Korean J. of Chem. Eng., 13(4), 404-408 (1996)

SHORT COMMUNICATION

MICROFILTRATION OF YEAST CELLS IN AN INTERNAL FILTER REACTOR

Woo Gi Lee, Beom Soo Kim* and Ho Nam Chang†

Department of Chemical Engineering and BioProcess Engineering Research Center, Korea Advanced Institute of Science and Technology, Taejon 305-701, Korea
*Department of Chemical Engineering, Konyang University, Nonsan 320-800, Korea
(Received 14 August 1995 • accepted 27 March 1996)

Abstract – Microfiltration was carried out in a newly-developed internal filter reactor system (stainless steel membrane filter, pore size=2 or 10 μm) using yeast cells of *Saccharomyces cerevisiae* ATCC 24858, industrial *S. cerevisiae*, and recombinant yeast RH 51. The filter performance was measured in terms of filtrate flux and retention coefficient of cell, and was highly influenced by agitation speed and cell concentration. Both gel polarization model and solid flux model failed to predict the filtration behavior in the internal filter system. An empirical equation was obtained to correlate filtrate flux as a function of agitation speed and cell concentration. Retention coefficient with a filter of 2 μm pore size was found more than 95%, and the filter was suitable for the yeast cell separation.

Key words: *Microfiltration, Internal Filter Reactor System, Yeast, Filter Performance, Gel Resistance*

INTRODUCTION

Enhancement of productivity of a bioprocess necessitates continuous operation of bioreactors with higher cell concentrations than are possible in conventional batch or continuous modes of culture. Membrane cell recycle has been effectively used to maintain high cell concentrations in bioreactors [Chang et al., 1994; Lee and Chang, 1987; Lee and Chang, 1990]. However, this process has limitations that need to be overcome prior to its industrial applications: (1) industrial substrates contain many particles which make pumping through external membrane device difficult; (2) oxygen supply and carbon dioxide removal may not be adequate while the broth is in the recycling loop; (3) sterilization of the external membrane device is difficult; and (4) recirculation of the broth requires pumps and additional energy for the operation. To overcome these problem, we developed an internal filter reactor system, which allowed microbial separation to be carried out inside the fermentor [Chang et al., 1993]. We have successfully employed this reactor system for ethanol production by *Saccharomyces cerevisiae* [Chang et al., 1993; Lee et al., 1994], and for the production of *Bacillus thuringiensis* spores [Kang et al., 1993]. Suzuki et al. [1994] used this type of reactor and introduced recovering system from membrane fouling.

There have been numerous reports on factors affecting filtration performance in cross-flow microfiltration of microbial cells [Kroner et al., 1984; Patel et al., 1987; Warren et al., 1991; Tanaka et al., 1993]. Despite several advantages of the internal filter system, however, there have been no studies on the characteristics of filtration performance of the new reactor system. In this study, we investigated the effects of operation conditions on filtration performance. From the experimental data, we suggested an empirical correlation equation predicting filtrate flux as a function of agitation speed and cell concentration. In addition, the conventional gel polarization and solid flux models were used to describe the filtration behavior in the system.

†To whom all correspondences should be addressed.

EXPERIMENTAL

1. Microorganisms

The yeast strains used in this study were *Saccharomyces cerevisiae* ATCC 24858, industrial *Saccharomyces cerevisiae* (Seoyoung Ethanol Industry, Korea), and recombinant yeast RH51 (Suwon University, Korea). These strains were maintained on slant containing 0.3% yeast extract, 0.3% malt extract, 0.5% bacto peptone, 2% glucose, and 2% agar at 4°C.

2. Filter Module

The filter module used in this study is shown in Fig. 1. The filter material was porous stainless steel with the pore sizes of 2 or 10 μm (Cuno Co., USA). The filter module consisted of 13 vertical cylindrical filter rods with inner diameter, outer diameter, and height of 7.5, 9.0 and 120 mm, respectively, and an upper frame of stainless steel. The total surface area of the filter module was ca. 440 cm^2.

