Separation by Microfiltration of *Rhodotorula Rubra* Cells from the Culture Broth

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Torularhodin is one of the carotenoid pigments produced by the yeast Rhodotorula sp., with a terminal carboxylic group considered now-a-days as a powerful antioxidant to be included in food and drugs formulations. The paper describes the separation phase for the preparation of the torularhodin, obtained from the Rhodotorula rubra yeasts cells. At the end of the bioprocess, the cell mass was concentrated by cross-flow microfiltration. Rhodotorula rubra was grown in 10 L stirred tank bioreactor in the MS3 medium. The final research stage was to estimate the membrane surface of the microfiltration module needed to perform all the separation operations applied on biomass separation from the broth for a maximum duration of 3.5 h and for 42 L culture broth (pilot scale).

Keywords: Rhodotorula rubra, torularhodin, microfiltration, membrane cleaning

Carotenoid pigments are obtained from microbial or vegetal sources [1] and the interest in their manufacture is increasing now-a-days because of their widespread use.

Carotenoid biosynthesis is a specific feature of the *Rhodotorula* species and *Phaffia* genera [2-3, 10], the torularhodin (pigment with a high-level antioxidant potential) being mainly biosynthesized by the *Rhodotorula* yeasts.

The membrane separation are being frequently used as methods for the sterile separation of many biological products for pharmaceutical use [4]; the main factor which limits the applicability and efficiency of these membranes is the time-alteration of their characteristics, phenomenon known as membrane fouling. From this point of view the culture media are typical examples of complex mixtures, which can heavily promote the membrane fouling. The phenomenon manifests itself through the reduction in time of the permeate flow as an effect of the membrane resistance increase. The mechanisms of membrane fouling usually include pore blocking, concentration polarization and cake formation [5-6].

The general equation (1) which describes the membrane separation is [7]:

$$J = \frac{\Delta p}{\mu\left(R_m + R_p + R_c\right)} \quad (1)$$

According to [8], the permeation fluxes under each of these cases may be given in table 1.

The comparison of the experimental data with the three proposed models allows distinguishing the dominant mechanism which determines the membrane fouling for a given system.

For the operation at a constant pressure difference, the initial level of the permeation flux mainly depends on the membrane resistance defined by R_m, as R_p and R_c are initially zero. With the proceeding of the microfiltration operation, pore blocking and cake formation will cause R_p and R_c to increase, and the microfiltration process can transfer from a membrane resistance-limited to a pore blocking resistance-limited or a cake resistance-limited process.

To assure the viability of a membrane separation process, the membrane fouling must be reduced as much as possible and this can be done through the use of new membrane materials, new modules configurations, the assurance of adequate flowing conditions, application of *in situ* or *ex situ* membrane regeneration procedures; these methods are to be used from case to case, individually or coupled.

In the present study the obtained experimental data had been used for the evaluation of the microfiltration module

membrane resistance-limited	pore blocking resistance-limited	cake resistance-limited
$J = \frac{J_o}{1 + J_o K_m t}$ (2)	$J = J_o \exp\left(-K_p t\right)$ (3)	$J^2 = \frac{J_0^2}{1 + J_0^2 K_c t}$ (4)
Eqs. (2)–(4) can be rewritten in a linearized form as:		
$\frac{1}{J} = \frac{1}{J_o} + K_m t$ (5)	$\ln J = -K_p t + \ln J_o$ (6)	$\frac{1}{J^2} = \frac{1}{J_o^2} + K_c t$ (7)

Table 1
THEORETICAL MODELS FOR MEMBRANE FOULING

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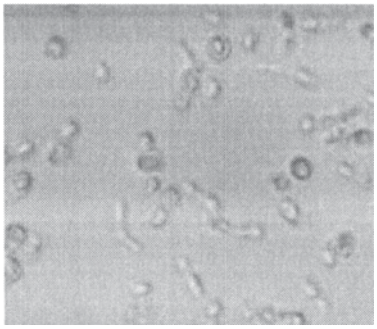

