Optimization of pulp concentration for bioethanol production from elephant grass (*Pennisetum purpureum*) using two commercial yeasts with addition of Tween 20

I Winarni¹, T K Waluyo¹ and G Pasaribu¹

¹Forest Product Research and Development Center, FORDA, Ciomas-Bogor 16610, Indonesia

E-mail: inawinarni@forda-mof.org

Abstract. The higher consumption of fuel, especially fossil fuels, will lead to depletion of fuel reserves and high air pollution, which causes environmental pollution. Therefore, it is necessary to find alternative fuels that are environmentally friendly and abundant availability in nature, so-called biofuels. One type of biofuel is bioethanol. Raw materials that can be produced into bioethanol are biomass or lignocellulosic material. Elephant grass is one type of lignocellulosic material that has high cellulose content (40.85%). The objective of this study was to determine the optimum pulp concentration by adding Tween 20 to the saccharification process with two types of commercial yeasts (fermipan and mauripan) in the fermentation process. The pulp variations used were 20, 25 and 30% w/v with Tween 20 concentrations 0% and 1% v/v. The results showed an increase in pulp concentration, and the addition of Tween 20 by 1% could increase the ethanol content compared to controls (Without Tween 20). The highest ethanol content was produced at 30% pulp concentration (13.09%) with addition 1% of Tween 20 in the saccharification process and fermipan yeast in the fermentation stage, and it could achieve ethanol content of 13.94%(v) when using Mauripan yeast.

1. Introduction
The need for gasoline is still very dependent on fossil fuels, which results in the depletion of the availability of fossil fuels. Therefore it is necessary to look for alternative sources of material that are renewable and environmentally friendly [1]. One of the alternative fuels that can be used is bioethanol, which can be produced from lignocellulosic material. One of the lignocellulose materials from agricultural waste that can be used as raw material for bioethanol production is elephant grass (*Pennisetum purpureum*). Nowadays, elephant grass is only used as fodder and is often considered a nuisance crop. Still, elephant grass contains high cellulose content (40.85%), which can be developed as a source of bioethanol production. The availability of elephant grass can be obtained continuously and abundantly because Indonesia has a tropical climate that facilitates the growth of elephant grass [2].

Conversion of lignocellulosic material into bioethanol is done through four main stages, such as pretreatment, hydrolysis, fermentation, and distillation, or ethanol separation. The pretreatment aims to reduce lignin content, reduce the level of crystallinity of cellulose, and expand the surface of biomass that makes the lignocellulose substrate easier to convert [3]. Research conducted on the effect of the pretreatment process has used: several concentrations of NaOH (0.2, 4, 6% w/v); temperature (130, 150, 170 °C) for (20, 40, 60 and 80 minutes) in the autoclave and in the production of bioethanol from...
elephant grass (*Pennisetum purpureum*), in which to increase cellulose lignin delignification can be done in the pretreatment process by varying NaOH, temperature and time [4]. The results showed that the highest glucose and bioethanol content were obtained at a concentration of 6% NaOH at a temperature of 130 °C with glucose content of 2.501% and bioethanol of 0.503% for 40 minutes and at a concentration of 6% NaOH at 40 minutes (temperature 130 °C ) with glucose content of 2.567% and bioethanol of 0.521%. Another research showed that the addition of 1 % until 3% concentration (v/v) Tween 20 as a surfactant in the bamboo pulp hydrolysis could increase the reducing sugar up to 90% and subsequently decreased [5].

Previous research on bioethanol production from the bamboo waste pulp through enzymatic hydrolysis, [6] stated that the increase in substrate concentration and the addition of surfactants could significantly increase reducing sugar levels in the hydrolysis process. [7] also stated that the addition of substrate concentration during hydrolysis would increase the ethanol content produced after fermentation.

Fermentation is influenced by several factors, one of which is the type of microorganism. *Saccharomyces cerevisiae* is one of the yeast species that has a very high ethanol conversion. This yeast is also known as bread yeast, which is widely circulated in the market with different trade names, such as Fermipan and Mauripan. Its presence in many markets makes these two yeasts effectively used in the fermentation process in bioethanol production. Previous researchers stated that the use of commercial yeast (*Saccharomyces cerevisiae*) was effective in the first six days of fermentation of elephant grass into ethanol, and after six days, the ethanol content decreased [8].