After the experiments the filter module was separated from the fermentor and cleaned with 1 N NaOH for several hours. It was washed and backflushed with distilled water prior to reuse. After these treatments, the filter performance returned to its original level.

3. Microfiltration of Yeast and Analytical Methods

The filter testing system consisted of a reservoir equipped with the filter module and two pumps with a filter chamber connected via a Tygon tube (Fig. 2). The reactor used in this study was a 1.5 L capacity closed vessel of 1.0 L working volume (Bioflo model C30, New Brunswick Scientific Co., USA). The pressure gauge was located between the reservoir and the pump. The fluid in the reservoir was removed through

the filter module by the suction pump and then filtrate was recirculated back to the reservoir for homogeneity.

Cultivated yeast cells in the fermentor were centrifuged and the aliquots were removed. The cells were remixed with a buffer saline solution which consisted of 8.5 g/L NaCl, 6 g/L NaH_2PO_4 and 3 g/L KH_2PO_4. Experiments were performed at pH 7 and room temperature (25°C). Samples were removed at above 5 minute intervals. A steady state was assumed when filtrate flux leveled off by assaying several successive samples.

The retention coefficient (R, in percentage) in defined as follows.

$$R = 100 \times \left(1 - \frac{C_f}{C_r}\right) \quad (1)$$

where C_f and C_r are concentrations of the cell in the filtrate and in the retentate, respectively. If the yeast cells are completely retained by the filter, the retention coefficient R is 1.

Cell concentration was measured using the spectrophotometer (Beckman DU-65, Fullerton, USA) at 570 nm. Cell dry weight was determined after centrifuging the cell suspension twice, washing in distilled water, and drying at 105°C for 1 day.

RESULTS AND DISCUSSION

The gel polarization model in cross-flow filtration has been used successfully for describing the pressure-independent behavior of the filtrate flux as a function of bulk cell concentration and linear velocity of the bulk fluid. It has not, however, been useful for predicting performance for suspensions of particles larger than a few microns. Nagata et al. [1989] suggested a new mass transfer model-solid flux model which assumes negligible back-diffusion of solids and sticky particles. In contrast to the graphical representation of J (filtrate flux) vs. ln C_b (bulk cell concentration) in the gel polarization model, the solid flux

Fig. 1. Stainless steel filter module.

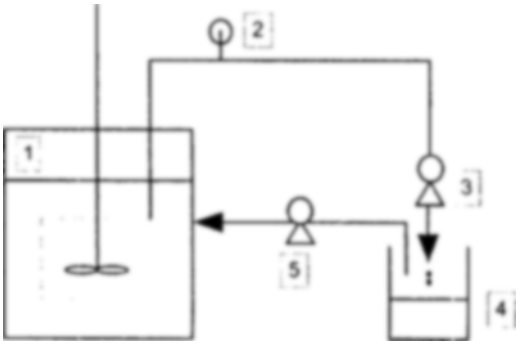

Fig. 2. Schematic diagram of experimental apparatus for the filter performance test.

(1) Fermentor equipped with filter module
(2) Pressure gauge
(3) Suction pump
(4) Filtrate chamber
(5) Recirculation pump

