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Process Fermentation of Filtrate Bamboo with Saccharomyces Cerevisiae and Zymomonas Mobilis

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Abstract. Fermentation is the process of the formation of ethanol from glucose by using enzymes. Bamboo is one of the materials containing glucose is high enough, that is previously done hydrolysis in advance. Bamboo used when the hydrolysis process of bamboo that does not include lignin and the pentose done process of pretreatment and not lignification. The purpose of this research is to produce ethanol as a raw material substitution of bioethanol, knowing pentose and dirt left in the bamboo. Therefore, need to be studied in the future, with the best process, that we used biological processes that can optimize the production of ethanol. The use of the enzyme (Saccharomyces Cerevisiae and Zymomonas Mobilis) is also significant because of the optimum enzyme conditions. Temperature, pH, and the yeast with optimal conditions when it can raise the level of his work. The fermentation process at temperature 25 C and 45 C, the filtrate is 500 ml solution of bamboo and the stirring speed of 200 rpm. The variable composed enzyme with a ratio (v/v) of 0.25 to 0.75. Resulting from the fermentation processed can produce ethanol with a yield 30.5% and 36% of the weight of the bamboo. The result of the process of fermentation obtained bioethanol with low ethanol yield of 10-15%, which requires the flash distillation process to obtain yield bioethanol technical 90-95%.

1. Introduction

Biomass from plants has declared as an alternative raw material for gasoline fuel substitution in the form of bioethanol, bioethanol obtained from biomass and bioenergy crops has proclaimed as one of the feasible alternatives as gasoline fuel [1]. Sustainable bioethanol from rice straw [2]. The technology for lignocellulose ethanol production relies mainly on pre-treatment, chemical or enzymatic hydrolysis, fermentation and product separation or distillation. An appropriate pretreatment strategy is essential for the efficient enzyme hydrolysis of lignocellulose biomass as lignin hinders the scarification process. Various pretreatment approaches have exploited in the past such as acid or alkali pretreatment, hydrogen peroxide pretreatment, steam explosion, liquid hot water, ammonia fiber expansion pretreatment, sodium chlorite pretreatment, and biological pretreatment [3].

The research conducted to evaluate acid pretreatment from hydroxide paper waste as material for bioethanol production, optimized sulfuric acid hydrolysis, fermentation process of hydroxide acid of paper waste by using Pichia Stipites. The ethanol content obtained at 77.54%. By one more distillation process, the ethanol content received at the level of 95-96% [4]. Chemical pretreatment of lignocellulose biomass with Sulphur (H_2SO_4) and phosphorus (H_3PO_4) acids used since they are relatively cheap and efficient in hydrolyzing lignocellulose, though the letter gives a milder effect and is more benign to the

environment. Hydrochloric (HCl) acid is more volatile and more natural to recover and attacks biomass better than H_2SO_4 [5]. Similarly, nitric acid (HNO_3) possesses good cellulose to sugar conversion rates [6]. However, both acids are expensive compared to Sulphur acid. Pretreatment of lignocellulose has received considerable research globally due to its affluence on the technical, economic and environmental sustainability of cellulose ethanol production. These paper reviews know, and emerging chemical pretreatment methods, the combination of chemical pretreatment with other ways to improve carbohydrate preservation reduce formation to degradation product, achieve high sugar yield at mild reaction conditions, reduce solvent loads and enzyme dose, reduce waste generation [7]. Technical and economic evaluation of bioethanol production from lignocellulose residues, a case of sugarcane and blue agave bagasse [8].

Initiatives of the future for lignin in biomass to bioethanol, pretreatment technologies to separate the main tree biopolymers (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 Sulphur acid and heated it at 140 0C for 10 min, and was ethanol yield 5.0 % no pretreatment [10]. Pretreatment followed with simultaneous scarification 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 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 g glucose / g cellulose [12]. Elements contained in the lignocellulose biomass of the plants are usually used lignocellulose biomass, a potential for bioethanol production globally. Agriculture (softwood), forestry (pretre[2]ment 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 recognized as a critical step of removing the hemicellulose fraction from the lignocellulose 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 techni[3]es in the absence of enzymes, acid or base catalyst. They obtained ethanol content below 15.6%. Bioethanol production from lignocellulosic bioma[2] involves different step such as pretreatment, hydrolysis, fermentation and ethanol recovery [26]. The technology for lignocellulosic ethanol production relies mainly on pre-treatment, chemical or enzymatic hydrolysis, fermentation and product separation or distillation. An appropriate pretreatment strategy is essential for the efficient enzyme hydrolysis of lignocellulosic biomass as lignin hinders the saccharification process. Various pre-treatment approaches exploited in the past such as acid or alkali pretreatment, hydrogen peroxide pretrea[1]ment, steam explosion, liquid hot water, ammonia fiber expansion pretreatment [27].