Based on these studies, an optimization study was proposed of bioethanol production from elephant grass by varying the pulp concentration and the addition of Tween 20 as a surfactant, and also used two different commercial yeasts for the fermentation process to produce maximum ethanol content.

### 2. Experiment

#### 2.1. Materials

Elephant grass was chopped about 2-3 cm in size and dried in the sun to reduce water content, and then it was mashed using a blender to get the sample in powder form. The sample was digested using 1% NaOH solution for delignification process in Autoclave. Digestion was kept at 121°C for one hour. The softened pulp was filtered and neutralized using hot water and dried in the oven for three hours. Elephant grass dried powder and pulp were analyzed for their chemical content, such as analysis of water content, ash, lignin, cellulose, solubility in hot water, solubility in cold water, and solubility in NaOH 1%.

Tween 20 is non-ionic surfactant; it has a chemical name polyoxyethylene sorbitan monolaurate. It is obtained from Merck, with a relative molecular mass of 1228, CMC of 0.059 mM, and HLB of 16.7 [9]. The surfactant chemical structure is shown in Figure 1.

![Figure 1. Chemical structure of Tween 20.](image_url)

The commercial cellulase from Novozyme was used in all enzymatic hydrolysis in this research. The concentration of cellulase enzymes used in the saccharification process was 10 FPU and had an
enzyme activity of 80 FPU/mL. Enzyme activity is needed to calculate the volume of cellulase enzymes needed in the hydrolysis process.

2.2. Procedure for enzymatic hydrolysis

One percent (1%) concentration of Tween 20 was dissolved in 100 mL of 0.05 M citric acid (C6H8O7) buffer at pH 4.8. Cellulase (10 FPU/g substrate) was added to the solution, and the mixture was stirred for one h at room temperature. Finally, several concentrations of substrate (20, 25, 30% w/v) were added to the solution, and the suspension was shaken at 50° C for 48 h. After saccharification, as much as 5 mL of saccharification product from each experiment was filtered. The reducing sugar concentration in the filtrate was measured by DNS (3,5-dinitrosalicylic acid) method. The rest of the saccharification product from each experiment was fermented by adding 1% (w/w) of two different commercial yeasts, namely Fermipan and Mauripan and 0.3% (w/v) of urea. The fermentation process was performed at room temperature (26-28°C) for 96 hours. The resulted fermentation broth was diluted twice with water then distilled until the distillate volume reached 100 mL, and ethanol concentration was determined using GC analysis.

3. Result and discussion

3.1. Physical and chemical characteristics of delignified elephant grass powder and pulp

Elephant grass powder was produced from elephant grass that had been ground using a blender and dried. Elephant grass powder was delignified using 1% NaOH. It is because NaOH solution can damage the structure of lignin in the crystalline and amorphous parts and partially separate hemicellulose [10]. There are differences in sample weights before and after delignification. Before delignification process, elephant grass powder was 300 g (dw), and after that, the weight became 160 g (dw). Physical changes occurred in the original elephant grass powder (brown), and the structure was harder before delignification process. The dry pulp became brownish-yellow, and the structure became softer, as shown in Figure 2.

![Figure 2](a) Powder and (b) dry pulp of elephant grass.

3.2. Chemical compound analysis

A chemical compound, such as water content, ash, lignin, and cellulose, which was ground into a powder, and dried pulp of elephant grass are listed in Table 1. In addition to animal feed, elephant grass is one type of grass that is underutilized and sometimes considered a nuisance plant. However, elephant grass contains high levels of cellulose so that it can be used as raw material for bioethanol [2].

The result showed that the water content in elephant grass dry pulp was 1.72%. Production of bioethanol prefers samples with low moisture content because it is an impurity substance that can decrease the speed of hydrolysis and fermentation [11].

Lignin is the main component of plant cell wall compilers, which is the second most abundant biopolymer after cellulose. Besides, it has a function as protective cellulose and hemicellulose and binding of other components so that a tree can stand upright [12]. The presence of lignin in the cell wall can inhibit enzymes from converting cellulose to become glucose, and without delignification
process, the enzymatic hydrolysis is very low (<20%) [13]. However, there is a lack of information about the influence of lignin on the adsorption of cellulases, along with the hydrolytic capacity of the cellulases adsorbed on lignin [14].

