

## RESEARCH ARTICLE



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# Comparison of Production Glucose from Starch and Cellulose Using Delignification and Hydrolysis Process

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Availability of starch (liquid waste rice flour) and cellulose (bulrush and bamboo) are available in substantial quantities in Indonesia. This research has a goal to produce glucose from starch, cellulose using delignification and hydrolysis process. Hydrolysis is a process for the breakdown of cellulose into glucose; it is a raw material which gets less attention after raw material analysis have starch, cellulose and glucose levels, have potential as glucose feedstock. The Bamboos used in the hydrolysis process is those that do not contain lignin and pentosan that previously performed pretreatment and delignification process. This research is to produce glucose as a feedstock for bioethanol, knowing pentosan and impurities remaining in bamboo solution. This study has the objective to produce glucose and glucose as well as an industrial prototype as a reference in developing bioethanol industry in Indonesia. Therefore it needs to be studied in the future, what is the best process that we can use, biology process or chemical process using NaOH, HCl, and H<sub>2</sub>SO<sub>4</sub> that can optimize glucose production. The enzyme treatment is also significant because the enzyme has an optimal condition, temperature and the degree of acidity (pH) and when the enzyme is in optimal conditions, it can increase its work rate. Variable remain in hydrolysis process is weight raw material 50 to 250 grams, temperature 45 °C, and stirring speed in control 200 rpm, enzyme volume on 1 to 10 ml, time 30 until 120 minutes. The comparison obtained can be used as a basis for the design of a prototype glucose production processes and equipment. Resulting from delignification and hydrolysis process are glucose, liquid waste rice flour on 9.98% glucose contain, bulrush on 37.9% glucose contain, and bamboo on 23.6% glucose contain. The results from hydrolysis process is glucose, and the next treatment fermented to obtain product bioethanol.

**Keywords:** Cellulose, Delignification, Glucose, Hydrolysis, Starch.

## 1. INTRODUCTION

Biomass from plants has been declared as an alternative raw material for gasoline fuel substitution in the form of bioethanol, bioethanol obtained from biomass and bioenergy crops has been proclaimed as one of the feasible alternatives as gasoline fuel.<sup>1</sup> Environmental and sustainability were bioethanol from rice straw.<sup>2</sup> The technology for lignocellulose ethanol production is primarily dependent on initial treatment, chemical or enzymatic hydrolysis, fermentation and distillation. A proper treatment strategy is required so that the enzyme hydrolysis of ligno-cellulosic biomass to be efficient because lignin prevents the saccharification process. There were numerous approaches for pretreatment process before such as acid or alkali pretreatment, pretreatment with hydrogen peroxide, steam explosion, liquid hot water, ammonia fiber expansion pretreatment, NaCl, and biological pretreatment.<sup>3</sup>

The research conducted to evaluate acid pretreatment from paper waste as material for bioethanol production, optimized sulfuric acid hydrolysis, fermentation process of HCl of paper waste by using *Pichia Stipitis*. The ethanol content was obtained of 77.54%. By one more distillation process, the ethanol content will be obtained at the level of 95–96%.<sup>4</sup> Chemical pretreatment of lingo-cellulosic biomass with H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub> acids are widely used since they are relatively cheap and efficient in lignocellulose, though the latter gives a milder effect and is more benign to the environment. HCl is more volatile and easier to recover and attacks biomass better than H<sub>2</sub>SO<sub>4</sub> similarly,<sup>5</sup> HNO<sub>3</sub> process good cellulose to sugar conversion rates.<sup>6</sup> However, both acids are expensive compared to H<sub>2</sub>SO<sub>4</sub>.

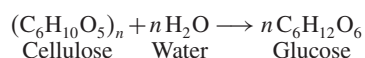
Pretreatment of lignocellulose has received considerable research globally due to its influence on the technical, economic and sustainability of cellulose ethanol production. This paper reviews already known and emerging chemical pretreatment methods, the combination of chemical pretreatment with other methods to improve carbohydrate preservation reduce formation

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to degradation of the product, achieve high sugar yield at mild reaction conditions, reduce solvent loads and enzyme dose, and reduce waste generation.<sup>7</sup> Technical and economical were evaluated bioethanol production from lingo-cellulosic residues, the case of sugarcane and blue agave bagasse.<sup>8</sup>

