Design Process of Ethanol Production by Extractive - Fermentation to Increase The Yield and Productivity of Ethanol

Ayu Ratna Permanasari, Ririn Indriani AR, Tri Widjaja, Ali Altway

Chemical Engineering Department, Faculty of Industrial Technology, Sepuluh Nopember Institut of Technology
Sukolilo – Surabaya - INDONESIA
***E-mail** : ay_ratna90@yahoo.com and kajur_tkimia@its.ac.id*

Abstract :

Ethanol is generally produced by batch fermentation process. However, this process will bring about accumulation of ethanol product which can poison the microorganism and lead to low productivity which finally results in an economical disadvantage. This study aims to increase the productivity of ethanol by using continuous fermentation in packed bed bioreactor and the fermentation is integrated by extraction process with recycle from the raffinate to the fermentor as an effort to increase the yield.

This study used immobillization technique by entrapment of mutated Zymomonas mobilis in κ-Carrageenan as the porous matrix and the continuous fermentation process was integrated with liquid-liquid extraction. This process used molasses as the raw material and Amyl-alcohol as the solvent for extraction process. The initial sugar concentration was 160,454g/L (17%). The raw material was prepared by sterilization and adding the nutrient as feed, and 100mL of the feed was added with 10 gr yeast extract and then mutated Zymomonas mobilis was innoculated. Starter was incubated during 15 hours before being used to make the immobilization bead from κ-Carageenan solution. Bead was incubated in the incubator shaker during 24 hours before it was used in the fermentor. Feed entered the fermentor with flow rate of 10mL/minutes, and broth from the fermentor was flowed to the extractor where the solvent was flowed counter currently. Raffinate was collected and flowed to the fermentor with recycle ratio 40%, 50%, 60%, and 70% with respect to the feed. Analysis of sugar reduction residue was performed using Dinitrosalisilic acid (DNS) method, while analysis of ethanol content was performed under Gas Chromatography (GC) method.

From the research, can be concluded that the raffinate recycle can increase the value of yield and productivity. The yield ethanol increase from 16,85% to 32,58% and the productivity increase from 66,25 g/L.h to 192,15 g/L.h with increasing recycle ratio from 0% (without recycle) to 50%. However, the ethanol yield and productivity decrease with increasing recycle ratio above 50%.

Keywords: ethanol, extractive-fermentation, immobillization cell, molasses, packed-bed bioreaktor

1. Introduction

Bioethanol (C_2H_5OH) are generally produced by batch fermentation process of sugar with the help of certain microorganisms. However, batch process has low productivity due to an accumulation of ethanol formed and it can poison to the microorganisms in the fermentor. Batch fermentation process of ethanol is limited by the presence of an inhibitor effect from ethanol itself, it can decrease the rate of product formation and concentration of biomass cells. Finally, batch fermentation process provide the maximum ethanol concentrations not more than 12% v/v **[1]**. After that, ethanol should be separated by distillation to get the higher consentration. One way to reduce these limitations is to separate the ethanol during ethanol itself is produced. One process that can be done is extractive fermentation.

To solve the inhibition problem to microorganism by the ethanol product in the batch fermentation, we need to do the fermentation in continue process. One method of continue fermentation often used is continue fermentation with immobilized cell in packed bed bioreactor. This method give the higher productivity than batch fermentation. However, because the reaction in the fermentor is short in time, there are much sugar that haven't convert to ethanol yet, it cause the ethanol yield is lower than batch process. Recently, integrated fermentation–separation systems have been used successfully to reduce end-product inhibition and thus, to improve overall process efficiency. The product can be removed by a membrane filtration process, by extraction, by evaporation or by adsorption **[2]**. Extractive fermentation is one methode that offer same advantages in fermentation and separation process such as solve the inhibition product and decrease the energy used in distillation process. To increase the ethanol yield from fermentation process, the raffinat from the extractor is turned back to the fermentor because it still have high sugar concentration and this sugar can be converted again to the ethanol.

2. Material and Methods

Molasses is an agro-industrial by-product often used in alcohol distilleries due to the presence of fermentative sugars, being an optimal carbon source for the microorganism metabolism. Sugar cane molasses is an abundant agro-industrial material produced in Brazil and other tropical countries and its low cost is an importantfactor for the economical viability of substances production by fermentation **[3]**.

