DEPENDENCE OF SACCHAROMYCES CEREVISIAE FILTRATION THROUGH MEMBRANE ON YEAST CONCENTRATION

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Abstract: Filtration of baker's yeast in an isotonic solution through a 0.45 micron membrane was investigated for yeast concentrations in the range 0.14 - 51 g/L at filtration pressures between 40 and 80 kPa. Yeast filtration through membranes depends on applied filtration pressure and on slurry concentration. It was found that for a yeast volume fraction in suspension above 0.06 the porosity of the yeast cake becomes weakly dependent on the suspension concentration. For highly diluted suspension the specific cake resistance approaches to the minimum value, which is sensitive to the filtration pressure. Correlation functions of the cake porosity and specific cake resistance were obtained in the investigated concentration range. It was found that the Kozeny-Carman coefficient is increased with increasing applied pressure. Both filtration pressure and slurry concentration can be subject of process control. In the range of moderate yeast concentration, manipulation of filtration pressure and of slurry concentration might increase the filtrate flux. Obtained results and assumptions made indicate that complex behavior of yeast cake at high slurry concentration can be further described within the framework of conventional model by increasing complexity of subsystems due to aggregation effect.

Keywords: membrane filtration, cake resistance, concentration, yeast

1. Introduction

Cell filtration from the liquid phase is widely spread in biotechnology, food and beverage industry, wastewater treatment, and medicine. In industry, bio-suspensions are characterized by is a broad range of cell concentrations.

Relationship of slurry concentration and specific cake resistance is referred for different suspended materials [1-6]. Because of the variety of solid phase properties, there is no general model to describe cake resistance vs. concentration, especially for bio-suspensions, where cells are compressible and differ in shape [7-12].

Among bio-suspensions, yeast suspensions are particularly important [13-15]. As yeast cells are similar in shape, the experimental dependence of cake resistance on biomass concentration was obtained. This dependence may be useful for filtration process control.

2. Background

Filtration law, cake hydraulic resistance, mode of interaction with filter media are depending on the physical and chemical properties of a bio-organic matter and may serve as information source for further process control [16].

For the cake filtration the relation between the filtrate flux J and specific filtrate volume can be written as

$$J = dq/dt = \frac{\Delta p}{\mathbf{m}[\mathbf{a}(c_m) \cdot x_m(c_m)q + R_m]}$$
(1)

where q is a specific filtrate volume, filtrate volume per unite of filtration area, m^3/m^2 ; t is the filtration time, s; Δp is the filtration pressure, kPa; **m** is the liquid viscosity, Pa \times s; $\mathbf{a}(c_m)$ is the specific cake mass resistance, m/kg; c_m is the solids mass volume fraction in suspension; $x_m(c_m)$ is the ratio of solid mass in the cake to the filtrate volume; R_m is the filter medium (membrane) resistance, 1/m. In equation (1) at least two parameters a and x_m depend on slurry concentration but, in addition, the filter medium resistance may be dependent on the concentration variation even if the size of suspended particles is larger than the membrane pore size. Based on micro filtration of a mono-dispersed suspension of latex particles, [17] determined that the fraction of unblocked pores increases with the solid concentration.

Using a membrane micro filter, Chang and Lee [18] investigated the influence of the physiological behaviour of an activated sludge on their separation process. Filtration probe method was used for monitoring and control antibiotic fermentation process [19]. Investigation of filtration properties of mycelia microbial broths showed that three parameters - ratio of cake mass to the filtrate volume, compressibility index, and Kozeny constant – correlate with the mycelium slurry age and morphology [20].

Culture broths of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* were filtered through Gelman 0.45-micron membrane at pressures below 200 kPa. Cells were characterised by the mean cell aspect ratio, L_{dm} , defined as the mean ratio of length to the equivalent cylindrical diameter [14,15,21]. Correlations of L_{dm} vs. cake porosity and specific cake resistance were found.