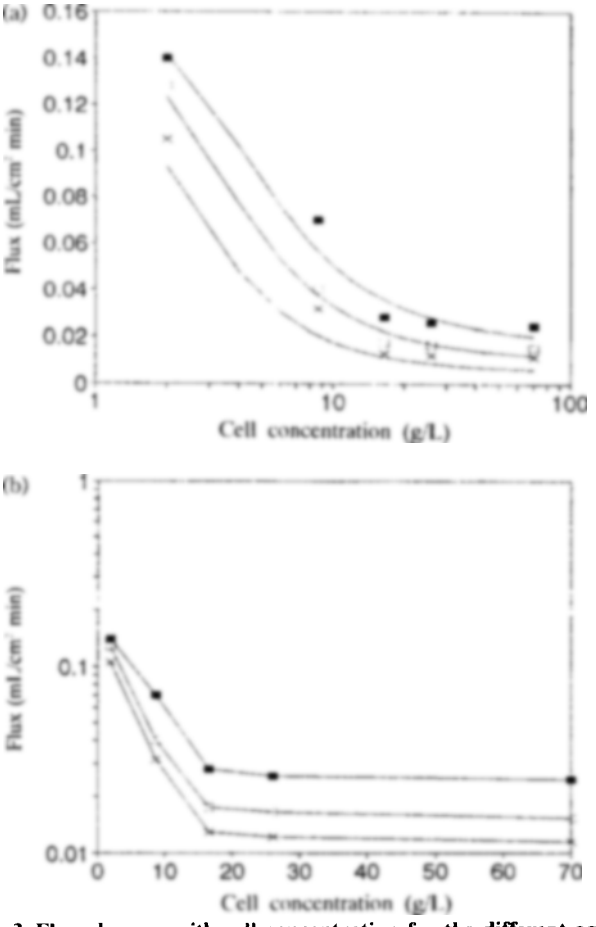

Fig. 3. Flux changes with cell concentration for the different agitation speed using *Saccharomyces cerevisiae* ATCC 24858; pressure difference=10 cmHg, pore size of filter=2 μm (■: 700 rpm, □: 400 rpm, X: 100 rpm).

(a) ln J vs. C_b
Data are plotted according to the gel polarization model. Solid lines indicate flux calculated from Eqs. (2) and (4).
(b) J vs. ln C_b
Data are plotted according to the solid flux model.

model gives a linear relationship in ln J vs. C_b.

To test whether the gel polarization model or the solid flux model is applicable to our internal filter reactor system, data are analyzed with each model and shown in Fig. 3(a) and (b). From the figures, it can be seen that both model fail to describe the filtration behavior in the internal filter system. Nagata et al. [1989] summarized that several different phenomena are responsible for the decline in filtrate rate during membrane filtration process. They divided the curve of J vs. ln C_b into five periods. In Fig. 3(a), two periods are identified with the boundary of a cell concentration of ca. 16.5 g/L. This phenomenon is very similar to that obtained with other cross-flow filtration devices [Nagata et al., 1989; Tanaka et al., 1993]. The first period below 16.5 g/L cell concentration is the period of multi-sublayer build-up and clogging. For this period, the gel polarization model is applicable as can be seen by the constant negative slope of the line which is equal to the mass transfer coefficient. The second period above that cell concentration is the period of densification of sublayers. After the sublayer growth has stabilized, the filtration rate declines rather slowly since the mass transfer coefficient is mainly affected by particle rearrangement rather than the net deposit of additional solids.

Since a comprehensive quantitative description of these phenomena is not available, attempts to obtain an empirical equation that predicts filtrate flux from cell concentration and agitation speed were made. Assuming a resistance model, the filtrate flux can be expressed as follows.

$$J = \frac{\Delta P}{R_g + R_m} \tag{2}$$

where J is filtrate flux (filtrate volume/time/membrane area), ΔP is transmembrane pressure drop, R_g is gel resistance, and R_m is membrane resistance. R_m is a constant that can be calculated from pure water flux, and in our membrane system, R_m is 56.8 cmHg cm^3/ml. Accordingly, R_g can be calculated from experimental flux data by the following equation.

$$R_g = \frac{\Delta P}{J} - R_m \tag{3}$$

Fig. 4(a) and (b) are plots of R_g with agitation speed and cell concentration, respectively. R_g decreased linearly with the logarithm of agitation speed. As the cell concentration increased, R_g increased to reach a constant value. From the relationships of Fig. 4(a) and (b), the following empirical equation can be derived.

$$R_g = (9.4 - 2.972 \log U)\ 10^{\frac{2.767 C_b}{2.743 + C_b}}$$
$$(2 < C_b < 70\ \text{g/L},\ 100 < U < 700\ \text{rpm},\ 1\ \text{L working volume}) \tag{4}$$

where U is agitation speed (rpm). The solid lines in Fig. 4(a) and (b) represent the values of R_g calculated from Eq. (4), which agreed relatively well to the experimental data. Also in Fig. 3(a), it is shown that the solid line by Eq. (4) fits the data well.

