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A New Spin Filter for High Density Culture and Ethanol Production by *Saccharomyces cerevisiae*

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Abstract A new spin filter consisting of 50 μm (nominal pore size) depth filters rolled on a stainless steel grid was developed, using *Saccharomyces cerevisiae* as a model suspension cell to evaluate the spin filter performance. In a 1.8-l fermentor with a rotation speed of 300 rpm and perfusion rate of 4 ml/min, a cell concentration of 49 g/l and ethanol concentration of 45 g/l from 100 g/l glucose could be obtained in a perfusion culture. The major mechanisms for cell separation used by the large-pore spin filter appeared to be centrifugal force and pivotal movement of the cells in the spin filter.

Key words: High density culture, spin filter, centrifugal force, pivotal movement, yeast

Perfusion culture of microorganisms is an efficient technique for obtaining high productivity of target biomolecules. The productivity of biological products generally increases with an increase in the cell concentration within the bioreactor. Various perfusion culture systems, in which the cells are retained in a bioreactor while spent medium is constantly replaced with fresh medium, have been used to elevate productivity. Perfusion systems for a high density culture can be divided into cell immobilization systems and cell separation systems. Depending on the position of the cell separation device, cell separation systems can be divided into internal- and external-cell separation systems. In internal-cell separation systems, the cell separation is achieved by size exclusion [1, 2, 8, 12, 13]. The advantages of an internal system include the avoidance of cell pumping, absence of uncontrolled volume creation, and easy scale-up [6]. However, this type of system has a critical problem of fouling, which requires a back-flushing operation and results in an inevitable variable flow of out-coming fluids [9].

A spin filter as an internal-cell separation system has been widely used for high density cultures of animal cells [3-7, 11, 15-17]. A spin filter is a rotating cylindrical filter mounted on the central shaft in a stirred tank reactor. In conventional spin filters, the cells are retained in the bioreactor, mainly by filtration-based cell separation using a rotating stainless steel screen. However, a spin filter has the inherent drawback of filter fouling by cell clogging on the surface of the filter screens, as is the case in other internal-cell separation systems [4, 5].

Accordingly, we developed a new spin filter to cope with the filter fouling problem. Polypropylene filters with a nominal pore size of 50 μm were used as the spin filter. The assembly of the spin filter was completed by rolling the filter material on a stainless steel grid support. The effects of the operating conditions - rotation speed of the filter and the perfusion rate of the new spin filter - on cell retention were investigated in the perfusion culture of yeast cells. The possible cell separation mechanisms used by the new spin filter are suggested.

Materials and Methods

Spin Filter

A large open stainless steel grid with a pore size of 0.5 cm was used as the support for the spin filter. The grid of the support was fabricated in a cylindrical shape and welded to a solid stainless steel bottom. The diameter and length of the support were 6.0 cm and 10.0 cm, respectively. The spin filter assembly was completed by rolling the filter material on the support. All parts of the spin filter assembly

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were made of 316 stainless steel. The thickness of the rolled filter was 6.0 cm. The filter material used in the current study was a polypropylene filter with a nominal pore size of 50 μm (Millipore Polygard-CR Cartridges, Millipore, Bedford, U.S.A.). The pore size of the filter was over 5 to 10 times larger than the yeast cells. The rolled filter matrix was fixed on the frame using a sterilizable silicon adhesive (Mega Copper Versachem Corporation, West Palm Beach, U.S.A.). The shape of the completed spin filter assembly was like a depth filter cartridge used in pre-filtration. The assembly was placed on the shaft to allow 3 cm of the filter to extend above the liquid level of the fermentor. A schematic diagram of the spin filter system is shown in Fig. 1.