Fig. 1. Microscopic morphology of the *Rhodotorula rubra* ICCF 209 yeast cells

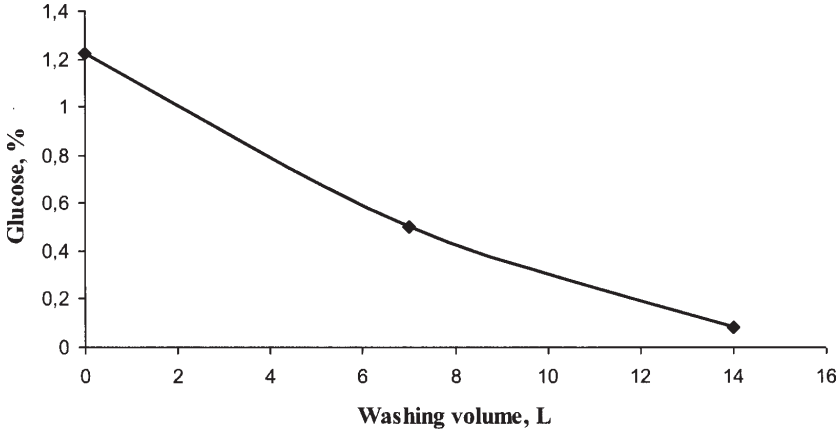

Fig. 2. The concentration of glucose in permeate by the continuous diafiltration

area needed to separate the yeast cells from a defined volume of the bioprocess broth.

Experimental part

Methodology

The choice of the membrane separation method must be taken into account, function of the characteristic dimensions of the components to be retained.

Microscopic morphology on Olympus U-CMAD 3, 500X shows spherical or elongated budding yeast cells or blastoconidia, 2.5-6.5 x 6.5-14.0 μm in size (fig.1).

In accordance with these characteristics of the *Rhodotorula rubra* yeast cells, the chosen separation method was the microfiltration, with a membrane with the pores dimension of 0.22 μm.

Experimental set-up

The yeasts cells are to be separated by microfiltration from 7 L of broth prior to cells disintegration to get the torularhodin by solvent extraction.

The filtration cartridge was Sartorius with the following characteristics: total filtration area of 0.1 m^2; polymer membrane from polysulfone; Hydrosart MF/UF - Sartocon Slice Cassette; the pores diameter of 0.22μm.

The glucose concentration was determined by the Luff-Schoorl method, in accordance with the EU Directive 79/796/EEC from 26 july 1979 referring to the analysis methods for testing certain sugars intended for human consumption [9].

Results and discussion

Washing of the biomass

The membrane washing with water through the process of continuous diafiltration, has the objective to remove the compounds from the bioprocess medium; at the same time the needed water volume is to be considered when quantifying the total filtration area in the scale up operation. The glucose concentration diminution, as an indicator of the membrane washing efficiency, is represented in the figure 2.

According to the figure 2 the glucose concentration decreases bellow the accepted limit after the use of 14 L of water, which means a specific consumption of 2 L of washing water/L of the broth.

The efficiency of the membrane washing is appreciated based on the glucose concentration diminution in the collected permeate, a value inferior to 0.1% being satisfactory.

The concentration of the biomass

After washing the suspension was concentrated to a volumetric ratio of concentration of VRC = 10, acquiring so a total volume of 0.7 L concentrated medium containing the yeasts cells.

In figure 3 it is represented the duration of each stage (washing and biomass concentration). It results a total duration of 165 min.

