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Characteristics in Crossflow Filtration Using Different Yeast Suspensions

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ABSTRACT

Characteristics of crossflow filtration of cell types were compared. Although the cell sizes were similar for the six yeast cells, the specific resistance of the cake measured in dead-end filtration and permeation flux were different. The flux decreased with increasing specific resistance. However, the dependencies of the steady-state flux on the shear stress were similar for all the yeast cells. The steady-state permeation flux of the yeast cell suspensions was correlated with the shear stress to the 0.6–0.9th power and with the cell concentration to the −0.3rd power regardless of the transmembrane pressure. The permeation flux in the unsteady state was well simulated by the correlation for the lift velocity of the cells obtained from the steady-state flux.

INTRODUCTION

Crossflow filtration is considered to be an efficient method to harvest microbial cells from fermentation broth (1, 2). Since the permeation flux is usually governed by the resistance of the cell layer formed on the membrane, it is quite important to investigate the factors affecting its formation. Much effort has been made to correlate the permeation flux with operational conditions such as circulation flow rate, transmembrane pres-

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sure, and cell concentration (3–6). However, only a little information is known on the effect of the cell properties on the permeation behavior. For this paper we cross-filtered six different yeasts to study this point. We measured the dependencies of the steady-state permeation flux on the shear stress acting on the cell layer, transmembrane pressure, and cell concentration for six yeast cell suspensions, and we discuss the causes for the differences among the cells. By using the correlations obtained from the experimental dependencies of the steady-state flux on the shear stress, the permeation flux during the unsteady-state was simulated.

EXPERIMENTAL

Microorganisms

Six kinds of yeast were used in this study. These were baker's yeast (Kanegafuchi Chemical Industry Co., Osaka, Japan), sake yeast IFO2347, wine yeast IFO2359, *Saccharomyces uvarum* IFO10010, *Zygosaccharomyces rouxii* IFO0505, and *Schizosaccharomyces pombe* IFO0358.

Cultivation

All the yeast cells were cultivated in a medium containing 0.3% yeast extract (Difco Laboratories, Detroit, MI, USA), 0.3% malt extract (Difco Laboratories), 0.5% Bactopeptone (Difco Laboratories), 0.5% NaCl, and 1% glucose. The pH of the medium was adjusted to 6.0 with 1 N HCl. The yeast cells were inoculated into 100 cm^3 of the medium contained in a 500-cm^3 shaking flask as a seed culture and cultivated at 30°C with reciprocal shaking (120 strokes per min) for 48 hours. Then, 80 cm^3 of the seed culture was inoculated into 4 dm^3 of the medium contained in a 5-dm^3 jar fermentor (MBF-500, Tokyo Rikakikai Co., Tokyo, Japan) as the main culture. An antifoaming agent (KM70, Shin-Etsu Chemical Industry, Tokyo) was added at 50 ppm. The aeration rate was 0.5 volume per volume per minute. The broth was agitated at 500 rpm by three turbine impellers, and cultivated for 24 hours at 30°C.

After cultivation, the cells were collected by centrifugation and washed with 0.9% NaCl solution. They were then suspended in a 0.9% NaCl solution and used for the measurement of the specific resistance and for crossflow filtration experiments. The cell concentration was expressed in wet weight of cells per unit volume of suspension.

Membrane

A microfiltration membrane, C045 (Toyo Roshi Co., Ltd., Tokyo), was used for both dead-end and crossflow filtration experiments. The mem-

brane was a screen-type filter made of cellulose acetate with a nominal pore size of 0.45 μm.

Measurement of Specific Resistance

The specific resistance was measured at 20°C in dead-end filtration by the steady-state method (7). A cell suspension in 0.9% NaCl solution was filtered with the aid of pressure from a nitrogen gas cylinder in a module with a filtration area of 38.1 cm^2 and a depth of 7 cm. After a continuous feeding of 0.9% NaCl solution, a cake formed on the membrane and the permeation flux reached a steady state. The specific resistance α was calculated from the permeation flux J, the transmembrane pressure ΔP, the viscosity of permeate μ_p, the membrane resistance R_m, and the weight of the cake per unit filtration area w, using the filtration Eq. (1):

$$J = \frac{\Delta P}{\mu_p(R_m + \alpha W)} \tag{1}$$

The viscosity of the permeate (0.9% NaCl solution) was 1.01×10^{-3} Pa·s at 20°C, which was measured with an Ostwald viscometer. The membrane resistance was calculated from the permeation flux in the filtration of a 0.9% NaCl solution. The value of R_m was 3.7×10^{10} m^{-1}.

