Saccharomyces Cerevisiae Immobilized in Alginate for Continuous Fermentation

Zhaohui Xing, Qi Zhang, Xueyi Shi, and Yan Lin

***Abstract*—*Saccharomyces cerevisiae* BY4742 cells were immobilized in alginate and applied for continuous fermentation. Immobilization of yeast cell showed technical and economic advantages over free cell system. In the fermentation, the initial glucose concentration was 110 g/L. The hydraulic retention time (HRT) of fermentation broth was set to 24 h with a re-activate time of 12 h after first incubation period 72 h. Ethanol yield and glucose utilizing ratio were analyzed for verifying the performance of continuous fermentation and the improvement of immobilized yeasts over free yeast cells for fermentation. Comparing dates in two experimental groups, the ethanol yield could reach to about 80% and the glucose utilizing ratio had the similar pattern with averagely 50%. The ethanol yield and glucose utilizing ratio had a significant improvement with the pre-activation and the re-activation.**

***Index Terms*—*Saccharomyces cerevisiae*, yeast immobilization, continuous fermentation, fermentation efficiency.**

I. Introduction

Because of global energy crisis with diminishing fossil fuels and environmental problems such as global warming and air pollution, growing attention has been devoted in the past few years to the ethanol production from renewable carbohydrate materials which is widely recognized as an alternative to fossil fuels with powerful economic, environmental and strategic benefits, and much research has focused on ethanol production using immobilized viable microbial cells in continuous systems [1], [2]. Traditional fermentation systems use freely suspended yeast cells in a batch bioreactor. The whole reactor volume is gradually fermented and subsequently removed from the reactor in which yeast cells can be adversely influenced by other parameters and inhibition can be caused either by product or substrate concentration [3]. During batch fermentation of free cells, product ethanol came out of the system with fermentation broth containing free cells, glucose and others impurities; it is hard to separate mixture components for ethanol only [4]. Recently the immobilized biocatalysts have been extensively investigated. Immobilized cells show many advantages over free cell in tolerance of temperature and pH, and can provide high cell activity in the reactors.

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There are several operational problems effecting continuous usability of continuous fermentation system. Continuous fermentation systems with immobilized yeast cells have a continuous flow of fermented medium into the fermenter and a corresponding continuous flow of fermented product out of the system, which means the nutrient utilization in, the single stage continuous stirred tank reactor (CSTR) for example, is lower than batch fermentation. Besides the designs of reactor system, such as the pre-activation time, the hydraulic retention time (HRT) and the reactor volume, have an impact on the experimental results [5].

The aim of this study was to obtain high ethanol production by immobilizing yeast cells in alginate, and apply these immobilized beads for continuous fermentation to test the fermentation performance of immobilized yeast [6], improve the designs of continuous fermentation according the experimental results, and offer reference for following development works.

II. Material and Methods

A. Yeast Strain and Culture Condition

S. cerevisiae BY4742 (originally from EURSCAF, Germany) used in this study was thermo tolerant strain selected and maintained in glycerol vials at -80 ℃ for use as a working stock in our laboratory (School of Environmental Science and Engineering, Shanghai Jiao Tong University, China). Active cultures for inoculation were obtained in 1 L Erlenmeyer flasks with 500 mL of growth medium containing 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract. The liquid medium was sterilized at 121 ℃ for 30 min. The pre-culture was performed in a rotatory shaker at 37 ℃ and 180 rpm for 16 h and then used to inoculate 500 mL baffled shake-flasks containing 250 mL of the above medium with the same condition. The cells were centrifuged at 3000 rpm for 3 min, the supernatant was decanted, and the cells were washed three times with sterile water [7].

B. Preparation for the Carriers

The obtained cells were re-suspended in sterile water and used as inoculum in the continuous fermentation. The cells count was determined at OD_{600}. Sodium alginate gel was obtained in 100 mL beaker with 45 mL sterile water containing 1.875 g sodium alginate. The medium was sterilized at 121 ℃ for 30 min.

C. Immobilization of Yeast

The immobilization in calcium alginate beads was carried out as followed: The yeast cells slurry (containing yeast 1.5 g) and the sodium alginates were mixed with sterile water to

final volume 300 mL, with 2.5% (w/v) sodium alginate and 2% (w/v) yeast cells slurry. The mixture obtained was extruded dropwise with sterile syringes into gently stirred 2% (w/v) $CaCl_2$ and kept for 4 h for stabilization. The beads obtained containing cells were stored at 4 ℃ and used for further studies.

D. Continuous Fermentation

Two control groups were used for the study. The beads in group 1 (G1) were direct used for fermentation. The beads in group 2 (G2) were activated before fermentation with active cultures for inoculation above. There were 2 reactors (1# and 2#) in each experiment group. Reaction conditions were as follows: the initial glucose concentrations were 110 g/L, with the cell concentration 4.6 g/L. The reactors were performed at a temperature of 35 ℃ and an initial pH of 4.0. Stirring speed was set as 180 rpm, and the incubation period was 144 h. Samples were token per 12 h or 24h. Two reactors contained fermentation broth 200 mL with 60 mL immobilized beads. The HRT of fermentation broth was set to 24 h with a re-activate time of 12 h after first incubation period 72 h.

