Transient and Stationary Operating Conditions on Performance of Lactic Acid Bacteria Crossflow Microfiltration

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A filtration rig equipped with a tubular alumina membrane was used to study the performance of crossflow microfiltration of *Lactobacillus helveticus*. Experiments were performed at constant permeation flux. High cell concentrations and fast transient conditions to the stationary J adversely affected permeability. Membrane fouling was due to a fast irreversible layer formation and to a reversible cell cake. This microbial deposit characteristics were dependent on the ratio permeation flux/wall shear stress, J/τ_w. Fouling was faster and more severe when J/τ_w was greater than a critical value of 1.15 $L^{-1} \cdot h^{-1} \cdot m^{-2} \cdot Pa^{-1}$. The disordered structure of this cell cake seemed to lead to a macromolecule deposit between the cells which adversely affected the membrane permeability. © 1996 John Wiley & Sons, Inc.
Key words: crossflow microfiltration • hydrodynamics • fouling • bioreactor • *Lactobacillus helveticus*

INTRODUCTION

Lactic acid bacteria are used extensively in many food fermentations to preserve, retard spoilage, and improve flavor and texture. Cultures of lactic acid bacteria are being increasingly used in agriculture as inoculants in the preservation of fodder as silage and in probiotic feed supplements. They also produce bioantagonists such as antibiotics and bacteriocins. They find commercial applications in the dairy industry, in preservation of sausages and meats, in pickling of vegetables, and in preparing fermented beverages.

Physical concentration of starters can be achieved using several techniques such as centrifugation, spray-drying, freeze-drying, or membrane processes.[19] Greater recovery of biomass by membrane techniques compared with centrifugation is one of the major advantages of filtration technology, which moreover: (i) obviates the production of aerosols that cause allergic reactions among employees; (ii) can produce cell free extracts not usually found in other techniques like centrifugation; (iii) provides a higher production rate unmatched by other techniques; and (iv) is economically feasible.[42] Besides, bioreactors, coupled with the cell recycling membrane process, require high cell viability and high permeate flux to remove lactic acid from the fermention broth to obtain increased lactic acid and cell mass production.[13,14,40]

Nonetheless, few reports describe the optimization of crossflow microfiltration (MF) and ultrafiltration (UF) of lactic acid bacteria. Higher MF flux was obtained with a 0.45 μm pore size membrane compared to 0.8 and 1.2 μm with a permeate enriched with a mixed culture of *Streptococcus diacetylactis, Streptococcus cremoris,* and *Leuconostoc citrovorum*.[32] Faigh et al.[17] aimed at maximizing culture activity (cell concentration) without cell injury, but their report was brief and without numerical data. Recent work underlines the relevance of both media composition[34] and microbial strain[26] and of membrane roughness on crossflow filtration of cell suspensions.[29]

Nevertheless, available information is scarce about the optimization of operating conditions during UF and/or MF of lactic acid bacteria.[43,44] A better management of the cell filtration operation is undoubtedly commercially important in improving membrane bioreactor performance.

This work focuses on the study of transient and stationary operating conditions of initial cell concentration, C_i, permeation flux, J, and ratio of permeation flux to wall shear stress, τ_w, on the performance of *Lactobacillus helveticus* MF.

MATERIALS AND METHODS

Calculations

Hydraulic Resistances

Calculation of hydraulic resistances of the membrane, R_m, of the overall fouling, R_f, and of the irreversible fouling, R_{if}, were done according to Darcy's law:

$$J = \frac{\text{TMP}}{\mu R} \tag{1}$$

where J is the permeation flux, μ is the dynamic viscosity (taken as $\mu_w = 0.62 \pm 0.01 \times 10^{-3}$ Pa · s [water] and $\mu_p = 0.75 \pm 0.05 \times 10^{-3}$ Pa · s [MF permeate] at 43°C), TMP is the transmembrane pressure, and R is the hydraulic resistance:

$$R = R_m + R_f = R_m + R_{if} + R_{rf}$$

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with:

R_m: clean membrane hydraulic resistance.
R_f: overall fouling hydraulic resistance.
R_{rf}: reversible fouling hydraulic resistance (fouling removed with a water rinsing of the membrane); this resistance includes concentration polarization.
R_{if}: irreversible fouling hydraulic resistance (fouling not removed after water rinsing).