Crossflow Filtration

Crossflow filtration was performed at 20°C with a thin-channel-type module as described elsewhere (8). The module was made of polycarbonate with a filtration area of 24 cm^2 (24 mm in width and 100 mm in length).

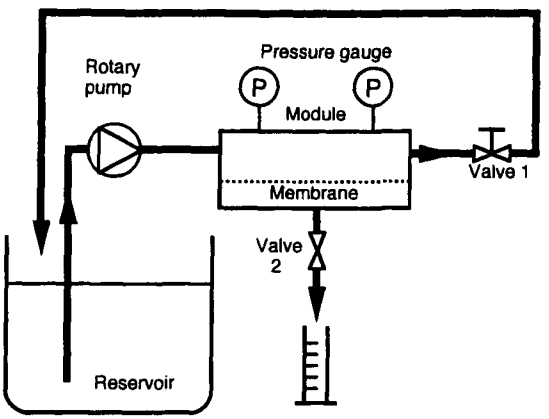

FIG. 1 Schematic diagram of the crossflow filtration unit.

A cell suspension was circulated with a rotary pump (RM10, Nakamura Metal Co., Osaka, Japan) equipped with a variable-speed drive (Ringcorn RXM-400, Shimpo Industry Co., Kyoto, Japan). The permeate was returned to the reservoir tank to keep the cell concentration constant (Fig. 1). After the experiment, the weight of the cake formed on the membrane was measured.

The circulation flow rate, the channel depth, the transmembrane pressure, and the cell concentration were changed in the ranges of 15–60 $cm^3 \cdot s^{-1}$, 2.4–5.6 mm, 25–98 kPa, and 5–100 $kg \cdot m^{-3}$ in wet weight, respectively. The linear velocity of the circulation flow ranged from 0.2 to 1.0 $m \cdot s^{-1}$.

Evaluation of Viscosity of Suspensions and Wall Shear Stress

Viscosity of cell suspensions, μ_s, was calculated by Einstein's equation (Eq. 2a):

$$\mu_s = \mu_0(1 + 2.5\phi) \tag{2a}$$

where μ_0 and ϕ are the viscosity of 0.9% NaCl solution (1.01×10^{-3} Pa·s at 20°C) and the volume fraction of the cell in the suspension, respectively (9).

The wall shear stress, τ, was calculated by using an equation derived for laminar flows in a rectangular duct as a friction factor (Eqs. 2b–2c) and Blasius's equation for turbulent flows (Eqs. 2d–2g), respectively (10):

$$\tau = \frac{32\,\mu_s q}{b^2(b + a)K} \qquad \text{(laminar flow)} \tag{2b}$$

$$K = \frac{16}{3} - \frac{1024b}{\pi^5 a}\left\{\tanh\left(\frac{\pi a}{b}\right) - \frac{1}{3^3}\tanh\left(\frac{3\pi a}{2b}\right)\right\} \tag{2c}$$

$$\tau = 0.0395\,\mathrm{Re}^{-1/4}\rho_s u^2 \qquad \text{(turbulent flow)} \tag{2d}$$

$$\mathrm{Re} = \frac{d_H u \rho_s}{\mu_s} \tag{2e}$$

$$d_H = \frac{2ab}{a + b} \tag{2f}$$

$$u = \frac{q}{ab} \tag{2g}$$

where μ_s, ρ_s, q, a, b, d_H, u, and Re are viscosity of suspension, density of

suspension, circulation flow rate, channel width, channel depth, hydraulic diameter, mean linear velocity, and Reynolds number, respectively (10). K is a constant determined by the a/b ratio. The effective channel depth, b, was calculated by Eq. (2h), taking the amount of the cake layer formed during crossflow filtration into consideration:

$$b = H - w/\rho_c \tag{2h}$$

where H and ρ_c are the channel depth of the module and the density of the cake, respectively. The value of ρ_c estimated from the weight and volume of the cake formed in dead-end filtration of baker's yeast was 1.15×10^3 kg·m^{-3}.

