Food Grade Ehanol Production with Fermentation Stem Sorghum and Distillation Process Using

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Abstract — **Sorghum is abundant in Indonesia, contains about 10% -12% of sugar in its stem which is the optimum sugar concentration in fermentation process for bioethanol production. Sorghum has a high potential to be developed as a raw material for food-grade ethanol production which can be used to support food-grade ethanol demand in Indonesia through a fermentation process. This research focused on the effect of microorganism varieties in the fermentation process which are mutant Zymomonas mobilis (A3), Saccharomyces cerevisiae and Pichia stipitis mixture. The Research for purification process are separated into two parts, distillation with steel wool structured packing and dehydration process using molecular sieve and eliminating impurities using activated carbon. The research can be concluded that the best productivity shown in continuous fermentation in the amount of 84.049 (g / L.hr) using the mixture of Saccharomyces cerevisiae and Pichia stipitis. The highest percentage of ethanol yield produced in batch fermentation using the mixture of Saccharomyces cerevisiae and Pichia stipitis that is equal to 51.269%. And for the adsorption, the best result shown in continuous fermentation by using Zymomonas Mobilis of 88.374%.**

Keywords: **Distillation, Ethanol, Pichia stipitis, Saccharomyces cerevisiae, Zymomonas mobilis.**

I. INTRODUCTION

Sorghum is a potential cerealia crop to be cultivated and developed. This crop has higher number of seed and biomass production than sugar cane. Sorghum has better composition than sugar cane. By squeezing the stem, the sap can gathered to be the main ingredient for bioethanol production. Tabel 1 shows the comparison between sorghum sap and sugar cane sap.

Tabel 1. Composition Sorghum Sap with Sugar Cane Sap

Composition	Sorghum Sap	Sugar Cane Sap
Brix (%)	13,60 – 18,40	12 – 19
Sucrose (%)	10 – 14,40	9 – 17
Reducing sugar (%)	0,75 – 1,35	0,48 – 1,52
Total sugar (%)	11 – 16	10 – 18

Bioethanol (C_2H_5OH) is obtained by fermentation process of simple sugar form / glucose which is contained in natural ingredients (plants) by using the help of certain microorganisms. This study is using batch and continuous fermentation with microorganisms *Saccaromyces cerevisiae*, *Zymomonas mobilis* A3 and *Pichia stipites*. Each of the species of microorganisms has very special advantages to be the fermentation agent. *Saccharomyces cerevisiae* is a single-cell microorganism lack of chlorophyll, including *Eumycetes* group. It grows well at 30 °C and pH 4.8. The optimum temperature for fermentation is between 28 –30 ^oC. The microorganism that used in this study is mutant *Zymomonas mobilis*. *Zymomonas mobilis* A3 has bigger morphology and less movement than common *Zymomonas mobilis*. The optimum pH for *Zymomonas mobilis* A3 is pH 4.5 [1]. *Pichia stipitis* has some advantages, which are more tolerable to high temperature and lower pH level than *Saccaromyces cerevisiae* and *Zymomonas mobilis*. In contradiction, *Pichia stipitis* cannot stand the high concentration of ethanol [2].

Fermentation process is separated into three kinds of types, batch, semi-batch, and continuous fermentation. In this study, the type of fermentation used are batch and continuous fermentation. Batch fermentation is fermentation by put the media and inoculums into the bioreactor and the product taken in the end of the fermentation. Continuous fermentation is using cell immobilization technique with kappa carrageenan. The batch fermentation process produces a maximum ethanol concentration of 12% v/v because the microorganism is not resistant to high ethanol concentration [3]. Usually in the form of spherical beads with diameter range from 0.3-3 mm. Yeast immobilization by trapping is a simple method and has high biomass concentration.

In batch process, concentration and productivity of ethanol produced low due to the inhibition of the ethanol formed in the fermenter cause will poison the microorganisms. The ethanol inhibition will decrease slowly or even stop the growth and production of microorganisms. Continuous fermentation is a solution that can be used to increase ethanol production rate [4]. Cell immobilization in packed bed reactor is continuos fermentation technique that often be used. However, when ethanol production rate increases, ethnaol inhibition on microorganisms will increase as well [5].

To separate the product of fermentation into pure ethanol, there was two separation methods to do in this study. There are distillation and adsorbtion processes. Distillation is a separation method based on the differences of volatility in a certain pressure and temperature. Component with more volatility tend to be in vapor phase

and the other component with less volatility tend to be in liquid phase. The pack bed that used is stainless steel wool.

