Original paper

Bioprocess Biosyst Eng 25 (2003) 309–314
DOI 10.1007/s00449-002-0310-0

Filtration of a bacterial fermentation broth: harvest conditions effects on cake hydraulic resistance

M. Meireles, E. Lavoute, P. Bacchin

Abstract The hydraulic resistance of cakes formed during the ultrafiltration of *Streptomyces pristinaespiralis* broths has been investigated for different harvesting conditions. *S. pristinaespiralis* broth was harvested after the point of microorganism activity declines (0-h aged broth) and afterwards held for different durations of up to 16 h (16 aged broths). Aging behavior occurring between the end of microorganism activity and harvest was compared for different acidification procedures (pH) and the mechanisms for which the hydraulic resistance of the cake is affected by aging have been investigated. For broths harvested under conditions where the acidification is fixed at pH 2 or 3, hydraulic resistance associated with cake build-up is directly determined by the interactions between the cells. Holding broths beyond 5 h contributes to a release of a soluble component from the cell surface. Enhanced cell surface interactions then turn the cake structure into a more open one and reduce the specific hydraulic resistance. For broths harvested under conditions where the acidification is fixed at pH 4, hydraulic resistance associated with cake build-up is both determined by cell interactions and cell morphology. The cause of the increase in specific hydraulic resistance with aging is due to the binding of a soluble component released by the microorganisms, which decreases the cell surface interactions.

Keywords Ultrafiltration, Cake hydraulic resistance, Biotechnology, Harvest time

Nomenclature

A	membrane area (m^2)
J	permeate flux ($m^3\ m^{-2}\ s^{-1}$)
J_{wf}	pure water flux ($m^3\ m^{-2}\ s^{-1}$)
k_o	Kozeny constant
m	dry mass of cake ($kg\ kg^{-1}$)
ΔP	transmembrane pressure drop (Pa)
R_c	hydraulic resistance of cake (m^{-1})
R_m	hydraulic resistance of membrane (m^{-1})
S_v	specific surface area (μm^{-1})
t	filtration time (s)
V	permeate volume (m^3)

Greek letters

α	specific hydraulic resistance per wet cake mass ($m\ kg^{-1}$)
ϵ	void fraction of cell cake
μ	viscosity of permeate (Pa s)
ρ	density of wet cell cale ($kg\ m^{-3}$)

Received: 28 August 2002 / Accepted: 19 November 2002
Published online: 8 February 2003

M. Meireles (✉), E. Lavoute, P. Bacchin
Laboratoire de Génie Chimique, UMR 5503,
Université Paul Sabatier, 118 route de Narbonne,
31062 cedex Toulouse, France
E-mail: meireles@chimie.ups-tlse.fr
Tel.: +33-561-558162
Fax: +33-561-556139

This work was supported by an industrial research program (Aventis Pharma, Vitry-sur-Seine, France).

1 Introduction

Fermentation processes are increasingly involved in the production of high value bioproducts such as pharmaceuticals. Separation of cells from soluble broth components may be achieved by conventional methods (rotating filter, sedimentation and centrifugation) but such recovery techniques must account for the often-labile nature of the bioproducts, the generally low concentration levels and the complex composition of the media. Membrane filtration is increasingly used for separation and concentration of cells from a fermentation broth as it offers advantages over classical processes. One of the main factors that greatly affects the operating costs is the magnitude of the permeate flux that can be achieved. When filtering fermentation broth the permeate flux can be severely limited by membrane fouling and cake formation.