Dead-end filtration method is often applied to estimate specific cake resistance in crossflow filtration. This method gives reasonable data for spherical and ellipsoidal-shaped cells, [9]. The advantage of the micro filtration method over cross-flow filtration test is that less segregated cake is formed by cells in comparison with cake formed in presence of a tangential velocity vector [12,13,22,23]. Dead-end filtration method has often served as standard procedure [24-27] and was used in the present work.

Role of slurry concentration in cake resistance. As was mentioned above, different dependences of cake resistance on concentration may be observed for different suspended matters. In particular, filtration of activated sludge in concentration range $5 - 50 \text{ kg/m}^3$ gave a linear dependence of a complex $(\mathbf{a} \cdot x_m)$ on solids volume fraction \mathbf{f} meaning a constant \mathbf{a} [3]. Here x_m is a ratio of solid mass in the cake to the filtrate volume, kg/m³, and \mathbf{a} is a specific mass cake resistance, m/kg.

For calcium carbonate the cake resistance was proportional to $(c_m)^{0.746}$ [2], whereas for a suspension of CaSO₄ the dependence **a** vs. concentration attains the maximum [4].

Relationship of filtrate flux and biomass concentration was observed for yeasts and bacteria by [28,29]. Authors [30] reported a permeability coefficient declining with cell concentration increasing in micro filtration of *B. polymyxa* broth through membrane.

For cultivated baker's yeast it was determined that a steady-state cross-flow filtration flux is related with yeast concentration as $\sim c^{-0.5}$ [8], where *c* is the yeast concentration, kg/m³. In turn, Schluep and Widmer [31] found that the specific cake resistance of *S. cerevisiae* with increasing yeast volume fraction **f** in suspension remained constant.

Authors [29] investigated filtration of *S. cerevisiae* through a tubular ceramic membrane with periodic back-flush membrane regeneration. In the range of yeast slurry concentration 5 - 80 g/L the optimum concentration was observed at 16 g of yeast /L where the mean flux increased about 2.5 times.

This brief overview shows the complicated nature of the cake resistance even in the case of a similar suspended matter.

In general, depending on a suspension type, the increase in slurry concentration may give rise to a decrease, to an increase or to a functional variation in the cake resistance [4,5,32]. A generalised empirical dependence of the specific cake resistance on solids mass fraction may be written in the form [33]

$$a(c_m) = a_0 + b_0(c_m)^k \exp(d_0 c_m)$$
(2)

where a_0 , b_0 , d_0 , and k are empirical coefficients. In many cases it is possible to assume $a_0 = 0$ and/or $d_0 = 0$ but other coefficients must be determined based on experimental dependence of **a** vs. c_m .

The purpose of this work is a more detailed investigation on yeast micro filtration in order to understand the influence of yeast concentration on the cake specific resistance and porosity.

3. Materials and Experimental Procedure

Commercially available *Saccharomyces cerevisiae* (baker's yeast) re-suspended in an isotonic solution of NaCl was used as model system [12]. The yeast cells were characterised by a narrow size distribution, with a measured average cell size of 5.8 microns, and spheroid shape, Figure 1.



Figure 1. View of baker's yeast suspended in isotonic solution used in experiments.

The studied range of yeast slurry concentration, c, was from 0.14 up to 51 g (yeast dry weight) per litre of isotonic solution.

The yeast volume fraction in the slurry f is a more representative parameter instead the mass-volume concentration c. The relationship between f and c was found to be

$$\mathbf{f} = (1 + 341.76/c)^{-1} \tag{3}$$

The measured f and calculated values using equation (3) are in good agreement (Figure 2).



Figure 2. Yeast volume fraction in suspension f vs. yeast concentration c, g (dry weight)/L, for baker's yeast in isotonic solution.

The yeast filtration properties vs. slurry concentration were measured by filtration in a cylindrical 32 mm diameter filter unit with a supported *Gelman* membrane (pore size 0.45 micron). Filtration pressure was constant during the filtration test. The yeast filtration properties were measured at filtration pressures of 40 and 80 kPa.