E. Analytical Method

Ethanol and reduce sugar samples were all diluted 10 times and analyzed by high performance liquid chromatography (HPLC LC-10AD, SHIMADZU, Kyoto, Japan) equipped with a refractive index detector (RID-10A, SHIMADZU, Kyoto, Japan). An Aminex HPX-87H column (Bio-Rad, USA) with a safe guard column operated at 65 ℃ using pure grade water as the mobile phase (0.8 mL/min) was used for the separation. In nutrient for continuous fermentation, only glucose can be fermented into ethanol by *S. cerevisiae* BY4742, so the maximum theoretical ethanol from glucose could be calculated according to the stoichiometric relationship represented by (1), i.e. 100 g of glucose can produce 51.1 g of ethanol. The ethanol yield was calculated according to (2). The glucose utilizing ratio is calculated according to (3).

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (1)$$

$$Y_E = \frac{C_{Eth}}{C_{G0} \times 0.511} \times 100\% \quad (2)$$

$$Y_{Glu} = \frac{C_{G0} - C_{Gt}}{C_{G0}} \times 100\% \quad (3)$$

where Y_E is the actual ethanol yield, C_{Eth} is the ethanol concentration in fermentation liquid, C_{G0} is the initial glucose concentration, and the 0.511 is the transformation coefficient of ethanol per gram glucose. Y_{Glu} is the glucose utilizing ratio, and CG_0 is the initial glucose concentration, C_{Gt} is the final.

III. Results and Discussion

A. Ethanol Yield in Continuous Fermentation

Ethanol yields in two reactors of two experimental groups were recorded to verify the influence of pre-activation and re-activation on ethanol production capacity of immobilized yeast cells in continuous fermentation. The ethanol yield of two reactors is presented in Fig. 1 and Fig. 2.

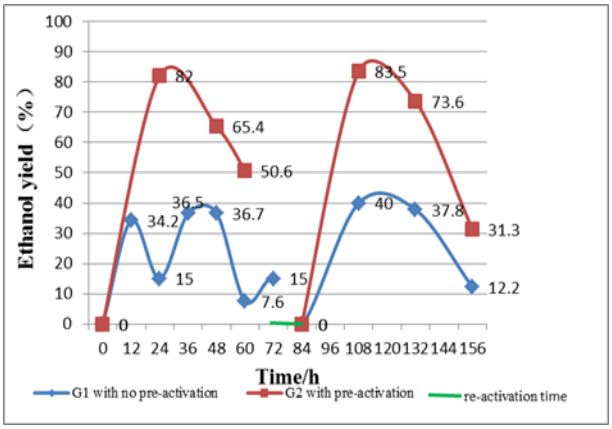

Fig. 1. Ethanol yield in 1# reactor at fermentation time of 0-156 h with 12 h re-activation time and initial glucose concentration of 108.78 g/L.

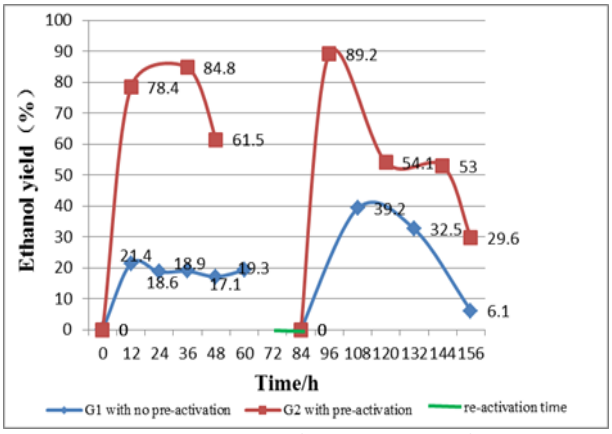

Fig. 2. Ethanol yield in 2# reactor at fermentation time of 0-156 h with 12 h re-activation time and initial glucose concentration of 102.99 g /L.

Effect of pre-activation in continuous fermentation: The fermentation was performed in two reactors with same fermentation conditions in each experimental group. The trends of ethanol of two experimental groups were similar, with a sharp rise occurring within the first 36 h followed by a significant reduction. The yeast cells retained relatively high fermentation activation after being immobilized in calcium alginate beads, and immobilization didn't damage active center of cell. From the comparison of two experimental groups, the ethanol in G2 was apparently higher than that in G1. Pre-activation before fermentation enhanced cells' biological activity.

Effect of re-activation in continuous fermentation: The highest ethanol yield, 89.2%, was obtained after re-activation time in 2# reactor of G2. The lowered ethanol yield obtained significant increase after reactivation. The trends of second period were similar to that in first period. After 72 h fermentation, the biological activity of cells reduced in reactors because of impurities, nutrition dead zones and other experiment reasons. Re-activation time of 12 h made the cell obtain original activity.

