ALCOHOLIC FERMENTATION WITH DIFFERENT INITIAL GLUCOSE CONCENTRATION USING IMMOBILIZED YEAST IN CALCIUM ALGINATE GEL

Bui Thanh Huyen, Le Van Viet Man
University of Technology, VNU-HCM
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ABSTRACT: *In this research, the growth and metabolic activities of Saccharomyces cerevisiae immobilized in alginate gel during alcoholic fermentation with different initial glucose concentration were studied. Starting from initial glucose concentration at 140 g/L, we observed that increasing this concentration to 220 g/L had little effect on the growth of immobilized yeast. Under the same conditions, the maximum biomass, glucose uptake rate, and final ethanol concentration obtained in the immobilized yeast cultures were always higher than those in the free yeast cultures. In 220 g glucose/L medium, the final ethanol concentration produced by immobilized yeast was very high (about 10.4-11.7% v/v). It can be concluded that immobilized yeast were less sensitive to high osmotic pressure and high ethanol concentration in medium than free yeast. Therefore, using immobilized yeast in alcoholic fermentation is potential to enhance ethanol productivity.*

1.INTRODUCTION

Ethanol has many uses in industry as well as in daily life. It is raw material in liquor production, a popular solvent in many industries and gradually becomes a popular environment friendly fuel. The development of the ethanol industry led to the demand for effective fermentation technique to improve ethanol yield.

Nowadays, all Vietnamese ethanol factories carry out the alcoholic fermentation in medium with initial substrate concentration 150 g/L. High gravity fermentation technology allows production of higher ethanol concentration without changing plant size. However, in the culture with high initial substrate concentration, yeast growth and metabolic activities were significantly inhibited due to the high osmotic pressure and high content of final products (including ethanol and other by-products) [1,3,12].

Optimistically, these disadvantages can be lessened by using immobilized yeast. It has been reported that immobilized yeast was less sensitive to the difficult fermentation conditions such as high temperature, low pH and high concentration of inhibitors [4,6,11]. Moreover, immobilized cells can be used for continuous fermentation or reused for many batch cycles [9]. Therefore, the combination of yeast immobilization and high gravity fermentation technology will be a good solution to ameliorate the ethanol production.

Among many cell immobilization methods with different carriers, cell entrapment in alginate gel is a popular method because of the simple immobilization procedure, the high cell density in the gel [7] and the non-toxic carrier [8].

In this research, the growth and metabolic activities of *Saccharomyces cerevisiae* immobilized in alginate gel during alcoholic fermentation with different initial glucose concentration were studied.

2.MATERIALS AND METHODS

2.1.Microorganism

A strain of *Saccharomyces cerevisiae* species from the collection of Microbiological Laboratory, Department of Food Technology, Ho Chi Minh City University of Technology was used.

2.2.Procedure of yeast immobilization

- Incubate *Saccharomyces cerevisiae* in shaken flasks at 30°C for 24 hours.
- Collect yeast cells by centrifugation (4°C, 6000 rpm, 15 min).
- Make a yeast suspension in sterilized water in which the cell concentration was 50×10^6 cells/mL.
- Mix this yeast suspension with an equal volume (1:1, v/v) of 30 g/L sodium alginate (Sigma, A-7128) solution.
- Extrude the mixture drop by drop by a syringe into 20 g/L $CaCl_2$ solution.

Gel formation was instantaneous but as far as gel hardening, the formed beads were immersed in the stirred $CaCl_2$ solution (using a magnetic stirrer) for at least 2 hours. The immobilization was carried out at ambient temperature. Each mL of alginate-yeast cell suspension formed 10 beads. The number of viable yeast cells in beads was about 25×10^6 cells/cm^3 beads. Diameter of beads ranged from 2.0 to 3.0 mm after immobilization. It was supposed that the bead diameter does not change during the fermentation.

After hardening, beads were washed by sterilized water to remove excess calcium ions. They were immediately used for experiments after entrapment.

Medium proposed by Strehaiano et al.[13] was used for yeast propagation and alcoholic fermentation. The composition of this medium was as follows (g/L): D-glucose – 150 to 250; $(NH_4)_2SO_4$ - 2; KH_2PO_4 - 5; $MgSO_4.7H_2O$ - 0.4; yeast extract - 1. The pH of the medium was 4.5.

Alginate solution, $CaCl_2$ solution and water used in the immobilization and culture medium were sterilized at 121°C for 15 minutes.

2.3.Alcoholic fermentation

Batch fermentation by immobilized yeast was carried out at 30°C, pH 4.5. The inoculating rate was 5×10^6 viable cells/mL. Immobilized yeast cells were used for 2 fermentation cycles (I, II). At the end of the first cycle, beads were separated from the culture and immersed in a 20 g/L $CaCl_2$ solution for 15 minutes for gel hardening. Then, they were washed by sterilized water and inoculated in the fresh medium. Fermentation was considered as completed if the decrease of glucose concentration in the culture was less than 1 g/L during 6 successive hours.

