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Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*

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Abstract

Fermentation of sugar by *Saccharomyces cerevisiae*, for production of ethanol in an immobilized cell reactor (ICR) was successfully carried out to improve the performance of the fermentation process. The fermentation set-up was comprised of a column packed with beads of immobilized cells. The immobilization of *S. cerevisiae* was simply performed by the enriched cells cultured media harvested at exponential growth phase. The fixed cell loaded ICR was carried out at initial stage of operation and the cell was entrapped by calcium alginate. The production of ethanol was steady after 24 h of operation. The concentration of ethanol was affected by the media flow rates and residence time distribution from 2 to 7 h. In addition, batch fermentation was carried out with 50 g/l glucose concentration. Subsequently, the ethanol productions and the reactor productivities of batch fermentation and immobilized cells were compared. In batch fermentation, sugar consumption and ethanol production obtained were 99.6% and 12.5% v/v after 27 h while in the ICR, 88.2% and 16.7% v/v were obtained with 6 h retention time. Nearly 5% ethanol production was achieved with high glucose concentration (150 g/l) at 6 h retention time. A yield of 38% was obtained with 150 g/l glucose. The yield was improved approximately 27% on ICR and a 24 h fermentation time was reduced to 7 h. The cell growth rate was based on the Monod rate equation. The kinetic constants (K_s and μ_m) of batch fermentation were 2.3 g/l and 0.35 g/l h, respectively. The maximum yield of biomass on substrate ($Y_{X/S}$) and the maximum yield of product on substrate ($Y_{P/S}$) in batch fermentations were 50.8% and 31.2% respectively. Productivity of the ICR were 1.3, 2.3, and 2.8 g/l h for 25, 35, 50 g/l of glucose concentration, respectively. The productivity of ethanol in batch fermentation with 50 g/l glucose was calculated as 0.29 g/l h. Maximum production of ethanol in ICR when compared to batch reactor has shown to increase approximately 10-fold. The performance of the two reactors was compared and a respective rate model was proposed. The present research has shown that high sugar concentration (150 g/l) in the ICR column was successfully converted to ethanol. The achieved results in ICR with high substrate concentration are promising for scale up operation. The proposed model can be used to design a lager scale ICR column for production of high ethanol concentration.

Keywords: Immobilized cell reactor (ICR); *Saccharomyces cerevisiae*; Ethanol fermentation; Encapsulated beads; Calcium alginate

1. Introduction

Due to the diminishing fossil fuel reserves, alternative energy sources need to be renewable, sustainable, efficient, cost-effective, convenient and safe (Chum and Overend, 2001). In the past decades, microbial ethanol production has been focused and considered as an alternative fuel for future since fossil fuel is depleted. Several microorganisms, including *Clostridium* sp., the well-known yeast ethanol producers, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are suitable candidates to produce ethanol (Flickinger and Drew, 1999; Gunasekaran and Raj, 2001).

Microorganisms under anaerobic growth conditions have the ability to utilize glucose by Embden–Mereyhof–Parnas pathway (Baily and Ollis, 1986). The phosphorylation of carbohydrates is carried out through the metabolic pathway; the end products are two moles of ethanol and carbon dioxide (Ingram et al., 1998).

During batch fermentation of *S. cerevisiae*, other influential parameters can adversely influence the specific rate of growth and inhibition can be caused either by product or substrate concentration. The viability of *S. cerevisiae* population, its specific rate of fermentation

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Nomenclature

S	substrate concentration, g/l	t	time, h
C	microorganisms cell concentration, g/l	A_t	activity of bacterium at time, t, U/g cell
K_s	Monod constant, g/l	A_0	initial activity of bacterium, U/g cell
μ	specific growth rate, g/l h	k_d	dissociation constant, h^{-1}
μ_m	maximum specific growth rate, g/l h		

and the sugar uptake rate are directly related to the desired medium condition (Holzberg et al., 1967). It has been reported by Nagodawithana and Steinkraus (1976), addition of ethanol in the cultured media was less toxic for *S. cerevisiae* than ethanol produced by cell bodies. This indicates that there are other metabolic byproducts, which can cause inhibition and may show impurity of ethanol produced in the fermentation system. Also the death rates were lower with addition of pure ethanol than the similar condition with the endogenously produced ethanol concentration (Nagodawithana and Steinkraus, 1976).

Use of biofilm reactors for ethanol production has been investigated to improve economics and the performance of fermentation processes (Vega et al., 1988). Immobilization of microbial cells for fermentation has been developed to eliminate inhibition caused by high concentration of substrate and product, also to enhance productivity and yield of ethanol production. Recent work on ethanol production in an immobilized cell reactor (ICR) showed that production of ethanol using *Z. mobilis* was doubled (Takamitsu et al., 1993). The immobilized recombinant *Z. mobilis* was also successfully used with high concentration of 12–15% sugar (Yamada et al., 2002).

The potential use of immobilized cells in fermentation processes for fuel production has been described previously. If intact microbial cells are directly immobilized, the removal of microorganisms from downstream product can be omitted and the loss of intracellular enzyme activity can be kept to a minimum level (Najafpour, 1990).