Marshall et al. [1] and Belfort et al. [2] have discussed some crucial factors that determine flux limitations owing to different mechanisms and nature of the fluid (particle or colloidal suspensions, macromolecular solutions). For the ultrafiltration of cells or microorganisms, both the build-up of a cell cake layer and internal fouling of the membrane by soluble broth components contribute to the hydraulic resistance of filtration. In some recent studies, critical factors for the cake layer hydraulic resistance such as cell morphology and particle surface properties have been identified. Nakanishi et al. [3] showed that filtration hydraulic resistance depends

on the size and shape of microorganisms, which indirectly determine the void fraction in the cake. For cross flow filtration, Tanaka et al. [4] revealed that rod-shaped particles orientate in the direction of flow. Cake filtration hydraulic resistance then appears to be higher than for cakes of ellipsoidal particles. McCarthy et al. [5] correlated the compressible nature of a cell cake to the aspect ratio of the cell present in the initial suspensions. Particle surface properties also play a role in hydraulic resistance to filtration. For submicron particles Bacchin et al. [6] described the effects of surface interactions on the rate of particle deposition in the cake. For cells, the link between the surface charge, cake morphology and cake hydraulic resistance has also been investigated by Ohmori et al. [7]. Interactions between cells and soluble broth components can also influence the hydraulic resistance to filtration. Because of the complex composition and the large amount of material in the fermentation broth, the nature of these interactions is very difficult to assess. Several authors [8, 9, 10] have provided evidence that for a mixture of protein and yeast cells, protein fouling of the membrane and total filtration hydraulic resistance are reduced in the presence of cells. This is attributed to the preventive effect of the cells towards both internal fouling and protein cake build-up on the membrane. However, for different types of mixture such as molasses and yeast [11], or protein and clay [12], the authors observe that the hydraulic resistance can be increased for the mixture or can depend on physicochemical conditions.

Broth harvesting conditions, including aging time, pH, temperature and stirring conditions, can influence both the morphology of cells and the release of intracellular components. It is well known that starving or damaged cells can release cell components. Recently Okanoto et al. [13] showed that exposure to nutrient poor broth during cell holding results in changes in filtration hydraulic resistance as a consequence of DNA release. Their hypothesis is that broth handling, in particular exposure to nutrient poor broth during cell holding/harvesting, may result in cell surface changes during aging. Changes in filtration hydraulic resistances were then attributed to the impact of DNA release on particle surface charge. Efforts have been made to determine which type of mechanism would thus contribute to an increase in filtration hydraulic resistance associated with a cake build-up. Very few studies have indeed investigated the effects of harvesting conditions on the aging behavior of a fermentation broth and the consequences in terms of an increase in fouling and the nature of fouling mechanisms. In this work we have investigated the effects of harvesting conditions on the filtration hydraulic resistance of *Streptomyces pristinaespiralis* broth. The objective was to determine whether holding broths in varying conditions, after the microorganism activity was stopped by an acidification of the media, would affect the filtration associated with a cake build-up. Additionally we aimed to determine whether the cause of any changes was the result of changes in the cell or was due to the release of components during this period of time.

2 Experimental

2.1 Fermentation broths

The fermentation broths used in this work were kindly provided by Aventis Pharma (CRP Vitry) from a *Streptomyces pristinaelis* strain. The seed culture medium contains raw matter from initial yeast cream and glucose. During the growth phase of the organism, 50% of sugar is absorbed by the biomass and 50% is degraded on CO_2 by respiration. An antibiotic is produced through a secondary mechanism. The rate of production is highest when the microorganism activity starts to decline. Fermentation is then stopped when the target product concentration no longer varies. This is achieved by fixing the pH in the fermentation tank around 4. (At the scale of industrial production, the control of pH in a fixed range is not trivial and sampling in the holding tank for different fermentations revealed that the pH value is actually in the range 2–4.)

The fermentation broth is then held at 17°C for a period, which depends on the availability of downstream separation steps. This holding time may last up to 16 h depending of downstream step availability (Fig. 1). The downstream process consists of several filtration steps designed to recover biomass and culture broth separately, which is ultimately refined. In this downstream process, the pH is maintained at a value of 3, which was shown to be an optimum between the target solubility, enzymatic stability and degradation. For this study, Aventis Pharma directly supplied fermentation batches from industrial 100 l reactors. Collected samples were filtered at 0 and 16 h "aging time" where "aging time" is the duration between harvest and filtration.