The pressure drop ΔP_L along the channel of the filtration module was measured using water instead of cell suspension, and the shear stress was calculated by Eq. (2i):

$$\tau = \frac{\Delta P_L ab}{2L(a + b)} \tag{2i}$$

where L is the length of the channel. The value obtained from Eq. (2i) agreed well with that calculated by Eqs. (2b)–(2h) (data not shown).

RESULTS AND DISCUSSION

Cake of Various Yeast Cells

Table 1 shows the long, short, and surface-average diameters of the yeast cells used in this study. The surface-average diameter was calculated

TABLE I
Cell Sizes, Specific Resistances, and Void Fractions of Cell Layers

Yeast cells	Cell sizes: Long diameter (μm)	Cell sizes: Short diameter (μm)	Cell sizes: Surface-average diameter (μm)	Specific resistance: α_0[a] (10^{11} m·kg^{-1})	Specific resistance: β[b] [10^6 m·kg^{-1}·Pa^{-1}]	Void fraction[c] (—)
Baker's yeast	5.3	4.7	4.9	3.3	2.8	0.24
Sake yeast	4.8	3.7	4.0	9.8	12.8	0.20
Wine yeast	4.5	4.0	4.2	2.1	5.6	0.29
S. uvarum	5.1	4.6	4.8	1.5	3.6	0.30
Zygo. rouxii	4.4	3.8	4.0	1.1	2.0	0.35
Schizo. pombe	7.1	3.7	4.3	2.4	4.3	0.27

[a] The specific resistance extrapolated to a transmembrane pressure of 0 kPa.
[b] The slope of the regression line.
[c] The void fraction calculated from surface diameter and α_0.

by assuming a yeast cell as an ellipsoid. Figure 2 shows the dependencies on the transmembrane pressure of the specific resistance of the yeast cell cakes measured in dead-end filtration. The dependencies of the specific resistance α on the transmembrane pressures ΔP were approximately expressed by $\alpha = \alpha_0 + \beta\Delta P$ at transmembrane pressures lower than 100 kPa with a regression coefficient higher than 0.99 for all the cells. The values of α_0 and β are shown in Table 1. The specific resistances were different although the cell sizes were similar. In particular, the specific resistances for the sake yeast were considerably higher. The void fractions of the yeast cell cake ϵ were calculated from α_0 and the surface-average diameter D using Eq. (3) derived from the Kozeny–Carman's equation (7) as summarized in Table 1.

$$\alpha_0 = \frac{180(1 - \epsilon)^2 D^2}{\epsilon^3 \rho_c} \tag{3}$$

The cells except baker's yeast and sake yeast showed the void fraction around 0.3. The sake yeast showed the lowest value of the void fraction. The structure near the cell surface would affect the void fraction of the cake (11).

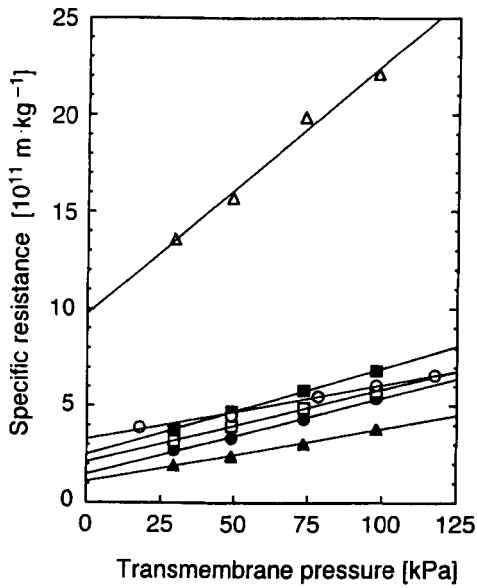

FIG. 2 Specific resistance of yeast cakes. (○) Baker's yeast, (△) sake yeast, (□) wine yeast, (●) *S. uvarum*, (▲) *Zygo. rouxii*, and (■) *Schizo. pombe*.