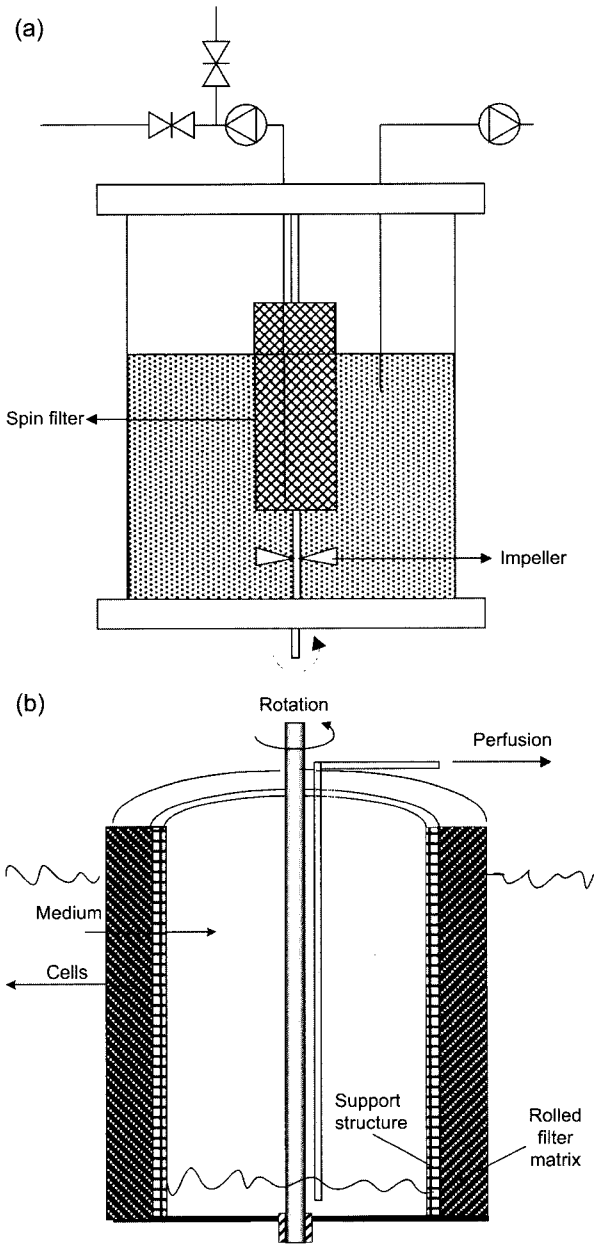

Fig. 1. (a) Schematic diagram of the experimental setup for the new spin filter operation. (b) Schematic diagram of the spin filter assembly.

Microorganism and Media

The *Saccharomyces cerevisiae* K35 was obtained from Bioprocess Engineering Lab. (Department of Chemical Engineering, Korea University, Seoul, Korea) and used in the present study as a model cell suspension to evaluate the performance of the spin filter in a perfusion culture. The growth medium for the seed and batch cultures contained 20 g/l yeast extract (Difco, Detroit, U.S.A.), 10 g/l bacto peptone (Difco, Detroit, U.S.A.), and 20 g/l glucose (Samchun Pure Chemical Industries Ltd., Bucheon, Korea). The production medium for the perfusion culture contained 100 g/l glucose, 10 g/l yeast extract, 5 g/l $(NH_4)_2SO_4$, 0.1 g/l $MgSO_4 \cdot 7H_2O$, and 1 g/l $Na_2HPO_4 \cdot 12H_2O$. The strain was stored at −70°C in a 30% (w/w) glycerol solution. To obtain inoculum, the frozen cells were transferred to YPD agar plates, which were stored at 4°C for a maximum of 1 month.

Performance of Spin Filter

The yeast cell slurry for the performance test of the new spin filter was obtained from the broth of batch cultures. Various operating conditions were examined by recycling the cells exiting from the inside of the filter. For cell recycling and sampling, a peristaltic pump (Masterflex 7518-00, Cole-Parmer Instrument Co., Barrington, U.S.A.) was used. The fermentor (Korea Fermentor Co., Incheon, Korea) used to evaluate the performance of the spin filter had a 1.8-l working volume. During the cell recycling, 4 ml of the culture broth was sampled from inside and outside of the spin filter, respectively, at the time passing 30 min after changing an operating condition. By measuring the optical density of the samples at 600 nm, the ratio of the perfusate/permeate cell density as the cell retention efficiency was calculated.