The slope of a plot of the measured water flux, J, versus TMP of a clean membrane and after the experiment gave the values of R_m and R_{if}.[38] R_f was calculated from TMP measurements during the experiments.[9] The hydraulic resistance of the reversible fouling was calculated according to: $R_{rf} = R_f - R_{if}$

When a cake builds-up, R_{rf} can be defined as R_d, the hydraulic resistance of the deposit. R_d can be defined[24] using:

$$R_d = \alpha M \tag{2}$$

where M is the mass of deposited layer per unit of membrane area (kilograms per square meter) and α is the intrinsic resistance of the deposit and is defined by the Carman–Kozeny expression as previously used[5]:

$$\alpha = \frac{180(1 - \epsilon)}{\rho_p d_p^2 \epsilon^3} \tag{3}$$

d_p is the mean diameter of particles being filtered, ρ_p is the particle density, and ϵ is the porosity of the deposit.

Taking into account the experimental error of each sensor (four pressure gauges [0.02 10^5 Pa each], temperature [0.4%], viscosity [1%], and permeation flux [3%]) and the relative standard deviation that characterizes the dispersion of results obtained from a series of measurements of the same variable, the calculated error amounted to ≈27% of the resistance. Nevertheless, consecutive measurements made on different days showed < 5% error in hydraulic resistance measurements, either for a clean membrane, for the overall or for the irreversible fouling. It was therefore concluded that this measured error is a better estimate of the actual error and was therefore used in the evaluation of fouling.

Wall Shear Stress

τ_w represents the forces applied by the fluid flowing tangentially to the membrane on an element of membrane area. It can be defined by a momentum balance (assuming that the fluid is incompressible, the flow is stationary and laminar Poiseuille-like and gravity is negligible), which leads to the following relationship:

$$\tau_w = \frac{d_h \Delta P}{4 L} \tag{4}$$

τ_w is related to the pressure drop, ΔP, and to the internal diameter, d_h, and length, L, of the tubular membrane. Such an expression can also be used in turbulent flow, taking into account the macroscopic properties of the fluid.

Filtered Volume

The filtered volume was calculated taking into account permeation flux and time data. The error was evaluated to be <3%.

Cell Suspension Preparation

Strains

Lactobacillus helveticus (CNRZ 303), a homofermentative DL lactic acid producer was used throughout this work. The cells were rods of 1 to 5 μm in length and 0.5 to 1.0 μm in diameter with a narrow size distribution. Two successive inoculations were made into the fermentation broth prior to inoculation of the bioreactor.

Medium

Sweet cheese whey was reconstituted from powder (65 $g \cdot L^{-1}$) (Préval, Montauban, France) with deionized water. The permeate was collected after UF using a composite membrane consisting of ZrO_2 filtering layer on a carbon support (M1 "Carbosep" membrane provided by TECH-SEP [Miribel, France]; molecular mass cut-off 150 kDa, inner diameter 6.0 mm, length 1.20 m, membrane area 1.67 m^2). Autolysed yeast extract (Biokar, Saint-Denis, France) and tryptone (Biomérieux, France) were both added at 10 $g \cdot L^{-1}$. The pH was adjusted to 7.0 with 10N NaOH before steam sterilization (120°C, 20 min) and subsequently adjusted to 6.0 before inoculation.

Growth Conditions

Two fermentors of 15 L (New Brunswick Scientific, New Brunswick, NJ) and 20 L, (Biolafitte, Saint Germain en Laye, France) were used. Cells were grown at 43°C under slow stirring. The pH of the broth was maintained at 6.0 ± 0.1 by the automatic addition of 10N NaOH.

Cell Suspension Concentration

Twenty liters of cells were centrifuged (7000g, 20 min, 20°C; Heraeus, Germany) at the end of the exponential growth phase at pH 4.3 ± 0.2. Cells were suspended in 1 L of the exhausted culture medium (same pH) to obtain a cell concentration of about 4×10^9 cfu $\cdot$ mL^{-1}, determined by optical density at 650 nm ($OD_{650\ nm}$) (see "Analyses" subsection).

