging from 5.92%. The cellulose content of sorghum bagasse powder after the pretreatment process was 71.94% (Table 4). Based on these data, it was concluded that the cellulose content was high enough to produce bioethanol which was quite high.

Table 4. Characteristics of sweet sorghum powder before and after delignification process on a scale of 50 kg raw materials

| Parameter                  | Before | Delignification |
|----------------------------|--------|-----------------|
| Lignin (%)                 | 17.13  | 5.92            |
| Cellulose (%)              | 37.42  | 71.94           |
| Hemicellulose (%)          | 9.04   | 6.91            |
| Ash content (%)            | 1.14   | 0.13            |

The glucose content produced in this saccharification process was around 9.5% (Figure 1). The contents of glucose and xylose produced during the saccharification process were increasing with the length of the saccharification process, but the optimum saccharification time was 44 hours (Figure 1).

The fermentation time needed for bioethanol production from sorghum bagasse was around 4 days (95 hours) (Figure 2). But the resulting ethanol content was 4.28% (Figure 2). In general, the alcohol content produced after the fermentation process with lignocellulose raw material ranges from 4-5% [18]. The higher alcohol content, the lower glucose content during the fermentation process (Figure 2). In this research, the alcohol content was produced about 82.8% with a yield of 13.7% (Table 5). From the pretreatment process until the distillation process takes 7 days.

**Figure 1.** Relation graph between glucose and xylose with saccharification time for bioethanol production from sweet sorghum bagasse
4. Conclusions
Bioethanol production process from sweet sorghum bagasse consisted of three stages, i.e. delignification of sweet sorghum bagasse powder used 10% NaOH solution (technical grade) at temperature of 120-130°C for 20 minutes, hydrolysis and saccharification process used xylanase and cellulase with ratio of 1% : 1% for 48 hours (2 days), and fermentation process by added 1% *Saccharomyces cerevisiae* for 4 days. These processes could produced 13.7% bioethanol with alcohol content of 82.8%.

5. References
[1] Zabed H, Sahu JN, Suely H, Boyce AN, Faruq G 2017 Renewable and Sustainable Energy Reviews. 71 475-501
[2] Bustaman S 2008 Jurnal Ekonomi Dan Pembangunan. 17 89-106
[3] Schlafle S, Senn T, Gschwind P, Kohlus R 2017 Bioresource Technology. 109-115
[4] Sebayang AH, Masjuki HH, Ong HC, Dharma S, Silitonga AS, Mahlia TMI, Aditiya HB 2016 RSC Advances. 6 14964–14992
[5] Knauf M, Moniruzzaman M 2004 Intl. Sugar J. 106(1263) 147–150
[6] Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL, Mielenz JR, Murphy R, Templer R, Tschaplinski T 2006 Science. 311 484–489
[7] Schubert C 2006 Nature Biotechnology. 24(7) 777–784
[8] Chen, Hongzhang, Qiu W 2010 Biotechnology Advances. 28 556–562
[9] Su MY, Tzeng WS, Shyu YT 2010 Bioresource Technology. 101 6669–6675
[10] Li X, Tau HK, Nghiem NP 2010 Bioresource Technology 101 5910-5916
[11] Alvira PE, Tomas-Pejo, Ballesteros M, Negro MJ 2010 Bioresource Technology. 101 4851-4861
[12] Gnansounou, E 2010 Bioresource Technology. 101 4842-4850
[13] Kartika AA, Mariana HS, Widjaya A, Mulyanto 2013 J Teknik Pomits. 2(1) 1-5
[14] Iranmahboob, Nadim JF, Monemi S 2002 Biomass and Bioenergy. 22 401-404.
[15] Kristina, Sari ER, Novia 2012 J Teknik Kimia. 3(18) 34-43
[16] Samsuri M, Gozan M, Mardias R, Baiquni M, Hermansyah H, Wijarnako A, Prasetya B, Nasikin M 2007 Makara Teknologi. 11(1) 17-24
[17] Retnoningtyas ES, Antaresty, Aylianawati 2013 Reaktor. 14(4) 272-276