Control samples with free cells were carried out in the same fermentation conditions. However, free cells were used for only one fermentation cycle.

2.4.Analytical methods

The yeast growth in immobilized yeast cultures was examined by 3 parameters: Yeast cell concentration within gel beads, yeast cell concentration outside beads, and yeast cell concentration in the whole culture, which were determined and calculated as described else where [5].

Yeast cell concentration was quantified by haemocytometry method, using a Thoma counting chamber. Dead cell concentration was determined by the methylene blue method [4].

Glucose concentration was quantified by a spectrophotometric method, using 3,5-dinitrosalycylic acid reagent [10]. Ethanol concentration was determined by a method based on distillation and density quantification [2].

2.5.Formulas

$R_G = \frac{[G]_i - [G]_f}{t}$, where R_G (g/h.L) is the glucose uptake rate, $[G]_i$ (g/L) is the initial glucose concentration in the culture, $[G]_f$ (g/L) is the final glucose concentration in the culture, and t (h) is the fermentation time.

3.RESULTS AND DISCUSSION

3.1.Immobilized yeast growth in media with different initial glucose concentration

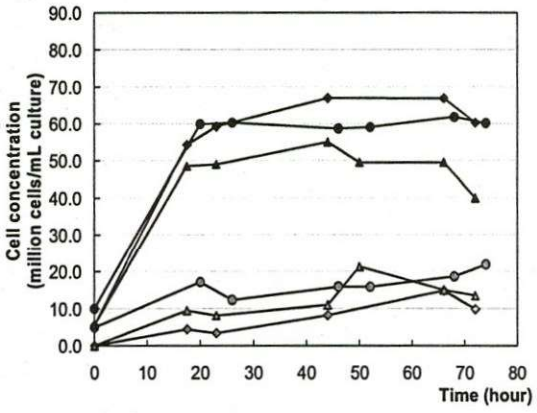
The growth of yeast in the immobilized cell cultures and free cell cultures with different initial glucose concentrations 140 g/L, 170 g/L, and 220 g/L are presented in Figure 1a, 1b, 1c respectively.

In general, the growth rate of immobilized yeast in cycle II was lower than that in cycle I. There may be 2 reasons for that.

Firstly, at the end of cycle I, immobilized yeast was in stress due to high ethanol concentration and low pH. So in cycle II, it took the yeast longer time to overcome the stress, adapt to new culture and continue budding.

Secondly, during the fermentation, the beads became porous because of the growth of yeast and the release of CO_2. Therefore, the immobilized yeast was more exposed to high osmotic pressure in cycle II than in cycle I.

However, the yeast growth rate and maximum biomass obtained in the immobilized cell cultures always higher than those obtained in the free cell cultures. Maybe, during the fermentation with high initial glucose contents using immobilized yeast, gradients of glucose concentration were formed through the gel beads. It helped the cell gradually get familiar with high osmotic pressure.



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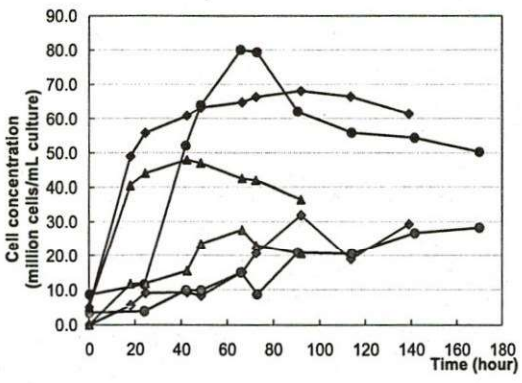

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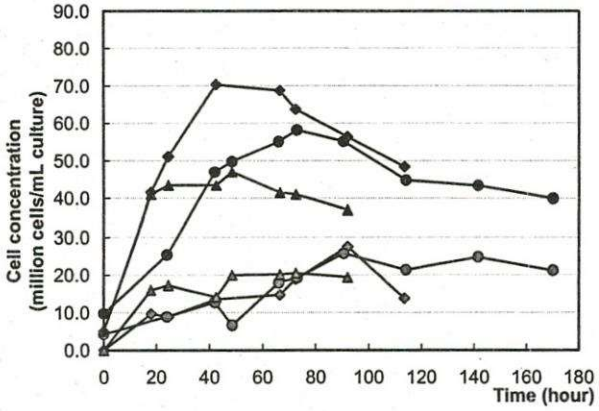


(c)

Fig. 1. Growth of yeast in immobilized yeast culture cycle I (◆ - Total cells, ◇ - Dead cell), cycle II (● - Total cells, ○ - Dead cell) and free yeast culture (▲ - Total cells, △ - Dead cell) during the fermentation at 30°C, initial pH 4.5 and initial glucose concentration 140 g/L (a), 170 g/L (b) and 220 g/L (c).

