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IMMOBILIZATION OF *Saccharomyces cerevisiae* IN RICE HULLS FOR ETHANOL PRODUCTION

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Abstract

The whole cell immobilization in ethanol fermentation can be done by using natural carriers or through synthetic carriers. All of these methods have the same purpose of retaining high cell concentrations within a certain defined region of space which leads to higher ethanol productivity. Lignocellulosic plant substance represents one of highly potential sources in ethanol production. Some studies have found that cellulosic substances substances can also be used as a natural carrier in cell immobilization by re-circulating pre-culture medium into a reactor. In this experiment, rice hulls without any treatment were used to immobilize *Saccharomyces cerevisiae* through semi solid state incubation combined with re-circulating pre-culture medium. The scanning electron microscopy (SEM) pictures of the carrier show that the yeast cells are absorbed and embedded to the rice hull pore. In liquid batch fermentation system with an initial sugar concentration of 50 g/L, nearly 100% total sugar was consumed after 48 hours. This resulted in an ethanol yield of 0.32 g ethanol/g glucose, which is 62.7% of the theoretical value. Ethanol productivity of 0.59 g/(L.h) is 2.3 fold higher than that of free cells which is 0.26 g/(L.h). An effort to reuse the immobilized cells in liquid fermentation system showed poor results due to cell desorption in the first batch which led to high sugar concentration inhibitory effect in the second batch fermentation. This might be solved by using semi solid fermentation process in the future work.

Keywords: ethanol, fermentation, immobilization, rice hulls, yeast cells

1. Introduction

The increase of world energy demand with rapid petroleum source depletion has made a common interest in seeking alternative fuels globally. Bioethanol made from lignocellulosic substances is considered as the feasible and sustainable alternative fuels because of its easy adaptability in existing engine and higher octane value compared to petroleum gasoline. Its expensive production in the commercial stage has made the world priority in searching for an effective and efficient conversion of lignocellulosic substances into fuel ethanol [1-4]. Immobilized cell technology has been suggested as an effective means for improving ethanol fermentation. The immobilization of cells leads to higher cell densities with consequent increases in reaction rates and productivity [5-10].

Ethanol production by immobilized cells has been extensively investigated for the last few decades. Cells have been immobilized on a variety of natural and synthetic supports. The most widely used synthetic carrier in immobilization methods is based on cell entrapment in gels, such as carrageen and Ca-alginate [7,8]. The main drawback of these systems is the lack of mass transfer between product and substrate in the system. Other disadvantages are the instability of Ca-alginate against the buffer solution and the disruption of

gel particles due to CO_2 evolution during fermentation, which also limits its usage [8,11].

One of the extensively used classes of natural support is lignocellulosic substances. Some lignocellulosic substances support investigation including sawdust, woodchips, rice husk, rice straw, sorghum straw, empty fruit bunches of oil palm tree and sugar cane bagasse. In this literature, the immobilization methods are all based on the recirculation of the concentrated yeast suspension through the reactor [8]. The advantage of lignocellulosic substance usage is the porosity of its surface which can facilitate the transmission of substrates and products between carriers and medium. The paper describes the possibility of using rice hulls as the natural carrier in cell immobilization.

2. Methods

Microorganisms and mediums. The pre-cultured commercial baker yeast from Korea was used in this work. The composition of the pre-culture medium for yeast was (in g/L): glucose, 15; sucrose, 15; yeast extract, 3; peptone, 5; malt extract, 3. The cells were cultivated in this medium in a controlled environment shaker at 30 ^oC for 20 hours in order to obtain high cell density. At the end of incubation period, cells were centrifuged aseptically and re-suspended in fresh pre-culture medium to be used as inoculum. The composition of the fermentation mediums (in g/L) was: glucose, 50; $MgSO_4$, 1; KH_2PO_3, 1. Then it was adjusted to pH 5.5 using citrate buffer solution and autoclaved at 121 ^oC for 15 minutes before used.

Immobilization. The yeast cells were immobilized in a bioreactor, with size 3 cm in diameter and 30 cm of height. The carriers were aseptically transferred to the sterilized equipment. The carrier volume was about 25-50% of the working volume of 170 mL. After the rice hulls were washed, they were sieved to remove other contaminants.The average size of the rice hullswas between 5-7 mm. There is no size reduction in this work. The cleaned rice hulls were then dried.

Rice hulls as much as 2.5 and 5 g were autoclaved at 121 ^oC for 15 min and then mixed with 50 ml pre-culture inoculums in which the concentration was $2x10^8$ cells/mL in flask. After 24 hours, the liquid from immobilizing reactor was discharged, filled with another 50 mL of fresh pre-culture medium, and kept for the next 24 hours before it was used for fermenting sugar.