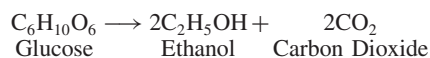
Initiatives will be future for lignin in biomass to bioethanol, pretreatment technologies to separate the three most important biopolymers (cellulose, hemicellulose, and lignin).<sup>9</sup> Pretreatment for hydrogen and bioethanol production from olive oil waste products was ethanol yield 5.4% treatment with 1.75 w/v H<sub>2</sub>SO<sub>4</sub> and heated at 140 °C for 10 min, and was ethanol yield 5.0% no pretreatment.<sup>10</sup> Pretreatment followed with simultaneous scarification and fermentation on bioconversion of microcrystalline cellulose for bioethanol production, the yield value of 67% bioethanol bioconversion.<sup>11</sup> A sustainable feedstock bioethanol production, cellulose hydrolysis was microwave irradiation using hydrochloric acid as catalyst, fermentation with yeast (*Saccharomyces Cerevisiae*), modest reaction conditions (2.38 M acid concentration), irradiation time 7 min, and yield of 0.67 grams glucose/grams cellulose.<sup>12</sup>

The glucose forming reaction from cellulose is as follows:



Elements contained in the lignocellulose biomass of the plants are usually used lignocellulose biomass are potentials for bioethanol production globally. Agriculture (soft wood), forestry (hardwood), and industrial waste are a major lignocellulose biomass for bioethanol production. The lingo-cellulosic biomass is one of the potential main sources for economic bioethanol production globally. Agricultural, forestry (soft and hardwoods) and industrial wastes are the major lingo-cellulosic biomasses.<sup>13</sup> The lingo-cellulosic biomass for bioethanol production was developed using inhibitors-tolerant *Saccharomyces Cerevisiae*, more than 4% (w/w) ethanol concentration was achieved, which corresponded to 72.3% theoretical yield of ethanol.<sup>14</sup> Bioethanol production using sodium hydroxide pretreated sweet sorghum bagasse without washing, ethanol theoretical yield from 44.06 ± 0.93% to 65.14 ± 0.91%.<sup>15</sup>

The fermentation process is affected by microorganisms that need proper nutrition to obtain a good result of fermentation. Good nutrition to supply microorganisms is nitrogen which can be obtained from the addition of NH<sub>3</sub>, ammonium salts, peptone, amino acids, and urea. Liquid nitrogen that is needed is 400–1000 gram/1000 lt. Phosphate is needed for 400 gram/1000 liter<sup>16</sup> in the fermentation process, glucose from fermentation process is converted into ethanol by the following reaction:



To increase the fermented sugar concentration after enzymatic scarification, conversion of glucose and xylose into ethanol needs a new fermentation technology.<sup>17</sup> The bioethanol production from lignocellulose biomass using process pretreatment, hydrolysis, fermentation and recovery of ethanol, was obtained by ethanol under 16% v/v, with the distillation process will again be derived ethanol 95–96% v/v. The research conducted bioethanol production from lignocellulose biomass by using pretreatment process, hydrolysis, and fermentation ethanol recovery. Therefore, ethanol content was obtained in the level below 16%, and by one more

distillation process, the ethanol content would be obtained at the level of 95–96% v/v.<sup>18</sup>

The research conducted by Ref. [16] about bioethanol production from agricultural waste using PROFER pretreatment method obtained ethanol content below 16%. Chemical pretreatment of ligno-cellulosic biomass with H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub> acids are widely used since they are relatively cheap and efficient in the hydrolysis of lignocellulose, though the latter gives a milder effect and is more benign to the environment. HCl is more volatile and easier to recover and attacks biomass better than H<sub>2</sub>SO<sub>4</sub>.<sup>19</sup> Chemical pretreatment of ligno-cellulosic biomass with H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub> acids are widely used since they are relatively cheap and efficient in the hydrolysis of lignocellulose, though the latter gives a milder effect and is more benign to the environment. HCl is more volatile and easier to recover and attacks biomass better than H<sub>2</sub>SO<sub>4</sub>.<sup>19</sup> The research conducted by Ref. [20] about ethanol production from sago pith waste (SPW) using microwave hydrothermal hydrolysis catalyzed by carbon dioxide, resulted in higher energy saving compared to previous techniques in the absence of enzymes, acid or base catalyst. They obtained ethanol content below 15.6%.