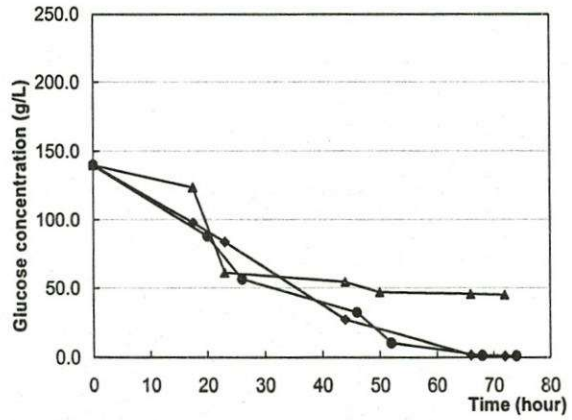
* Total cells included viable cells and dead cells.

3.2.Metabolic activities of immobilized yeast cultured with different initial glucose concentration

The influence of initial glucose concentration on metabolic activities of yeast was evaluated by the overall glucose uptake rate and final ethanol concentration (Table 1). The changes of glucose concentration during the fermentations were shown on Figure 2.

In the immobilized cell cultures, cycle II was usually longer than cycle I and the increase in initial glucose concentration led to in the increase in fermentation time. Besides, when the initial glucose content increased, the glucose uptake rate decreased. However, the changes in the immobilized cell cultures were not as significant as in the free cell cultures.

In comparison with the free cell cultures, the immobilized cell cultures always lasted longer but the residual glucose concentrations were much lower. In addition, the final ethanol concentrations obtained by immobilized cells were about 8.4 – 11.7 %v/v, which were twice as high as those obtained by free yeast in the similar conditions. Probably, the immobilized cells were protected from the undesirable changes of the culture during the fermentation (the decrease of pH and the accumulation of ethanol). These changes might cause the diminution in metabolic activities of the free cells.



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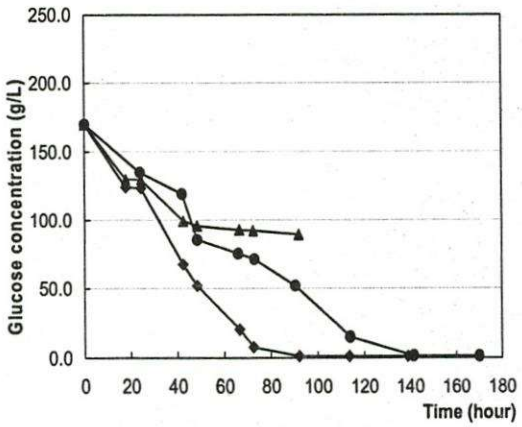

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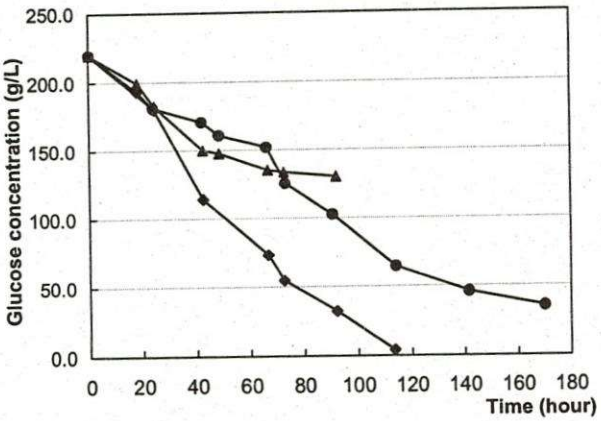

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Fig. 2. Kinetics of glucose content in the immobilized cell culture cycle I (◆), cycle II (●) and free cell culture (▲) at 30°C, initial pH 4.5 and initial glucose concentration 140 g/L (a), 170 g/L (b) and 220 g/L (c).

Table 1. Characteristics of alcoholic fermentation by immobilized and free cells at different initial glucose concentrations (140, 170, and 220 g/L).

Characteristic	Glucose uptake rate (g/L.h)			Final ethanol concentration (%v/v)		
Initial glucose concentrations (g/L)	140	170	220	140	170	220
Immobilized yeast - Cycle I	1.93	1.83	1.90	8.4	10.4	11.7
Immobilized yeast - Cycle II	2.04	1.19	1.09	8.6	10.4	10.4
Free yeast	1.43	0.72	0.98	4.7	4.7	5.6

3.CONCLUSION

The obtained result in this study confirmed the protective effect of alginate carrier on yeast cells during alcoholic fermentation. Therefore, immobilized yeast was more resistant to high osmotic pressure, low pH media and high ethanol concentration. Those characteristics made it possible to obtain high ethanol concentration in the culture with high initial glucose concentration. In conclusion, using immobilized yeast in alcoholic fermentation is potential to enhance ethanol productivity without significant changes in the plant size.