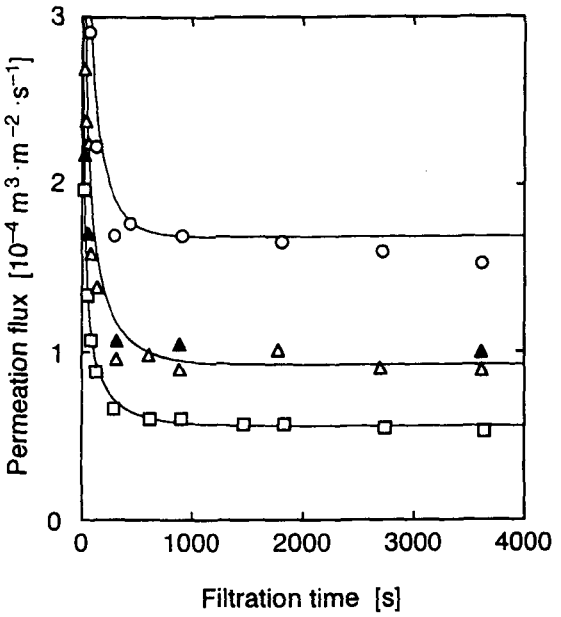

FIG. 3 Time courses of crossflow filtration of yeast suspensions. Circulation flow rate, channel depth, transmembrane pressure, and cell concentration were 30 $cm^3 \cdot s^{-1}$, 2.4 mm, 49 kPa, and 20 $kg \cdot m^{-3}$, respectively. (○) *Zygo. rouxii*, (△, ▲) baker's yeast, and (□) sake yeast. The filled triangles shows the permeation flux for the baker's yeast cell suspension calculated from the weight of cake and the specific resistance measured in dead-end filtration (Eq. 3). Solid lines show the flux calculated from the differential equation including the lift velocity (see text for details).

Courses of Permeation Flux in Crossflow Filtration

Figure 3 shows the typical courses of permeation flux during crossflow filtration, where suspensions of *Zygo. rouxii*, baker's yeast, and sake yeast were cross-filtered. The circulation flow rate, the channel depth, the transmembrane pressure, and the cell concentration were 30 $cm^3 \cdot s^{-1}$, 2.4 mm, 49 kPa, and 20 $kg \cdot m^{-3}$, respectively. The permeation flux rapidly decreased at the beginning of the filtration and reached a nearly constant value (steady state) after 1000 seconds. However, the permeation flux gradually reduced even after 1000 seconds of crossflow filtration when the circulation flow rate exceeded 60 $cm^3 \cdot s^{-1}$ or the cell concentration was higher than 100 $kg \cdot m^{-3}$ (data not shown). The gradual reduction of flux would be due to the gradual deposition of the cells which were broken under high shear stress onto the membrane (12). The experimental steady-

state flux agreed well with the value calculated by Eq. (1) using the amount of cake formed on the membrane per unit filtration area and the specific resistance measured in dead-end filtration as described previously (13).

Dependence of Steady-State Permeation Flux on Shear Stress

Figure 4 shows the relationship between the steady-state flux and shear stress acting on the cake surface in crossflow filtration of yeast suspensions. The cell concentration was 20 $kg \cdot m^{-3}$ for all the experiments. In the case of baker's yeast cells, the circulation flow rate, the channel depth, and the transmembrane pressure were varied. For the other yeast cells, the steady-state flux was measured at a transmembrane pressure of 49 kPa with the channel depth of 2.4 mm varying the circulation flow rate. As shown in Fig. 4, the steady-state flux was correlated with an equation including only shear stress, regardless of the transmembrane pressure:

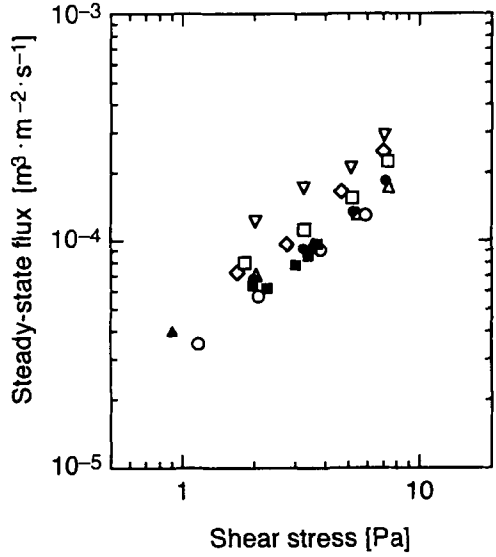

FIG. 4 Dependence of the steady-state permeation flux on the shear stress in crossflow filtration of yeast cell suspensions. (●, ▲, ■) Baker's yeast, (○) sake yeast, (△) wine yeast, (□) *S. uvarum*, (▽) *Zygo. rouxii*, and (◇) *Schizo. pombe*. In crossflow filtration of baker's yeast cell suspension, circulation flow rate (●), channel depth (▲), and transmembrane pressure (■) were changed in crossflow filtration of baker's yeast cell suspension in the ranges of 15–60 $cm^3 \cdot s^{-1}$, 2.4–5.6 mm, and 25–98 kPa, respectively. In the other cells, only the circulation flow rate was varied from 15 to 60 $cm^3 \cdot s^{-1}$ at the cell concentration of 20 $kg \cdot m^{-3}$, the channel depth of 2.4 mm, and the transmembrane pressure of 49 kPa.

$$J = k_1 \tau^n \quad (4)$$

where k_1 is the permeation flux at a shear stress of 1 Pa and n is the exponent for the dependence of the steady-state flux on the shear stress.

From the data shown in Fig. 4, k_1 and n are summarized in Table 2. The regression coefficients for the relationship between J and τ were more than 0.96 for all the cells. The exponents were similar for all the cells used in this study. However, the values of k_1 varied. Although the reason for the variation of k_1 is not clear, one possible reason is that the steady-state permeation flux depends on the structure of the cake. The calculated void fraction in Table 1 would reflect the structure at the surface layer of the cake where no appreciable transmembrane pressure was acting. There was a tendency for the steady-state flux to become lower with decreasing void fraction (Tables 1 and 2).

Several investigators have studied the dependence of the steady-state flux on the shear stress using yeast cells (1, 3–6). In all the previous studies the flux was correlated with the shear stress by a relationship similar to Eq. (4). The values of the exponent n ranged from 0.4 to 1.0. The n values obtained in this study using different kinds of yeast cells ranged between 0.63 and 0.88. In the shear-induced diffusion model (14, 15) and the surface transport model (16), the exponent is 1.0. Our result is slightly lower than the values predicted by these models.

Dependence of Steady-State Permeation Flux on Cell Concentration

Figure 5 shows the dependence of the steady-state flux on the cell concentration in the crossflow filtration of suspension of four yeast cells

TABLE 2
Steady-State Flux and Its Dependence on Shear Stress in Crossflow Filtration of Yeast Suspensions[a]

Yeast cells	Steady-state flux[b] ($m^3 \cdot m^{-2} \cdot s^{-1}$)	Dependence of flux on shear stress[c] (—)
Baker's yeast	4.2	0.63
Sake yeast	3.1	0.80
Wine yeast	4.2	0.69
S. uvarum	4.9	0.73
Zygo. rouxii	7.6	0.67
Schizo. pombe	3.1	0.88

[a] At a cell concentration of 20 $kg \cdot m^{-3}$.
[b] At a shear stress of 1 Pa.
[c] The exponent for the dependence of the steady-state flux on shear stress.