Table 1. The chemical compound of powder and pulp of elephant grass.

| Parameter (%) | Raw material (%) | Dry pulp (%) |
|---------------|------------------|--------------|
| Water content | 11.9             | 1.72         |
| Ash content   | 12.8             | 5.52         |
| Lignin content| 12.4             | 2.90         |
| Cellulose content | 52.3        | 73.2         |

The delignification process of elephant grass powder into dry pulp can increase cellulose content and reduce water, ash, and lignin content, as shown in Table 1. The results showed that cellulose content in elephant grass powder after being delignified increased by 21%. The lignin delignification process contained in the pulp has been separated from cellulose so that the content of cellulose increases (73.3%). Dawson and Boopathy [15] stated that optimum cellulose hydrolysis would succeed if 50% or more of lignin had been removed from the lignocellulose material. In the production of bioethanol, ash content is also expected to be low because ash is an impurity that can reduce the speed of hydrolysis and fermentation [11]. Thus, delignification must be performed to reduce lignin content and cellulose crystallinity. Also, it is to shrink the size of the biopolymer using fragmentation, grinding, milling, and mechanical interactions to make it more easily hydrolyzed, thus increasing the ethanol content.

The main ingredient of bioethanol production is cellulose. Cellulose is a beta glucose polymer with a β-1,4 bond between its glucose units. Cellulose also functions as a structural material in plant tissue in the form of a mixture of homologous polymers. It is usually accompanied by other polysaccharides and lignin in varying amounts. High cellulose content indicates that the pulp has the potential to be further processed into bioethanol, with pretreatment, hydrolysis, and fermentation. Samples with higher cellulose content will give much more mass of sugar C6 (sugar with six carbon atoms) than samples with lower cellulose content [11].

3.3. The effect of Tween 20 and substrate concentration

Analysis of reducing sugar content in elephant grass pulp is to determine the content of glucose monomer produced after the hydrolysis process. Figure 3 shows the value of reducing sugar of elephant sugar pulp without Tween 20 (A) and 1% (v/v) Tween 20 (B) as a surfactant. The results showed that an increase in reducing sugar content occurred in the addition of 1 mL tween 20 in the hydrolysis process. The difference of all treatments showed that the content of reducing sugar with the addition of 1% (v/v) of tween 20 was higher than 0% Tween 20 or control. It proved that the use of surfactant (Twee 20) could increase reducing sugar content. The previous research by [16] examined the effect of several surfactants on enzymatic hydrolysis of the newspaper and found Tween to be among the best performers, with two times higher conversion at 80 h than without surfactant. From the previous work, it was concluded that non-ionic surfactants could enhance cellulose performance to overcome the negative effect of cellulose crystalline [17],[18],[19]. Another research by [20] stated that Tween 20 surfactant had a positive effect on the hydrolysis process by accelerating the reaction and by increasing the action of enzymes in converting cellulose to glucose. Therefore, reducing sugar content with the addition of surfactants will be higher without surfactants. [6] say that reducing sugar content will increase with the addition of surfactants as much as 1 mL in each treatment variation (20, 25, and 30% w/v substrate), compared to without the addition of surfactants. It is because the addition of surfactants makes it easier for the substrate to be hydrolyzed to produce reducing sugars. The addition of surfactants with high concentrations is also likely to prevent contact between cellulose and cellulase enzymes so that it will reduce the rate of conversion.
Figure 3. Reducing sugar content of dry pulp of elephant grass without Tween 20 (A) and 1% Tween 20 (B).

The addition of pulp concentration to the hydrolysis process also significantly increased the reducing sugar content. The pulp concentrations used were 20, 25 and 30% (w/v). The analysis results of reducing sugar content (Figure 3) showed the highest increase in reducing sugar content produced at 30% (w/v) of pulp concentration. It is because the higher the substrate concentration, the higher the total sugar produced [21]. The results of this study are also the same as [22] that said an increase in substrate concentration would increase reducing sugar content.

Figure 4. Ethanol Content of dry pulp of elephant grass using (A) Fermipan and (B) Mauripan commercial yeast.