Recently, immobilized biomass activity has been given more attention, since it has been acknowledged to play a significant role in bioreactor performance (Gikas and Livingston, 1997). Frequently, immobilized cells are subjected to limitations in the supply of nutrients to the cells. Thus, because of the presence of heterogeneous materials such as immobilized cells, there is no convective flow inside the beads and the cells can receive nutrients only by diffusion (Riley et al., 1996). Immobilization of cells to a solid matrix is an alternative means of high biomass retention. The cells divide within and on the core of the matrix (Senthuran et al., 1997). Alginate is widely used in food, pharmaceutical, textile, and paper products. The uses of alginate utilized in these products are for thickening, stabilizing, gel and film forming. Sodium alginate is a linear polysaccharide, normally isolated from many strains of marine brown seaweed and algae. Thus the name alginate, the copolymer consists of two uronic acids or polyuronic acid. It composed of primarily of D-mannuronic acid (M) and L-glucuronic acid (G). Alginic acid can be either water-soluble or non-water soluble depending on the type of the associated salt. Interchanging of sodium ions with calcium ions in the solution may follow solidification of sodium alginate in calcium chloride solution. The sodium salt, other alkaline metals and ammonia are soluble in water, whereas the polyvalent cations salts, e.g., calcium, are not water-soluble, except the magnesium ions.

The purpose of this research was to obtain high ethanol production with high yield of productivity and to lower the high operating costs. The ICR column fermentor was used with *S. cerevisiae* using an entrapment technique utilizing alginate as a porous wall to retain the yeast cells. The effect of initial glucose concentration on the production of ethanol by *S. cerevisiae* was evaluated. The yield for large scale ethanol production was compared to the ethanol produced in batch fermentation using the same microorganism, *S. cerevisiae*.

2. Methods

2.1. Experimental reactor system

The ICR was a plug flow tubular column, constructed with a nominal diameter of 5 cm, ID of 4.6 cm, Plexiglas of 3 mm wall thickness and 85 cm length. The medium was fed to the ICR column from a feed tank located above the column. A variable-speed Master flex pump, model L/S easy load (Cole-Parmer, Vernon Hills, IL, USA) was used to transfer feed medium from a 20-liter polypropylene autoclaveable Nalgene carboy (Cole-Parmer, Vernon Hills, IL, USA), the carboy serving as reservoir. The effluent from the column was collected in a 20-liter polypropylene autoclaveable carboy serving as product reservoir. A flow breaker was installed between the column and feed pump, which prevented the growth of microorganism and contamination of feed line and feed tank. The samples from the ICR column were taken

from the inlet and outlet compartments of the column. A 16 h culture was harvested at exponential growth phase and mixed with 2% sodium alginate. The slurry of yeast culture was converted to droplet form while it was dripped into a 6% bath of calcium chloride using a 50 ml syringe. Once the slurry was added to the bath, beads of calcium alginate with entrapped cells were formed (Najafpour, 1990). The bed consisted of uniformly packed 5 mm beads. The solidified beads were transferred to the column. About 70% of the column was packed. The extra space was counted for bed expansion by the fresh media. The void volume was measured by volume of distilled water pumped through the bed. The packed ICR column was used in continuous mode for the duration of fermentation. The fresh feed was pumped in an upflow manner; while sugar and ethanol concentration was monitored during the course of continuous fermentation. The working volume of ICR after random packing was 740 ml. The bed volume was about 660 ml. The experimental set-up of ICR is shown in Fig. 1. There was no evidence of cell leakage from the beads to the surrounding media, the matrix was permeable to substrate and product, the cell growth and glucose conversion were monitored in the ICR. Overgrowth of beads after a few days of operation was controlled. Carbon dioxide was purged to eliminate overgrowth of beads. The major overgrowth occurred at the entrance region, about the first 30 cm length of the column where the sugar concentration was very high. High sugar concentration of 25, 35, 50, and 150 g/l were used.

The microbial overgrowth was controlled with carbon dioxide passed through the bed. There was a maximum 30% increase in the beads diameter at the lower part of column, where the glucose concentration was dominating in this region. The void volume was measured by passing sterilized water. In addition to carbon source, the feeding media consisting of 1 g/l yeast extract was pumped from the bottom of the reactor, while the flow rate was constant for a minimum duration of 24 h.

A seed culture of *S. cerevisiae* ATCC 24860 (American Type Culture Collection, Manassas, VA, USA) was grown in a media of 5 g of glucose, and 0.5 g of yeast extract, respectively, and 1.5 g of KH_2PO_4 and 2.25 g of Na_2PO_4 phosphate buffer up to a total volume of distilled water, 500 ml. The media was sterilized at 121 °C for 15 min. The stock culture of the microorganisms was