Values of the filtration pressure were chosen based on experimental conditions reported in the above mentioned publications for *S. cerevisiae* as well as on the value of the so-called critical transmembrane pressure when further pressure increase does not affect permeate flux, [34].

The membrane pore size was smaller than the cell size and filtration follows the cake formation law. Therefore, the specific cake mass resistance a was determined using a linear function derived from equation (1) [27]:

$$t/q = Aq + B \tag{4}$$

where $A = \mathbf{n} \cdot \mathbf{a} \cdot x_m / (2\Delta p)$, and $B = \mathbf{n}R_m / \Delta p$. For *Gelman* membrane, measured R_m was $1.2 \cdot 10^{10}$ 1/m what is close to referred by [21], $R_m = 1.7 \cdot 10^{10}$ 1/m.

As an example, the dependence of t/q on q for $\Delta p = 40$ kPa is shown in Figure 3. The line slope corresponds to coefficient A. Then, if the parameter x_m is known, the specific cake resistance **a** may be obtained.



Figure 3. Dependence of t/q on q for different yeast concentration c. Applied filtration pressure is 40 kPa.

The ratio x_m of cake solid mass to the filtrate volume was calculated by a formula mentioned in [27]:

$$x_m = c_m \mathbf{r} / (1 - mc_m) \tag{5}$$

where c_m is the solid mass fraction in the slurry, kg/kg, \mathbf{r} is the liquid phase density, kg/m³, and m is the ratio of wet cake mass to the mass of solid in the cake. Parameter m may be represented in the form $m=1/c_{mc}$, where c_{mc} is the maximum solid mass fraction in the cake. The small difference between real cell density in isotonic solution and the solution density allows assuming $c_m \approx \mathbf{f}$ [8].



Figure 4. Dependence of x_m and m on yeast volume fraction f for filtration pressure 40 and 80 kPa. 1 – Equation (5) when m = 1.4; 2 – Equation (5) when m = 1.2; and 3 – Linear approximation by equation (6).

Dependences of x_m and m on the yeast volume fraction in the slurry are given in Figure 4. The ratio m depends on filtration pressure because of cake compressibility as well as slurry concentration. Decreasing of m with increasing f means denser cell packing in the cake for higher f. Nevertheless, due to the low sensibility of relation (5) to the parameter m in the range of m = 1.2 - 1.4, dependence x_m on f is not much different for lower (m = 1.2) and higher (m = 1.4) limits for both filtration pressures in the investigated concentration range (Figure 4). For f < 0.06 the relationship x_m vs. f may be approximated by the linear dependence:

$$x_m = 1109.1 \cdot \mathbf{f} \tag{6}$$

For a slurry concentration f > 0.06 - 0.07, equation (5) is preferable because of the growing deviation from linear approximation described by eq. 6. Cake porosity data may give more precise information about parameter m.

4. Results and Discussion

4.1. Cake porosity

The cake porosity is an important characteristic of the filtration process. Results obtained experimentally for cake porosity are shown in Figure 5 together with some published values. As may be seen from the graph, the cake porosity depends on concentration and filtration pressure. Cake has denser packing when slurry concentration f increases. Two regions of the cake porosity behaviour are seen in Figure 5: the region of intensive decrease in porosity for low slurry concentration, and the region of quasi-constant porosity when f > 0.06.



Figure 5. Dependence of the cake porosity e on volume fraction of yeast f for filtration pressure 40 and 80 kPa. Points – experiment, Curves 1 and 2 – approximation by equation (7). Arrows: A – value from [10], B – adopted from [21].

For both filtration pressures the dependence of e on f is described by the following fitting function

$$\boldsymbol{e} = 0.23 \operatorname{arctg}\left(\frac{b_1}{\boldsymbol{f} + 0.03}\right) + c_1 \tag{7}$$

where for $\Delta p = 40$ kPa, $b_1 = 0.00905$; $c_1 = 0.22$, and for 80 kPa $b_1 = 0.0105$; $c_1 = 0.2$. Application of other functions, for instance exponential, gives significant deviations in the modelling of **a** at **f** > 0.02.

