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Bioehanol Production from Bulrush with A Combination of Chemical and Biological Process

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***Abstract*—Bulrush is a plant that is available abundantly however it has not been utilized well. During this time, it was utilized just as animal feedstock, even it is considered as weeds. Bulrush has cellulose, glucose, and starch content that can be used as material of ethanol production. This research was aimed to review hydrolysis process, fermentation process, batch distillation process, and search for alternative material for bioethanol production. In bioethanol making process, three processes were done such hydrolysis process biologically by using enzyme and chemically by using HCl.While fermentation process used *Saccharomyces Cerevisiae* and batch distillation. After those three processes were done, high level of bioethanol content was obtained in 95%-96% and it could be concluded that bulrush can be used as alternative material of bioethanol production.**

***Keywords*—bioethanol, bulrush, hydrolysis, fermentation, batch distillation**

I. 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 alternative as gasoline fuel [1]. Environmental sustainability of bioethanol from rice straw[2]. The technology for lignocellulose ethanol production relies mainly on pre-treatment, chemical or enzymatic hyhrolysis, fermentation and product separation or distillation. An appropriate pretreatment strategy is essential for the efficient enzyme hydroysis of lignocellulosic biomass as lignin hinders the saccharification process. Various pretreatment approaches have been exploited in the past such as acid or alkali pretreatment, hydrogen peroxide pretreatment, steam explosion, liquid hot water, ammonia fiber expansion pretreatment, sodium chorite pretreatment, and biological pretreatment [3].

The research conducted to evaluate acid pretreatment from hydroside paper waste as material for bioethanol production, optimized sulfuric acid hydrolysis, fermentation process of hydroside acid 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 in the level of 95-96% [4]. Chemical pretreatment of lignocellulosic biomass with Sulphuric (H_2SO_4) and phosphoris (H_3PO_4) acids are widely used since they are relatively cheap and efficient in hydrolysing lignocellulose, though the letter gives a milder effect and is more benign to the environment.

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Hydrochloric (HCl) acid is more volatile and easier to recover and attacks biomass better than H_2SO_4 [5], similarly, nitric acid (HNO3) posseses good cellulose to sugar conversion rates [6]. However, both acids are expensive compared to sulphuric acid.

Pretreatment of lignocellulose has received considerable research globally due to its effluence on the technical, economic and enviromental sustainability of cellulose ethanol production. This paper reviews know and emerging chemical pretreatment methods, the combination of chemical pretreatment with other methods to inprove carbohydrate preservationreduce formation to degradation product, achieve high sugar yield at mild reaction conditions, reduce solvent loads and enzyme dose, reduce waste generation [7]. Technical and economical avaluation of bioethanol production from lignocellulosic residues, case of sugarcane and blue agave bagasses [8].

Initiatives of the future for lignin in biomass to bioethanol, pretreatment technologies to separate the tree main biopolimers (cellulose, hemicellulose, and lignin) [9]. Pretreatment for hydrogen and bioethanol production from olive oil waste products was ethanol yield 5.4 % treatment with 1.75 w/v sulphuric acid and heated it at 140 ^oC for 10 min, and was ethanol yield 5.0 % no pretreatment [10]. Pretreatment followed with simultaneous saccharification and fermentation on bioconversion of microcrystalline cellulose for bioethanol production, the yield value of 67 % bioethanol bioconversion [11]. A sustainable feedstock bioethanol production, cellulose hydrolysis was microwave irradition using hydrochloric acid as catalyst, fermentation with yeast (Saccharomyces cerevisiae), modest reaction conditions (2.38 M acid consentration), irradition time 7 min, and yield of 0,67 g glucose / g cellulose [12].
The glucose forming reaction from cellulose is as following:

$$(C_6H_{10}O_5)_n + n\,H_2O \longrightarrow nC_6H_{12}O_6$$

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

The fermentation process is affected by microorganism that needs good nutrition in order to obtain a good result of fermentation. Proper nutrition to supply microorganisms is nitrogen which can be obtained from the addition of NH_3, 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 lt [16] in the fermentation process, glucose from fermentation process is converted into ethanol by the following reaction:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

To increase the fermented sugar concentration after enzyme saccharification, conversion of glucose and xylose into ethanol needs a new fermentation technology [17]. 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, fermentation and ethanol recovery. Therefore, ethanol content was obtained in the level below 16%, and by one more distillation process the ethanol content would be obtained in the level of 95-96% v/v [18].