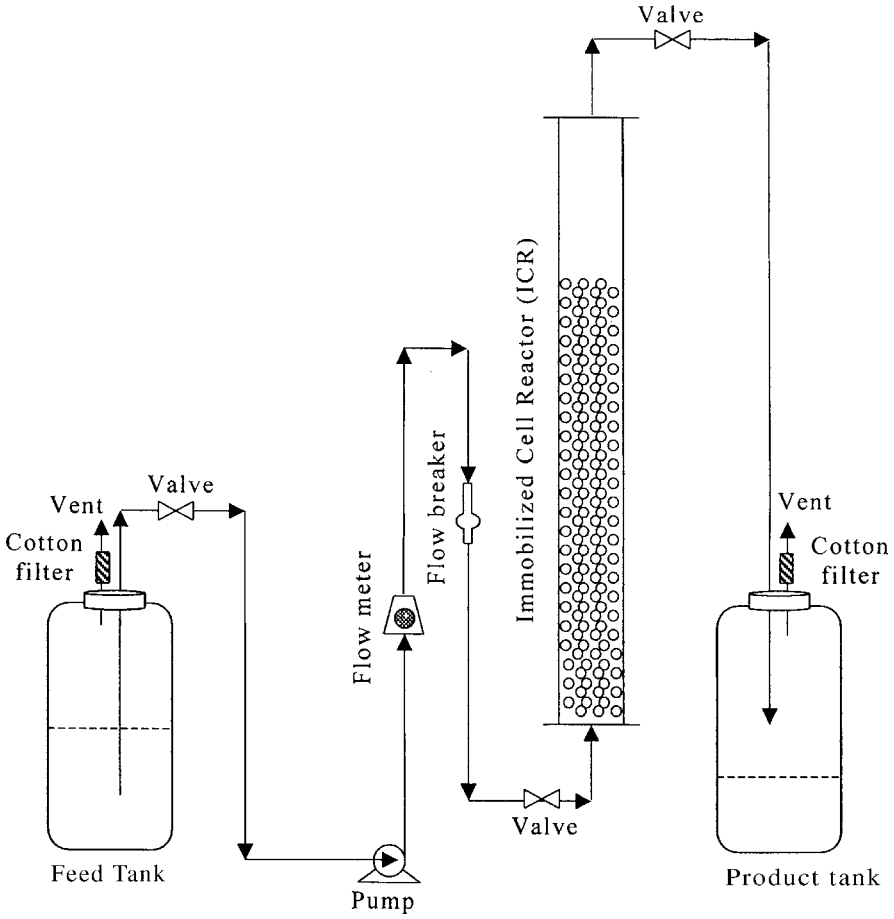

Fig. 1. Schematic diagram of ICR experimental setup.

transferred to the broth media for preparation of seed culture.

Sodium alginate (Fisher Scientific, Manchester, UK) was prepared by dissolving 10 g of powder form in 500 ml of distilled water. A separate solution of 120 g of calcium chloride was made in 2 l distilled water. Sodium alginate and calcium chloride solution were autoclaved at 121 °C for 15 min. The sterilized sodium alginate solution and the high cell density of the grown seed culture were thoroughly mixed. Beads were prepared by droplet from a pipette about 5 mm diameter in a sterilized calcium chloride solution. The cell density of seed culture for bead preparation was 3.1 g/l. The wet and dry weights of a 16 h incubated beads sample were 3.28 and 0.5 g, respectively. The moisture content of the beads was 85%.

2.2. Glucose concentration determination

To determine the concentration of inlet and outlet glucose in the ICR, a reducing chemical reagent, 3,5-dinitrosalicylic acid 98% solution (DNS) was used. The DNS solution was prepared by dissolving 10 g of 3,5-dinitrosalicylic acid in 2 M sodium hydroxide solution. A separate solution of 300 g sodium potassium tartrate solution was prepared in 300 ml of distilled water. The hot alkaline 3,5-dinitrosalicylate solution was added to sodium potassium tartrate solution. The final volume of DNS solution was made up to 1 l with distilled water. The calibration curve was prepared with 2 g/l glucose solution (Summers, 1924; Miller, 1959).

2.3. Ethanol detection

Ethanol production in the fermentation process was detected with gas chromatography, HP 5890 series II, (Hewlett-Packard, Avondale, PA, USA) equipped with flame ionization detector (FID) and GC column Porapak QS (Alltech Associates Inc., Deerfield, IL, USA) 100/120 mesh. The oven and detector temperature were 175 and 185 °C, respectively. Nitrogen gas was used as a carrier gas. Isopropanol was used as an internal standard.

2.4. Yeast cell dry weight and optical density

In batch fermentation, approximately 2 ml sample was harvested every 2 h. The absorbance of each sample during batch fermentation was measured at 620 nm using spectrophotometer, Cecil 1000 series (Cecil Instruments, Cambridge, England). The cell dry weight was obtained using a calibration curve. The cell dry weight was proportional to cell turbidity and absorbance at 620 nm. The cell concentration (dry weight) of 2.1 g/l was obtained from the 24 h culture broth, the free cell samples with absorbance of 1.6 at 620 nm.

2.5. Electronic microscopic scanning of immobilized cells

For electronic microscopic scanning (SEM) micrographs, samples were taken from fresh beads and 72 h beads from the ICR column. The samples were dipped into liquid nitrogen for 10 min, then freeze-dried for 7 h in the Freeze Drier, EMITECH, model IK750, Cambridge, UK. The sample was fixed on the aluminium stub and coated with gold palladium by Polaron machine model SD515, EMITECH, Cambridge, UK, at 20 nm coating thickness. Finally the sample was examined under SEM using Stereoscan model S360 brand SEM-Leica Cambridge, Cambridge, UK.

2.6. Statistical analysis

The size of beads was uniform and consistent, the mean size of beads with 3% alginate and based on measurement of 20 samples, and the mean value for the beads diameter was 4.85 mm, with standard deviation of 0.3 and the calculated variance was 0.1. The standard deviation was less than 5%. The data for batch fermentation experiment with 50 g/l glucose presented in Figs. 4–6 for batch was replicated three times. The data for ICR with 25, 35, 50 and 150 g/l glucose that were conducted at wide range of flow rates are shown in Figs. 7–11, were repeated in additional runs. The standard deviations of collected data for the batch experiments were approximately 5% and the standard deviation for the ICR experimental data with sugar concentration of 50 g/l was approximately 10%. The statistical analysis of the obtained data was carried out in a spreadsheet, Microsoft, Excel 2000. The error analysis for ICR's data with substrate concentration of 150 g/l was slightly higher but it was in the range of 10–12%.