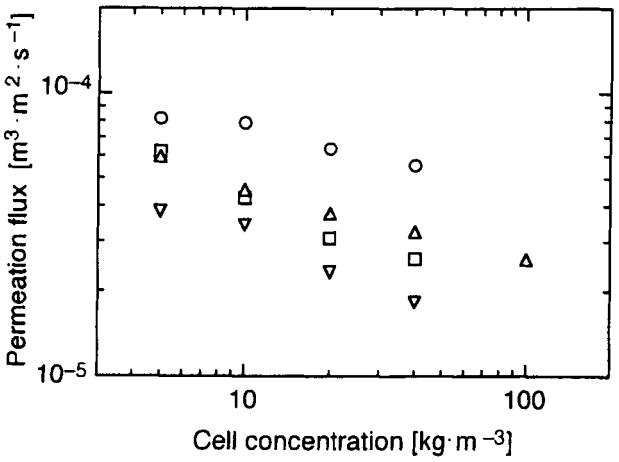

FIG. 5 Dependence of the steady-state permeation flux on the cell concentration in crossflow filtration of suspensions of yeasts. The permeation fluxes at the shear stress of 1 Pa were plotted. (○) *Zygo. rouxii*, (△) baker's yeast, (□) wine yeast, and (▽) sake yeast.

(baker's yeast, *Zygo. rouxii*, wine yeast, and sake yeast) at a specified constant shear stress of 1 Pa. The dependence on the cell concentration was similar for all the cells tested. The flux was proportional to the -0.32nd power of the cell concentration. This dependence on the cell concentration is consistent with the shear-induced diffusion model which predicts the -0.33rd power dependence. However, the result does not agree with the surface transport model predicting the 0th power dependence (1, 14–16).

Simulation of Flux in Unsteady State of Crossflow Filtration

The simulation of the permeation behavior during the unsteady state of crossflow filtration is useful to optimize the operational conditions, especially when the filtration is operated in a backwashing mode. We calculated the permeation flux in the unsteady state from the dependencies of the steady-state permeation flux on the operational conditions. The cake formation rate dw/dt is equal to $\rho_p CJ/(\rho_s - C)$ in dead-end filtration where C, ρ_p, and ρ_s are the cell concentration in wet weight, the density of the permeate, and the density of the cell suspension, respectively. In the crossflow filtration, the rate of cake removal should be taken into account. Using the lift velocity $V_L(C, \tau)$ (17, 18), the cake formation rate

in crossflow filtration is

$$\frac{dw}{dt} = \frac{\rho_p C}{\rho_s - C}(J - V_L(C, \tau)) \tag{5}$$

Since the cake formation rate is zero at a steady state, the lift velocity becomes coincident with the steady-state permeation flux. Then Eq. (6) is obtained from the experimental steady-state flux.

$$v_L(C, \tau) = kC^{-0.32}\tau^{0.73} \tag{6}$$

The exponents for the concentration and shear stress were the averages for the yeasts obtained in this study as shown previously. The constants k for *Zygo. rouxii*, baker's yeast, wine yeast, and sake yeast were 1.6×10^{-4}, 1.0×10^{-4}, 9.0×10^{-5}, and 8.9×10^{-5} $m^3 \cdot m^{-2} \cdot s^{-1}$, respectively.