Adsorbtion proces is separated into two parts, molecular sieve dehydration and activated carbon. Molecular sieve is zeolite adsorber with uniformly small pores. Pores capture molecules that pass selectively depend on the molecule's size. 3A molecular sieve with ±3 angstroms pore size used in this study. Activated carbon has black color, tasteless and odorless, powder form and granular, which build an extremely smooth structure so adsorption surface area can reach 300-3500 cm^2 / g.

II. MATERIAL AND METHODS

A. Materials and Equipments

Materials that used were: sorghum stem sap, *Saccaromyces cerevisiae*, *Zymomonas mobilis* A3, *Pichia stipites*, carrageenan, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, yeast extract, KCl, NaCl, DNS, molecular sieve, and activated carbon.

The equipment was: packed bed bioreactor, peristaltic pump, Gas Chromatography (GC), incubator shaker, autoclave, separator funnel, flask and distillation and adsorption columns.

B. Methodology

The food-grade ethanol is obtained by fermentation process. First the sap of sorghum stem is sterilized by autoclave at 121^{o}C for 15 minutes. The initial sugar concentration is 100g / L. Then make a starter with mutant *Zymomonas mobilis* cultured as much as 3 oses into 100 mL of sap with nutrient medium (1 g (NH4) 2SO4, KH2PO4 1 g, MgSO4.7H2O 0.5 grams, 10 grams of yeast extract) [6]. Then it cultured in an incubator shaker at 30^{0}C for 8 hours. Furthermore, for continuous fermentation is done by dissolving 10 g into 450 mL of distilled water, then heating at 70^{0}C until it begins to form into a gel (heating for 15 minutes) and cooling until 50^{0}C. Then mix 50 ml starter with 450 ml of carrageenan solution. Then mold it into beads in 1000 mL of KCl 3.5%. Then wash the beads with 0.85% NaCl solution and incubated in an incubator shaker for 24 hours. Put cell immobilization beads into fermenter. Peristaltic pump flowed sterile sap into the fermenter (bioreactor packed bed), and flow out from fermenter into broth storage tank. For batch fermentation, sap that has been pretreatment and starter as much as 10% of the reactor volume are put into 1,8L fermenter. The fermentation process is to be done for 90 hours. After 90 hours, the fermented broth distilled twice at 80^{o}C to obtain the ethanol. First dehydrate process with 3A molecular sieve at 80°C and then adsorbtion with activated carbon to remove impurities.

C. Analysis

Analysis for initial sugar concentration performed twice, with HPLC and DNS method. In fermentation process, reducing sugar analysis has to be done every 6 hours for 90 hours with DNS method.

Ethanol content analysis performed by Gas Chromatography. The samples that used are the broth, distillate 1, the distillate 2 and adsorption product.

III. RESULT AND DISCUSSION

Analysis of intial sugar concentration is using HPLC and DNS method. In this study, the fermentation process has to be done for 90 hours to make the reduction of residual sugar can decrease until steady state. Figure 1 shows the result of ethanol concentration (%) from batch and continuous fermentation for each variety of microorganisms.

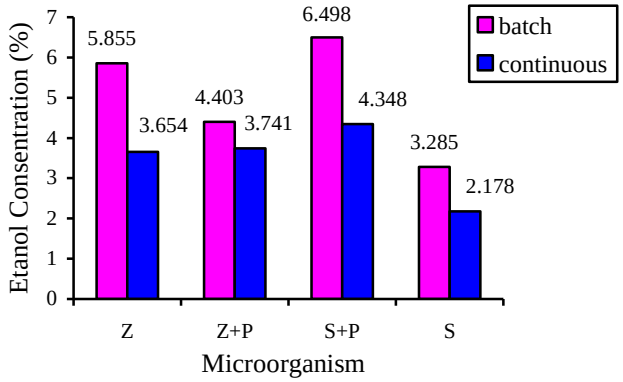

Figure 1. Comparison of Ethanol Concentration for Batch and Continuous Fermentation for Each Variety of Microorganisms

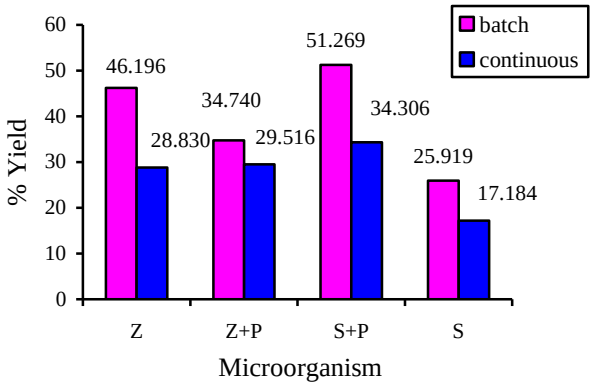

Figure 2. Comparison of Yield (%) for Batch and Continuous Fermentation Process for Each Variety Microorganisms

Yield is ethanol concentration compared to glucose concentration that consumed. In this study, the yield of batch fermentation is higher than continuous fermentation which is shown on figure 2.