Compared with Saccharomyces cerevisiae, the ethanol yield and productivity of Zymomonas mobilis are higher, because less biomass is produced and a higher metabolic rate of glucose is maintained through its special Entner–Doudoroff pathway. It was reported that the ethanol yield of Z. mobilis could be as high as 97% of the theoretical yield of ethanol to glucose, while only 90–93% can be achieved for S. cerevisiae. Also, as a consequence of the low ATP yield, Z. Mobilis maintains a higher glucose metabolic flux, and correspondingly, guarantees its higher ethanol productivity, normally 3–5 folds higher than that of S. cerevisiae **[4]**. Mutated Zymomonas mobilis is Zymomonas mobilis which is mutated with hydroxylamine solution and produce Z. mobilis strain that have higher morfology and less movement than general Z.mobilis. Mutated Z.mobilis can stay in condition with range pH 4-5 and high temperature until 50^0C.

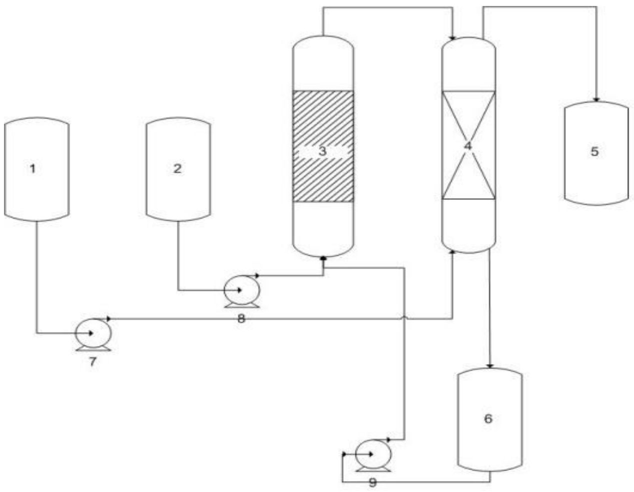
The entrapment of immobilised cells within a porous polymeric matrix such as calcium alginate or κ-carrageenan, along with some others, has been studied extensively. Polymeric beads are usually spherical with diameters ranging from 0.3 to 3.0 mm. Immobilising yeast cells using entrapment is a relatively simple method and a high biomass concentration is facilitated **[5]**. In this research we used κ-carrageenan as the supporting mattrice.

Several researchers have ranked solvent classes based on experimental comparisons of performance: carboxylic acids > alcohols > esters > amines > ketones > ethers > hydrocarbons. Alcohols are one of the better classes of solvents. However, prediction of the relative performance of different alcohols has had mixed results. It has been shown that the ethanol capacity of the alcohol decreases as molecular weight increases. Structural differences such as branch position and size have an effect. In addition, we have shown that position of the hydroxyl group has a significant effect on performance due to the extended hydrogen-bond structure of the solvent molecules. Regarding the toxicity of alcohols to yeast, lowmolecularweight alcohols (C2–C10) are toxic or inhibitory (to yeast growth and/or ethanol production), while higher molecular weight alcohols are not **[6]**.

2.1. Experimental

Molasses with sugar concentration 17% (160,454 g/L) was added by nutrient such as 5,19 g $(NH_4)_2SO_4$, 1,53 g KH_2PO_4 dan 0,55 $MgSO_4.7H_2O$ for each one liter and than, it was sterilized in the autoclaf for 15 minutes. Fermentor used is packed bed bioreactor with immobilized cell and supporting matrice κ-Carrageenan.

Starter used in this research is mutated Z.mobilis which was innoculated in 100mL feed with 10g yeast extract and it was incubated in the shacker incubator for 15 hours. After that, starter was mixed in carageenan solution and formed by noozle with diameter 2mm into 3,5% KCl solution. This immobilized cell was called bead. Bead was incubated again in the shacker incubator for 24 hours.



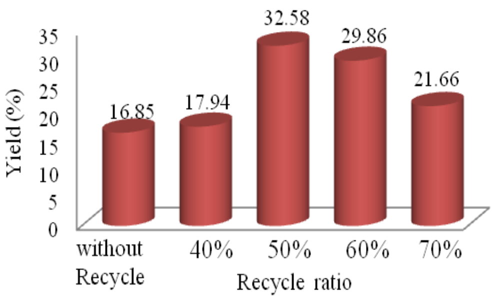
1. Solvent tank
2. Feed thank
3. Fermentor
4. Extractor
5. Extract tank
6. Raffinat thank
7. Solvent peristaltic pump
8. Feed peristaltic pump
9. Raffinate peristaltic pump

Figure 1. Simple schematic of extractive-fermentation

Bead was placed in the fermentor and feed was flowed with flow rate 10 mL/minutes and broth from fermentor was flowed to the extractor where the solvent (amylalcohol) was flowed counter currently. Raffinate was collected and flowed to the fermentor with recycle ratio 40%, 50%, 60%, and 70% with respect to the feed. Analysis of sugar reduction residue was performed using Dinitrosalisilic acid (DNS) method, while analysis of ethanol content was performed under Gas Chromatography (GC) method. Simple schematic of extractif fermentation is shown in Figure 1.