2.2 Filtration system

The filtration experiments were carried out using a 300 ml stirred cell (41.8 cm^2 filtration area) (Amicon, France) with 100,000 dalton mol. wt cut-off polyethersulfone membranes (Techsep, St Maurice de Beynost, France). Before first use, membranes were

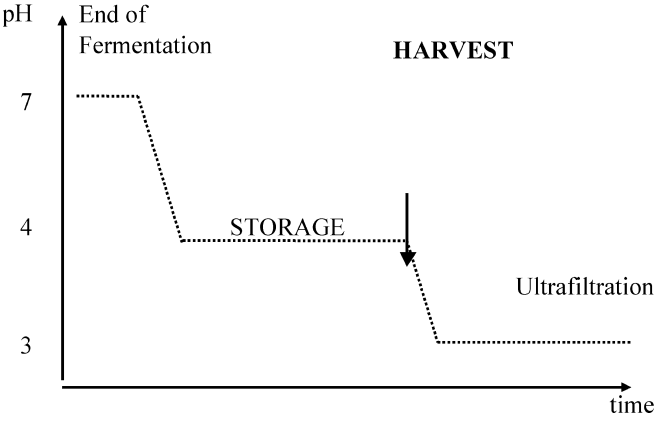

Fig. 1. Scheme of the first steps for downstream processing of the fermentation broth

rinsed with distilled water to remove any trace of preservatives. A compacting procedure was then carried out by filtration of distilled water for an hour at a transmembrane pressure of 2 kPa. This procedure is necessary to ensure that the membrane porous structure did not vary when filtration tests were carried out at transmembrane pressures lower than 200 kPa. Before each experiment, membranes were pre-fouled according to the following procedure: 300 ml of broth was concentrated three-fold at a transmembrane pressure of 100 kPa and was then kept in contact with the membrane for 12 h. This procedure was used to ensure that no interactions between soluble components and internal structure of porous membrane occur during the course of subsequent filtration. It was followed by the measurement of the flux of deionised water at ΔP = 100 kPa. Filtration tests were subsequently carried out according to the following procedures. Broth (150 ml) was introduced in the filtration cell and then filtration started. The experiments were conducted at constant room temperature (20°C) under constant pressure conditions by applying pressurized air (200 kPa). Cumulative permeate weight was recorded on a balance as a function of time and filtration flux was deduced from these variations. Filtration was stopped when cumulative permeate weight reached a value of 60 ml. Cleaning procedure was then performed (Ultrasil 10 8 g/l) at high temperature (30–35°C).

2.3
Optical microscopy

Morphological characteristics were observed with an optical microscope (Axiolab A 6 Reflected Light Microscope Zeiss, Zeiss) equipped with a video camera (Camera CCD-IRIS Sony, Sony Corp., Japan). Prior to each observation, broth samples were first centrifuged and then diluted with deionised water for correct spreading on slides. After air drying of the slides, Ziehl's carbon Fuschin was poured on the slide and rinsed with water for 1 min. Magnification of 1,000× was used with an oil-immersed lens.

2.4
Protein assays

Protein concentration of supernatants obtained from the centrifugation of broths samples achieved at 6,000 rpm and 4°C during 30 min (Refrigerated Centrikon, Kontron Instruments) was assayed by the Coomassie G-250 Protein Assay (Biorad, Germany) against serial dilution of a BSA standard.

2.5
Dry cell mass in slurry

To measure the dry cell mass in slurry, harvested broth samples were washed with 8 g/l Nacl. Washing was carried out by centrifugation at 4,000 g with three cycles of resuspending in approximately the original volume of 8 g/l Nacl. A dry cell mass in slurry was then obtained by drying at 105°C (Moisture Analyser HR 73, Metter Toledo, Switzerland). The dry cell mass in slurry is expressed in g of dry cell mass/g of suspension.