Microfiltration

Experimental Rig

The MF rig described in detail by Daufin et al.[10] could be operated either at controlled permeation flux (J) or at controlled transmembrane pressure (TMP). The constant J was maintained during the experiment by a progressive decrease of the pressure in the permeate compartment through a permeate bleed valve (the mean retentate pressure, P_r, was maintained constant by the slit of the retentate pressure valve), so as to ensure a required increase in TMP owing to the increase of fouling. Thus both TMP and R_f described fouling. The pressure of the permeate compartment could be set as a static counterpressure (CPSTAT), or as a dynamic counterpressure (CPDYN) achieved by circulating the permeate co-current to the retentate to maintain an equal TMP profile along the filtering path.[37]

The rig was equipped with extra independent controls (retentate temperature [T], retentate tangential flow rate [v], mean retentate pressure [P_r]) to enable to master filtration procedures by setting variables to the desired values through any potential pathway. It allowed real-time data monitoring and storing by a computer through a multichannel analyzer (μMac 4000, Analog Devices, Norwood, MA)

Membrane

The rig was equipped with a single membrane tube, which is a composite membrane consisting of an α-alumina filtering layer on an alumina support (''Membralox'' membrane provided by SCT [Bazet, France]; inner diameter 7.0 mm, length 0.75 m, membrane area 1.54×10^{-2} m^2). The mean pore diameter of this membrane is 0.2 μm (manufacturer's information). It was previously demonstrated that this type of membrane has a pore size distribution between 0.06 and 0.21 μm, whatever method was used: permporometry biliquid or gas–liquid.[30] The same membrane was used for all experiments. Prior to the first one, the new membrane was conditioned (1.0% [v/v] HNO_3, 2 h, 70°C). The hydraulic resistance of this membrane using water was $R_m = 0.17 \pm 0.02 \times 10^{12}$ m^{-1} (mean of six runs).

Cleaning

Before each experiment the membrane was cleaned according to the sequence as follows:

(i) Alkaline cleaning with hypochlorite solution containing 1.0 g · L^{-1} active chlorine and pH adjusted to 11.0 using 10N NaOH (T = 50°C; 40 min, v = 7.0 m · s^{-1}, $P_r = 3.0 \times 10^5$ Pa, and J = 400 L · h^{-1} · m^{-2}).
(ii) Water rinse with 0.2 μm of filtered tap water after 5- and 2-μm filters in series until water pH was reached (≈10 min).
(iii) Acid cleaning with HNO_3 (0.03 moL · L^{-1}), pH 1.2 (same as in i).
(iv) Filtered water rinse (≈10 min) (same as in ii).

Rinsing and Water Flux

Before the experiment, and after the cleaning sequence, the water flux was measured for R_m calculation. The experimental conditions of the water flux measurement were: filtered tap water (0.2 μm); T = 43°C; v = 4.5 m · s^{-1}; and retentate pressure (P_r) = 2.1×10^5 Pa. TMP was measured at four J values in the range 0 to 300 L · h^{-1} · m^{-2}. At each given TMP value J was allowed to stabilize for 5 min before data were recorded.[21]

At the end of an MF experiment, the membrane was rinsed with filtered water at 43°C for 30 to 35 min by increasing v to the test tangential flow rate (6.0 m · s^{-1}) in three cycles, and water flux was measured, as indicated above, to calculate R_{if}.

Cleaning, rinsing, and water flux measurements were performed in CPSTAT or CPDYN according to MF experiments.

MF Experiments

MF experiments were performed at a constant temperature (43 ± 1°C), mean tangential flow rate (v = 6.0 ± 0.1 m · s^{-1}), and mean retentate pressure ($P_r = 3.5 \pm 0.1 \times 10^5$ Pa). The permeate was recycled so as to maintain a constant volume concentration ratio (VCR = 1) in the retentate compartment.

Five liters of suspension were prepared in advance and 2 L were used to flush the water out of the loop.

TMP = $f(J)$: The first experiment was performed with the CPSTAT mode during 176 min with an initial suspension concentration of $C_i = 14 \times 10^9$ cfu · mL^{-1}. After the water flush, v and P_r were set to their stationary values as already described in detail.[10,21] When v and P_r reached their corresponding values, TMP was first increased step by step up to a maximum value of 2.9×10^5 Pa, and then decreased gradually. At each given value of TMP, J was measured during 10 min (time previously evaluated to be close to the steady state). This experiment was aimed at assessing the operating conditions to be used in further experiments.

MF at constant stationary J preceded by various transient operating conditions: Transient operating conditions consisted of two permeation flux (J) increase regimes. During the''fast'' transient regime, J was increased (by gradual opening of the permeate valve) in a linear way to its set value of 130 L · h^{-1} · m^{-2} in 5 min, while, during the ''slow'' transient regime, J was increased to the same stationary value in 30 min.

MF performed at different stationary J: After the water flush, v, P_r, and the CPDYN mode of operation were set and regulated at the desired level. When the stationary val-

ues were reached, J was increased in 5 min to its set value, in a linear way.

