CHARACTERIZATION OF O_2 TRANSFER CAPABILITY IN THE AEROBIC BIOREACTOR FOR CAROTENOID PIGMENTS FORMATION

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Partea experimentală a fost realizată în scopul de a obține un amestec de pigmenți carotenoidici printre care şi torularhodina (considerat ca fiind un antioxidant util) într-un bioproces aerob utilizând drojdia Rhodotorula rubra ICCF 209. S-a demonstrat necesitatea unui exces ridicat de oxigen dizolvat în faza de creştere (~50% din valoarea de saturație) în bioreactorul aerob.

Pentru a cuantifica capacitatea de transfer interfazic al oxigenului în condițiile de parametri de agitare-aerație studiați în bioreactor este importantă cunoaşterea coeficientului volumetric de transfer de masă, kLa.

Experimentele au fost efectuate într-un bioreactor (Bioengineering, AG), 100 L (42 L volum util) cu parametrii controlați şi înregistrați de computer.

Pentru determinarea kLa s-a aplicat metoda dinamică (apă, fără reacție), cu variația parametrilor: turația şi debitul de aer disipat în mediu (viteza superficială a gazului).

The experimental work done to prepare carotenoid pigments mixture with torularhodin content (considered as a useful antioxidant) in aerobic bioprocess with Rhodotorula rubra ICCF 209 yeast demonstrated the need of high excess of dissolved O2 during the phase growth (~50% of the saturation value) in the aerobic bioreactor.

In accordance to this finding it was needed to characterize the O2 transfer capability of the bioreactor by kLa coefficient determination.

The experiments were carried out in 100 L (42 L working volume) bioreactor (Bioengineering AG), with computer-controlled and recorded parameters.

The kLa value was dynamically determined under model condition (water, no reaction) with variation of the parameters-stirrer speed and gas flow rate.

Keywords: *Rhodotorula rubra*, k_La, carotenoid pigment, bioprocess

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1. Introduction

Carotenoids are important natural pigments, displaying yellow, orange and red colours, widely found in micro organisms and plants. Industrial carotenoid pigments such as β-carotene and torularhodin are used as natural food colorants or food additives. Several studies have shown that carotenoids combat various types of cancer and other diseases due to their antioxidant and/or provitamin A properties [1].

Facing the growing economic significance of carotenoids, due to their use as food colorants, nutritional supplements, in cosmetics or in human therapy as antioxidants, much interest has been dedicated to new supplies of this class of pigments. In particular, the development of carotenoid-producing bioprocesses is regarded as a competitive solution, as it can provide important quantities of pigments such as torularhodin and β-carotene produced by *Rhodotorula rubra* or astaxanthin from *Phaffia rhodozyma* without facing the typical problems generated by the weather dependency of the agriculture production.

Filamentous fungi, yeasts and some species of bacteria, algae and lichens can produce carotenoids. Among microbial sources of carotenoids, yeasts such as *Sporobolomyces* and *Rhodotorula* are of commercial interest [2-4].

The experimental work done to prepare carotenoid pigments mixture with torularhodin content (considered as a useful antioxidant) in aerobic bioprocess with *Rhodotorula rubra* ICCF 209 demonstrated the need of high excess of dissolved O_2 during the growth phase (~30 - 50% of the saturation value) in the aerobic bioreactor, in agreement with other researchers' findings [5-6].

In accordance to this idea, it was necessary to characterize the O_2 transfer capability of the bioreactor by k_La (the volumetric coefficient of O_2 transfer) coefficient determination.

The oxygen transfer represents the most important characteristic to be considered for the design and operation of the bioreactor mixing-sparging devices. It can be described and analyzed by using k_La. The k_La values are influenced by a lot of factors, such as: (a) geometrical and operational characteristics of the vessels; (b) media composition: (c) type, concentration and morphology of cultivated micro organism [7].

2. Experimental set-up

The experiments were carried out in 100 L (42 L working volume) bioreactor (Bioengineering AG), with computer-controlled and recorded parameters.

The bioreactor has mechanical stirring (Rushton impeller) and the main parameters (temperature, pH, mixing speed, air flow rate) are controlled, but foam level and dissolved oxygen are only monitored.

The k_La value was dynamically determined under model condition (water, no reaction) with the variation of the parameters-stirrer speed and gas flow rate. The saturation curves obtained with the pO_2 electrode continuous determination system are used to determine the k_La value.

The k_La coefficient was measured based on the balance equation of the O_2 in the liquid phase by the application of the dynamic pressure method (as a measuring method which is a priori less sensitive to the above limitations).

3. Results and discussions

3.1. Determination of the k_La

The rate of oxygen transfer across the gass-liquid interface may be expressed using a mass transfer coefficient which characterizez the liquid resistance to transfer.