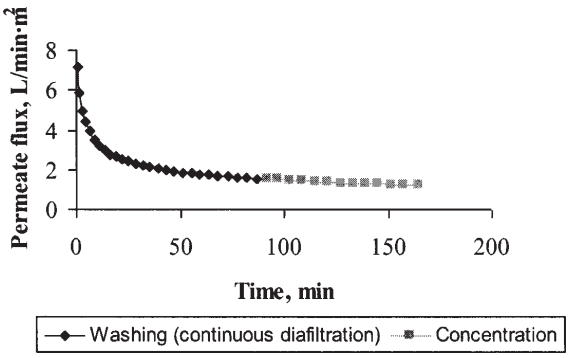

Fig. 3. Permeate flux for each phase (washing and biomass concentration)

Fouling and regeneration of the membrane

The fouling of the membrane during successive stages of biomass separation is obvious, fact set off by the time variation of the permeate flux (fig. 3).

The reduction of the permeate flux is not linear, which can be explained due to different involved mechanisms. Therefore for the first 87 min of the washing stage, realized at a constant pressure difference of $\Delta p = 1.15$ bar, the experimental data compared with the models defined by the relationships (5)-(7) lead to the following conclusions

- in the first 10 min from the beginning of the biomass washing operation the reduction of the permeate flux is due to the pores fouling (probably determined by constituents with dimensions smaller than the *Rhodotorula rubra* cells, fact proven by the correlation of the experimental data with the model described in the relationship (6) (fig. 4,a);
- in the next 77 min the formation of a precipitate layer takes place on the surface of the membrane due to the progressive covering of its surface with cells from the yeast suspension, which introduces the correlation of the experimental data with the model corresponding to the relationship (7) (fig. 4,b);

The influence of the flowing conditions upon the permeation speed was studied. It was found an initial rise

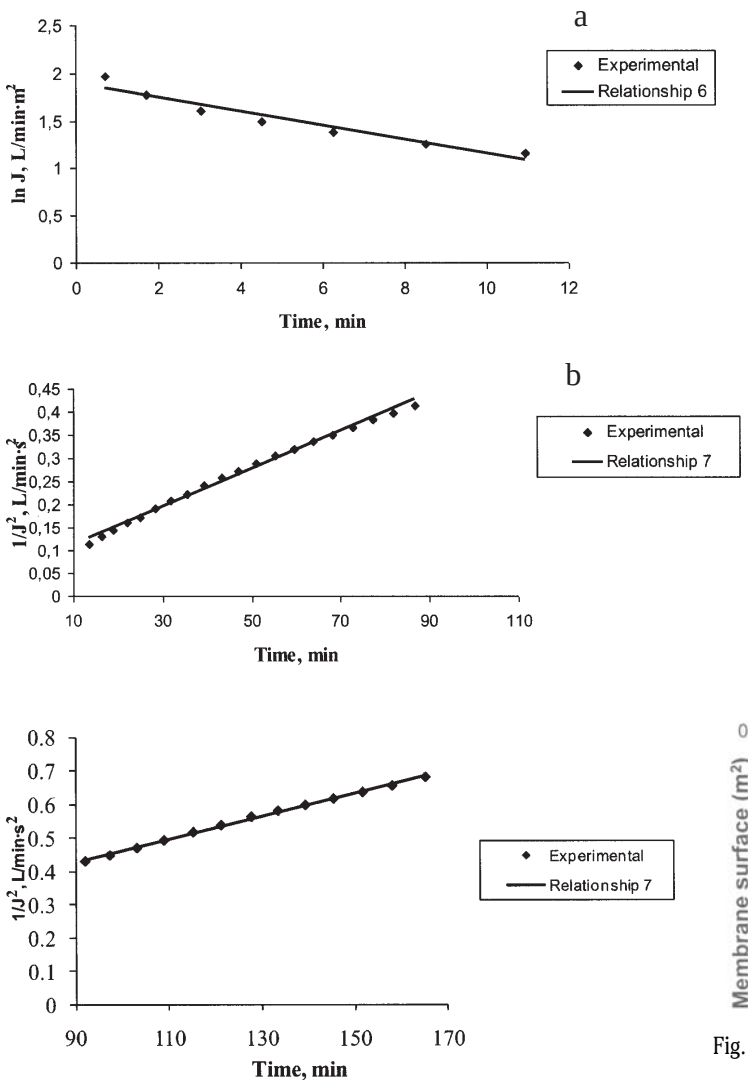

Fig. 4. The mechanisms for the membrane fouling in the washing phase of the biomass:
a – pores fouling;
b - the formation of the precipitate