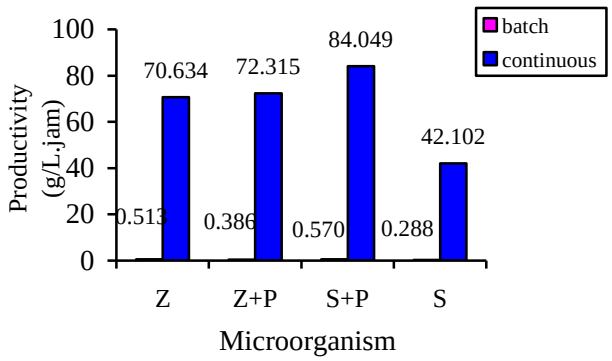

Figure.3 Comparison of Productivity (g / L.hr) for Batch and Continuous Fermentation Process for Each Type Microorganisms

Ethanol productivity is ethanol concentration produced per unit time. The amount of ethanol productivity depends on the amount of ethanol concentration produced, higher ethanol concentration that produced, the ethanol productivity will be higher as well.

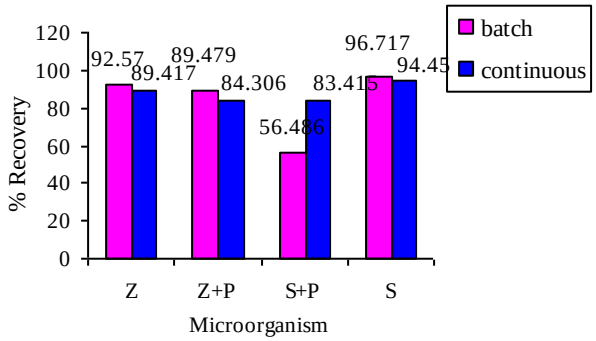

Figure 4. Comparison of % Recovery for Batch and Continuous Fermentation Process for Each Variety Microorganisms

Prosentase of recovery is the ethanol mass in distillate toward initial ethanol concentration.In Figure 4 shown that % recovery for batch fermentation higher than continuous fermentation for each variety of microorganisms. This result is appropriate with the theory because in batch fermentation, the fermentation process is more homogenous due to the mixing process so that more substrate converted into ethanol.

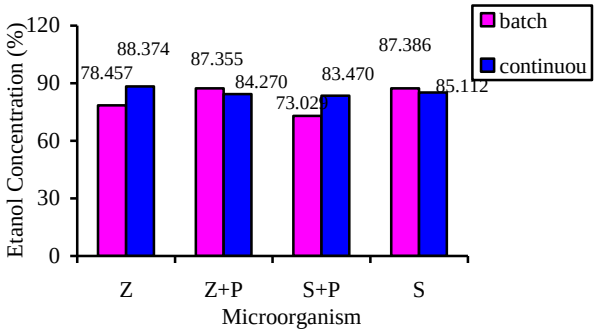

Figure 5. Comparison of Ethanol Concentration for Batch and Continuous Fermentation Process for Each Variety Microorganisms

Separation method for ethanol-water mixture is better to be done in vapor phase with good controlled condition. In this study, the separation done in vapor phase so that the water can be trapped in molecular sieve.

Activated carbon selected for adsorbtion due to its characteristic that can remove and adsorb impurities very well. Besides, activated carbon is more economical than other absorbents.

IV. CONCLUSION

1. The fermentation and distillation process with variations of microorganisms provide the best productivity results that are shown in continuous fermentation in amount of 84.049 (g / L.hr) using mixture of *Saccharomyces cerevisiae* and *Pichia stipitis*.
2. The highest % ethanol yield is produced by batch fermentation using *Saccharomyces cerevisiae* and *Pichia stipitis* in amount of 51.269%.
3. The final results of adsorption, the best data shown in continuous fermentation by microorganisms *Zymomonas mobilis* in amount of 88.374%.

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