Because the experiments were performed at various constant J values and could not be compared through TMP values, we decided to use R_f as a criterion for comparison. However, R_f depends on R_m, which can vary between experiments.[21] This is why the fouling evolution versus time was assessed by calculating the commonly accepted normalized value of R_f, R_f/R_m.

The combination of stationary tangential flow rate and permeation flux on MF performance was studied, with the following operating conditions: J = 25, 60, 83, 100, and 130 ± 1 L · h^{-1} · m^{-2}; v = 6.0 ± 0.1 m · s^{-1}.

The influence of concentration on MF performance was assessed with two cell concentrations: 1×10^9 cfu · mL^{-1} and 5×10^9 cfu · mL^{-1}. Stationary J was 130 L · h^{-1} · m^{-2}.

Reproducibility

It was shown that controlled operating conditions were reproducible with our rig[10]: standard deviation of permeation flux, $SD_J < 3\%$; tangential flow rate, $SD_v < 1\%$; and volume concentration ratio, $SD_{VCR} < 2\%$. Due to the well-characterized membrane resistance (R_m), the reproducibility of the evolution of fouling versus filtered volume was satisfactory: standard deviation of R_f and $R_f/R_m < 5\%$.[21]

Analyses

Total cell mass was determined by OD measurements (650 nm, Beckman, DU 7400, Gagny, France) correlated with cell concentration (r^2 = 0.99). Lactose was determined by HPLC (Waters Associates, Milford, MA) equipped with a refractometer: separation took place in a 7.7 × 300 mm stainless-steel column (μ spherogel carbohydrate, Beckman, Gagny, France) at 80°C with water as eluent (0.6 mL · min^{-1}). Lactic acid concentration was measured using an HPLC system equipped with a UV detector (214 nm). The ion-exchange column (6 × 300 mm, Aminex A_6, Biorad, Richmond, CA) was operated at ambient temperature with 0.01N H_2SO_4 (1 mL · min^{-1}) as eluent. Cell viability was evaluated after plating on the medium supplemented with Agar 15 g · L^{-1} (Difco) in Petri dishes and growth for 2 days at 43°C. Each given value was the average of six different determinations.

Density was determined using a density-meter (Haake DM48, Karlsruhe, Germany), and dynamic viscosity with a viscosimeter (Haake D8). All the cell slurries used during these studies had newtonian properties.

RESULTS

Cell Viability and Permeate Sterility

The cell viability was 100% after 170 to 230 min of MF run, but the number of cells decreased by a factor of 2 to 10 after 360 min of filtration. Longer runs (550 min) presented an even larger loss of viability: from 1×10^9 to 5×10^6 cfu · mL^{-1}.

Whatever the operating parameters, all the cells were retained in the retentate stream: the permeate samples withdrawn during the MF experiments were sterile after incubation at 43°C for 2 to 4 days.

Permeation Flux Versus Transmembrane Pressure

The evolution of permeation flux versus transmembrane pressure showed a hysteresis loop between increasing and decreasing TMP (Fig. 1).

At lower pressures, an increase of TMP (from 0.1 to 0.3 × 10^5 Pa) induced a sharp increase of J which reached 120 L · h^{-1} · m^{-2} for TMP = 0.3 × 10^5 Pa. The permeation flux was much lower than the pure water flux. At constant TMP below 0.3 × 10^5 Pa, J was stable with time. According to Figure 2, the normalized fouling hydraulic resistance, R_f/R_m was steady during that period: $R_f/R_m \approx 5$.

Above TMP = 0.3 × 10^5 Pa, for each TMP increment, J rose quickly up to a maximum value and then decreased with time. J was not further improved by TMP increase (Fig. 1): the almost-steady J value obtained at each TMP increment, after 10 min of filtration, was ~120 L · h^{-1} · m^{-2} (Fig. 1). R_f/R_m increased sharply from 5 to around 70 (Fig. 2).

When TMP decreased step by step from 2.9 × 10^5 Pa, a linearly decreasing flux was observed (r^2 = 0.99) (Fig. 1). During this phase, J remained stable in the course of time and was smaller than the value recorded during the phase of increasing TMP. R_f/R_m decreased slightly from 70 down to a final value of 60, which is 12 times higher than the R_f/R_m value obtained during the TMP increase (Fig. 2).