[3] Bioethanol production from the liquid waste of rise flour using fermentation by Saccharomyces, a ma[3]mum of 23.8% glucose and 40.5% ethanol yield, the developed technique for liquid waste of rise flour resulted in higher energy saving compared to the previous method in the absence of enzymes, acid or base catalyst [28]. hardwood), and ind[3]trial waste are a significant lignocellulose biomass for bioethanol production. The lignocellulose biomass is one of the potential main sources for economic bioethanol production globally. Agricultural, forestry (soft and hardwoods) and industria[1]astes are the major lignocellulose biomasses [13]. 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 [1]onducted bioethanol production from lignocellulose biomass by using the pretreatment process, hydrolysis, fermentation, and ethanol recovery. Therefore, ethanol content obtained in the level below 16%, and by one more distillation process the ethanol content would receive at the level of 95-96% v/v [18].

The research conducted by [19] about bioethanol production from agricultural waste using PROFER Cellulosic or second generation (SG) bioethanol produced from lignocellulosic biomass (LB) in three main steps: pretreatment, hydrolysis, and fermentation. Pretreatment involves the use of physical

processes, chemical methods, physic-chemical 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 holo-cellulose [29, 30], reduction of cellulose crystallinity [31, 32], an 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 the addition of acids or enzymes such as cellulose. 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 content [37, 38], optimum mixing [39], substrate and end-product concentration, enzyme activity, reaction conditions such as pH and temperature [40, 41].

From the previous research, it knows bioethanol from cellulose resulted in good bioethanol. The study was to search alternative material, review hydrolysis process, fermentation process to gain bioethanol product with a high level of ethanol. The originality of this research was the second generation that was bulrush, by using two methods (hydrolysis and fermentation) simultaneously, used two enzymes [Saccharomyces Cerevisiae (SC) and Zymomonas Mobilis (ZM)], and technical ethanol production with the level of 10-15% as the technical ethanol.

2. Experimental

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

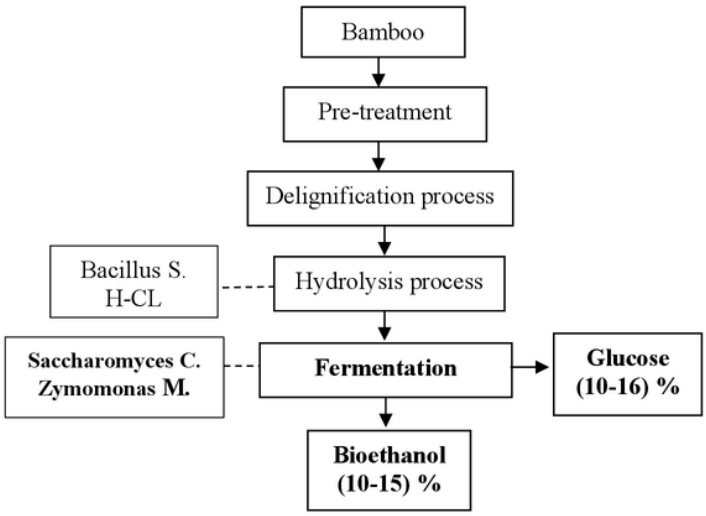

Figure 1. Glucose and bioethanol production flow used fermentation process

The pieces and refined fiber of bamboo with the approximate length of 5 cm and polished thread 200 mesh done to obtain the high level of glucose and cellulose during the hydrolyzed process by Bacillus and H-Cl. The quality bioethanol product determined by various influencing parameters such as the acidity (pH), the volume ratio of H-Cl to bamboo, the volume ratio of Bacillus Subtitles to the filtrate, the volume ratio of the enzyme (Saccharomyces C. and Zymomonas M.) to the filtrate, and fermentation time. Laboratory analysis did the quality analysis of raw materials and bioethanol product. The study conducted on the instrumentation and gravimetric analysis by using Gas Chromatography

(GC) and Spectrophotometer, which analyzed items were the concentration of bamboo, glucose, ethanol, H-Cl, and impurities.

Hydrolysis process in Figure 1 done in stable condition: temperature of 30 C, water volume in 7 liters, and hydrolysis time in 1 hour with 200 rotations per minute (RPM). For the changing condition: bamboo weight of 50, 100, 150, 200, 250 (grams), the ratio of bacillus to filtrate volume 1:2; 5:4; 10:7 and H-Cl solution volume 10, 20, 30, 40, 50 (ml). The level of glucose in hydrolysis filtrate yield was analyzed before the fermentation process done previous research [42]. Fermentation process in Figure 1 done in stable condition: filtrate bamboo ratio of the varies Saccharomyces C. and Zymomonas M.: 5, 9, 13 (% v/v), fermentation time 4, 6, 8, 10, 12 days. Filtrate rate influences the residual glucose levels, obtained maximum residual glucose levels (1,3 - 3) %, and this is because in the tank hydrolysis reactor and the amount of filtrate starter Saccharomyces C. and Zymomonas M. in still little so that the fermentation process is not optimal. With the increasing amount of filtrate hydrolysis and starter Saccharomyces Cerevisiae then the smaller the residual glucose, because it fermented into ethanol.