The production bioethanol from cashew apple juice was fermentation by microorganism *Saccharomyces Cerevisiae* Y2084 and Vin 13. The maximum ethanol concentration achieved by Y2084 was 65.00 g/L and by Vin 13 was 68.00 g/L, and fermentation time was 2 days.<sup>21</sup> Bioethanol from paper fiber residue using diluted NaOH, the fermentation process with *Saccharomyces Cerevisiae*. The fermentability of the hydrolysis decreased sharply for hydrolysis produced at a temperature higher than 50 °C, the ethanol in monosaccharide hydrolysis was found to be 34.06 g/L and ethanol yield was 0.097 g/g.<sup>22</sup> Simultaneous bio hydrogen and anaerobic fermentation with Immobilized sludge for production bioethanol with Continuous Stirred Tank Reactor (CSTR), the concentration H<sub>2</sub> production rate 10.74-mile mole/h. L and ethanol production rate 11.72-mile mole/h. L.<sup>23</sup>

Bioethanol production from lignocellulosic biomass involves different step such as pretreatment, hydrolysis, fermentation and ethanol recovery.<sup>18</sup> There were numerous approaches for pretreatment process before such as acid or alkali pretreatment, pretreatment with hydrogen peroxide, steam explosion, liquid hot water, ammonia fiber expansion pretreatment, NaCl, and biological pretreatment. Bioethanol production from sago pith waste (SPW) using Hydrolysis by carbon dioxide, a maximum of 43.8% glucose and 40.5% ethanol yield, the developed technique for SPW resulted in higher energy saving compared to previous methods in the absence of enzymes, acid or base catalyst.<sup>20</sup>

Cellulosic or second generation (SG) bioethanol is produced from lignocellulosic biomass (LB) in three main steps: pretreatment, hydrolysis, and fermentation. Pretreatment involves the use of physical processes, chemical methods, physicochemical processes, biological methods, and several combinations thereof to fractionate the lignocellulose into its components. It results in the disruption of lignin seal to increase enzyme access to holocellulose,<sup>24,25</sup> reduction of cellulose crystallinity,<sup>26,27</sup> increase in the surface area,<sup>28,29</sup> porosity<sup>30,31</sup> of pretreated substrates, resulting in increased hydrolysis rate. In hydrolysis, cellulose and hemicelluloses are broken down into monomeric sugars via the addition of acids or enzymes such as cellulase. Enzymatic hydrolysis offers advantages over acids such as low energy

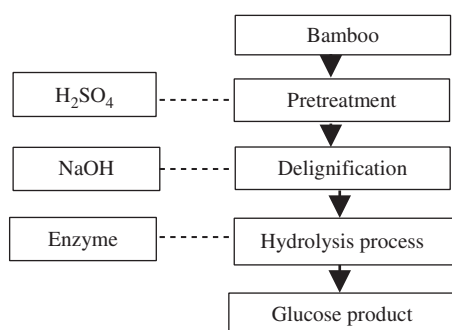


Fig. 1. Glucose production flow used pretreatment, delignification, and hydrolysis process.

consumption due to the mild process requirement, high sugar yield, and no unwanted wastes. Enzymatic hydrolysis of cellulose affected by properties of the substrate such as porosity, cellulose fiber crystallinity, and degree of polymerization, as well as lignin and hemicellulose content,<sup>32,33</sup> optimum mixing,<sup>34</sup> substrate and end-product concentration, enzyme activity, reaction conditions such as pH and temperature.<sup>35,36</sup> Numerical and experimental bioethanol production from liquid waste of rice flour with process hydrolysis, fermentation and batch distillation with batch process.<sup>37,38</sup>

From the previous research, it was known that cellulose from bulrush and bamboo resulted in good cellulose. The aim of this study was to search material bulrush and bamboo from Indonesia with pretreatment process, and delignification process to gain cellulose product with a high level of cellulose. The originality of this research was the second generation that was bulrush and bamboo, by using two processes (pretreatment and delignification) simultaneously, technical cellulose production with combination acid ( $H_2SO_4$ ) and base (NaOH), degradation lignin levels, and pentosan levels as the substitution material of glucose, as for liquid waste rice flour does not require delignification process, process scarification is required.