3
Effects of harvesting on filtration hydraulic resistance associated with cake build-up

Filtration theory was used to relate the rate of flux decline to the hydraulic resistance associated with the cake build-up. For dead-end unstirred filtration, it is generally assumed that the permeate flux at any time t, is described by Darcy's law for flow through two porous media [14] (cake and membrane) in series:

$$J = \frac{\mathrm{d}V}{A\mathrm{d}t} = \frac{\Delta P}{\mu(R_\mathrm{m} + R_\mathrm{c})}, \quad (1)$$

The membrane hydraulic resistance R_m can be obtained from the pure water flux through the pre-fouled membrane just before filtration, J_wf, as:

$$R_\mathrm{m} = \frac{\Delta P}{J_{\mathrm{w,f}} \cdot \mu}, \quad (2)$$

For a constant transmembrane pressure, integration of Eq. (1) gives:

$$\frac{t}{V/A} = \frac{1}{J_\mathrm{w}} + \frac{\mu R_\mathrm{c}}{2\Delta P}\left(\frac{V}{A}\right), \quad (3)$$

Figure 2 shows typical plots of $t/(V/A)$ versus V/A during filtration. Data analysis based on Eq. (3) assumes this plot to be linear. Some authors have reported that this plot was not always linear implying a differing period for the early cake build-up mechanism caused by the contact between broth components and the membrane material and a subsequent period caused by cake deposition. This was ruled out in this study as a static pre-contact procedure was used before filtration.

Table 1 summarizes the results from filtration runs in terms of cake hydraulic resistance R_c for a filtered volume of 60 ml. A specific nomenclature is used where for instance, the sample B2–5 is a broth held at pH 2 during a 5-h aging period. The comparison of data shows that the hydraulic resistance of the cake filtration is almost halved after a minimal aging time of 5 h for the broth held at pH 3, and 7 h for broth held at pH 2, whereas for broth held at pH 4, the hydraulic resistance decreases at an early stage and then increases up to 80% of the initial value.

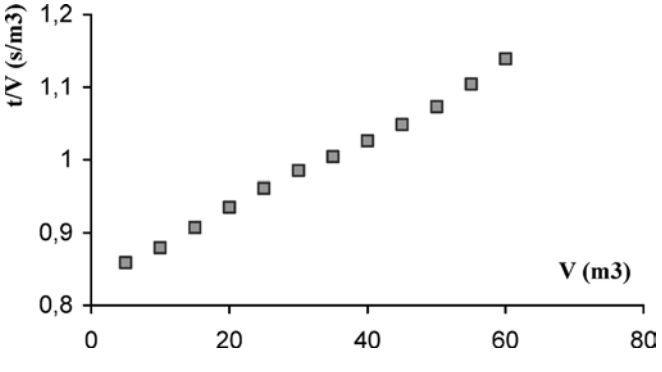

Fig. 2. Treatment of filtration data: example of a plot of $t/(V/A)$ vs. V/A during filtration of B2–5 broth. The transmembrane pressure is equal to 100 kPa

Table 1. Cake hydraulic resistances obtained from $t/(V/A)$ vs.V/A slopes or B2, B3 and B4 samples for different aging times

Sample filtered	Resistanceof the cake $R_c \times 10^{10}$ (m^{-1})
0 h aging sample	
B2–0	3.12
B3–0	2.04
B4–0	2.02
5 h aging sample	
B2–5	3.04
B3–5	1.90
B4–5	1.00
7 h aging sample	
B2–7	3.00
B3–7	0.70
B4–7	0.90
9 h aging sample	
B2–9	0.90
B3–9	0.68
B4–9	1.50
13 h aging sample	
B2–13	0.10
B3–13	0.62
B4–13	1.80
16 h aging sample	
B2–16	0.11
B3–16	0.64
B4–16	1.85

The decrease in hydraulic resistance associated with a cake build-up observed for broths aged at pH 2 or 3 or in the early stage for broth aged at pH 4 can be related to a change in the cell morphology, to a modification of the interaction between cells and broth soluble components or to a modification of soluble components during the aging process.