The research conducted by [19] about bioethanol production from agricultural waste using PROFER pretreatment method obtained ethanol content below 16%. The purpose of dilute acid pretreatment is the removal of hemicelluloses and the recovery of the sugar component. Among all pretreatment methods, the acid pretreatment methods of biomass with dilute sulfuric acid has long been recognized as a critical step of removing the hemicellulosic fraction from the lignocellulosic substrate to economize the biological conversion of cellulosic biomass to ethanol [20]. The research conducted by [21] 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 of bioethanol from cashew apple juice, bioethanol consentration evaluate with fermentation by microorganism Saccharomyces cerevisiae Y2084 and Vin 13. The maximum ethanol concentration achieved by Y2084 was 65.00 g/L and by Vin13 was 68.00 g/L, and fermentation time was 2 days [22]. Bioethanol produvtion from paper fibre residue using diluted NaOH and the fermentation process with microorganism Pinicillium Chrysogenum and Saccharomyces Cerevisiae. The fermentability of the hydrolysate decreased strongly for hydrolysate produced at temperature higher than 50 ^oC, The ethanol consentration of monosaccharide hydrolysate was found to be 34.06 g/L and ethanol yield was 0.097 g/g [23]. Simultaneous biohydrogen and anaerobic fermentation with Immobilized sludge for production bioethanol with continous stired tank reaktor (CSTR), the hight H_2 production rate (10.74 mmol/h.L) and ethanol production rate (11.72 mmol/h.L) [24].

The equation (1) is a model of Differential-Algebraic-Equations (DAEs) for simple batch distillation of multi-component systems, that there is no phase which forms two liquids, the vapor composition was calculated by using BUBL T equation [25].

Bioethanol production from lignocellulosic biomass involves different step such as pretreatment, hidrolysis, fermentation and ethanol recovery [26]. The technology for lignocellulosic ethanol production relies mainly on pre-treatment, chemical or enzymatic hyhrolysis, fermentation and product separation or distillation. An appropriate pretreatment strategy is essential for the efficient enzyme hydroysis of lignocellulosic biomass as lignin hinders the saccharification process. Various pre-treatment approaches have been exploited in the past such as acid or alkali pretreatment, hydrogen peroxide pretreatment, steam explosion, liquid hot water, ammonia fiber expansion pretreatment [27]. Bioethanol production from sagu pith waste (SPW) using Hydrolysis by carbon dioxide, a maximum of 43.8% glucose and 40.5% ethanol yield, the develoved technique for SPW resulted in higher energy saving compared to previous techniques in the absence of enzymes, acid or base cataluyst [28].

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, physico-chemical processes, biological methods, and several combinations there of to fractionate the lignocellulose into its components. It result in the disruption of lignin seal to increase enzyme access to holocellulose [29, 30], reduction of cellulose crystallinity [31, 32], and increase in the surface area [33, 34] and porosity [35, 36] of pretreated substrates, resulting in increased hydrolysis rate. In hydrolysis, cellulose and hemicelluloses are broken down into monomeric sugars via addition of acids or enzymes such as cellulase. Enzymatic hydrolysis offers advantages over acids such as low energy 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 fibre crystallinity, and degree of polymerization, as well as lignin and hemicellulose ontent [37, 38], optimum mixing [39], substrate and end-product consentration, enzyme activity, reaction conditions such as pH and temperature [40, 41].

From the previous research, it was known that bioethanol from cellulose resulted good bioethanol. The aim of this research was to search alternative material, review hydrolysis process, fermentation process, and distillation batch process to gain bioethanol product with high level of ethanol. The originality of this research was the second generation that was bulrush, by using three processes (hidrolysis, fermentation, and batch distillation) simultaneously and technical ethanol production with level of 95-96% as the substitution material of bioethanol.

II. Experimental

From the result of laboratory analysis, it was known that ethanol forming elements were cellulose, glucose and starch. The average concentration of cellulose was 48%, glucose was 5 % and starch was 20%.