Analytical methods. Cell biomass concentration was determined based on the NREL LAP-008 [12] using the absorbance at 600 nm with a UV-visible spectrophotometer and converted to dry cell concentration on the basis of a corresponding standard curve. Cell number was determined using a haemocytometer. The yeast cells were separated from the support by washing rice hulls with distilled water and centrifuging the collected liquid.

The concentration of ethanol was determined by DS 6200 Gas Chromatograph (Donam Instrument, Inc.) with DB-1 column and a flame ionization detector. The chromatogram was run at 100 ^oC oven temperature and 120 ^oC detector and injection temperature using air as carrier gas and H_2 as a flaming gas.

To measure glucose concentration, the sample solution was diluted then determined by the dinitrosalicylic acid method [13,14]). Samples of absorbance were measured using UV Spectrophotometer HACH, US/DR-4,000U at 575 nm.

Electron microscopic scanning. A JSM-5400 Scanning Electron Microscope (JEOL) was used to take images of yeast cells immobilized in rice hull pores. The samples were soaked in 3.5% glutaraldehyde for 6 hours, and dried by treatment with 70% ethanol, followed by overnight retention of the samples in desiccators for the removal of moisture [8].

3. Results and Discussion

Immobilization of yeast cells to rice hulls. The method which is commonly used in cell immobilization by using lignocellulosic substances as the carrier is by re-circulating pre-culture medium through the reactor. This system can be cultivated in a form of semi solid which makes the immobilized cell concentration increase [8]. It can be seen from Table 1. Effect of rice hulls mass/inoculums volume ratio to immobilize yeast cells.

By using 5 g of rice hulls and 50 mL of inoculums, the immobilized cell concentration is 0.18 g DCW (dry cell weight)/g DRW (dry rice hull weight). Cell concentration decreases to 0.12 g DCW/g DRW when 2.5 g of rice hulls are used for immobilizing the cells. The insufficient rice hull surface area for yeast to absorb might be the cause for this decrease. From Figure 1.SEM pictures of rice hull pores filled with yeast cells after immobilization, we can see that rice hulls have a large number of pores which can be used by yeast cells to stick and to absorb.

The comparison of batch fermentation in free cell system and immobilized cell system. The main advantage of immobilized cell technology is the increase of cell population density which is actually aided by flocculating process. This action makes the productivity of the fermentor increase by two or three times [2,11].

It is showed in Fig. 2. Viable cell concentration of *S. cerevisiae* in fermentation broth which using

inoculums as much 10% of fermentation medium and same initial sugar concentration of 50 g/L. Viable cell concentration in the fermentation broth after 6 hour in immobilized system was 0.9 x 10^7 unit cells/mL, it is 1.8 fold higher than the free cells system with 0.5 x 10^7 unit cells/mL.

This experiment proves the advantage of cells immobilization by increasing the ethanol productivity in liquid fermentation (Fig. 3).

In the free cell system, a 72-hour fermentation of the yeast cells consumes 79.53% of the total sugar, while immobilized cells system takes only 48 hours to consume 99.1% of the total sugar. The ethanol concentration and yield for immobilized cell system are 15.65 g/L and 0.32 g ethanol/g sugar consumed respectively. Compared to the free cell system, the ethanol concentration and yield in immobilized cell system are higher by 2.43 and 2 times which are 6.45 g/L and 0.16 g ethanol/g sugar consumed, respectively. Ethanol productivity is 0.26 g/(L.h) for free cells and 0.59 g/(L.h) for immobilized cells, and it is 2.3 times higher than that of the free cell system.

Table 1. Effect of Rice Hulls Mass/Inoculums Volume Ratio to Immobilize Yeast Cells

Inoculum volume (mL)	Rice hulls mass (gr)	Immobilized cell concentration (gDCW/gDRW)
50	2.5	0.12
50	5	0.18

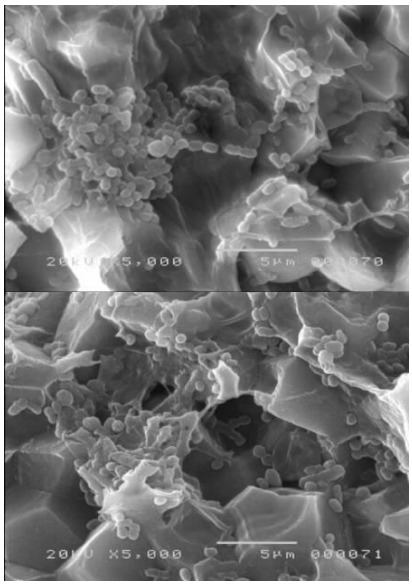


Fig. 1. Scanning Electron Microscope (SEM) Pictures of Rice Hull Pores Filled with Yeast Cells After Immobilization (5 g Rice Hulls/50 mL Inoculums)