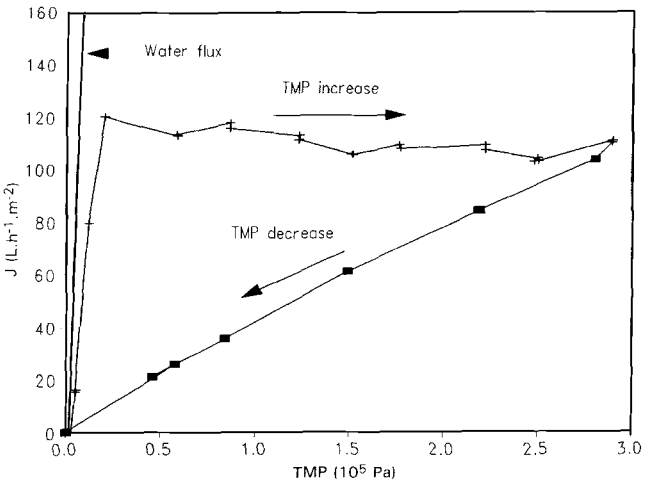

Figure 1. Permeation flux (J) versus transmembrane pressure (TMP). Operating conditions: $C_i = 1.4 \times 10^{10}$ cfu · mL^{-1}; v = 6.0 m · s^{-1}; $P_r = 3.5 \times 10^5$ Pa; T = 43°C. J were registered after 10 min of filtration at a given TMP value. Static mode operation (CPSTAT, see Materials and Methods).

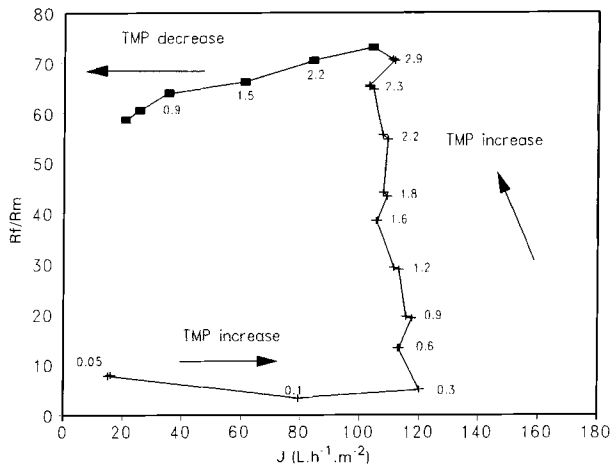

Figure 2. Normalized fouling hydraulic resistance (R_f/R_m) versus permeation flux (J). Operating conditions: same as Figure 1. TMP values ($\times 10^5$ Pa) are indicated for each TMP increment. J were registered after 10 min of filtration at a given TMP value.

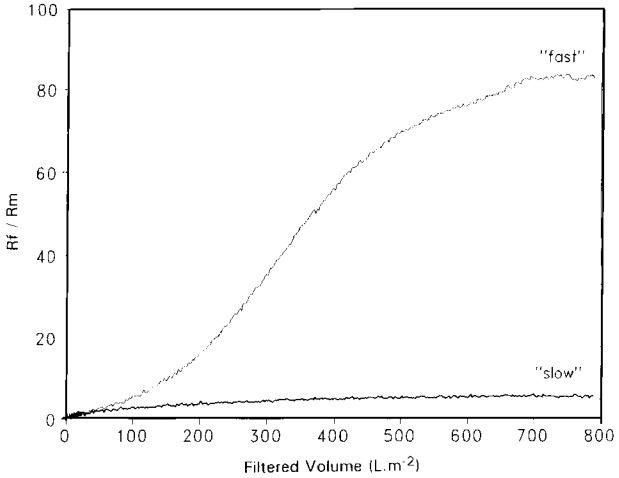

Figure 3. Normalized fouling hydraulic resistance (R_f/R_m) versus filtered volume for two transient regimes of permeation flux increase to J = 130 L · h^{-1} · m^{-2}. "slow": 5 min; "fast": 30 min. Operating conditions: C_i = 6 ± 2 × 10^8 cfu · mL^{-1}; v = 6.0 m · s^{-1}; P_r = 3.5 × 10^5 Pa; T = 43°C. Dynamic mode operation (CPDYN, see Materials and Methods).

At the end of this J versus TMP experiment, the reversible fouling, R_{rf}, represented 87% of the overall fouling hydraulic resistance and R_{if} = 1.55 × 10^{12} m^{-1}.

MF Performance Versus Time

Effect of Initial Cell Concentration

R_f/R_m was greatly influenced by the initial cell concentration, C_i. With the higher concentration (5 × 10^9 cfu · mL^{-1}) R_f/R_m reached ≈90 after 145 min of filtration, while at the same time, with a lower concentration (1 × 10^9 cfu · mL^{-1}), R_f/R_m was five times lower. With this latter experiment, filtration lasted more than 550 min. With both C_i concentrations, R_{rf}/R_f reached 96% at the end of these constant J experiments.