A change in the cell morphology is evident in the optical micrographs shown in Fig. 3. For the broth held at pH 4, micrographs show a drastic change from a structure of hyphae in pellets at 0 h (Fig. 3: broth B0–4) to a hatched and divided network at 16 h (Fig. 3: broth B16–4). Changes also appear for broths held at pH 2 or 3. However in these conditions, it is almost indisputably caused by a decompaction of the hyphae more than a lysis of the filaments. Long and tangled filaments are evidenced in micrographs of B2–16 and B3–16 broths. A modification of cell charge during aging might be an explanation for these changes. The protein assays of supernatants of the broths hold at different pH values show that the soluble protein concentration changes during the aging are most significant for broths aging at pH 4.0 (Table 2).

As pointed out earlier, the changes in protein content might be affected by cell starvation and damaged. Okamoto et al. [13] have shown that an exposure to

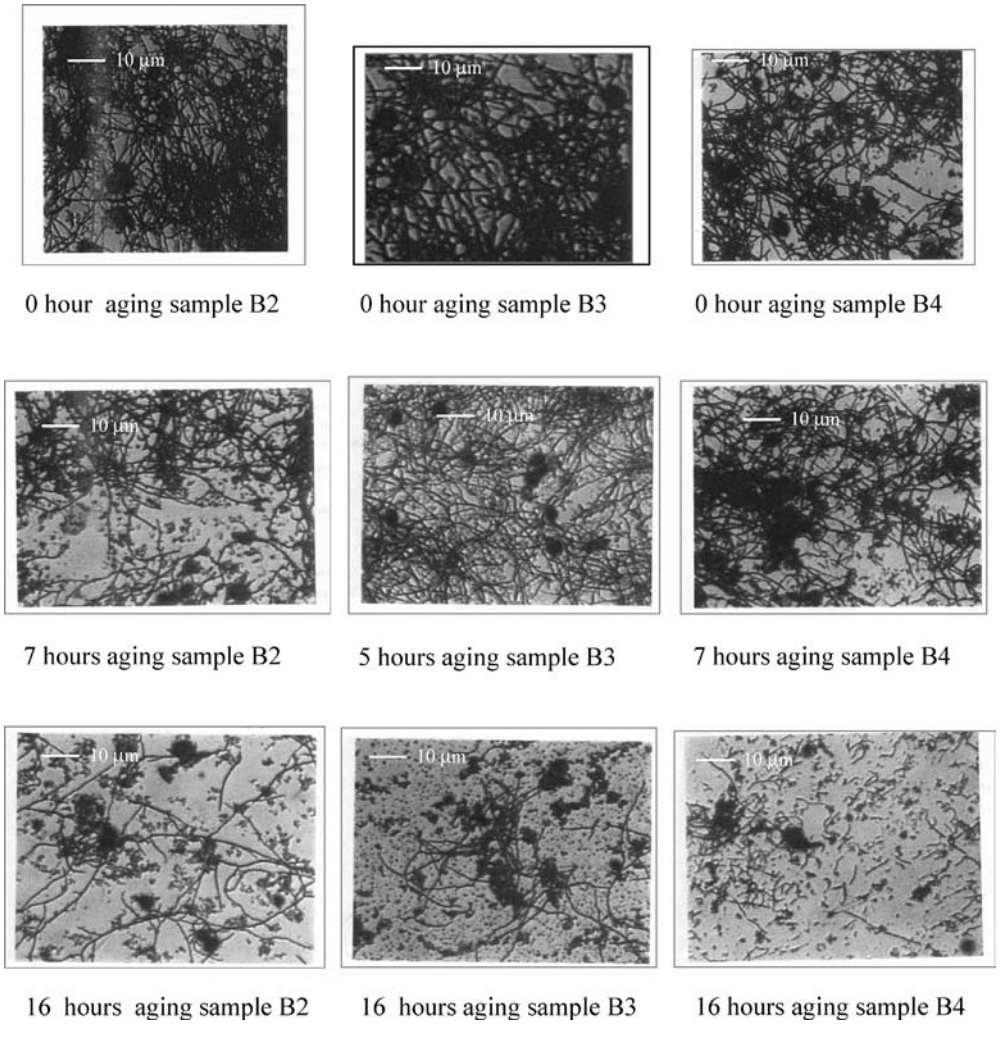

Fig. 3. Optical microscope images of broths samples for different procedures of acidification and for different aging times