Cake is more compact for higher filtration pressure because of a cake compressibility effect. Cake compressibility was determined through the cake resistance as a function: $a = a'(\Delta p)^n$ where a' is a coefficient, and n is a compressibility index. For the range of filtration pressures 40 - 80 kPa the average value of the obtained compressibility index n = 0.7 coincides with those measured by [8] for cultivated baker's yeast.

4.2. Specific cake resistance

Conventionally, if the pore size is assumed to be constant, the increase in specific cake resistance results in porosity decrease [34]. For the estimation of specific cake resistance,

Kozeny-Carman relationship is widely used [7,8] in the form [31,35,36]: $\mathbf{a} = 180(1 - \mathbf{e})/(\mathbf{r}_c d_p^2 \mathbf{e}^3)$, where number 180 is a coefficient for granular bed packing, \mathbf{r}_c is the solid phase density, and d_p is the particle diameter. In detail, the specific cake resistance may be written as

$$\boldsymbol{a} = \frac{36K \cdot (1-\boldsymbol{e})}{\boldsymbol{r}_c d_p^2 \boldsymbol{e}^3} = \frac{36K_0 T^2 \cdot (1-\boldsymbol{e})}{\boldsymbol{r}_c d_p^2 \boldsymbol{e}^3}$$
(8)

where *K* is the Kozeny-Carman coefficient that includes a pore tortuosity *T* and a coefficient K_0 related with pore (particle) shape and dependent on porosity [12,21,37-39]. For granular beds *K* is assumed to be constant and around K = 4.2 - 5.0 due to the relatively narrow range of granular bed porosity. However, for yeast cells this parameter is variable [12,39].

The yeast specific cake resistance demonstrates both dependencies on filtration pressure and slurry concentration as shown in Figure 6 (points marked with arrows were borrowed from [15]). We observe an increase of a with f growth. However, this dependence is complex and experimental data were fitted with two different approaches, as seen in Figure 6:

1) Fitting experimental data by formula (2), curves 1 and 2;

2) Application of equation (8) with incorporated fitting function (7), curves 3 and 4.

For the full range of investigated slurry concentration, equation (2) may be represented as the function

$$\boldsymbol{a} = a_0 - 1.7 \cdot 10^{11} \exp(-43 \cdot \boldsymbol{f}) \tag{9}$$

with $a_0 = 3.2 \cdot 10^{11}$ and $4.3 \cdot 10^{11}$ m/kg for $\Delta p = 40$ and 80 kPa, respectively (Figure 6, curves 1 and 2). When $\mathbf{f} = 0$ according to the equation (9) we have, respectively, $\mathbf{a}_0 = 1.5 \cdot 10^{11}$ and 2.6 10^{11} m/kg.



Figure 6. Dependence of the yeast specific cake resistance **a** on slurry concentration f. Filtration pressure 40 and 80 kPa. Points are experimental data, where arrows mark values borrowed from [15]. Curves 1 and 2 correspond to function (9), obtained from formula (2); 3 and 4 are calculated by equations (8) and (7). Curves 5 and 6 are calculated by equation (8) and (7) assuming tortuosity variation.

Both filtration pressure and slurry concentration can be process controlled. In the range of moderate yeast concentration, manipulation of filtration pressure and slurry concentration may reduce the cake resistance.

Equation (9) fits satisfactory to experimental data. It must be admitted that the volume of filtered slurry must be large enough to form a cake on the membrane even for small f. Coefficient a_0 corresponds to specific cake resistance for infinitely diluted suspension, depends on filtration pressure, and has significant contribution in correct fitting procedure. Therefore, the boundary condition $a_0 > 0$ is important for the modelling of diluted suspension long run filtration.