B. Glucose Utilizing Ratio in Continuous Fermentation

Glucose utilizing ratios of two reactors of experimental groups were studied to demonstrate biological activity changes of yeast cells. The glucose utilizing ratios of two reactors is presented in Fig. 3 and Fig. 4.

Effect of pre-activation in continuous fermentation: In each experimental group, the glucose utilizing ratios had a significant superiority in G2 with pre-activation than that in

G1. The biological activity of immobilized yeast cells tended to be strengthened, and within first 36 h, the ratio reached the highest, 95.93%, which indicated that pre-activation cells could metabolize glucose effectively. With the increase of fermentation time, the glucose utilizing ratios of yeast had a significant decline, which in G1 fell from about 30% to 15% and in G2 fell from about 80% to 50%. That indicated one-step activation might not maintain the high activity for long time because of variables in reactors.

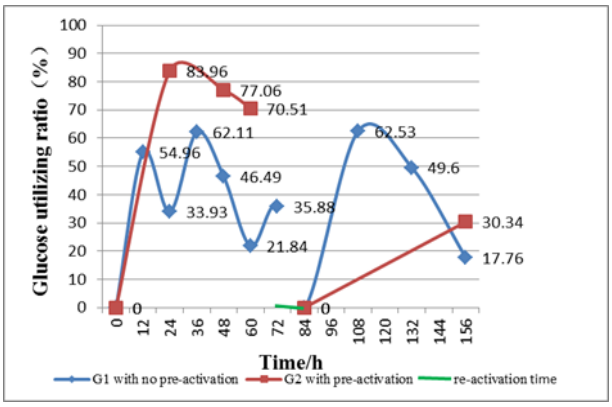

Fig. 3. Glucose utilizing ratio in 1# reactor at fermentation time of 0-156 h with 12 h re-activation time and initial glucose concentration in G1 was 109.78 g/L.

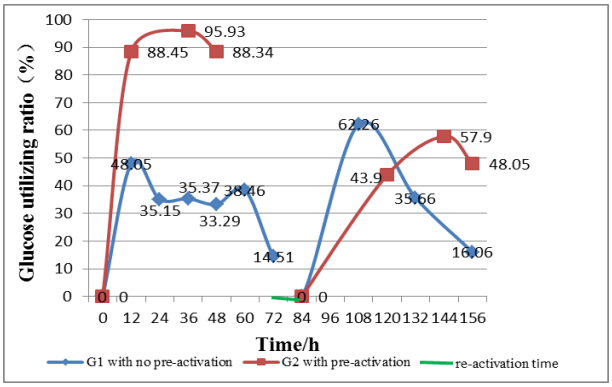

Fig. 4. Glucose utilizing ratio in 2# reactor at fermentation time of 0-156 h with 12 h re-activation time and initial glucose concentration in G2 was 102.99 g/L.

Effect of re-activation in continuous fermentation: the glucose utilizing ratios of two reactors in each experimental group had a significant reduction after about 36 h. After re-activation, the activity of immobilized yeast cells was regained with a rise trend of glucose utilizing ratio in the second period. At the end of each fermentation period, the glucose metabolism of yeast cells became inefficient, due to ethanol concentration in reactor, impurities after fermentation and mass transfer problems, which reduced the glucose utilizing ratio. After re-activation, yeast cells came alive due to the appropriate growth environment which stimulated the biological activity center.

IV. Conclusion

In this study, an effective method for immobilization of the yeast *S. cerevisiae* BY4742 was applied in continuous fermentation. The ethanol yields and glucose utilizing ratios were analyzed in two groups of continuous fermentation reactors. Immobilized yeast had high ethanol fermentation activity but the performance was affected by many experiment factors, especially the biological activity of yeast [8]. Comparing date in G1 and G2, the ethanol yield could reach to about 80% in G2 with pre-activation; the glucose utilizing ratio had the similar pattern. Optimizing the growth condition showed satisfactory results [9], activation before the fermentation can significantly improve the efficiency of yeast producing ethanol; besides with the efficiency falling, yeast cells restore fermentation activity though re-activation. After re-activation, the glucose utilizing ratio could reach averagely 50%. Ethanol yield within first 24-36 h could reach averagely 80% with pre-activation and re-activation. Activation enhanced the biological activity of cells thus improved the fermentation performance in continuous fermentation.

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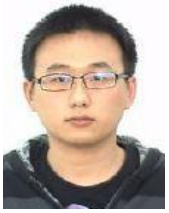

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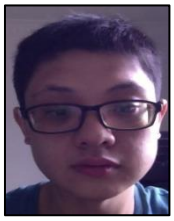

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Mr. Zhang performed well both in study and academic research so that he was recommended for a direct entry into postgraduate studies in SJTU without being required to sit any entrance examinations. He has been awarded the Tung OOCL Scholarship and the First Prize of SJTU academic scholarships in 2013.

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