Perfusion Culture with New Spin Filter

The fermentor equipped with the spin filter was inoculated with 150 ml of a preculture from 500-ml shake flasks and started as a batch culture. The continuous operation was started in the later part of the exponential growth phase. For feeding the medium and sampling, a peristaltic pump (Masterflex 7518-00, Cole-Parmer Instrument Co., Barrington, U.S.A.) was used. Operating conditions determined by the experiments evaluating the performance of the spin filter were used in the perfusion culture. The rotation speed of the spin filter was 300 rpm and the perfusion rate was 4 ml/min. The temperature of the culture broth was controlled at 30°C. Four milliliters of the culture broth was sampled from the inside and outside of the spin filter, respectively. The dry cell weight was determined by washing the cells

in distilled water and then drying them at 80°C for 24 h. The ethanol concentration in the supernatant of the perfusate was determined by gas chromatography (YongLin M600D, Anyang, Korea) with a flame ionization detector and a Porapak Q80/100 column, using 1% (v/v) isopropanol as the internal standard.

RESULTS AND DISCUSSION

Effects of Operating Conditions on Cell Separation

The cell separation was influenced by both the rotation speed of the spin filter and the perfusion rate. As shown in Fig. 2, the cell concentration of the perfusate was the lowest at 300 rpm, which indicated that the cells were well separated at intermediate rotation speeds. The optimum cell retention within the test range of rotation speed was obtained at 300 rpm. At the rotation speed of 300 rpm, the ratio of the perfusate/retentate cell density was 0.45, which indicated that 45% of cells in the broth of the fermentor went outside of the spin filter study through the filter matrix. To the best of our knowledge, this is the first study to show that cell retention efficiency is affected by the rotation speed of the spin filter. In previous studies, the rotation of the screen has been found not to increase the cell retention. The direction of the cell movement was not found to be correlated with the rotation speed of the spin filters [10, 14]. The conventional spin filters not only exhibited cell retention in the stationary state, but also showed better retention than in the rotation of the filter. The rotation was necessary to prevent clogging of the filter

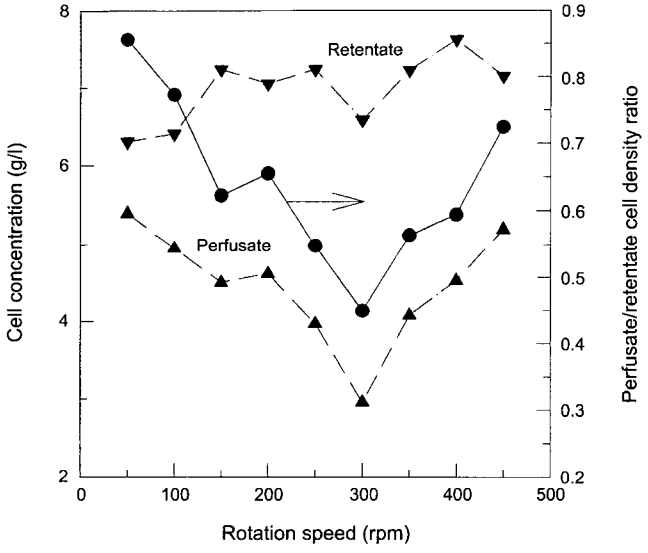

Fig. 2. Effect of rotation speed of the spin filter on cell retention efficiency during recycling of yeast cells.
The perfusion rate was 4 ml/min. ▲ and ▼, cell concentrations; ●, perfusate/retentate cell density ratio.