## 2. METHODOLOGY

Laboratory analysis did the quality analysis of raw materials and cellulose product. The analysis was conducted on the instrumentation and gravimetric analysis by using Atomic Adsorbent Solvent (AAS) and Spectrophotometer, which analyzed items were the concentration of cellulose, lignin, pentosan, extractive substances, water, ash, and  $SiO_2$ .

Procedure of pretreatment and delignification process: starch (liquid waste rice flour) and cellulose (bulrush and bamboo) is dried under the Sun, weight bamboo is 50–250 grams, as many as 3 liter aquadest mixed with 200 ml of  $H_2SO_4$  2N, then inserted into the tool hydrolysis, in the heater with temperature 25–125 ( $^{\circ}C$ ), and hydrolysis time is 30–120 minutes. In this process, the lignin will be separated from bamboo with filtering tools, so a layer of cellulose will be processed hydrolysis with free lignin. The procedure of hydrolysis process: Sample of weight bamboo 50 grams, that has become powder was added with 3-liter of water, and NaOH 2N with the variation of concentration of 2–10 ( $\%v/v$ ), heated at a temperature 25–125 ( $^{\circ}C$ ), then filtered and washed with water until neutral pH.

The procedure of hydrolysis process: the fibers obtained from the process of pretreatment is taken as much as 50–250 grams and added water volume 800 ml is then inserted into the tool

hydrolysis, the process of hydrolysis using free variable volume enzyme of cellulose hydrolysis and time. So the experiment was performed for 30 until 120 minute times with a variable that has been defined earlier. The temperature maintained at 45  $^{\circ}C$ , because the enzyme of cellulose can work optimally at temperatures around 40–50  $^{\circ}C$  and the stirring round of speed 200 rpm. After it is filtered and the filtrate is obtained from the solution of starch (liquid waste rice flour) and cellulose (bulrush and bamboo) has done its hydrolysis, the filtrate was tested to find out how glucose content contained therein.

## 3. EXPERIMENTAL RESULT

Figure 2 showed the effect of glucose levels on the volume expansion of bacillus, the higher the volume of bacillus, the higher the glucose level. Addition of Bacillus volume above 9% will result a constant value since its performance is declining and the Bacillus die over time, thus recfuiring regeneration. While the addition between 3–9% v/v gave a maximum increase of glucose level since it is the growth phase of Bacillus and will optimality decompose starch into glucose.

Figure 3 showed hydrolysis process is done by the various weight of bulrush: 50, 100, 150, 200, and 250 (grams) with the addition of the various HCl volume varied: 10, 20, 30, 40, 50 ml. After the extraction process is complete then the solids and filtrate are obtained, the filtrate will be processed by the fermentation process to obtain ethanol concentration and solids can be used as compost. Filtrate pH was measured according to the terms fermentation process that is approximately 4.5; limited to a minimum pH of 3.5 because, in that condition, Saccharomyces Cerevisiae were still survived. To maintain pH 4.5, the addition of NaOH is done, if filtrate pH is under 4.5, and the addition of citric acid is done, if the pH of the filtrate is above 4.5.

Figure 4 showed hydrolysis from bamboo used condition: temperature of pretreatment on 100  $^{\circ}C$ , volume water on 3 L, Volume 2N  $H_2SO_4$  on 200 ml, weight bamboo on 100 gram, Weight bamboo 50 gram, pH of Hydrolysis on 4.5, and hydrolysis temperature on 45  $^{\circ}C$ . The condition is changed, hydrolysis Time (minutes): 30, 60, 90, 120 and volume enzyme cellulose (ml): 4, 6, 8, 10,12.

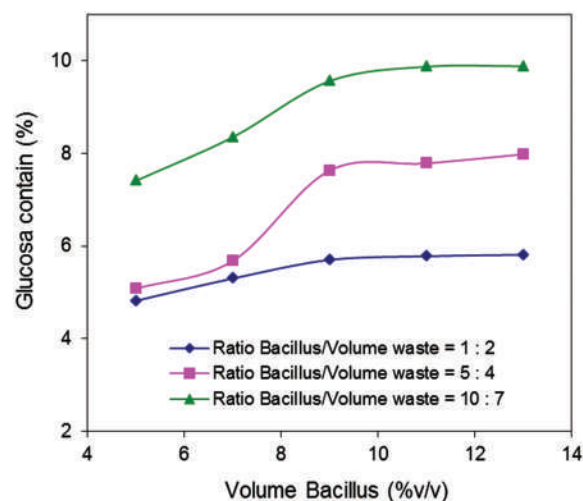


Fig. 2. Effect of the additional volume bacillus addition to glucose contains in the liquid waste rice flour.