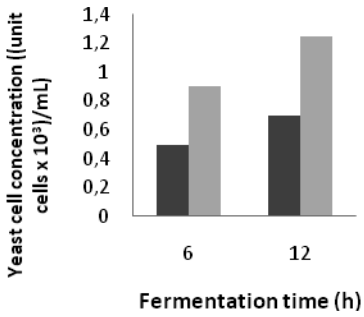
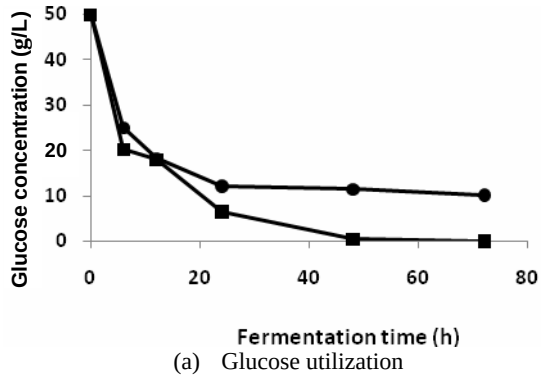
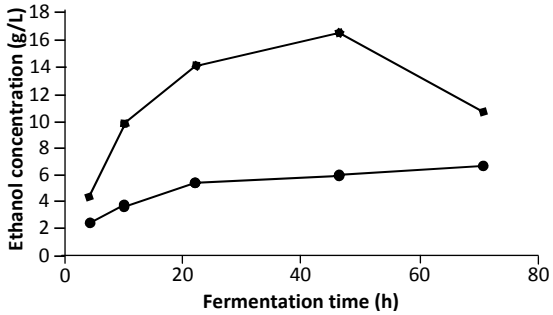


Fig. 2. Viable Cell Concentration of *S. cerevisiae* in the Fermentation Broth During 12 Hours First Batch Fermentation Using Free Cell (■) and Immobilized Cells (■)



(a) Glucose utilization



(b) Ethanol production

Fig. 3. Comparison of the Fermentation Kinetics Between Immobilized Cells System (■) and Free Cell System (•)

Reuse of immobilized cells in a batch process for production of ethanol. Rice hulls were used in the first batch fermentation, filtered and transferred to a fresh fermentation medium. In this new system, the immobilized cells show poor performance for liquid fermentation. The ethanol concentration after 3 hour fermentation was 2% v/v, and then it gradually decreased (Fig. 4). This possibly happened because the cells which were immobilized in the rice hulls were leaking into fermentation broth of the first batch, hence reducing the number of cells attached at the rice hull pores. This phenomenon is supported by data shown in Fig. 2. and Fig. 5.

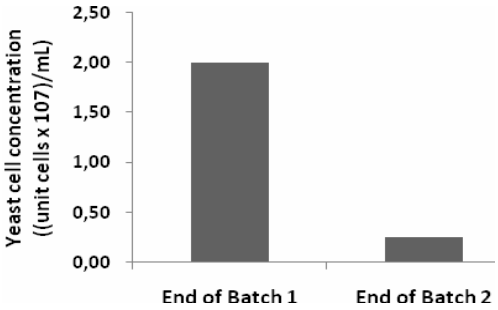


Fig. 4. Ethanol Production Using Immobilized Cells in 2nd Batch Fermentation System

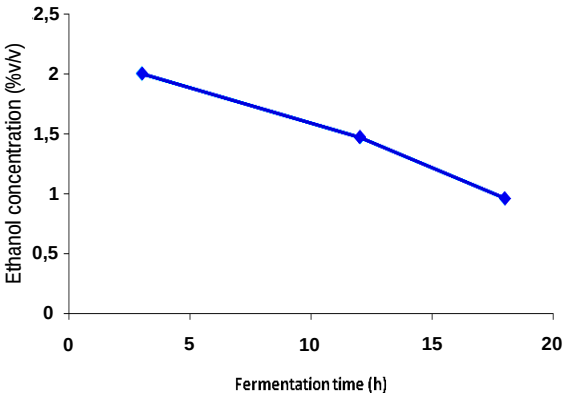


Fig. 5. Viable Cell Concentration of *S. cerevisiae* at the end of 1st and 2nd Batch Fermentation

The cell concentration in the first batch fermentation broth increased at the end of fermentation; it reached 2×10^7 unit cells/m, 8 times higher compared to the 2nd batch which is only 0.25×10^7 unit cells/mL. This low concentration of yeast cell is insufficient to convert available sugar into ethanol. In accordance with the literature, high concentration of glucose proves inhibitory to the yeast cell growth [15].

4. Conclusion

Immobilization of baker's yeast cells to rice hulls was done in semi solid form to achieve firm cell immobilization. Rice hulls have the potential as a carrier for cell immobilization with several advantages such as: low carrier cost and simple procedure of immobilization. By using immobilized cells, fermentation process could be done faster compared to the free cell form with ethanol productivity more than 2 times higher. Liquid fermentation system is unsuitable for repeated batch fermentation using immobilized cell form. There should be a further investigation for applying the immobilized cells in semi solid state fermentation.

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