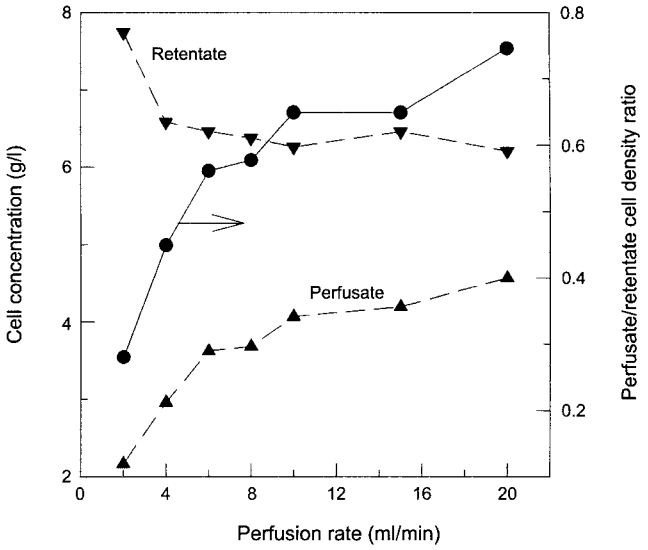

Fig. 3. Effect of perfusion rate on cell retention efficiency during recycling of yeast cells.
The rotation speed of the spin filter was 300 rpm. ▲ and ▼, cell concentrations; ●, perfusate/retentate cell density raio.

screen. Previous results indicate that the centrifugal force does not contribute to the cell retention in conventional spin filters [10, 14]. In the surface filters used for the conventional spin filters, there is a certain amount of mixing across the screen, and due to the relative motion of the fluid and the screen surface, this mixing breaks down the centrifugal field of the surface on the spin filter. It was presumed that the centrifugal force generated by rotating the filter contributed to the cell separation in the new spin filter design. Assuming that the fluid within the matrix of the filter clung to the matrix, thereby not being disturbed by the bulk mixing, Stoke's law can be applied to predict the direction of cell motion. As expected from the force balance of the cell motion, the higher the perfusion rate, the higher was the perfusate/retentate cell density ratio (Fig. 3). The best cell retention was obtained at a 2 ml/min perfusion rate. However, this perfusion rate is too low to be used for the operation of a perfusion culture of yeast. Thereafter, when considering productivity and cell retention efficiency, a perfusion rate of 4 ml/min was chosen as the operating condition for the perfusion culture.

Perfusion Culture of Yeast Cells

In the perfusion culture of yeast, the cell concentration of the perfusate from the new spin filter was lower than 7 g/l throughout the perfusion culture (Fig. 4). The cell concentration inside the fermentor increased linearly with time after 32 h. This result showed that the yeast cells were effectively rejected from the filter matrix. At around 70 h, the perfusion rate decreased to 2.4 ml/min. The operation of the fermentor was stopped at 72 h because of filter fouling caused by the

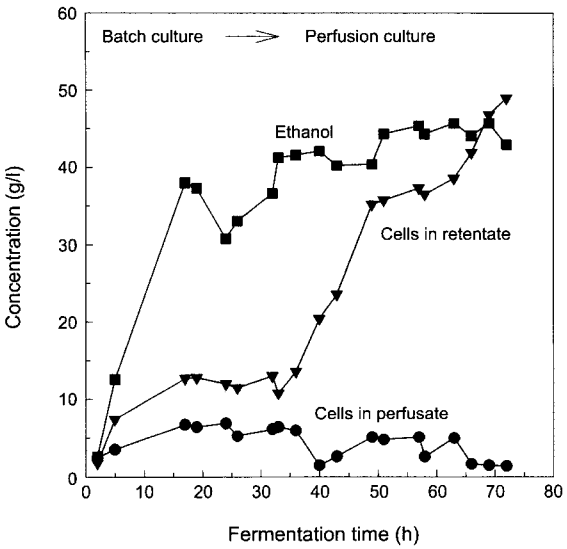

Fig. 4. Cell retention during perfusion culture of yeast cells at rotation speed of 300 rpm and perfusion rate of 4 ml/min.