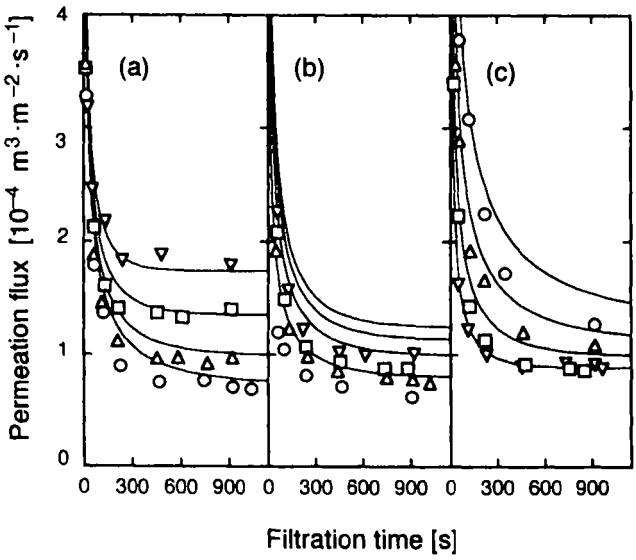

FIG. 6 Courses of permeation flux during crossflow filtration of baker's yeast cell suspension. The channel depth was 2.4 mm. Solid lines show the flux calculated from the differential equation including the lift velocity (see text for details). (a) Effect of circulation flow rate at the transmembrane pressure of 49 kPa and cell concentration of 20 $kg \cdot m^{-3}$: (○) 15 $cm^3 \cdot s^{-1}$, (△) 30 $cm^3 \cdot s^{-1}$, (□) 45 $cm^3 \cdot s^{-1}$, and (▽) 60 $cm^3 \cdot s^{-1}$. (b) Effect of transmembrane pressure at the circulation flow rate of 30 $cm^3 \cdot s^{-1}$ and cell concentration of 20 $kg \cdot m^{-3}$: (○) 25 kPa, (△) 49 kPa, (□) 74 kPa, and (▽) 98 kPa. (c) Effect of cell concentration at the transmembrane pressure of 49 kPa and circulation flow rate of 30 $cm^3 \cdot s^{-1}$: (○) 5 $kg \cdot m^{-3}$, (△) 10 $kg \cdot m^{-3}$, (□) 20 $kg \cdot m^{-3}$, and (▽) 40 $kg \cdot m^{-3}$.

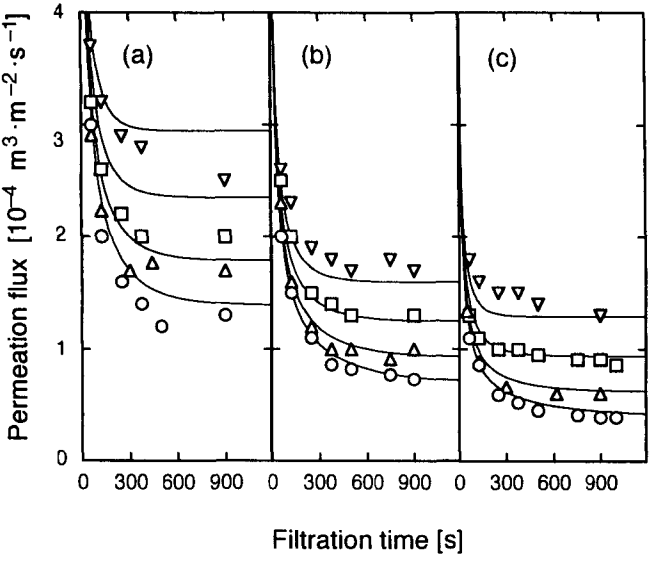

FIG. 7 Permeation flux during unsteady state in crossflow filtration of suspensions of *Zygo. rouxii* (a), wine yeast (b), and sake yeast (c) with different circulation flow rates. The channel depth, transmembrane pressure, and cell concentration were 2.4 mm, 49 kPa, and 20 kg·m^{-3}, respectively. Solid lines show the flux calculated from the differential equation including the lift velocity (see text). Circulation flow rate: (○) 15 cm^3·s^{-1}, (△) 30 cm^3·s^{-1}, (□) 45 cm^3·s^{-1}, and (▽) 60 cm^3·s^{-1}.

The differential equation (Eq. 5) was solved for w by the Runge–Kutta method with the initial condition of $w = 0$ at $t = 0$. Then the permeation flux was calculated by using Eq. (1). The simulated results are shown by solid lines in Figs. 3, 6, and 7, respectively, and agree well with the experimental permeation flux. Thus, the flux during the unsteady state could be estimated from the rate of cell removal obtained from steady-state fluxes and the specific resistance of the cell layer.

CONCLUSIONS

In crossflow filtration of suspensions of six different yeasts, the dependence of the steady-state permeation flux followed the shear-induced diffusion model. The difference in the specific resistance that would reflect the surface cake structure affected the permeation flux. The permeation flux during the unsteady state of crossflow filtration could be simulated with the lift velocity predicted from the behaviors of the steady-state flux.

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