3. Results and discussion

3.1. Evaluation of immobilized cells

In preparation of immobilized cells, 1.5%, 2%, 3% and 6% of alginate were used. By pressing them manually, the hardness and rigidity of beads were tested. The physical criteria of the prepared beads are summarized in Table 1. The suitable alginate concentration based on activity of the beads for ethanol production was 2%. The weight percentage of alginate was related to substrate and product penetration into the beads and return to bulk of fluid. Beads with low alginate (1.5%) were too soft and easily breakable. The soft beads were pressed once it was loaded in ICR column, therefore unable to be used successfully, also the soft beads faced problems

Table 1
Physical properties and appearance of *S. cerevisiae* beads

Alginate (wt.%)	1.5	2	3	6
Beads diameter (mm)	5	4.9–5	4.8–4.9	4.5
Growth, diameter expansion after 72 h	50–60%	25–30%	20–25%	No expansion
Diffusion problem	Nil	Nil	Nil	May exists
Cell activity	Active	Fully active	Fully active	Partially active
Physical appearance	Easy to break	Flexible, hard enough to stand	Flexible and hard	Very hard
Stability	Not stable	Good stability	Stable and rigid	Very rigid

such as overgrowth and expansion of beads diameter when grown in sugar solution. The high alginate beads (6%) were very hard and almost unbreakable by pressing manually. It was very rigid therefore diffusion was the most probable cause since ethanol production declined. The 2% alginate was used since the beads were strong enough to hold the weight of packing in the ICR column. The packed beads were used in ICR column for the duration of 10 days. The prepared beads were refrigerated for two weeks and activated in sugar solution. The storage condition and ICR column were non-sterile; therefore, there were chances of contamination in the packing of ICR and in activation process.

A series of electronic micrographs were taken from the fresh and 72 h immobilized beads. The outer and inner surfaces of the beads are shown in Figs. 2 and 3, respectively. These micrographs were used as a comparative indicator for yeast growth on the surface of the solid support (2% calcium alginate). There was no apparent leakage of cells from the beads into the bulk of fluid. It was also apparent that by 72 h in ICR, the yeast grew on the outer surface. Therefore the active sites were potentially available for ethanol production without diffusion problems. The outer surface of fresh and 72 h immobilized cell beads at magnifications of 300 and 2000 are shown in Fig. 2. A visual comparison between fresh and 72 h beads indicates that the cells were present on the surface, with a few contaminants from a bacillus type organism as shown in Fig. 2d. This may have occurred during transferring stage.

The inner surfaces of the beads before and after use were compared. The cells were initially trapped inside the beads and after 72 h, the cells apparently migrated from the inner side to the surface. The micrographs of inner side of beads before and after use, in magnification of 300 and 2000 are shown in Fig. 3. After 72 h the cells appeared to have formed new colonies on the surface of alginate layer. By contrast the surfaces were completely covered with colonies after 72 h of ethanol production in ICR.

3.2. Batch fermentation

Ethanol fermentation in batch experiments was carried out in triplicates with 50 g/l of glucose solution as sole carbon source for *S. cerevisiae*. The purpose of batch experiment was to compare the amount of glucose concentration and ethanol production in batch fermentation and immobilized cells reactor (ICR). The concentration of glucose was gradually decreased while the cell density and ethanol production were increased for a duration of 27 h, shown in Fig. 4. There was a lag phase of 4–6 h; the glucose consumption was low at this stage. Subsequently the concentration profile markably decreased during batch fermentation after 8–16 h. Since the cell density was initially low the trend of sugar consumption was also low. The resulting cell growth curve from batch experiment was a typical sigmoidal shape (S-shape). The maximum cell density of *S. cerevisiae* in batch fermentation was 13.7 g/l with 50 g/l glucose concentration. The average yield of biomass and product on substrates ($Y_{X/S}$ and $Y_{P/S}$) were 33% and 32% respectively. The rate of ethanol productivity for 24 h was 1.4 g/l h.

Langmuir–Hanes plot based on Monod rate equation is presented in Fig. 5. The Monod kinetic model can be used for microbial cell biocatalyst and is described as follows:

$$\frac{S}{\mu} = \frac{K_s}{\mu_m} + \frac{S}{\mu_m}$$

The terms K_s and μ_m are defined as the Monod constant and maximum specific growth rate, respectively. The data generated in this study was linearly fitted with the model, as a function of concentration produced during the exponential phase versus time (Fig. 5). From the plot, the maximum specific growth rate and Monod constant were determined to be 0.35 g/l h and 2.23 g/l, respectively. The large value of the Monod constant may suggest that at high concentration of substrate, more influence on the [CS] dissociation occurred than [CS] formation.

3.3. Relative activity

The economics of an immobilized cells process depend on the lifetime of the bacterium and a continued level of clean product delivered by the fixed cells. It is important to eliminate the free cells from the downstream product without the use of any units such as