Table 2. Soluble proteincontent for supernatants issued from B2–16, B3–16 and B4–16 broth

Sample	Protein contentin the supernatant (mg/ml)
16 h aging sample	
B2–16	0.21
B3–16	0.24
B4–16	0.42

Table 3. Specific area and void fraction of cake for B2–16, B3–16 and B4–16 broth

Sample	Specific area S_v (μm^{-1})	Void fractionε
16 h aging sample		
B2–16	8.1	0.36
B3–16	7.9	0.38
B4–16	8.0	0.45

nutrient poor broth during cell holding can induce changes in the broth components produced.

Based on our observations it is proposed that the changes in the cell during aging are largely responsible for variations in filtration hydraulic resistance associated with the cake build-up. At this stage of the study, we do not really have more information about the biochemical and physicochemical reactions leading to this cell change. However we observed for the conditions when there was no filament lysis, an abundance of yeasts in the aged broth whereas they were quite scarce when hatched filaments were observed in the broth. We propose an hypothesis that when broths are harvested and held at pH 4, biological activity is not completely halted and the aging process results in filament lysis accompanied by an increase in soluble protein content.

4
Mechanisms increasing cake hydraulic resistance

Based on our observations of the changes in the cell morphology and in the protein content during aging, one can consider different mechanisms for hydraulic resistance associated with cake build-up:

1. Alteration of cake structure by changing cell morphology or compaction of the hyphae.
2. Alteration of cake structure by deposition of soluble components within the cake.

An estimation of the impact of an alteration of cake structure on hydraulic resistance can be estimated through the Kozeny–Carman equation [15]:

$$\frac{R_c A}{\rho m} = \frac{k_0(1-\varepsilon)^2}{\varepsilon^3} S_v^2, \qquad (4)$$

where R_c is the hydraulic resistance associated with the cake build-up, k_o is the Kozeny constant commonly taken as 5, ρ is the density of cell assumed as 1,000 kg m^{-3}. The cake mass parameter, m, was calculated from dry cell weight in the broths (around 0.05 g/g on average) and from cumulative permeate weight (60 ml) for each experiment. S_v, the specific surface area of cell, and ε, the void fraction of the cake, have been determined for 16-h aged broths by Lavoute et al. (in preparation), Table 3.

From these data, Eq. (3) would predict for 16-h aged broths an hydraulic resistance of 2.09×10^9 m^{-1} for B2–16, 1.6×10^9 m^{-1} for B3–16 and 2.1×10^9 m^{-1} for B4–16 when experimental filtrations give respectively 1×10^9, 6.4×10^9 and 1.8×10^{10} m^{-1}. From these calculations, we might infer that the cell changes during aging can quantitatively account for the decrease in hydraulic resistance observed for B2 and B3 broths. The cause of the decrease in hydraulic resistance that occurs for B2 and B3 broths held beyond 5 h might be determined by a change in the interaction between the aged cells. Release of a component from the cell surface after 5 h of aging would enhance the magnitude of charge repulsion between cells and as a consequence decrease the specific hydraulic resistance of B2- and B3-aged broths. For broth aged at pH 4, the value predicted for the cake hydraulic resistance is much lower than those obtained from the experiments. Even though a change in cell morphology occurs, the void fraction of the cake is still too low to quantitatively account for the increase in hydraulic resistance after 7 h of aging. As pointed out earlier, changes in soluble protein content are most significant for these conditions. Alteration of cake pore structure can thus be promoted by soluble component deposition within the cake. Assessment of Eq. (3) for the void fraction that would justify cake hydraulic resistance obtained from B4–16 gives a value of 0.15. However the amount of soluble component is too low to account for a 30% decrease in void fraction by protein deposition. An alternative explanation would be a modification of interactions between cell and a soluble component binding at the cell surface not present at the earlier stage of aging. This is supported by the fact that the lysis can induce the release of different components than those initially present in the broths. Reduced magnitude of cell surface charge caused by soluble component binding increases hydrophobicity and decreases charge repulsion predisposing cells to further aggregation [7]. If the aggregation state changes with aging through the increase in soluble protein content, it is possible that no changes have been observable by optical microscopy in the suspended broths since the ratio cell density over protein content is low. Scanning electron microscopy of cakes could clear that point. However, assessment of how the preparation of samples, including how solvent exchange and sputter coating should be handled, would be required to ensure that no changes in cake structure occur during sample preparation.