The product of the cellulose hydrolysis process was used in the fermentation for ethanol production. A microbe that is commonly used in the fermentation process is *Saccharomyces cerevisiae* because it has a high alcohol tolerance level [23]. Ethanol content obtained from this research varied depending on the concentration of the substrate added to the hydrolysis process. The addition of 1% (v/v) Tween 20 as a surfactant at all treatment of elephant grass pulp resulted in increased content of ethanol, both fermented with yeast Fermipan and Mauripan (Figure 4). Ethanol content produced from fermentation using Fermipan was not much different from Mauripan yeast. It is because the two yeasts have the same composition, which contains *Saccharomyces cerevisiae*. What distinguishes these two yeasts is that Mauripan is cheaper than Fermipan. On the other hand, ethanol content increases by increasing the concentration of the substrate added to the hydrolysis process and also by the addition of 1% (v/v) tween 20 as a surfactant.

The highest ethanol content obtained by the use of Fermipan and Mauripan when fermentation process. 30% (w/v) substrate concentration and also the addition of 1% Tween 20 during the
hydrolysis process are equal to 13.09% and 13.94%, respectively. This result is following the results of research conducted by [5] and [24]. Meanwhile, ethanol content without surfactant (control) showed lower values in all treatments. These results also are following the research conducted by [5] and [20].

3.4. Cellulose conversion

The percentage of cellulose conversion in the treatment of several pulp concentrations without addition Tween 20 (0%) and using 1% tween 20 during the hydrolysis process is shown in Figure 5. The results showed that cellulose conversion increased with the increase of substrate concentrations and the addition of 1% tween 20 during the hydrolysis. It is due to the growing amount of substrate added during hydrolysis. Also, the more cellulose is converted to sugar. Increasing excessive substrate concentration can also reduce enzyme performance because enzyme activity has reached its maximum limit, or it can be said the condition where all enzymes have been saturated with substrates [25]. The more substrates added to the hydrolysis, the longer it takes to convert cellulose to glucose. It lacks cellulose because there is no more addition of enzyme concentration in the solution, so there are still many celluloses that have not been converted into glucose [20]. Researchers stated that the addition of surfactants to the hydrolysis process could increase the conversion of cellulose to glucose with low enzyme concentrations [26]. The percentage conversion of cellulose (Figure 5) in all treatments has an average value of 60%. It proves that the conversion of cellulose into glucose is good enough.

4. Conclusion

Based on the results of this study, it can be concluded that the increased substrate concentration of dried pulp elephant grass (Pennisetum purpureum) and addition of 1% (v/v) tween 20 during the hydrolysis process can increase reducing sugars and ethanol content after the fermentation process, compared to without the addition of Tween 20 (control). The highest ethanol content was produced from the treatment of 30% (w/v) substrate concentration, with the addition of 1% (v/v) Tween 20 during the hydrolysis process. The addition of Fermipan and Mauripan in the fermentation process was equal to 13.09% and 13.94%, respectively. On the other hand, the results showed that Mauripan as commercial yeast produced higher ethanol content than Fermipan in all treatments.

5. References

[1] Wiraatmaja I 2010 Physical characteristics test of biogasoline as an alternative fuel to replace a pure engine (in Indonesian) J. Ilm. Tek. Mesin 4: 145–154

[2] Sari N K 2009 Bioethanol from elephant grass (Pennisetum purpureum) by batch distillation (in Indonesian) Jurnal Teknik Kimia 8: 94–103
[3] Qi B, Chen X and Wan Y 2010 Pretreatment of wheat straw by nonionic surfactant-assisted dilute acid for enhancing enzymatic hydrolysis and ethanol production Bioresour. Technol. 101: 4875–4883

[4] Hasan A, Amiruddin and Kusmiyati 2014 Effects of alkaline pretreatment on bioethanol production from elephant grass (Pennisetum purpureum) (in Indonesian) Prosiding Seminar Rekayasa Kimia dan Proses Semarang pp 1-14

[5] Winarni I and Maulidina R 2018 Bioethanol production from bamboo pulp using enzymatic saccharification with several concentration of surfactant bioethanol production from bamboo pulp using enzymatic saccharification with several concentration of surfactant International Proceeding Conference of Biomass p 209

[6] Alberthys N R 2017 Utilization of bamboo waste for making bioethanol through enzymatic hydrolysis process (in Indonesian) [Thesis] (Bogor: Institut Pertanian Bogor)

[7] Bardant T B, Abimanyu H and Faesal A 2012 Study on response surface methodology (RSM) of water hyacith alkaline pretreatment and enzymatic hydrolysis Proceeding of The 2nd Korea-Indonesia Workshop & International Symposium on Bioenergy from Biomass p 130–134