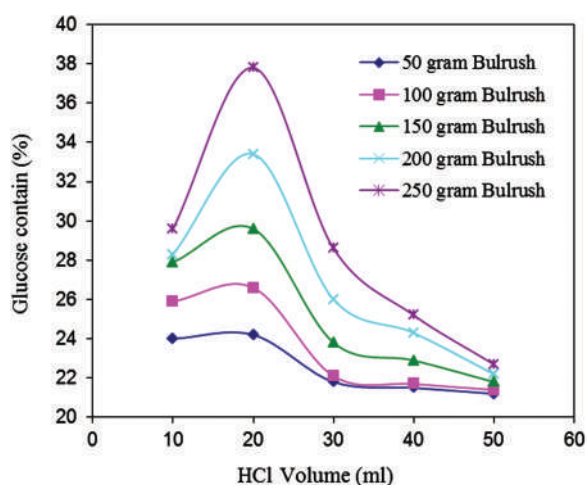


Fig. 3. Effect of the addition HCl volume on glucose contains in the bulrush.

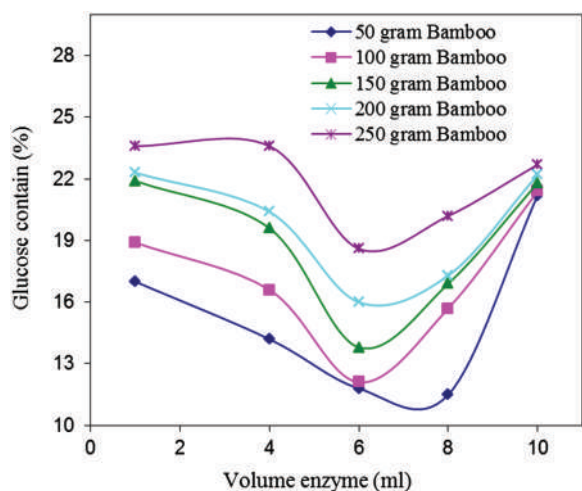


Fig. 4. Effect of the addition enzyme volume on glucose.

#### 4. CONCLUSION

Based on the aim of research in reviewing of process pretreatment, delignification, and hydrolysis, and also searching for alternative material of glucose product. Resulting from delignification and hydrolysis process are glucose, liquid waste rice flour on 9.98% glucose contain, bulrush on 37.9% glucose contain, and bamboo on 23.6% glucose contain. The results from hydrolysis process is a glucose, and the next treatment fermented to obtain product bioethanol.