Effect of Transient Operating Conditions

Figure 3 shows the drastic difference of R_f/R_m evolution versus the filtered volume, between the two transient regimes' "fast" (5 min) and "slow" (30 min) J increase. With the "fast" procedure, R_f/R_m increased sharply while with the "slow" one fouling was small and slow. After 550 min of filtration the "fast" transient regime led to a final fouling 15 times higher than the "slow" regime.

At the end of the experiments, R_{if} values were of the same order of magnitude ("slow" regime: R_{if} = 0.31 × 10^{12} m^{-1}; "fast" regime: R_{if} = 0.47 × 10^{12} m^{-1}). R_{rf} reached 96% of R_f, when the overall fouling was higher (R_f = 11.30 × 10^{12} m^{-1} at the end of the experiment of the "fast" transient regime) compared with only 64% of R_f in the "slow" procedure (R_f = 0.86 × 10^{12} m^{-1}).

Effect of Stationary Permeation Flux

According to Figure 4, with J < 83 L · h^{-1} · m^{-2}, R_f/R_m was stable during the 6 h of filtration. Fouling increased slowly with J = 100 (b) and sharply with 130 L · h^{-1} · m^{-2} (a). Table I, which gives R_{rf} and R_{if} values for each experiment, shows that, for a similar filtration time, R_{if} values were higher for experiments with higher R_f whatever the filtered volume. R_{if} represented about two to five times the R_m values.

Values of R_f/R_m assessed at the end of each experiment, versus J/τ_w, showed a critical value of J/τ_w over which fouling increased drastically (Fig. 5). When J/τ_w < around 1.15 L · h^{-1} · m^{-2} · Pa^{-1}, R_f/R_m remained smaller and increased slowly with J/τ_w increase.

DISCUSSION

Cell Viability

Most of the experimental data on crossflow microfiltration of bacteria suggest little or no loss of viability.[25,27] Nonetheless, loss of 20% of viability was noticed during *Lactobacillus acidophilus* MF.[33] The cell viability could be impaired by zones of high shear stress (which depends on type of pumps, rig configuration,[23] and operating conditions) or modifications of the physico-chemical environment or with recombinant cells. It was previously shown that the shear stress alone is rarely involved in loss of cell viability when pH is controlled, even if the residence time is high: 1,000 h runs[7] or 25,000 passages in a gear pump[12] induced no cell death. Chan et al.[6] showed that a Reynolds number around 62,000 could induce *Escherichia coli* cell disruption after a residence time of 105 min.

In the retentate compartment of our experimental rig, there were high shear zones like the retentate pressure valve, which induced a high pressure drop through its small hydraulic diameter (around 3 mm); at the used retentate

Table I. Operating conditions and hydraulic resistances of experiments performed with various permeation fluxes.

	Experiment					Coefficient of variation
	a	b	c	d	e	(%)
Ci, 10^9 cfu.ml^{-1}	5	2	3	5	5	30
J, L.h^{-1}.m^{-2}	130	100	83	60	25	3
τ_w, Pa	72.5	74.5	73.0	72.0	70.0	4
J/τ_w, L.h^{-1}.m^{-2}.Pa^{-1}	1.78	1.35	1.15	0.84	0.36	7
Rm × 10^{12} m^{-1}	0.15	0.20	0.15	0.17	0.15	5
Final Rf × 10^{12}m^{-1}	14.00	12.20	0.53	0.45	0.25	5
Rif × 10^{12} m^{-1}	0.49	0.76	0.37	0.15	0.18	5
Filtration time, min	144	362	364	361	362	2
Filtered volume, L.m^{-2}	312	603	509	364	157	3

flow rate (780 L · h^{-1} to ensure 6 m · s^{-1}), the Reynolds number could be estimated to be higher than 132,000 which corresponded to a shear stress of 2.6 10^5 Pa. Because pH was not controlled during all our MF experiments, the acidification with the shear stress increased by residence time could be responsible for the cell death.

Fouling Mechanisms

According to the results presented above, fouling could be divided into two different layers: a reversible one, which is the main contributor to the overall fouling increase; and an irreversible one.