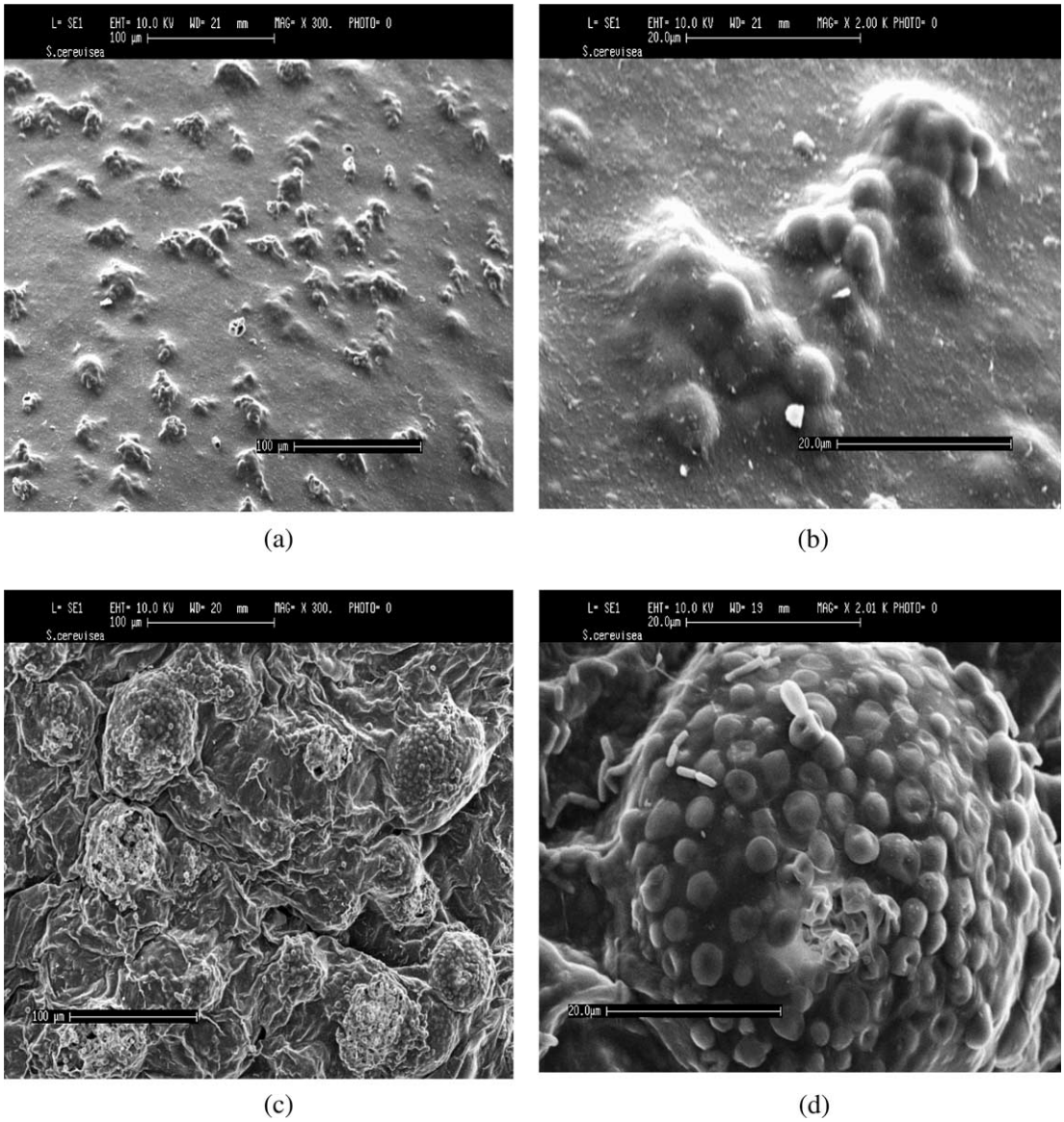

Fig. 2. Electronic photomicroscope of the outer surface of immobilized *S. cerevisiae* beads: (a) outer surface of the fresh beads with magnification of 300 μm; (b) outer surface of the fresh beads with magnification of 2000 μm; (c) outer surface of the used beads after 72 h with magnification of 300 μm; (d) outer surface of the used beads after 72 h with magnification of 2000 μm.

centrifuge or filtration processes. Since the cells are retained in the ICR, the activity of intracellular enzymes may play a major role. It is assumed that the deactivation of the enzyme at constant temperature following a first-order equation is shown below (Yuan et al., 2002):

$$A_t = A_0 \exp(-k_d t)$$

where A_t and A_0 are activities of enzymes at time, t and initial time zero, respectively. Also k_d is the dissociation constant.

The plot of relative activity versus time in batch fermentation with free cells is shown in Fig. 6. The value of k_d was 0.36 h^{-1} (Fig. 6). According to the first-order dissociation rate constant, the halflife of the *S. cerevisiae* ($\tau_d = \ln 2/k_d$) in batch fermentation suspended cells with 50 g/l of glucose was calculated to be 1.95 h. The free cells were apparently completely deactivated after 60 h in batch operation. The data indicate that the free cells were deactivated rapidly compared to immobilized cells when used in a continuous mode for more than seven days.

3.4. Reactor set-up

The volume of reactor without beads was 1.4 l. The column was loaded with the solidified uniform beads of *S. cerevisiae*. The void volume of the reactor was 660 ml when it was packed with immobilized beads. The growth