[8] Nasution H, Dewi R S and Hasibuan 2016 Bioethanol from elephant grass (Pennisetum purpureum scumach) using the acid hydrolysis method and fermentation by Saccharomyces cerevisiae (in Indonesian) J. Pendidik. Kim. 8 p 144–151

[9] Eriksson T, Börjesson J and Tjerneld F 2002 Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose Enzyme Microb. Technol. 31: 353–364

[10] Gunam I, Buda K and Guna I M Y S 2010 effect of delignification treatment with naoh solution and rice straw substrate concentration on cellulase enzyme production from Aspergillus niger NRRL A-II (in Indonesian) Biologi 14: 55–61

[11] Sokanandi A, Pari G, Setiawan D and Saepuloh 2014 Chemical components of ten types of wood less known as possible use as raw materials for making bioethanol (in Indonesian) Jurnal Penelitian Hasil Hutan 32: 209–220

[12] Anindyawati T 2009 Prospects for enzymes and lignocellulosic wastes for bioethanol production (in Indonesian) Selulosa 44: 49–56

[13] Lynd L R 1996 Overview and evaluation of fuel ethanol from cellulosic biomass : technology, economis, the enviroment, and policy Technol. Econ. Enviroment Policy 21: 403–465

[14] Li Y, Sun Z, Ge X and Zhang J 2016 Effect of lignin and surfactant on adsorption and hydrolysis of cellulosases on cellulose Biotechnol. Biofuels 9: 1–9

[15] Dawson L and Boopathy R 2008 Cellulosic Ethanol production from sugarcane baggase without enzymatic saccharification BioResources 3: 452–460

[16] Park J W, Takahata Y, Kajiuchi T and Akehata T 1992 Effects of nonionic surfactant on enzymatic hydrolysis of used newspaper Biotechnol. Bioeng 39: 117–120

[17] Berlin A, Gilkes N, Kurabi A, Bura R Tu M B, Kilburn D and Saddler J N 2005 Evaluation of noble fungal cellulase preparations for ability to hydrolyze softwood substrates - evidence for the role of accessory enzyme Enzyme Microb. Technol. 37: 175–184

[18] Boussaid A and Saddler J N 1999 Adsorption and activity profiles of cellulosases during the hydrolysis of two Douglas fir pulps Enzyme Microb. Technol. 24: 138–43

[19] Winarni I, Koda K, Waluyo T K, Pari G and Uraki Y 2014 Enzymatic Saccharification of soda pulp from sago starch waste using sago lignin-based amphipathic derivatives J. Wood Chem. Technol. 34: 157–168

[20] Winarni I and Bardant TB 2017 Bioethanol from sengon wood waste with high concentration substrate method (in Indonesian) Jurnal Penelitian Hasil Hutan 35: 231-242

[21] Hawuswiwa E, Sutrisno A K, Wardani and Ningtyas D W 2015 The influence of cassava paste concentration (Manihot esculenta) and fermentation time on the process of making cassava wine (in Indonesian) Jurnal Pangan dan Agroindustri 3: 147–155

[22] Kumar S, Gajjala L K S and Banerjee R 2017 Simultaneous pretreatment and saccharification of bamboo for biobutanol production Ind. Crops Prod. 101: 21–28
[23] Sassner P, Martesson C, Galbe M and Zacchi G 2008 Steam pretreatment H2SO4 impregnated salix for production of bioethanol *Bioresour. Technol.*

[24] Bardant TB, Indiyanti and Selviyanti T 2012 Study of response surface methodology (RSM) on the effect of span 85 in high substrate loading enzymatic hydrolysis of palm oil EFB *Proceeding of The 2nd Korea-Indonesia Workshop & International Symposium on Bioenergy from Biomass* p 230–37

[25] Bahri, Sayful M, Mirzan and Hasan M 2012 Characterization of amylase enzyme from glutinous corn seed sprouts (*Zea mays ceratina* L) *J. Nat. Sci.* 1: 132–143

[26] Parnthong, Jatuporn and Suratsawadee K 2014 statistical optimization for application of nonionic surfactant in enzymatic hydrolysis of palm fiber for ethanol production *Int. J. Chem. Eng. Appl.* 5: 123–30