The cutting of bulrush with approximately length of 5 cm was done in order to obtain the high level of glucose and cellulose during hydrolyzed process by bacillus and HCl. The quality of bioethanol product was determined by various influencing parameter such as the acidity (pH), the volume ratio of HCl to bulrush, and the volume ratio of Bacillus to filtrate, SC starter concentration, fermentation time, and batch distillation time.

The quality analysis of raw materials and bioethanol product was done by laboratory analysis. The analysis was conducted on the instrumentation and gravimetric analysis by using Gas Chromatography (GC) and Spectrophotometer, which analyzed items were the concentration of starch, glucose, ethanol, HCl, crude protein level, and N, P, K, Ca, Mg, S.

A. Procedure of Hydrolysis Process

Hydrolysis process in **Figure 1** was done in stable condition : temperature of 30 ^{o}C, water volume in 7 liters, and hydrolysis time in 1 hour with 200 rotations per minute (RPM). For the changing condition: bulrush weight of 50, 100, 150, 200, 250 (grams), the ratio of bacillus to filtrate volume 1:2 ; 5:4 ; 10:7 and HCl solution volume 10, 20, 30, 40, 50 (ml). The level of glucose in hidrolysis filtrate yield was analyzed before the fermentation process was done.

B. Procedure of Fermentation Process

The addition process of citrate acid and NaOH to the glucose yield from hydrolysis process which is unqualified the requirements was done. Then citrate acid was added to the hydrolysis filtrate yield which would be fermented until reach the approximate fermentation acidity (pH) of 4,5. Next, starter was put into the solution that would be fermented in anaerobic condition then sealed tightly the bottle and observed during certain time. In fermentation process such **Figure 1**, the stable conditions were the temperature of 30 ^{o}C, acidity (pH) of 4,5 ; the volume of fermentation was the same amount as the volume of hydrolysis process filtrate. The changing condition were fermentation time 4, 5, 6, 7, 8 (days), starter 8, 10, 12 (%), then the ethanol content was analyzed.

C. Procedure of Distillation Process

The yield of fermentation was put in distillation flask in order to obtain the ethanol from glucose. Batch distillation showing in **Figure 1** was conducted on temperature of 78 ^{o}C, completed by total condensor and gas stove heating. After the bottom solution volume remained 10% from its initial volume, the distillation process was stopped then the ethanol content was analyzed.

III. Result and Discussion

A. Quality Raw Materials

Bulrush using as a study material was derived from bulrush crops in the surrounding area. Assessment method was done by doing a survey and laboratory analysis to obtain some data about the quality and quantity of the available bulrush. The expected result was data about the quality and quantity of bulrush before processing to be an ethanol.

Based on the results of laboratory analysis, it was known that ethanol forming elements were cellulose, glucose and starch. The average concentration of cellulose was 48.1 %, glucose was 4.8% and starch was 20.4%. If the entire cellulose can be hydrolyzed completely, it will be obtain the glucose levels of 53%.

The cutting of bulrush with approximately length of 5 cm was done in order to obtain the high levels of glucose and cellulose before it was hydrolyzed by bacillus and HCl solution. Bulrush should be made in powder form, so cellulose can be hydrolyzed perfectly. However that process took an higher cost. Besides, bulrush in the powder form could suffer the physical destruction, thus causing the damage of glucose group. The drying process of bulrush was naturally done first in the room temperature. The drying process was done in an oven at 100^{0}C for 3 hours. This was done for cost savings. The drying process aimed to reduce the water content in ethanol. Water level that was permitted by Standart National Indonesia (SNI) was 1%.

The decreasing of pH from pretreatment material was affected by the addition of HCl volume 7%v/v because the requiring pH for fermentation process was 4,5. Before doing the hydrolysis process, the pH of filtrate was measured according to the terms of fermentation process that is approximately 4.5. To obtain pH 4.5, the addition of Na-OH was done if pH of the filtrate was under 4.5 and the addition of citric acid if the filtrate pH was above 4.5.

B. Hydrolysis Process

In acid hydrolysis, HCl or H_2SO_4 was commonly used in certain level. Hydrolysis was usually done in a special tank made of stainless steel or copper pipe connected to the heating ducts and exhaust pipes in order to regulate the air pressure (Kuhad *et al.*, 2010). The cellulose content of bulrush could be converted into glucose by concentrated acid hydrolysis process with certain concentration.