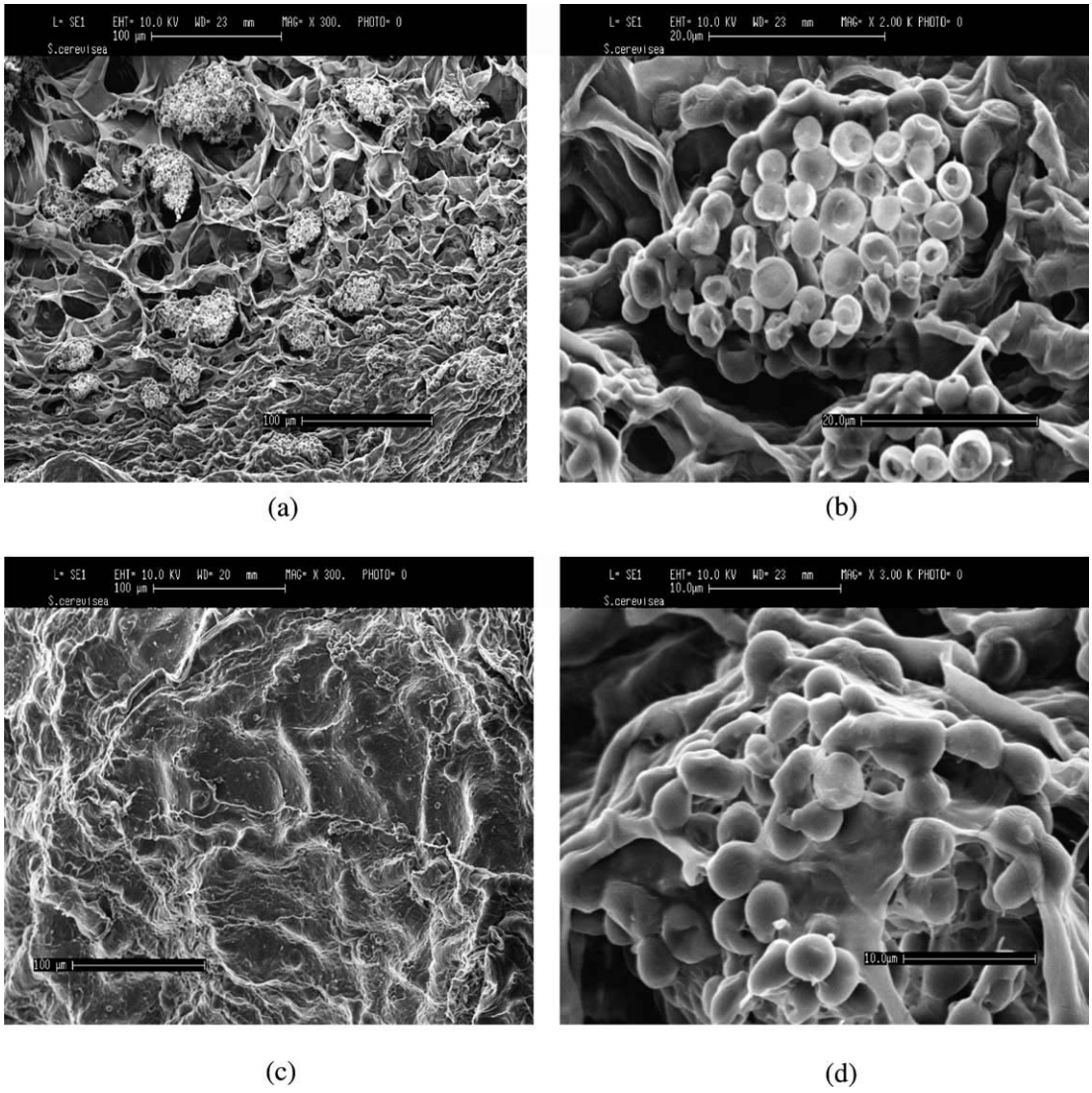

Fig. 3. Electronic photomicroscope of the inner surface of immobilized *S. cerevisiae* beads: (a) inner surface of the fresh beads with magnification of 300 μm; (b) inner surface of the fresh beads with magnification of 2000 μm; (c) inner surface of the used beads after 72 h with magnification of 300 μm; (d) inner surface of the used beads after 72 h with magnification of 2000 μm.

of beads with different proportions of column packing is shown in Fig. 7. A fresh feed of 10 g/l glucose solution was pumped from the bottom of the reactor. The optimum amount of packing obtained, was in the range of 65–70% of the reactor volume. The trend of the collected data resembles the growth curve of yeast in suspended cell culture. The diameters of beads were increased with the increase of *S. cerevisiae* cell density, which indicates that *S. cerevisiae* had grown within the solid support. Under these circumstances substrate would be easily consumed at the solid surface coated with immobilized yeast cells. Thus, it was expected that substrate concentration at the surface would be less than the concentration of substrate in the bulk fluid. The main objective was to determine whether substrate penetration into beads occurred. Since the matrix of beads was quite porous, it was assumed that the concentration gradient was the major force that influenced the mass transfer process in immobilized cells of *S. cerevisiae*. Therefore, the immobilized-yeast system was preferred compared to free cells in the solution. Moreover, economical aspects of immobilized yeast must be considered as it eliminates the need for the extra unit for free cell removal from product stream. The other advantages of immobilization system are that, the substrate may not be accumulated on the surface of beads and there was no evidence of cell leakage from the beads.

3.5. Effect of high concentration of substrates on immobilized cells

The fermentation was performed with various sugar concentration to increase product concentrations. The initial sugar concentrations were 25, 35 and 50 g/l. The

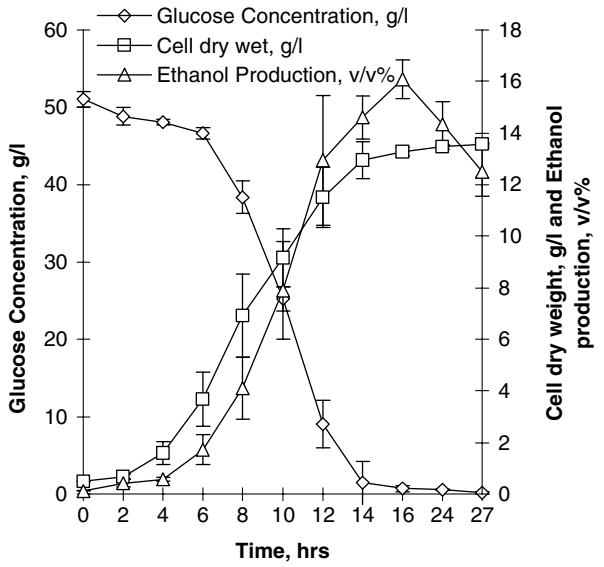

Fig. 4. Glucose concentration, cell density and production of ethanol in batch fermentation with initial concentration of 50 g/l glucose versus time.

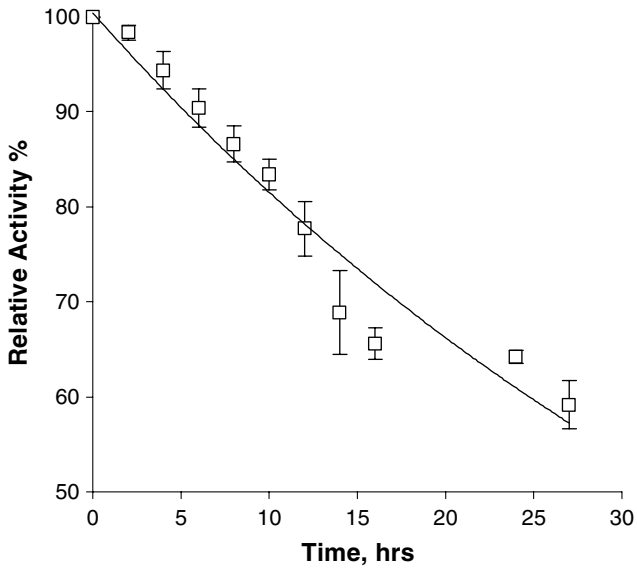

Fig. 6. Relative activities of *S. cerevisiae* in batch fermentation.

