Effect of Substrate Concentration (Glucose) on Ethanol Fermentation Continue with Immobilized Fixed Bed Fermenter for 2/3 Mesh Pumice Anchoring Size

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Abstract. The need for ethanol which is increasing at all times demands development related to ethanol production, especially those produced through the fermentation process both development in terms of raw materials and processes. Ethanol fermentation process continues to have problems one of them is wash out or the carrying of microorganisms into the product stream which causes the number of microorganisms to continue to decrease in the fermenter. One way to solve the problem of wash out is to tether the microorganism first to the anchoring medium (immobilized cell) and choose the suitable type of fermenter. The purpose of this study was to determine the effect of glucose concentration and the best conditions on the ethanol fermentation process continue with immobilized cell fixed bed fermenter to the value of ethanol concentration and yield ethanol produced as well as the percent of microorganisms still carried into the product streams. Based on the results of the study, the best conditions were obtained with a residence time of 2 days with the ethanol concentration of 1.20%v/v, the yield of fermented ethanol was 37.75%w/w, and % number of cells released was 0.45% at a glucose feed concentration of 150g/L. Fermentation is carried out under conditions of immobilized cell using microorganisms from the type of yeast Saccharomyces cerevisiae. In this study the variables that were considered fixed were the expansion 1/2 height of 1.47m column height, temperature of 25-30°C, and a pH of 5.5.

1 Introduction

Ethanol is an organic compound in the form of a colorless liquid which has many uses [1]. In industry, ethanol is used as raw material for the chemical industry, as a solvent, as an antiseptic or hand sanitizer, and as an alternative fuel mixture. The need for ethanol is increasing from year to year both in Indonesia and in the world because ethanol is being used more and more [2].
According to data released by International Experience, in 2019, the level of world ethanol production has reached 87 million tons per year worldwide. The United States itself still ranks first, as the largest ethanol producing country in the world. The world's ethanol production, which reaches 87 million tons per year, is used by many industries, from the energy industry to alternative fuels [3].

In the production of ethanol by fermentation one of the obstacles occurs in the continuous fermentation process because there are still many microorganisms carried into the product stream (wash out) which causes the number of microorganisms in the fermenter to decrease at any time and can cause microorganisms to accumulate in the product tank as well as efficiency the fermentation process decreases so that immobilized cell conditions can be an alternative [4].

This research was conducted to determine the effect of glucose concentration and the best conditions in the continuous ethanol fermentation process with an immobilized cell fixed bed fermentor on the value of the ethanol concentration and yield of ethanol produced as well as the percentage of microorganisms that are into the product stream.

2 Research Methodology

2.1 Experimental Approach

The process of making ethanol from glucose is carried out by continuous fermentation in a fixed bed column fermentor. Fermentation was carried out under immobilized cell conditions using a microorganism from a type of yeast, namely Saccharomyces cerevisiae. The mooring media used in immobilized cell conditions was 2/3 mesh size pumice with adsorption mooring method. The bed height used was $\frac{1}{2}$ of the 1.47m column height, the time was 2 days, the temperature was 25-30°C, the pH was 5.5, and the glucose feed concentrations were 80 g/L, 150 g/L and 200 g/L.

2.2 Research Tool

![Diagram of Fixed Bed Immobilized Fermenter Column](image1.png)

![Photo of Immobilized Fixed Bed Fermenter Column](image2.png)

Fig. 1. (a) Schematic of Fixed Bed Immobilized Fermenter Column and (b) Photo of Immobilized Fixed Bed Fermenter Column
2.3 Research Procedure

The following is the research procedure carried out:

![Research Procedure Diagram]

**Fig. 2.** Research Procedure

2.4 Analysis

The analysis that needs to be carried out in the fermentation of glucose into ethanol is an analysis of ethanol concentration, glucose concentration, and number of cells (microorganisms). Analysis of glucose concentration and ethanol concentration was carried out using the refractometric method while the analysis of the number of cells was carried out using the counting chamber method.