The rate of oxygen transfer from air bubbles to liquid in a batch stirred bioreactor is given by the following relationship:

- for oxygenation:

$$\frac{d(V_L \cdot C_o)}{dt} = k_L a\left(C_{oi} - C_o\right) \cdot V \tag{1}$$

- for de-aeration (deoxygenation):

$$\frac{d\left(V_L \cdot C_o\right)}{dt} = k_L a\left(C_o - C_{oi}\right) \tag{2}$$

or, after integration

- for oxygenation:

$$\ln \frac{C_{oi} - C_o}{C_{oi} - C_{o,o}} = -\frac{k_L \cdot a}{1 - \varepsilon_G} \cdot t \tag{3}$$

- for de-aeration (deoxygenation):

$$\ln \frac{C_o}{C_{o,o}} = -\frac{k_L \cdot a}{1 - \varepsilon_G} \cdot t \tag{4}$$

where *a* is the bubble surface area per unit volume of the gas-liquid mixture (m_i^2/m^3) and ε_G is the gas holdup (i.e., the volume fraction of the gas-liquid mixture occupied by yhe gas) [8]. Thus, (1- ε_G) is the volume fraction of the gas-liquid mixture occupied by the liquid.

V – volume of dispersion, L ; $V_L = V(1-\varepsilon_G)$, L

C_{oi} – the saturation concentration of the oxygen in the liquid, g/L, mol/L;

$C_{o,o}$ - initial concentration of oxygen in water before starting the aeration (oxygenation) or the de-aeration (deoxygenation) experiment.

k_L – the volumetric coefficient of O_2 transfer, m/s .

The change in dissolved oxygen concentration, C_o in the liquid phase was detected using a pO_2 electrode.
The saturation concentration of the oxygen in the liquid, C_{oi} was obtained from the Henry's Law [9].
Also, the oxygen composition in the liquid immediately adjacent to the gas-bubble interface cam be considered constant, at C_{oi} (the value for air-saturated water) in the oxygenation case and equal to zero for the deoxygenation case.

Table 1

The studied domain of stirrer speed and air flow

	D_{VG},		*Temperature,*	
n, min^{-1}	***L/min***	***P, atm***	***^oC***	***ε_G***
300	40	1,5	37	0,076
250	30	1,5	37	0,0467
200	20	1,5	37	0,025

A first objective is to evaluate the group of parameters, $\frac{k_L \cdot a}{1-\varepsilon_G}$ from the slope of ln (C_{oi}-C_o) versus time ot the ln C versus time plots.

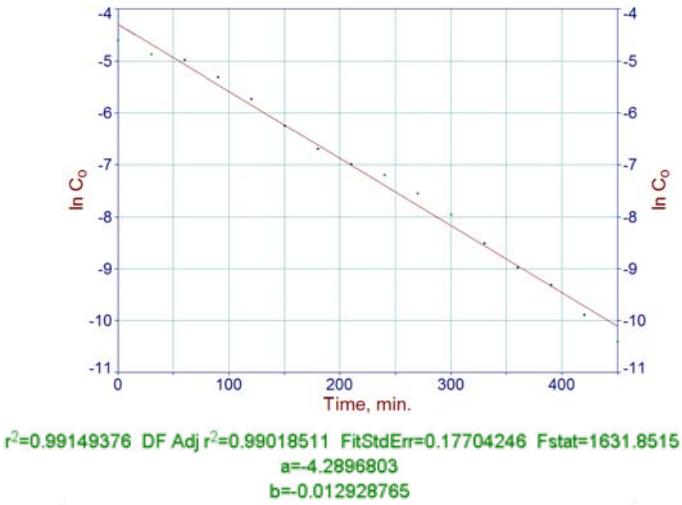


Fig. 1. ln C versus t plot for deoxygenation at 40 L/min nitrogen flow rate

Fig. 1 is a plot of lnC versus t for deoxygenation runs at the corresponding nitrogen flow rate of 40 L/min. These plots are also linear conforming to Eq. 4. and indicate an increase in $\frac{k_L \cdot a}{1-\varepsilon_G}$ with an increase in the gas flow rate.

Fig. 2 is a plot of ln (C_{oi}-C_o) versus t (Eq. 3) for the oxygenation of water. The plot is linear and, conform to Eq. 3, has a negative slope. The slope for the large gas flow rate is more negative than it was expected because $\frac{k_L \cdot a}{1-\varepsilon_G}$ increases with an increase in the Reynolds number.