2. Results and Discussion

Bamboo using as a study material derived from bamboo crops in the surrounding area. Assessment method is done, by doing a survey and laboratory analysis to obtain some data about the quality and quantity of the available bamboo. The expected result was data about the quality and bamboo quantity before processing to be ethanol. Based on the results of laboratory analysis, it is known, that ethanol forming elements were cellulose and glucose. The average concentration of cellulose was 48.1 %, glucose was 4.8%, and impurities. If the entire cellulose hydrolyzed completely, it will be obtaining the glucose levels of 53%.

The results of fermentation process with ratio enzyme again filtrate cellulose as:

Table 1. Ethanol level and yield on fermentation process

Ratio Filtrate Sellulose (%v/v)	Fermentation time (day)	Glucose level Fermentation (%)		Ethanol Level Fermentation (%)	
		SC	ZM	SC	ZM
5	4	4.82	5.76	10.5	10.0
	6	5.30	5.23	11.0	12.3
	8	5.70	5.27	9.0	13.4
	10	5.78	5.54	12.5	10.3
	12	5.81	5.77	11.0	10.3
9	4	5.08	5.90	12.0	9.1
	6	5.68	5.76	14.5	13.2
	8	7.63	6.03	14.0	14.9
	10	7.78	6.78	15.0	13.5
	12	7.98	7.91	14.0	12.6
13	4	7.41	6.88	14.0	13.0
	6	8.35	7.95	14.5	13.7
	8	9.56	8.77	15.0	14.5
	10	9.87	9.05	14.5	12.3
	12	9.88	9.35	13.0	12.8

The pieces and refined fiber of bamboo with an approximate length of 5 cm and polished thread 200 mesh were done to obtain the high levels of glucose and cellulose before it hydrolyzed by Bacillus

and H-Cl solution. Bamboo should be made in powder form so that cellulose hydrolyzed perfectly. However, that process took a higher cost. Besides, bulrush in the powder form could suffer the physical destruction, thus causing the damage of the glucose group. The drying process of bulrush was naturally done first in the room temperature. The drying process was done in an oven at 1000C for 3 hours. These done for cost savings. The drying process aimed to reduce the water content in ethanol. The 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 H-Cl volume 7%v/v because the requiring pH for fermentation process was 4,5. Before doing the hydrolysis process, the pH of filtrate measured according to the terms of the fermentation process that is approximately 4.5. To obtain pH 4.5, the addition of Na-OH done if the pH of the filtrate was under 4.5 and the addition of citric acid if the filtrate pH was above 4.5. Filtrate rate influences the residual glucose levels, for a number of starter Saccharomyces C. 5, 9, and 13% v/v, obtained maximum residual glucose levels (1,3 - 3) %, this is because in the tank hydrolysis reactor and the amount of filtrate starter Saccharomyces C. still little, so that the fermentation process is not optimal. With the increasing amount of filtrate hydrolysis and starter Saccharomyces C. then the smaller the residual glucose, because it fermented into ethanol.

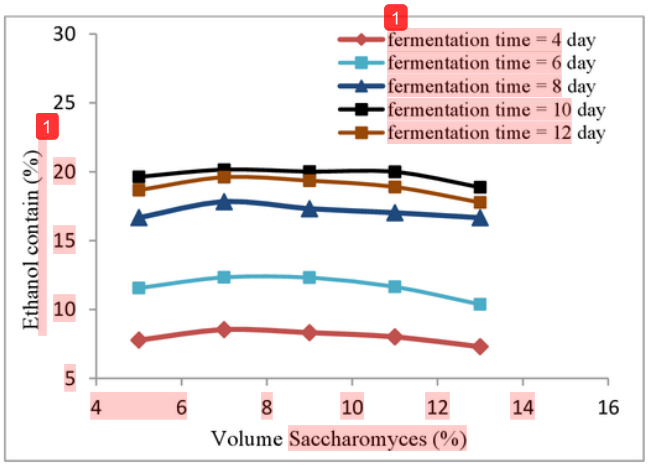

Figure 2. Effect Saccharomyces volume on the remaining ethanol contain

After analysis glucose levels in the rest of the fermentation process, with the addition of Saccharomyces C. 7 % of the volume of fluid (filtrate) showed small residual glucose levels compared to the addition of starter 5, 11 and 13 %. These are because the preliminary research has been conducted by following the Journal and the acquisition of 7% of the fluid volume. Filtrate rate influences the residual glucose levels, for a number of starter Saccharomyces C. 5, 9, and 13%, obtained maximum residual glucose levels (1.5-10) %, this was due to the amount in the tank reactor filtrate hydrolysis and starter Saccharomyces C. still little, so that the fermentation process is not optimal. With the increasing amount of filtrate hydrolysis and starter Saccharomyces C. then the smaller the residual glucose, because it fermented into ethanol.

5. Conclusion

Fermentation process from raw materials (bamboo) to produce bioethanol, glucose levels obtained in the fermentation process as (5-10) % for filtrate cellulose, levels of ethanol in the fermentation process equal 10 to 15 %. The Saccharomyces C. had higher glucose and bioethanol levels results of Zymomonas M., but durability Zymomonas M. stronger in a fermentation process.

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