Figure 2 showed hydrolysis process was done by the various weight of bulrush: 50 100, 150, 200, and 250 (grams) by the addition of the various HCl volumes: 10, 20, 30, 40, 50 ml. After the extraction process was finished thus the solid and filtrate were obtained. The filtrate will be processed by the fermentation process to obtain ethanol concentration and solids can be used as compost. The effect of pH was essential in the fermentation process so filtrate must be measured for pH in the minimum level of 3,5 until the maximum level of 4,5, because SC can be survived on that range of pH. To maintain pH in 4.5, the addition of NaOH would be done if filtrate pH was under 4.5 and the addition of citric acid would be done if the pH of the filtrate was above 4.5.

The effect of glucose concentration in 20 ml HCl volume to bulrush weight was higher, on 250 grams bulrush and 20 ml HCl volume will be gained the glucose concentartion of 37,8%. Therefore, before fermentation process was done, the maximum level of optimum glucose concentration was 16%. If the glucose level was higher than 16%, the dilution would be done, if the glucose level was lower than 16%, the addition of pure glucose would be done.

Figure 3 showed the effect of glucose level to bacillus volume. The greater amount of an additional bacillus volume, the greater glucose level would be gained. The Bacillus volume between 3-9 (%v/v) showed the increasing of glucose concentration, because that condition was growing phase of Bacillus, so the cellulose containing in bulrush turned into glucose. On the Bacillus volume above 9%, the graph showed constatnt, this was caused by the decreasing of Bacilus performance by time to time then they finally died, so it was necessary to regenerate Bacillus. The yield glucose concentration on hidrolysis process by using Bacillus was 10%, while by using HCl was 37%, so the glucose concentration by using Bacillus was lower than by using HCl, because chemical process for glucose concentration was higher than biological process, however biological process was more enviromental friendly.

C. Fermentation Process

The using fermentation process was the fermentation process which is not used oxygen, in other word, anaerobic process. To control the production of ethanol from sugar was quite complex because the concentration of substrate and oxygen influenced yeast metabolism, cell survival, cell growth, cell division, and ethanol production. The selection of a suitable SC, high concentration tolerance, and substrate were an essential point for improving the ethanol concentration and yield. The most important points in fermentation were starter preparation, inoculation process, until it was completely ready to be put into fermentation tank.

Figure 4 showed the best filtrate fermentation process from bulrush hydrolysis result was in 250 grams bulrush, 8, 10, and 12 (%) starter additions (liquid SC), fermentation time of 4,5,6,7, and 8 days. The addition of 10% starter of liquid volume showed the higher ethanol concentration than 8-12% starter addition. Then, the remaining glucose level was analyzed. The addition of 10% starter showed the lower remaining glucose level compared to the addition of 8-12% starter. After 6 days, the remaining glucose level was higher, because Bacillus performance became weaker and SC regeneration was done. After fermentation process was done on 250 grams bulrush, 10% SC starter, and 6 days, it was gained ethanol concentration of 31,7%, ethanol concentration in our research was higher than the research conducting by Balat *et al*., (2008), Nibedita *et al.,* (2012), and Saravana *et al*., (2014) which was under 16%.

D. Batch Distillation Process

Batch distillation process was conducted on temperature of 78^0C, completed by total condensor and gas stove heating, after bottom solution volume remained 10% from initial volume, batch distillation was stopped.

Table 4 showed the best result of batch distillation process from fermentation process was on 10% SC starter and 6 days fermentation time. After hidrolysis and fermentation process were done on 250 grams bulrush, 10% SC starter and 6 days fermentation time, it was obtained 96% ethanol concentration and 33,3% ethanol yield. Ethanol concentration from our research result was higher than the research conducting by Alok *et al*., (2012), 77, 54% and ethanol yield resulting in our research was economically profitable.

IV. Conclusion

Based on the aim of research in reviewing hidrolysis process, fermentation process, and batch distillation process, and also searching for alternative material of bioethanol product. The gaining glucose level in the hydrolysis process was 37.8%, ethanol level in the fermentation process was 31,7%, ethanol level in the batch distillation process was 31,69%. The bulrush as material using to product bioethanol, ethanol concentration of 96% fulfilled the requirement as technical ethanol economically, which was 95% and ethanol yield level of 33,3% was enable to be commercialized in pilot plan scale.