5
Conclusions

For broths harvested in conditions where the acidification is fixed at pH 2 or 3, hydraulic resistance associated with cake build-up is directly determined by the cell interactions. Holding broths beyond 5 h contributes to a decrease in specific hydraulic resistance. This decrease could then be related to a release of a soluble component from the cell surface. Enhanced cell surface interactions would then decrease and turn the cake structure into a more open one with a reduced hydraulic resistance.

For broths harvested in conditions where the acidification is fixed at pH 4, hydraulic resistance associated with cake build-up is also determined by both cell interactions and cell morphology. Optical microscopic observations give evidence that aging directly affects cell morphology through a lysis for broths held beyond 7 h. However, this does not contribute to a decrease in cake void fraction compliant with the order of magnitude for hydraulic resistance. The cause of the increase in hydraulic resistance with aging is most likely due to the binding of a soluble component released by the microorganisms that decreases the cell surface interactions.

References

1. Marschall AD, Munro PA,Trägardh G (1993) The effect of protein fouling in microfiltration and ultrafiltration on permeate flux, protein retention and selectivity:a literature review. Desalination 91:65–108
2. Belfort G, Davis RH, Zydney AL (1994) The behavior of suspensions and macromolecular solutions in cross flow microfiltration. J Membr Sci 96:1–37
3. Nakanishi K, Takadoro T, Matsuno R (1987) On the specific hydraulic resistance of cakes of microorganisms. Chem Eng Commun 62:187–201
4. Tanaka T, Abe K, Akasawa H, Yoshida H, Nakanishi K (1994) Filtration characteristics and structure of cake in cross flow filtration of bacterial suspension. J Ferment Bioeng 78:455–461
5. McCarthy AA, O'Shea DG, Murray NT, Walsh PK, Foley G (1998) Effect of cell morphology on dead end filtration of the dimorphic yeast *Kluyveromyces marxiamus* var.*marxiamus* NRRLy2415. Biotechnol Prog 14:279–285
6. Bacchin P, Aimar P, Sanchez V (1995) Model for colloidal fouling of membranes. AIChE J 53:223–228
7. Ohmori K, Glatz CE (1999) Effects of pH and ionic strength on microfiltration of *C. glutamicum*. J Membr Sci 153:23–32
8. Arora N, Davis RH (1994) Yeast cake layers as secondary membrane in dead end microfiltration of bovine serum albumin. J Membr Sci 92:247–256
9. Davis RH, Kuberkar VT (1999) Effects of added yeast on protein transmission and flux in cross flow membrane microfiltration. Biotechnol Prog 15:472–479
10. Güell C, Czekaj, Davis RH (1999) Microfiltration of protein mixtures and the effects of yeast on membrane fouling. J Membr Sci 155:113–122
11. Tanaka T, Kamimura R, Fujiwra R, Nakanishi K (1994) Crossflow microfiltration of yeast broth cultivated in molasses. Biotechnol Bioeng 43:1094–1101
12. Causserand C, Jover K, Aimar P, Meireles M (1997) Modification of clay cake permeability by adsorption of protein. J Membr Sci 137:31–44
13. Okamoto Y, Ohmori K, Glatz CE (2001) Harvest time effects on membrane cake hydraulic resistance of *Escherichia coli* broth. J Membr Sci 190:93–106
14. Darcy H (1856) The public fountains of the city of Dijon. Hist I'Acad Roy Sci 1733, 351
15. Carman PC (1938) Fluid flow through a granular bed. Trans Inst Chem Eng 15:150–167