This irreversible phenomenon was prominent during the first few minutes of MF since R_f (observed with TMP $\leq 0.3 \times 10^5$ Pa [Fig. 2]) was constant regardless of the large flux increase. Irreversible fouling could be attributed simultaneously to the adsorption and entrapment of fermentation broth components, such as proteins, mineral salts, polysaccharides, cell debris (such as cell walls, nucleic acids, etc.), and cells.[8,34] Those components have been shown to be foulants of ceramic membranes.[32,34]

Because cells were totally rejected by the membrane, due to their size, compared to the largest pore size of the membrane (0.21 μm),[30] it is likely that reversible fouling was built as a cake of microbial particles accumulated at the membrane surface. Some molecules (such as proteins, peptides, salts, etc.) could induce concentration polarization and participate to the reversible fouling, but because the major parts of these molecules (residual lactose, organic acids, salts, and small peptides) passed through the membrane, this effect is assumed to be insignificant. The characteristics of the cell deposit depended on the J/τ_w ratio, where J governs the convective transfer toward the membrane and τ_w the erosion[2,41] and shear-induced diffusion[4,11,16] away from the membrane.

When R_{rf} ($= R_d = \alpha M$) increased, both α and M were supposed to increase. A cell cake is compressible when TMP increases.[3] Moreover, entrapment of medium compo-

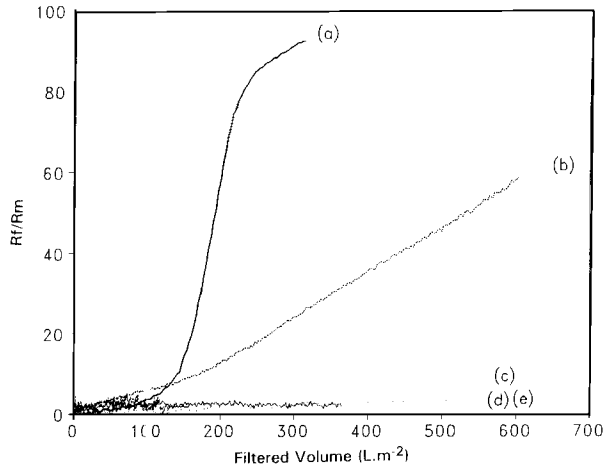

Figure 4. Normalized fouling hydraulic resistance (R_f/R_m) versus filtered volume for experiments performed with different permeation fluxes J (130, 100, 83, 60, and 25 L · h^{-1} · m^{-2}). (a)–(e): see Table I. Operating conditions: v = 6.0 m · s^{-1}; P_r = 3.5 × 10^5 Pa; T = 43°C. Dynamic mode operation (CPDYN, see Materials and Methods).

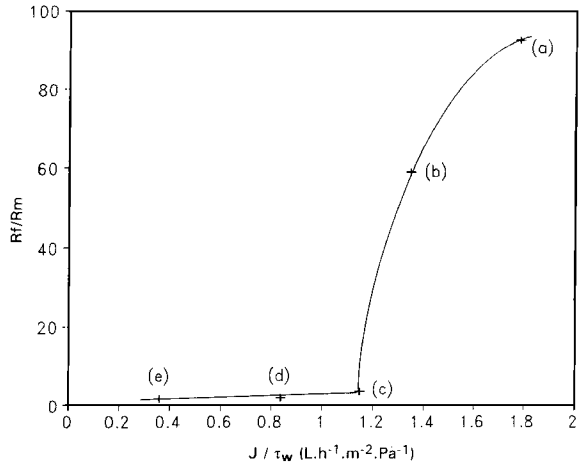

Figure 5. Normalized fouling hydraulic resistance (R_f/R_m) measured at the end of the experiment versus the ratio J/τ_w. (a)–(e): see Table I. Operating conditions: same as in Figure 4.

nents in the cake structure increases M^1, and decreases ϵ, leading to a further increase in α. The thicker and more disorganized deposit favored by the cell rod shapes[39] of *L. helveticus* allowed more macromolecules from the medium (and especially from the yeast extract) to be trapped and to compact the cake, leading to a fast fouling increase. Tanaka et al.[39] have shown, by scanning electron microscopy, that the *E. coli* cell layer close to the outer cake surface was oriented along the tangential circulation flow direction in crossflow filtration while close to the membrane, they were deposited at random. Our assumption is that the cell cake layer could be considered as a net which was more or less clogged by broth components depending on the hydraulic parameters of the crossflow filtration. A greater cell number reinforced the trends observed due to the enhanced amount of cells and solutes transferred to the membrane.

When TMP was decreased, R_d remained constant and likely did the deposit characteristics (α and M). This irreversibility could be explained by the fact that the cells are maintained in a consolidated state in such a way that they cannot be removed by erosion or shear-induced diffusion.