3 Result and Discussion

3.1 The Effect of Sampling Time on Fermented Ethanol Concentration in Immobilized Cell Conditions with Variations in Glucose Bait Concentration with 2/3 Mesh Pumice Stone Anchoring Size

![Graph: Effect of Sampling Time on Fermented Ethanol Concentration]

**Fig. 3.** Effect of Sampling Time on Fermented Ethanol Concentration in Immobilized Cell Conditions with 2 Days Fermentation Time at Various Concentrations of Glucose Bait with 2/3 mesh Pumice Stone Attachment Size for 25 mL Sampling Volume

**Fig. 3.** showed that there was an effect of variations in glucose concentration on the value of the fermented ethanol concentration. The higher the glucose feed concentration, the fermented ethanol concentration also increases until the steady state time limit is marked by no more changes in the ethanol concentration value. The time to reach a steady state condition for ethanol concentration is the same because the time data for sampling is carried out in a predetermined time range, although actually it can be different for the time to reach the steady state [5].
Based on the graph above, at a variation of glucose concentration from 80 g/L to 150 g/L, the concentration of fermented ethanol increased, but at a concentration of 150 g/L to 200 g/L it decreased. This is due to the possibility of substrate inhibition. Substrate inhibitor itself is a compound that can inhibit the reaction rate of an enzyme. Inhibitors work by binding to the enzyme so that the enzyme becomes damaged or does not match the substrate [6].

3.2 Effect of Sampling Time on Consumed Glucose Mass in Immobilized Cell Conditions with Variations of Glucose Concentration with 2/3 Mesh Pumice Stone Attachment Size

![Graph](image)

**Fig. 4.** Effect of Sampling Time on Consumed Glucose Mass for Immobilized Cell Conditions with 2 Days Fermentation Time at Various Concentrations of Glucose Bait with 2/3 mesh Pumice Stone Attachment Size for 25 mL Sampling Volume

**Fig. 4.** showed the effect of sampling time on the mass of glucose consumed. The longer the sampling time, the mass value of glucose consumed will increase. At various glucose concentrations of 80 g/L, 150 g/L and 200 g/L the mass value of glucose consumed increased, but at a glucose concentration of 200 g/L produced the highest mass of glucose consumed. This is due to the possibility of substrate inhibition. The relationship between the mass value of glucose consumed and the number of microorganisms, namely the microorganisms themselves play a role in producing enzymes and these enzymes will later convert glucose into ethanol [7], but can be seen in the graph above that the small value of glucose consumed caused by the small number of microorganisms and the lack of activity of microorganisms when producing enzymes.

3.3 Effect of Sampling Time on % Yield (Mass of Ethanol Compared to Mass of Glucose Consumed) with Immobilized Cell Conditions on Variations of Glucose Bait Concentration with 2/3 Mesh Pumice Stone Anchor Size

![Graph](image)
Fig. 5. The Effect of Sampling Time on % Yield (Mass of Fermented Ethanol Compared to Consumed Glucose Mass) with Immobilized Cell Conditions on Variations of Glucose Feed Concentration with 2/3 Mesh Pumice Stone Anchor Size

Fig. 5. showed the effect of sampling time on the % yield of fermented products produced with a pumice mesh anchoring size of 2/3. The longer the sampling time, the resulting %yield increases until the steady state time limit is marked by no more changes in the %yield value of the fermented products produced [8]. The time to reach steady state conditions for the % yield produced is the same because the time data for sampling is carried out in a predetermined time range even though the actual time can be different for the time to reach steady state.

Based on the graph above, at varying glucose concentrations from 80 g/L to 150 g/L the % yield of fermented products increased, but at concentrations from 150 g/L to 200 g/L decreased. This is due to the possibility of substrate inhibition. In addition, comparing fermented ethanol products with consumed glucose, the higher the ethanol product, the higher the % yield produced. At varying glucose concentrations from 80 g/L to 150 g/L the % yield of the fermented product increased because the ethanol concentration of the fermented product was high, but at a concentration of 150 g/L to 200 g/L it decreased. This was due to the low concentration of fermented ethanol produced at a glucose concentration of 200 g/L.