Fig. 5. The fouling mechanism in the concentration stage of the biomass

of the flux of permeate (relationship (1)), which determines an increase of the yeasts cells layer deposit on the membrane surface; but the final result is the reach of a new stationary state (permeation flux virtually constant) as an effect of the realised equilibrium between the flux of solute towards the membrane surface and that moved by the rejected current (tangential flow). Due to operating procedure reasons, this increase of the permeate flux is also valid in the initial phase of the biomass concentrating stage; further on the flux presents a decrease tendency conforming to the relationship (7) (fig. 5).

As a consequence of the above mentioned facts, the membrane regeneration was done through washing it both at the surface, and in the pores (taking into account the characteristics of this fouling mechanism) with NaOH 1 N (10 min.) and H_2SO_4 2.5% (10 min.).

Scaling-up the process of membrane separation

The scaling-up to the next stage (pilot scale) was done on the basis of the experiments carried out, demonstrating the membrane separation is a superior solution to the procedure currently applied, and meaning centrifugation.

The calculation hypotheses used to estimate the membrane surface of the microfiltration module needed to perform all the separation stages applied on biomass separation from the broth for a maximum duration of 3.5 h and for 42 L culture broth, are as follows:

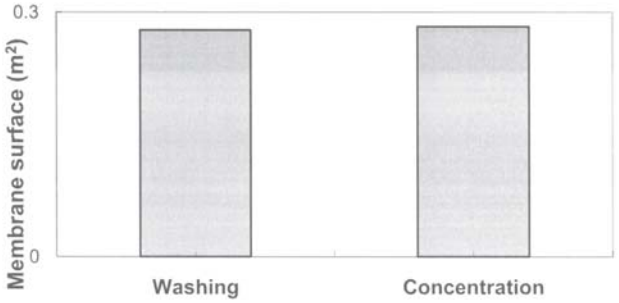

Fig. 6. The needed membrane area during each stage of biomass separation

- the stages of the total cycle of the biomass separation at the pilot scale are the same as those defined for the lab study;
- the values of the permeation fluxes through the membrane, obtained on the basis of the experimental study, are reproducible at this next scale;
- the washing liquid volumes to be used are proportional to the processed suspension volume;
- the duration ratios (reported to the total processing cycle) of each working stage at this scale are equal with the ones determined by the experimental study.

The scaling-up study determined a necessary membrane area of 0.3 m^2 (fig. 6) for the microfiltration module to be used to separate the yeasts cells from 42 L broth (pilot scale).

Conclusions

The paper presented the study of the separation by microfiltration of the *Rhodotorula rubra* yeasts cells from 7 L bioreactor cultivation broth.

Experimental data demonstrated there is a heavy membrane fouling due to both the pore blockage and the building-up of a precipitate coat on the membrane surface. A membrane regeneration protocol was consequently proposed, which proved to be adequate.

The scaling-up procedure evaluated a necessary membrane area of 0.3 m^2 for the microfiltration module applied to separate the yeasts cells from 42 L broth (pilot bioreactor scale).

Notation

J – permeation flux ($m^3 m^{-2} s^{-1}$)
J_0 – initial permeate flux ($m^3 m^{-2} s^{-1}$)
Δp – pressure difference (Pa)
R – membrane resistance (m^{-1})
R_m – resistance due to concentration polarization (m^{-1})
R_p – resistance due to pore blocking (m^{-1})
R_c – resistance due to cake formation (m^{-1})
K_m, K_p, K_c – system parameters relating to membrane resistance, pore blocking resistance, and cake formation resistance, respectively
t – time (s)

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Manuscript received: : 5.09.2011