high cell density. Although the decreased perfusion rate due to filter fouling was easily restored to the initial perfusion rate by raising the rotation speed of the filter, a bleeding operation was still required for a stable and long-term operation. A further study on the long-term operation of a perfusion culture including bleeding is currently in progress. The average ethanol concentration of the perfusate was 44. 9 g/l, which corresponded to 88% of the theoretical ethanol yield. At 72 h, the cell concentration reached 49 g/l, which was about 5-times higher than that attained in a conventional continuous culture of yeast without a cell separation device. Although the cell concentration by the new spin filter system was lower than those by the other filtration-based cell retention culture systems, the new system has an advantage in that it does not require a back-flushing operation which is indispensable for filtration-based cell separation systems. As spin filter systems are easy to scale-up, the system developed in the current study would appear to be more favorable than other filtration-based cell separation systems. However, the drawback of the proposed system is that it has a limited filter rotation speed and perfusion rate. To obtain a higher centrifugal force and fulfill the oxygen demand of an aerobic culture, the rotation speed must be higher. In this case, perfusion may be impossible because of the vortex developed in the inside of the spin filter unit. As such, the rotation speed cannot be freely increased to the desired value. One of the disadvantages of the proposed spin filter is that the perfusion rate is relatively low. Since the driving force for the movement of the culture broth is acquired by the hydraulic head, this is not enough to support high perfusion rates. However, these operating limits can be overcome by using the perfusion method applied in the Rotorfermentor [10]. Here, the cell-free perfusate is removed by the driving force generated from the difference between the pressure of the inside and outside of the rotating microporous membrane in the Rotorfermentor. If this method is applied to remove the cell-free perfusate, the perfusate rate in continuous mode of operation can be increased to the ranges used in the general operation of a cell retention culture. Conclusively, incorporation of a depth filter as the filter material in a cell retention culture could produce a stable operation without the need for back-flushing. Accordingly, the new spin filter developed in the current study could be widely used in high density cultures of suspension cells that are not self-aggregated and do not stick to the porous mass of the depth filter.

Cell Retention Mechanisms

Although many mechanisms might have contributed to the cell separation in the new spin filter system, it would appear that cell rejection was achieved by three combined effects: collision, centrifugal force, and pivotal movement of the cells. The principal retention mechanisms in the stationary depth filter are random adsorption and mechanical entrapment throughout the depth of the filter matrix. As such, the first contribution was capturing and some rejection based on the collision of the cells with the filter material. Even though the nominal pore size of the tested filter was over 5 to 10 times larger than the cell size, the cells were rejected toward the outside of the filter. Assuming that non-hydrodynamic attraction between the cells, the packing materials, and the axial direction (perpendicular to radial direction) force acting on the cell motion were negligible, it was highly likely that cells collided with the filter matrix and some of the cells were rejected. Once the cells made contact with the surfaces of the filter matrix, some of the cells remained attached to those surfaces or stayed in the spaces within the filter matrix, in the absence of outward direction force from the filter.

The second contribution was the lift drag due to the centrifugal force generated by rotating the filter. Rotating the filter can be viewed as producing a cross flow, while the perfusion can be viewed as permeation [16]. When the spin filter was rotated at a higher rotation speed, there was an increased cell retention efficiency within the fermentor. From this result, it is suggested that cells moved outward due to the centrifugal force. This can be explained by the fact that the undisturbed centrifugal field in the matrix of the spin filter was protected against fluid turbulence (or bulk mixing by an agitator). However, the outer part of the centrifugal field in the filter matrix was disturbed by fluid turbulence at rotation speeds higher than 300 rpm, indicating that the centrifugal force was more effective under laminar flow of the culture broth.

The third contribution seemed to be the pivotal movement of the cells in the porous mass of the filter. Because the

nominal pore size of the filter was quite large (50 μm) compared to the cell size, the liquid and cell movement became perpendicular to the radial direction, when the filter was rotated. This movement caused the cells to move toward the outside of the filter. The pivotal movement allowed the cells to have a momentum, thereby enabling them to overcome the liquid flow toward the inside of the filter. Accordingly, the pivotal momentum combined with the centrifugal force appeared to be the main mechanism of cell separation in the proposed spin filter system.

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