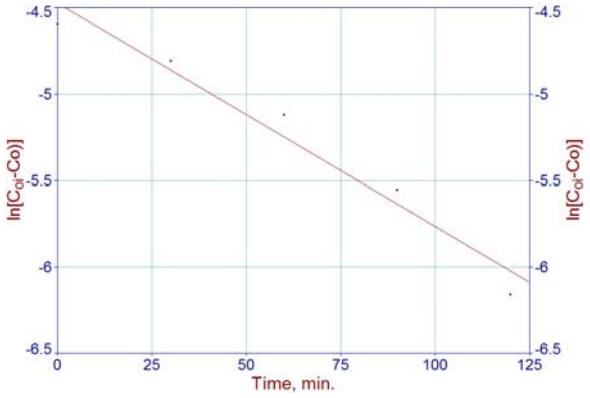


Fig. 2. ln (C_{oi}-C) versus t plot for oxygenation at 40 L/min air flow rate

Fig. 3 is a plot of lnC versus t for deoxygenation runs at the corresponding nitrogen flow rate of 30 L/min. These plots are also linear, conforming to Eq. 4. and indicate an increase in $\frac{k_L \cdot a}{1-\varepsilon_G}$ with an increase in the gas flow rate.

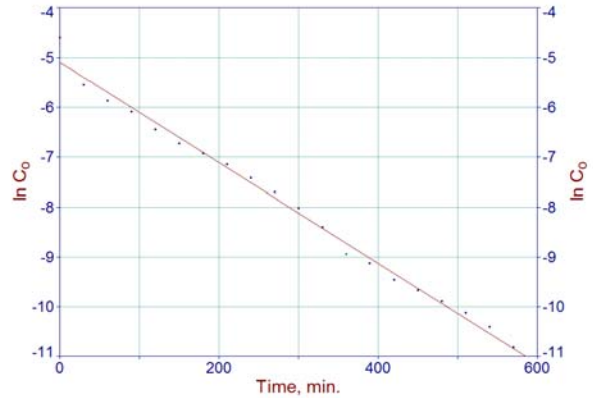


Fig. 3. ln C versus t plot for deoxygenation at 30 L/min nitrogen flow rate

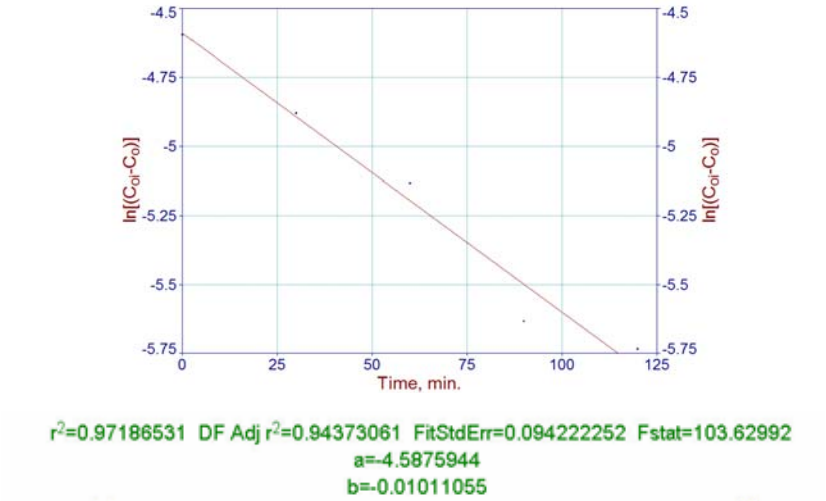


Fig. 4. ln (C_{oi}-C) versus t plot for oxygenation at 30 L/min air flow rate

Fig. 4 is a plot of ln (C_{oi}-C_o) versus t (Eq. 3) for the oxygenation of water. The plot is linear and conform to Eq. 3, having a negative slope.

Fig. 5 is a plot of lnC versus t for deoxygenation runs at the corresponding nitrogen flow rate of 20 L/min. These plots are also linear conforming to Eq. 4.