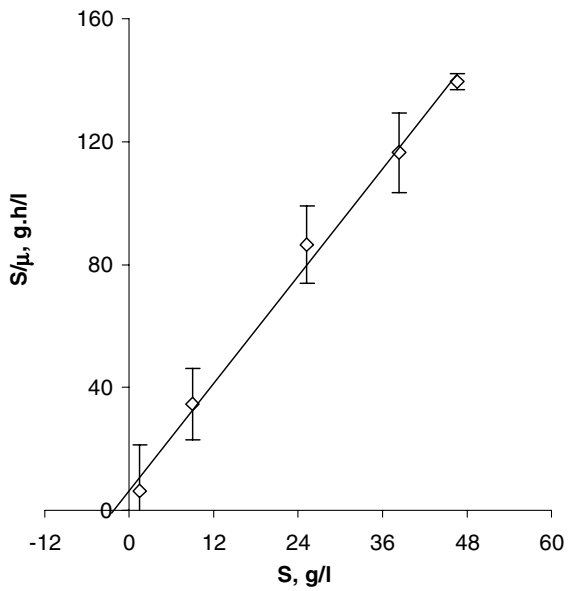

Fig. 5. Kinetic model for batch fermentation, Langmuir–Hanes plot.

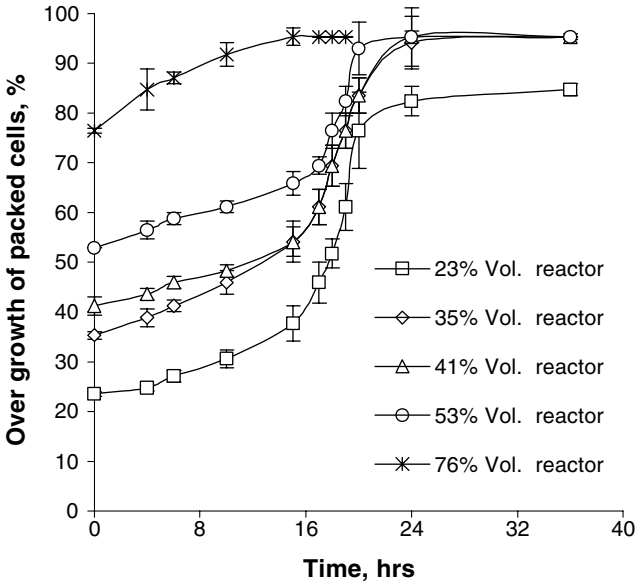

Fig. 7. Percentage, growth of immobilized cell with various initial beads loading.

sugar consumption profile in the ICR is presented in Fig. 8. The sugar consumption trends of various glucose concentrations were similar, with a sharp reduction of substrate occurring within the first 3 h. A range of 55–75% of the sugars was reduced within 3 h of retention time. A 6 h of retention time indicated that this was the most suitable time to achieve high sugar consumption. A longer retention time was required for higher sugar concentration of up to 150 g/l in the ICR column (7 h) shown in Fig. 11. The amount of cell immobilized with calcium alginate was determined by the cell dry weight of immobilized cells and the assumption that 98% of the beads were considered to be loaded with active cells. The beads measurements were carried out prior to loading in the ICR column.

Conversion of glucose versus dilution rate, used in the continuous fermentation process with immobilized *S. cerevisiae* is shown in Fig. 9. As the sugar concentration increased the conversion may have decreased. At very high sugar concentration, the conversion of sugar was decreased. The maximum sugar conversion at 6 h retention time was obtained from 74.3%, 80.5%, and 88.2% for 25, 35, 50 g/l glucose concentrations, respec-

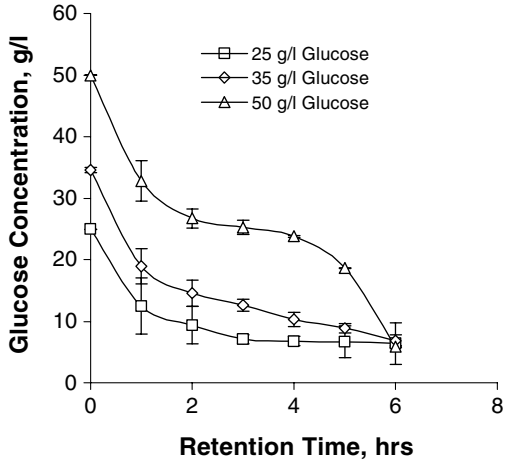

Fig. 8. Consumption of glucose in immobilization cell column.

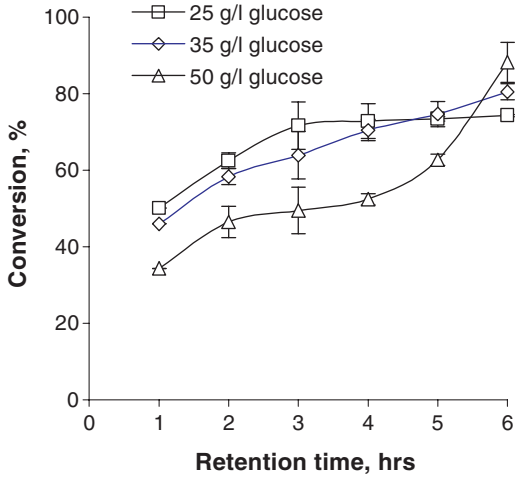

Fig. 9. Conversion versus retention time in immobilized cell column.