3.4 Effect of Substrate Inhibitor on Fermentation Ethanol Concentration Produced in Immobilized Cell Conditions with 2/3 Mesh Pumice Stone Fastening Size

**Fig. 6.** Effect of Glucose Bait Concentration on Fermented Ethanol Concentration Produced in Immobilized Cell Conditions with 2/3 mesh Pumice Stone Anchoring Size at the Time of Sampling at the 90th Hour.

Fig. 6. shows the relationship between the concentration of fermented ethanol at various glucose feed concentrations. From the results of the study, at glucose concentrations of 80 g/L, 150 g/L, and 200 g/L and the resulting fermented ethanol concentrations were respectively 1.07%v/v, 1.20%v/v, and 0.87%v/v. Judging from the concentration of ethanol produced from the fermentation, it is strongly influenced by the concentration of glucose feed.

Based on the graph above, at varying glucose concentrations from 80 g/L to 150 g/L the concentration of fermented ethanol increased, but at concentrations of 150 g/L to 200 g/L it decreased. This is due to the possibility of substrate inhibition. According to [9] at a certain
concentration of glucose, the concentration of ethanol as a result of distillation decreases because glucose becomes an inhibitor and high plasmolysis occurs [10] so that the plasma membrane is released from the cell wall to its environment, as well as the inhibitory nature of glucose against cells that causes the fermentation rate to decrease. In this study, the maximum concentration of ethanol occurred at various concentrations of 150 g/L but decreased at variations of glucose concentrations of 200 g/L.

3.5 The Effect of Glucose Concentration on the % of Cells Released with the Size of 2/3 Mesh Floating Stone Fixing Media

Table 1. Comparison of the % Number of Released Cells at Various Concentrations of Glucose with The Size of 2/3 Mesh Pumice Anchoring Media

<table>
<thead>
<tr>
<th>Condition</th>
<th>80 g/L</th>
<th>150 g/L</th>
<th>200 g/L</th>
<th>Loose Cells (%) at 90 hours (steady state)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tethered cells</td>
<td>2.47x10⁹</td>
<td>-</td>
<td>2.78x10⁹</td>
<td>-</td>
</tr>
<tr>
<td>Loose Cells (%)</td>
<td>1.25x10³</td>
<td>0.45</td>
<td>1.05x10³</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.35x10³</td>
<td>0.54</td>
</tr>
</tbody>
</table>

From Table 1, shows the effect of sampling time on the % number of loose cells. The longer the sampling time, the % number of loose cells will decrease. It can be seen that the longer the sampling time for the same glucose concentration, the % number of released cells decreases.

Based on the graph above, the % number of cells released for varying glucose concentrations from 80 g/L to 150 g/L decreased but increased to 200 g/L as time went by. Based on these data it can be concluded that the % number of released cells is influenced by the high concentration of glucose which causes inhibition of the substrate.

4 Conclusion

Based on the results of the research that has been done, it can be concluded as follows:

1. In pumice anchoring media with a mesh size of 2/3 the concentration value of ethanol and % yield of ethanol produced by fermentation of glucose consumed increased for glucose feed concentrations from 80 g/L to 150 g/L but decreased to 200 g/L while the % amount cells released from the glucose feed concentration of 80 g/L to 150 g/L decreased but increased to 200 g/L due to substrate inhibition.

2. The best conditions for pumice anchoring media with a mesh size of 2/3 with variations in glucose concentrations of 80 g/L, 150 g/L, and 200 g/L obtained the fermented ethanol concentration value of 1.20%v/v, the value % yield of fermented ethanol on glucose consumed was 37.75% w/w, and % number of released cells was 0.43% in steady state conditions obtained at a glucose feed concentration of 150 g/L.
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References