3. Result and Discussion.

Figure 2 saw the yield and ethanol productivity of ethanol before and after the recycle raffinate. Ethanol yield with 0% recycle ratio (without recycle) is lower than ethanol yield with recycle ratio. Because the raffinat still had some of sugar concentration, when it was turned back to the fermentor, Sugar concentration the fermentor would increase (from fresh feed and raffinate). Sugar concentration increased and also the ethanol yiled. The higest ethanol yield, 32,58%, is gotten from extractive fermentation with recycle ratio 50% , whereas increasing recycle ratio (60% and 70%) decreased the yield. The reason for this decrease in the overall fermentation performance was possibly product and substrate inhibition. Roukas studied continuous ethanol production from carob pod extract in a packed-bed bioreactor and found that ethanol concentration and ethanol productivity increased significantly with the increase in initial sugar concentration up to 20%, but theoretical ethanol yield decreased with the increase of initial sugar concentration from 10% to 25%. They stated that above a critical substrate concentration, decreased water activity and the proceeding of plasmolysis caused a decrease in the rates of fermentation **[7]**. Beside that, the greater recycle ratio will cause more and more contact between solven and the microorganisms in the fermentor, and the accumulation solvent diffused inside the bead can poison the microorganisms.



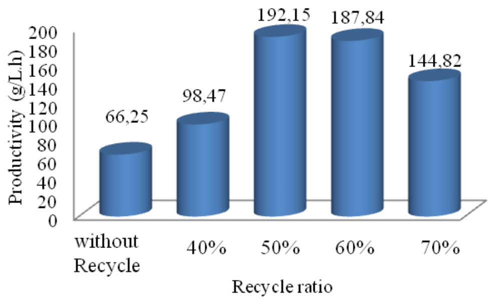


Figure 2. The effect of recycle ratio to the ethanol (a) yield and (b) productivity

The higest productivity is in 50% recycle ratio. It give the maximum productivity 192,15g/L.h with dillution rate 3,676 h^{-1}. Increasing recycle ratio above 50% caused the decresing of productivity. It was caused by lower ethanol concentration. If the dillution rate increase, the residence time of fermentation process would decrease so that the fermentation process is short in time. Too short fermentation process would produced the low ethanol concentration and when flow rate was increased in order to get a higher dilution rate, the peristaltic feed pump was found to be incapable of withstanding the level of backpressure caused by the CO_2 gas and the reactor was therefore shut down **[7]**.

4. Conclusion

From the research, can be concluded that the raffinate recycle can increase the value of yield and productivity. The yield ethanol increase from 16,85% to 32,58% and the productivity increase from 66,25 g/L.h to

192,15 g/L.h with increasing recycle ratio from 0% (without recycle) to 50%. However, the ethanol yield and productivity decrease with increasing recycle ratio above 50%.

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References

[1] Minier M, Goma G. 1982. Ethanol Production by Extractive Fermentation, Biotechnology and Bioengineering, vol. XXIV, Pp. 1565-1579
[2] Kim CH. Kim SW. Hong SI. 1999. An Integrated fermentation-separation process for the production of red pigment by Serratia sp. KH-95. Process Biochemistry, 485-490
[3] Cazetta M.L., Celligoi M.A.P.C., Buzato J.B. and Scarmino I.S., 2007, Fermentation of Molasses by Zymomonas mobilis : Effects of Temperature and Sugar Concentration on Ethanol Production, Bioresource Technology, 98, 2824-2828
[4] Bai FW, Anderson WA, Moo-Young M. 2008. Research review paper Ethanol fermentation technologies from sugar and starch feedstocks, Biotechnology Advances, 26, 89-105
[5] Pilkington PH. Margaritis A. Mensour NA. Russel I. 1997. Fundamentals of immobilised yeast cells for continuous Beer Fermentation: A Review.Institute of Brewing and Distilling, Vol. 104, pp. 19-31
[6] Offeman RD. Stephenson SK, Franqui D. Cline, JL. 2008. Extraction of ethanol with higher alcohol solvents and their toxicity to Yeast, Separation and Purification Technology, 63: 444–451
[7] Goksungur Y, and Zorlu N. 2000. Production of Ethanol From Beet Molasses by Ca-Alginate Immobilized Yeast Cells in a Packed-Bed Bioreactor. Turk J Biol, 25: 265-275