The most significant improvement of filtrate flux is obtained by keeping the filter as free from deposits as possible by employing sufficiently high shear force. In this study, a way to minimize deposits on the surface of the filter is to increase the agitation speed (Fig. 5), as was shown in Fig. 4(a) that the gel resistance decreased with agitation speed. Since the cell concentration used in Fig. 5 is 2 g/L [first period in Fig. 3(a)], the gel polarization model can be applied to describe the filtration behavior. From the slope of the line in Fig. 5, the relationship between the mass transfer coefficient (k) and agitation speed (U) as a hydrodynamic factor can be obtained, and it was expressed as follows.

$$k \propto U^{0.25}$$
$$(C_b < 16.5\ \text{g/L},\ 100 < U < 700\ \text{rpm},\ 1\ \text{L working volume}) \tag{5}$$

The agitation speed in the reactor must be optimized, because too high agitation speed bring about a high energy consumption. This agitation speed requirement may vary depending on the application.

The effect of the pore size of filter on filtrate flux is shown in Fig. 6. Pressure difference and agitation speed in the reactor were controlled at 10 cmHg and 700 rpm, respectively. No significant difference was shown in filtrate flux in 2 μm and 10

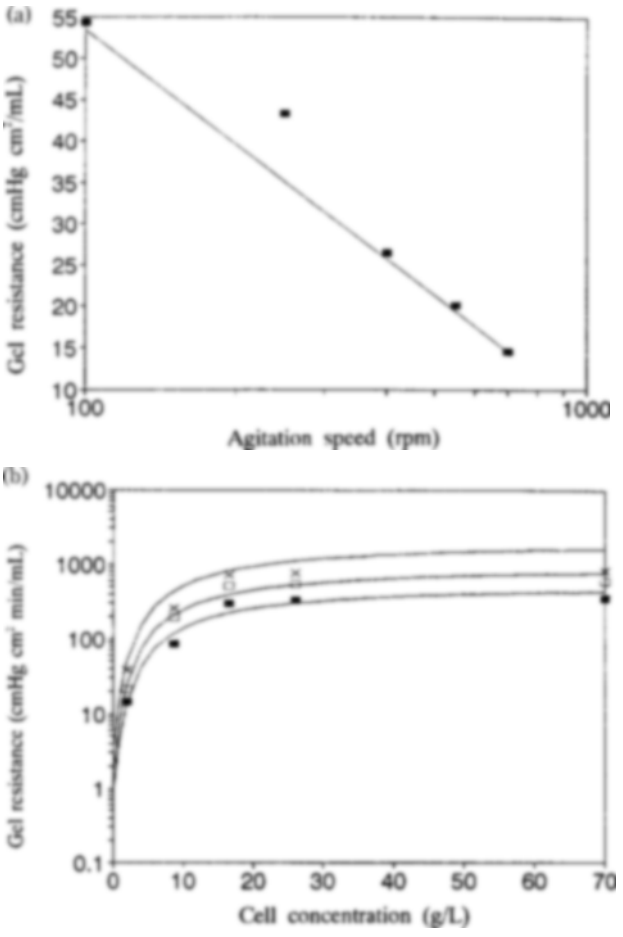

Fig. 4. Gel resistance as a function of (a) agitation speed and (b) cell concentration. Solid lines indicate gel resistance calculated from Eq. (4). Symbols in Fig. 4(b) are the same as in Fig. 3.