Application of equation (8) with incorporated fitting function (7) is useful for verification of the permeability k and Kozeny-Carman coefficient K dependences on concentration and filtration pressure (curves 3 and 4 in Figure 6).

The following average values of the Kozeny-Carman coefficient were obtained for low and moderate yeast concentration: $\Delta p = 40$ kPa, $36 \cdot K = 204$, hence, $K \approx 5.67$; $\Delta p = 80$ kPa, $36 \cdot K = 272$, and $K \approx 7.55$ [12]. As can be seen, the coefficient K has a tendency to increase with increasing applied filtration pressure. In the range of moderate concentrations model (8) deviates from experimental values of **a**. One of the reasons for the observed effect is the tortuosity dependence on cake porosity [39]. Conventionally, relationship of tortuosity T and **e** is represented by $T = 1/e^n$, where n = 0.4 - 0.5 for spherical particles. In this case, the specific cake resistance (8) takes the form $\mathbf{a} = \{72 \cdot (1-e)\}/(\mathbf{r}_c d_p^2 e^{3+2n})$ (K_0 assumed to be 2.0) and is shown in Figure 6 as curves 5 (n = 0.5) and 6 (n = 0.4). It is interesting to admit that a large n value for higher filtration pressure might be related with the squeezing of some cells which block some pores. If this happens, then the flow pathway increases and the relation T vs. **e** is stronger. Nevertheless, starting from moderate concentrations the model overestimates the cake resistance.

The gradual increase in concentration increases the gap between predicted and measured a (Figure 7); this result allows speculating that lower experimental a is the result of the process whose description follows.



Figure 7. Deviation of predicted a_{mod} from experimental a_{exp} vs. concentration f. Dashed lines correspond to the investigated range of concentration where maximum deviation from experiment $a_{mod} / a_{exp} \approx 1.4$.

Increasing suspension volume concentration leads to increase a probability of cell aggregation. After being incorporated in the cake, some aggregates remain aggregated, even when they are subject to squeezing (see Figure 8, sketch).

The effect of interaction energy on floc structure was modeled by Cohen [40] who found that the influence of a dimensionless energy of particle interaction on floc structure decreases with decrease in the aggregate size. Moreover, relatively small aggregates with particle number $N \le 12$ have high probability to build dense packing.

Investigation of aggregation and flocculation [41-43] shows that flocs have fractal structure and their porosity in general is defined as $e = 1 - (N)^{\frac{d_F - 3}{d_F}}$, where d_F is the floc fractal dimension. For instance, it was found for activated sludge $d_F = 2$, hence, $e \propto 1 - N^{-0.5}$ [41]. In the work [42] the upper limit of d_F is given as 2.8. Simulation of aggregation confirms that the fractal dimension of aggregates and, hence, their compactness increases with increasing suspension concentration [43].

Therefore, in our case, overlapped cells in the cake may be considered, in the first approach, as a quasi-particle of diameter d_{ps} with volume equal total aggregate volume $d_{ps} = N^{1/3}d_p$, where N is the cell number in the aggregate. Two dimensional simulation was done to confirm this assumption. The resulting graph is shown in Figure 8: in the concave part of the curve, marked by an arrow, a significant increase in probability of cells aggregation was observed. Simulated and experimental profiles of the dependence e on f are quite similar. We may therefore assume that aggregated particles may function a bigger particles and work with a binary packing model [39,44,45].



Figure 8. Sketch of 2-dimensional (2-D) cake build up simulation: 2D porosity e_{2D} vs. 2D concentration f_{2D} (curve) and sketch of overlapped cells (grey) distributed within the cake in the region of packing porosity marked by arrow.

Binary packing model considers the cake as a binary particle mixture of two sizes: d_p and $d_{ps} = N^{1/3}d_p$, so average particle diameter in the cake is, Figure 9a,

$$d_{av} = \{x_{ps} / (N^{1/3}d_p) + (1 - x_{ps}) / d_p\}^{-1}$$
(10)

where x_{ps} is the fraction of *N*-agglomerated cells in the mixture. For simplicity we shall ignore the aggregate porosity.