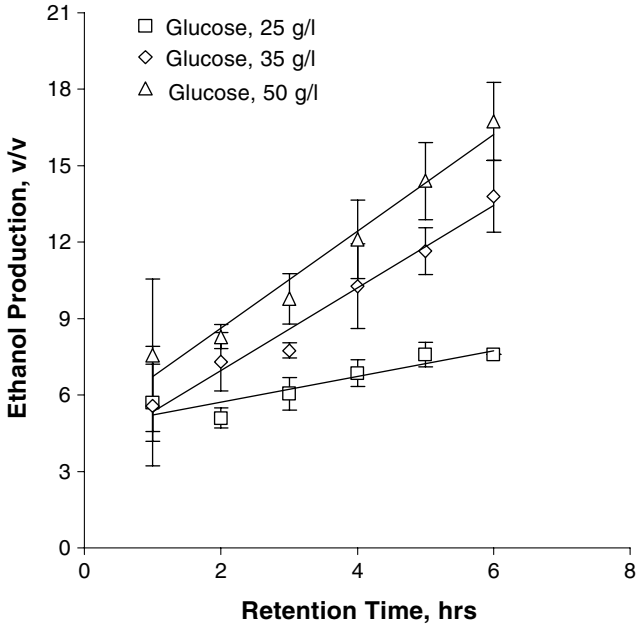

Fig. 10. Ethanol production versus retention time in immobilized cell column.

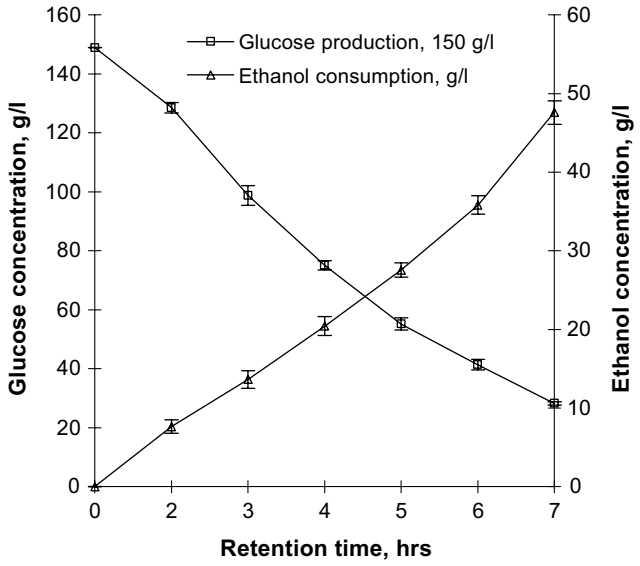

Fig. 11. Glucose concentration and ethanol production versus retention time in ICR with initial substrate concentration of 150 g/l glucose.

tively. At high concentration of sugar, conversion was highly influenced by dilution rate. The conversion decreased to less than 10% once the dilution rate approached wash out. The sugar conversion at low sugar concentration appeared to be much less sensitive to feed dilution rate. The conversion for 25 g/l glucose was constant at 74% for a retention time of greater than 3 h (Fig. 9). The trend of the data with 50 g/l sugar showed that conversion increased with respect to retention time. At a higher sugar concentration, it required a longer retention time to achieve higher conversion.

The reactor productivity was obtained by dividing final ethanol concentration with respect to sugar concentration at a fixed retention time. It was found that the rates of 1.3, 2.3, and 2.8 g/l h for 25, 35, 50 g/l of glucose concentrations were optimal. The ethanol productivities with various substrate concentrations were linearly dependent on retention time (Fig. 10). The proportionality factor may have increased while the substrate concentration was increased. As the sugar concentration was doubled, the slope of the line for ethanol productivity with 50 g/l sugar was increased 5-fold. These results indicated that the ICR column has high capacity to produce very high concentrations of ethanol. The final ethanol concentrations with 25 and 50 g/l of glucose were 7.6% and 16.73% v/v, respectively.

High glucose concentration 150 g/l was used in continuous fermentation using immobilized *S. cerevisiae*; and the obtained data sugar consumption and ethanol production with respect to retention time are shown in Fig. 11. As the retention time was gradually increased the glucose concentration dropped, while the ethanol concentration profile yielded an increasing trend. The maximum ethanol concentration of 47 g/l was obtained with retention time of 7 h. The yield of ethanol production was approximately 38% compared with batch data where only 8% improvement was achieved.

4. Conclusions

Continuous ethanol production in an ICR was successfully carried out with high sugar concentration. In batch fermentation, when the concentration of glucose was 50 g/l, substantial substrate inhibition strongly occurred. The advantage of immobilized cells reactor was that the substrate inhibition of substrate and product were not apparent even with 150 g/l glucose solution in the fresh feed. The ICR system exhibited a higher yield of ethanol production (38%) compared to the batch system. The ICR column gave a high performance to processing feed with concentrated sugar. Most of ICR experimental runs resulted in glucose consumption of 82–85%. The results indicated that the immobilization of *S. cerevisiae* possesses the capacity not only to utilize high concentration of sugar but also to yield higher ethanol productivities during the course of continuous fermentation. The ethanol production in ICR column was increased by 5-fold, as the glucose concentration was doubled from 25 to 50 g/l. It is clear from the new findings in the present investigation that there would be potential application for utilizing concentrated feed with higher rate of ethanol production as the cell was loaded into the gel matrices of sodium alginate shown in the SEM micrographs had eliminated the free cells from the ethanol product stream.

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