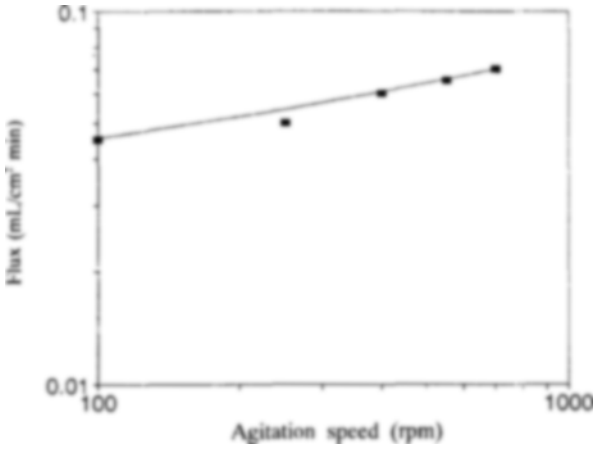

Fig. 5. Effect of agitation speed on filtrate flux using *Saccharomyces cerevisiae* ATCC 24858; cell concentration=2 g/L, pressure difference=5 cmHg, pore size of filter=2 μm. Solid line indicates flux calculated from Eqs. (2) and

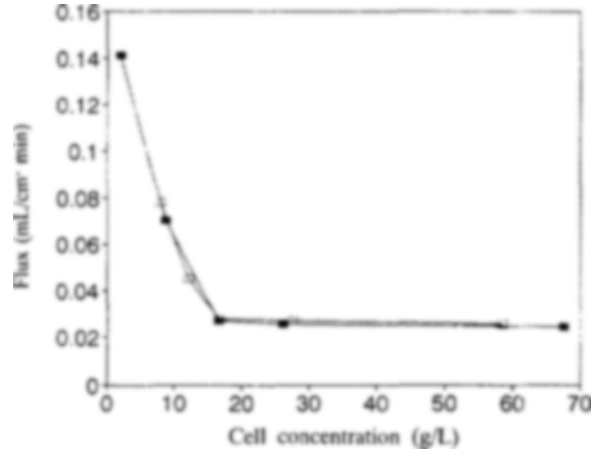

Fig. 6. Flux changes with cell concentration for the different pore size of filter using *Saccharomyces cerevisiae* ATCC 24858; pressure difference=10 cmHg, agitation speed=700 rpm (■: 2 μm, □: 10 μm).

μm filter, while the retention coefficients of yeast cells in 2 μm pore size filter were higher than those of 10 μm filter [Chang et al., 1993]. Therefore, the filter of 2 μm pore size was suitable than 10 μm pore size filter for yeast cell separation.

Fig. 7 shows retention coefficients for three different yeast cells as a function of cell concentration at agitation speed of 700 rpm and pressure difference of 10 cmHg. Retention coefficients for three different yeast cells were a bit different due to difference in cell size and morphology. *Saccharomyces cerevisiae* ATCC 24858 had the highest retention coefficients among the yeast cells tested. All retention coefficients for three different yeast cells were found more than 99.5% above cell concentration of 50 g/L.

CONCLUSIONS

1. The empirical correlation of filtrate flux as a function of agitation speed and cell concentration in the internal filter reactor system was expressed as follows.

Fig. 7. Retention coefficient changes with cell concentration for the different yeast cells; pressure difference=10 cmHg, agitation speed=700 rpm, pore size of filter=2 μm (■: ATCC 24858, □: Industrial, X: RH 51).

$$R_g = (9.4 - 2.972 \log U)\ 10^{\frac{2.767 C_b}{2.743 + C_b}}$$
$$(2 < C_b < 70\ g/L,\ 100 < U < 700\ rpm,\ 1\ L\ working\ volume)$$

2. When the cell concentration was less than 16.5 g/L, the gel polarization model could be applied to describe the filtration behavior. The relationship between the mass transfer coefficient and agitation speed was expressed.

$$k \propto U^{0.25}$$
$$(C_b < 16.5\ g/L,\ 100 < U < 700\ rpm,\ 1\ L\ working\ volume)$$

3. The pore size of filter used in this study has a little effect on steady state filtrate flux.

4. The retention coefficient with the filter of 2 μm pore size was found more than 95% and the filter was suitable for yeast cell separation.