Yeast cells in aggregates display denser packing than flocs and fractal dimension approaches 3.0. The value of d_F in the range 2.7 – 2.8 [42] looks reasonable for yeast aggregates (Figure 9a), where the dashed line corresponds to the cake porosity level at $\mathbf{f} \sim 0.06 - 0.16$ and respectively, N is above 7.0.



Figure 9. Role of aggregation in specific cake resistance. (a) Effect of aggregate's fractal dimension and N on aggregate porosity. (b) Dependence of $(d_{av}/d_p)^2 \sim a_{mod}/a_{exp}$ on the volume fraction of aggregates in cake x_{ps} and degree of aggregation N.

If the aggregation effect includes part of the dispersed phase for $\mathbf{f} \sim 0.06 - 0.16$, the overall porosity \mathbf{e} stabilization may be observed due to interplay of the following factor s (we limited this discussion to $\mathbf{f} \sim 0.06 - 0.16$):

- 1. The probability of obtaining dense packed floc decreases with increase in concentration [40]. In reality we have distribution of flocs by size, density and, therefore, by porosity.
- 2. Considering aggregates as large particles means that packing porosity decreases with increasing d_{ps} but simultaneous increasing in d_{av} leads to slowing down the increase in cake resistance.

The dependence of $(d_{av}/d_p)^2 \sim \mathbf{a}_{mod}/\mathbf{a}_{exp}$ on the volume fraction of aggregates in cake x_{ps} and degree of aggregation N is shown in Figure 9b, where dashed lines corresponds to the cake resistance deviation at $\mathbf{f} \sim 0.14$. Estimations made are within the range of the observed cake resistance gap between experiment and conventional model. Nevertheless, the obtained results and the assumptions made indicate that complex behavior of yeast cake at high slurry concentration can be described within the framework of conventional binary or ternary models by increasing the complexity of subsystems due to aggregation effect.

This may be a subject for further investigation for precise filtration control at large cell concentration.

Even the direct observation of experimental results shows the importance of specific cake resistance and concentration relationship. Figure 10 represents filtration time t needed to obtain 1 mm yeast cake thickness on the *Gelman* membrane.



Figure 10. Filtration time t needed for obtaining of 1 mm yeast cake thickness on the *Gelman* membrane (0.45 micron). 1 – filtration pressure 40 kPa, and 2 – filtration pressure 80 kPa. A higher filtration pressure gives a lower filtration time, but due to the cake resistance behaviour a more concentrated slurry can be filtered at the same time *t* and lower pressure.

5. Conclusion

Filtration of baker's yeast in an isotonic solution through a 0.45 microns membrane was investigated for yeast concentrations in the range 0.14 - 51 g/l and filtration pressure values of 40 and 80 kPa. Yeast filtration depends on applied filtration pressure and slurry concentration. It was found that for yeast volume fraction in suspension above 0.06 the porosity of yeast cake weakly depends on suspension concentration.

The specific cake resistance of highly diluted suspension approaches to the minimum value, which is sensitive to the filtration pressure.

Kozeny-Carman coefficient in the specific cake resistance relationship is increased with increasing applied filtration pressure. It means that simultaneously with porosity decreasing the tortuosity increasing of cake pores may affect specific resistance due to the cake compression. This statement needs further investigation on highly compressible materials.

Both filtration pressure and slurry concentration can be process controlled. In the range of moderate concentrations, the manipulation of filtration pressure and slurry concentration may increase the filtrate flux. Similar effect on filtrate flux increasing may be reached, for example, by increasing the filtration pressure and decreasing the slurry concentration or vice versa.

Obtained results and made assumptions indicate that complex behavior of yeast cake at high slurry concentration can be further described within the framework of conventional binary or ternary models by increasing the complexity of subsystems due to aggregation effect. This may be useful for precise filtration control at large cell concentrations.

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