Effect of J/τ_w Ratio on the Structure of the Reversible Deposit

Transient J/τ_w *Ratio*

The transient procedure to set to the stationary J and J/τ_w altered the overall performance of the filtration device as already described.[15,35] With *Propionibacterium acidipropionici,* a start-up procedure with no permeation during 8 h allowed doubling of the steady state permeate flux.[8] Nevertheless, this point is not usually taken into account, the plants are often oversized, the filtration times are reduced, and the number of cleaning sequences are increased. These facts have large negative impacts on the economics of the entire process.

The "fast" J/τ_w transient regime quickly forced a large amount of cells toward the membrane, resulting in a disordered structure of the cell deposit.[20,36] With a high permeation flux, as compared with the erosion rate, Mackley and Sherman[31] observed that each particle that reached the filtering layer was stopped at its impact point. This supports the assumption that, with the "fast" transient regime, the convection forces are stronger and particles have less time and space to organize. The deposit composed of less dense structure would be then capable of entrapment of medium molecules.

On the contrary, the "slow" transient regime induced a more organized deposit. In a microbial suspension, smaller particles are preferentially deposited during crossflow filtration as predicted by the study of the forces acting on a particle as it approaches the membrane.[3,18] The deposited cell layer is more dense with a smaller capability of entrapping macromolecules or medium components in the cake structure.

Critical J/τ_w *Ratio*

Previous considerations regarding the role of J/τ_w during transient operating conditions may be taken into account for the interpretation of MF performance under stationary conditions as well. They bring about the explanation why there was a critical J/τ_w value (namely 1.15 $L \cdot h^{-1} \cdot m^{-2} \cdot Pa^{-1}$ in our experiments) below which tangential erosion overcame the convection of cells toward the membrane, resulting in small involvement of the reversible fouling in the overall fouling. At high values of J/τ_w (>1.15 $L \cdot h^{-1} \cdot m^{-2} \cdot Pa^{-1}$) operating time was sharply reduced owing to prominent convective arrival of matter compared with erosion. In MF of skim milk, which aims at separating casein micelles from whey proteins[28] and in MF of pretreated whey, which aims at clarifying whey,[22] fouling was reported to increase faster and MF operation time was dramatically shortened when J/τ_w was over a critical value at around 1.0 $L \cdot h^{-1} \cdot m^{-2} \cdot Pa^{-1}$. Why the critical values J/τ_w are so close together remains difficult to understand at the present time and will be further investigated.

CONCLUSIONS

To reach the best performance during such MF and to ensure a high cell viability, much attention has to be paid to pH control, to residence time, and to properly design the retentate compartment with no zone of high shear that might cause physical cell alteration.

During lactic acid bacteria microfiltration, the reversible fouling dominates the separation and its characteristics depend on J and τ_w. Consequently, the operating conditions must be carefully selected and controlled so as to ensure that J/τ_w is inferior to a critical J/τ_w value for transient conditions as well as for stationary conditions. Then, more efficient operation (lower fouling) and longer operating times will be achieved.

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NOMENCLATURE

C_i	initial concentration of the suspension ($g \cdot L^{-1}$)
d_h	hydraulic diameter of the membrane (m)
d_p	mean diameter of the particle (m)
J	permeation flux ($m \cdot s^{-1}$ or $L \cdot h^{-1} \cdot m^{-2}$)
L	length of the membrane tube (m)
M	mass of the deposit layer ($kg \cdot m^{-2}$)
OD	optical density
P_r	mean retentate pressure (Pa)
R	hydraulic resistance (m^{-1})
R_d	hydraulic resistance of the deposit (m^{-1})
R_f	overall fouling hydraulic resistance (m^{-1})
R_{if}	irreversible fouling hydraulic resistance (m^{-1})
R_m	membrane hydraulic resistance (m^{-1})
R_{rf}	reversible fouling hydraulic resistance (m^{-1})

SD	standard deviation
T	temperature (°C)
TMP	transmembrane pressure (Pa)
v	mean tangential flow rate ($m \cdot s^{-1}$)
VCR	volume concentration ratio

Greek symbols

α	intrinsic resistance of the deposit ($m \cdot kg^{-1}$)
ΔP	pressure drop along the membrane (Pa)
ϵ	porosity of the deposit
μ	dynamic viscosity (Pa · s)
μ_w	dynamic viscosity of the water (Pa · s)
μ_p	dynamic viscosity of the permeate (Pa · s)
ρ_p	density of the particle ($kg \cdot m^{-3}$)
τ_w	shear stress at the membrane wall (Pa)

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