LÊN MEN CỒN BẰNG NẤM MEN CỐ ĐỊNH TRONG TRONG CÁC MÔI TRƯỜNG CÓ NỒNG ĐỘ GLUCOSE BAN ĐẦU KHÁC NHAU

Bùi Thanh Huyền, Lê Văn Việt Mẫn
Trường Đại học Bách Khoa, ĐHQG-HCM

TÓM TẮT: *Trong nghiên cứu này, chúng tôi khảo sát sự sinh trưởng và hoạt tính của nấm men Saccharomyces cerevisiae cố định trong gel alginate trong quá trình lên men cồn với nồng độ glucose ban đầu của môi trường tăng dần. Khi tăng nồng độ glucose ban đầu từ 140 g/L lên 200 g/L, chúng tôi nhận thấy sự thay đổi nồng độ glucose không ảnh hưởng đáng kể đến sự sinh trưởng cũng như hoạt tính của nấm men cố định. Trong cùng điều kiện thí nghiệm, quá trình lên men bằng nấm men cố định luôn cho lượng sinh khối cực đại, tốc độ lên men và nồng độ cồn cuối cao hơn quá trình lên men với nấm men tự do. Trong môi trường có nồng độ glucose ban đầu là 220 g/L, nấm men cố định tạo được nồng độ cồn cuối rất cao (khoảng 10.4-11.7% v/v). Có thể kết luận rằng nấm men cố định trong gel alginate ít mẫn cảm với áp suất thẩm thấu cũng như nồng độ cồn cao của môi trường. Vì vậy, sử dụng nấm men cố định trong sản xuất cồn từ môi trường có nồng độ chất khô ban đầu cao là một phương pháp hiệu quả để nâng cao sản lượng cồn.*

REFERENCES

[1]. Almeida RB., Alameida e Silva JB., Lima UA., Silva DP., Assis AN., *Evaluation of fermentation parameters during high-gravity beer production,* Braz. J. Chem. Eng., 18 (4): 459-465 (2001)

[2]. AOAC, *Official Methods of Analysis of AOAC International,* 15th edition, Maryland, AOAC International, (1990)

[3]. Arrizon J., Gschaedler A., *Increasing fermentation efficiency at high sugar concentrations by supplementing an additional source of nitrogen during the exponential phase of the tequila fermentation process*, Can. J. Microbiol., 48: 965-970 (2002)

[4]. ASBC, Methods of analysis, 8th edition, *Minnesota: The American Society of Beer Chemists* (1992)

[5]. Bui TH., Le Van VM., *Growth and metabolic activities of immobilized yeast cells in clcium alginate gel during alcoholic fermentation,* Proceedings of Regional Symposium on Chemical Engineering 2005, 1: 153-157 (2005)

[6]. Desimone MF., D'Aquino M., Diaz LE., *Ethanol tolerance in free and sol-gel immobilized Saccharomyces cerevisiae,* Biotechnol. Lett., 24 (19): 1557-1559 (2002)

[7]. Dömény Z, Šmogrovičová D, Gemeiner P, Šturdík E, Pátková J, Malovíková A, *Continuous secondary fermentation using immobilized yeast,* Biotechnol. Lett., 20: 1041-1045, (1998)

[8]. Göksungur Y, Zorlu N, *Production of ethanol from beet molasses by Ca-alginate immobilized yeast cells in a packed-bed bioreactor*, Turk. J. Biol., 25: 265-275 (2001)

[9]. Klein J., Kressdort B., *Polymers for the immobilization of whole cells and their application in biotechnology,* Angew. Makromol. Chem., 166: 293-309 (1989)

[10]. Miller GL., *Use of dinitrosalicylic acid reagent for determination of reducing sugar,* Anal. Chem., 3: 426-428 (1959)

[11]. Norton S., Watson K., D'Amore T., *Ethanol tolerance of immobilized brewers' yeast cells,* Appl. Microbiol. Biotechnol, 43 (1): 18-24 (1995)

[12]. Oliveira C., Rosa MF., Cabral JMS., Aires-Barros RM., *Effect of extraction and enzymatic esterification of ethanol on glucose consumption by two Saccharomyces cerevisiae strains: a comparative study,* J. Chem. Technol. Biotechnol., 76: 285-290 (2001)

[13]. Strehaiano P., Mota M., Goma G., *Effect of inoculum level on kinetics of alcoholic fermentation*, Biotechnol. Lett., 5: 135-140 (1983)