TABLE 4. ETHANOL CONSENTRATION AND YIELD ON DISTILLATION BATCH

Time fermentation	Starter SC	Ethanol consentratio distillation	Ethanol yield
[day]	*[%]*	*[%]*	*[%]*
4	8	91.5	30.5
5		93	31.8
6		94	32.3
7		92.5	31.6
8		91	32.3
4	10	92	30.2
5		94.5	31.8
6		96	33.3
7		95	32.6
8		94	32.1
4	12	94	30.4
5		94.5	32.8
6		95	33.1
7		94.5	32.9
8		93	32.6

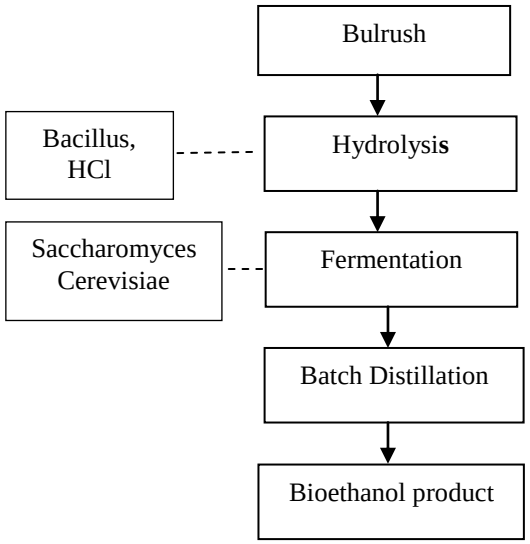

Figure 1. Bioethanol production flow used hydrolysis process, fermentation process, and batch distillation

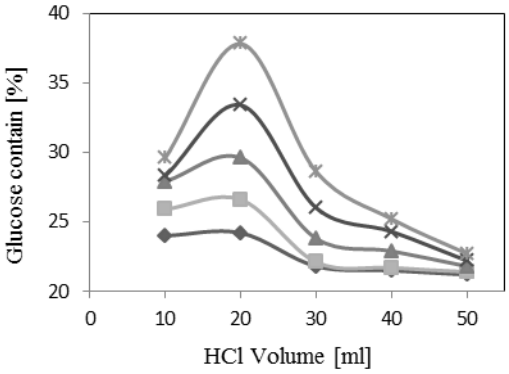

Figure 2. Changes glucose contains with addition HCl volume in bulrush (50 gram bulrush: ♦, 100 gram bulrush: ■, 150 gram bulrush: ▲, 200 gram bulrush: x, 250 gram bulrush: *)

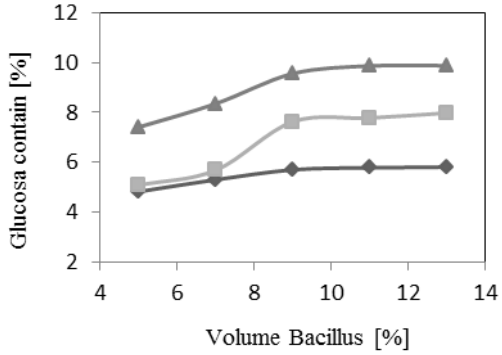

Figure 3. Changes glucose contains with addition volume bacillus in bulrush, (ratio bacillus/volume filtrate = 1:2: ♦, ratio bacillus /volume filtrate = 5:4: ■, ratio bacillus/volume filtrate = 10:7: ▲)

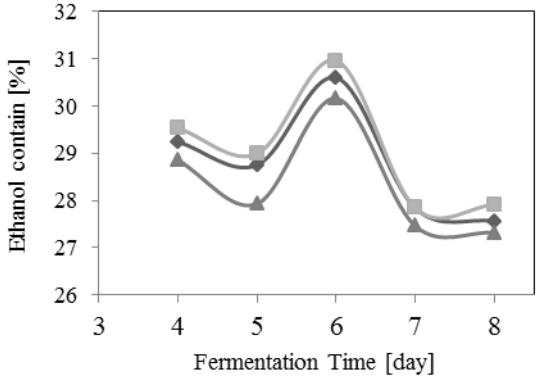

Figure 4. Changes glucose contains with fermentation time in bulrush (200 gram bulrush: ♦, 250 gram bulrush: ■, 100 gram bulrush: ▲)

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