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#### References and Notes

1. A. Demirbas, *Applied Energy* 88, 17 (2011).
2. R. Singh, M. Srivastava, and A. Shukla, *Renewable and Sustainable Energy Reviews* 54, 202 (2016).
3. A. Kumar, L. K. Singh, and S. Ghose, *Bioresour. Technol.* 100, 3293 (2009).
4. A. K. Dubey, P. K. Gupta, N. Garg, and S. Naithani, *Carbohydr. Polym.* 88, 825 (2012).
5. A. Demirbas, *Energy Sources* 30, 27 (2008).
6. M. Tutt, T. Kikas, and J. Olt, *Agronomy Researct Biosystem Engineering* 1, 269 (2012).
7. E. C. Bensah and M. Mensah, *Research Article* 10, Article ID 719607 (2013), <http://dx.doi.org/10.1155/2013/719607>.
8. I. Barrera, A. Myriam, H. Alizadeh, and A. Allieri, *Chem. Eng. Res. Des.* 107, 91 (2016).
9. N. Brosse, M. Nasir, M. Ibrahim, and A. A. Rahim, *Review Article* 10, Article ID 461482 (2011), DOI: 10.5402/2011/461482.
10. F. Battista, G. Mancini, B. Ruggeri, and D. Fino, *Renewable Energy* 88, 401 (2016).
11. Y. Liu, H. Zhou, S. Wang, K. Wang, and X. Su, *Bioresour. Technol.* 182, 289 (2015).
12. I. N. Pulidindi, B. B. Kimchi, and A. Gedanken, *Renewable Energy* 71, 77 (2014).
13. A. Limayem and S. C. Ricke, *Progress in Energy Combustion Science* 38, 449 (2012).
14. H. Z. Chen and Xiaoguo, *Renewable and Sustainable Energy Reviews* 57, 468 (2016).
15. M. Yu, J. Li, S. Chang, L. Zhang, and S. Li, *Fuel* 175, 20 (2016).
16. N. Sarkar, S. K. Ghosh, S. Bannerjee, and K. Aikat, *Renewable Energy* 37, 19 (2012).
17. K. Karimi, S. Kheradmandinia, and M. J. Taherzadeh, *Biomass Bioenergy* 30, 247 (2006).
18. M. Balat, H. Balat, and O. Cahide, *Progress in Energy Combustion Science* 34, 551 (2008).
19. R. C. Kuhad, R. Gupta, Y. P. Khasa, and A. Singh, *Bioresour. Technol.* 101, 8348 (2010).
20. S. K. Thangavelu, A. S. Ahmed, and F. N. Ani, *Applied Energy* 128, 277 (2014).
21. E. D. Deenanath, K. Rumbold, and S. Iyuke, *Research Article* 11 (2013), Article ID 107851, <http://dx.doi.org/10.1155/2013/107851>.
22. G. S. Geetha and A. N. Gopalakrishnan, *E-Journal of Chemistry* 8, 1951 (2011).
23. W. Han, Z. Wang, H. Chen, X. Yao, and Y. Li, *Research Article* 5 (2011), Article ID 343791, doi:10.1155/2011/343791.
24. J. S. Lim, Z. Abdul Manan, S. R. W. Alwi, and H. Hashim, *Renewable and Sustainable Energy Reviews* 16, 3084 (2012).
25. Y. Z. Pang, Y. P. Liu, X. J. Li, K. S. Wang, and H. R. Yuan, *Energy and Fuel* 22, 2761 (2008).
26. J. Gabhane, S. P. M. P. William, A. N. Vaidya, K. Mahapatra, and T. Chakrabarti, *Biomass Bioenergy* 35, 96 (2011).
27. Y. Kim, R. Hendrickson, and N. S. Mosier, *Bioresour. Technol.* 99, 5206 (2008).
28. J. S. Lee, B. Parameswaran, J. P. Lee, and S. C. Park, *J. Sci. Ind. Res.* 67, 965 (2008).
29. Y. Li, R. Ruan, and P. I. Chen, *Transactions of the American Society of Agricultural Engineers* 47, 821 (2004).
30. P. Harmsen, W. Huijgen, L. Bermudez, and R. Bakker, *Tech. Rep.*, Biosynergy, Wagenin-gen UR Food and Biobased Research 1184 (2010).
31. J. W. Lee and T. W. Jeffries, *Bioresour. Technol.* 102, 5884 (2011).
32. J. S. Van-Dyk and B. I. Pletschke, *Biotechnol. Adv.* 30, 1458 (2012).
33. J. D. McMillan, *Renewable Energy* 10, 295 (1997).
34. D. M. Lavenson, E. J. Tozzi, N. Karuna, T. Jeoh, R. I. Powell, and M. J. Mc Carthy, *Bioresour. Technol.* 111, 240 (2012).
35. G. Radeva, I. Valchev, S. Petrin, E. Valcheva, and P. Tsekova, *Carbohydr. Polym.* 87, 1280 (2012).
36. Y. Sun and J. Cheng, *Bioresour. Technol.* 83, 1 (2002).
37. N. K. Sari, S. Sutyono, E. Luluk, E. Dira, P. Wesen, and S. H. Tatik, Bioethanol production from liquid waste of rice flour with batch process, *MATEC Web of Conferences* (2016), Vol. 58, DOI: 10.1051/mateconf/20165801003.
38. N. K. Sari, I. N. Abdi, P. Wesen, and R. Dewati, Numerical of bioethanol production from liquid waste of rice flour by distillation process, *MATEC Web of Conferences* (2016), Vol. 58, DOI: 10.1051/mateconf/20165801014.

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