ACKNOWLEDGEMENT

This work was supported by grant-in-aid from the Ministry of Trade, Industry, and Energy of Korea, and the Korea Science and Engineering Foundation.

NOMENCLATURE

C_b : bulk cell concentration [g/L]
C_f : cell concentration in filtrate [g/L]
C_r : cell concentration in retentate [g/L]
J : filtrate flux [ml/min/cm^2]
k : mass transfer coefficient [ml/min/cm^2]
R : retention coefficient [%]
R_g : gel resistance [cmHg cm^2/ml]
R_m : membrane resistance [cmHg cm^2/ml]
U : agitation speed [rpm]
ΔP : transmembrane pressure drop [cmHg]

REFERENCES

Chang, H. N., Lee, W. G. and Kim, B. S., "Cell Retention Culture with an Internal Filter Module: Continuous Ethanol Fermentation", *Biotechnol. Bioeng.*, **41**, 677 (1993).

Chang, H. N., Yoo, I. K. and Kim, B. S., "High Density Cell Culture by Membrane-Based Cell Recycle", *Biotechnol. Adv.*, **12**, 467 (1994).

Kang, B. C., Lee, S. Y. and Chang, H. N., "Production of *Bacillus thuringiensis* Spores in Total Cell Retention Culture and Two-Stage Continuous Culture Using an Internal Ceramic Filter System", *Biotechnol. Bioeng.*, **42**, 1107 (1993).

Kroner, K. H., Schutte, H., Husdedt, H. and Kula, M. R., "Cross Flow Filtration in the Down Sream Processing of Enzymes", *Proc. Biochem.*, **19**(Apr.), 68 (1984).

Lee, C. W. and Chang, H. N., "Kinetics of Ethanol Fermentations in Membrane Cell Recycle Fermentors", *Biotechnol. Bioeng.*, **29**, 1105 (1987).

Lee, W. G., Lee, Y. S., Chang, H. N. and Chang, Y. K., "A Cell Retention Internal Filter Reactor for Ethanol Production Using Tapioca Hydrolysates", *Biotechnol. Techniques*, **8**, 817 (1994).

Lee, Y. L. and Chang, H. N., "High Cell Density Culture of a Recombinant *Escherichia coli* Producing Penicillin Acylase in a Membrane Cell Recycle Fermentor", *Biotechnol. Bioeng.*, **36**, 330 (1990).

Nagata, N., Herouvis, K. J., Dziewulski, D. M. and Belfort, G., "Cross-Flow Membrane Microfiltration of Bacterial Fermentation Broth", *Biotechnol. Bioeng.*, **34**, 447 (1989).

Patel, P. N., Mehaia, M. A. and Cheryan, M., "Cross-Flow Membrane Filtration of Yeast Suspensions", *J. Biotechnol.*, **5**, 1 (1987).

Suzuki, T., Sato, T. and Kominami, M., "A Dense Cell Retention Culture System Using a Stirred Ceramic Membrane Reactor", *Biotechnol. Bioeng.*, **44**, 1186 (1994).

Tanaka, T., Kamimura, R., Itoh, K., Nakanishi, K. and Matsuno, R., "Factors Affecting the Performance of Crossflow Filtration of Yeast Cell Suspension", *Biotechnol. Bioeng.*, **41**, 617 (1993).

Warren, R. K., Macdonald, D. G. and Hill, G. A., "Cross-Flow Microfiltration of *Saccharomyces cerevisiae*", *Proc. Biochem.*, **23**, 337 (1991).