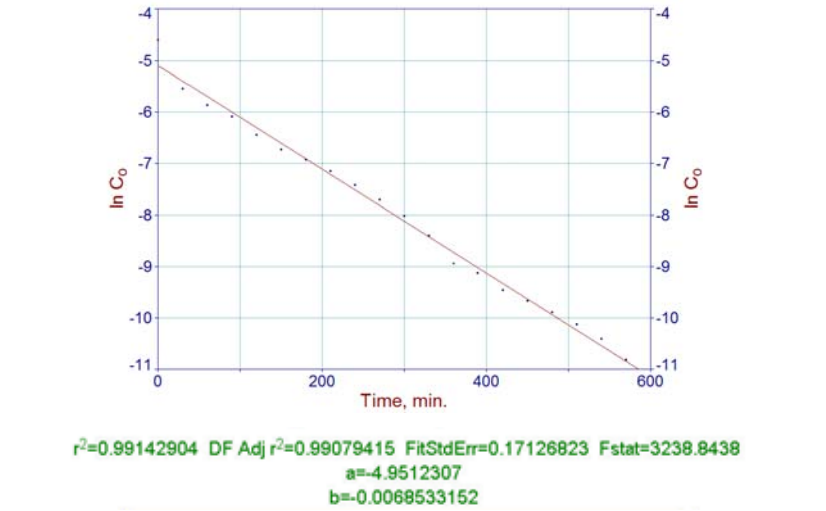


Fig. 5. ln C versus t plot for deoxygenation at 20 L/min nitrogen flow rate

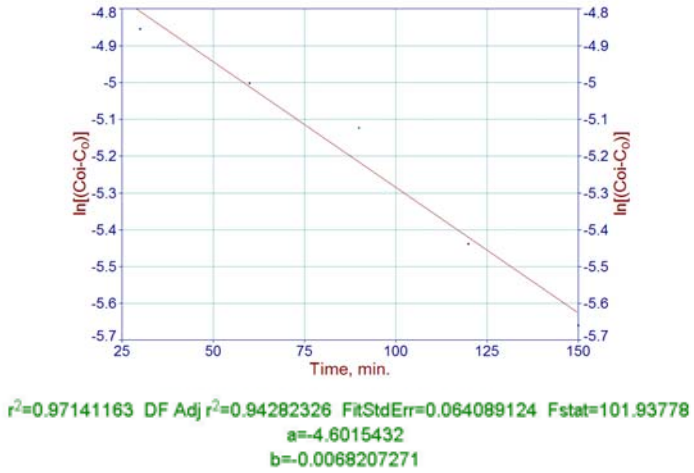


Fig. 6. ln (C_{oi}-C) versus t plot for oxygenation at 20 L/min air flow rate

Fig. 6 is a plot of ln (C_{oi}-C_o) versus t (Eq. 3) for the oxygenation of water. The plot is linear and conform to Eq. 3, having a negative slope.

Table 2

Values of k_La determined for the studied domain of stirrer speed and air flow

n, s^{-1}	D_{VG}, L/min	k_La, s^{-1}
5	40	0.0129
4.17	30	0.0097
3.33	20	0.0067

3.2 Power consumption

The specific power consumption (P/V_l) is the parameter which indicates the turbulence degree and media circulation in bioreactor and quantifies the contributions of the rotation speed and the power input. Moreover, as it was mentioned in the literature, the energy dissipated by mechanical agitation is not dependent on the stirrer geometry. The Reynolds number defines the turbulence degree of the agitated liquid and is done by the relationship:

$$\mathrm{Re} = \frac{\rho_L n d_a^2}{\eta_L} \tag{5}$$

where:

- diameter of the circle described by the stirrer, d_a = 0,2 m;
- stirrer speed, n, s^{-1}

- the liquid density, ρ_L and the liquid viscosity, η_L for water at 37 °C
- air flow, D_{VG}, L/min

For Re > 10000 [10]

$$P = 6\rho_L n^3 d_a^5 \tag{6}$$

and one can calculate P_g - the power consumption for the aerated medium as follows:

$$\lg\frac{P_g}{P} = -192\left(\frac{d_a}{D}\right)^{4,38}(\mathrm{Re})^{0,115}\left(\frac{d_a n^2}{g}\right)^{1,96 d_a/D}\frac{D_{VG}}{d_a^3 n} \tag{7}$$

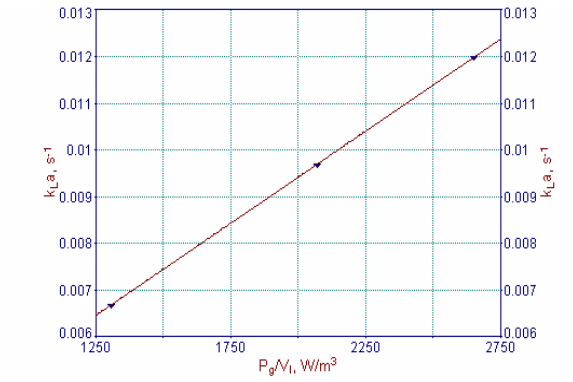
where: V_l = 42 L, liquid volume; D = 0.4 m, tank diameter; g = 9.81 m/s²;

The characteristic relationship obtained in accordance with the experimental data is:

$$k_L a = 0.0035(P_g/V_l)^{0.42}(v_G)^{0.4} \tag{8}$$

where: $v_G = \frac{4D_{VG}}{\pi D^2}$, gas velocity, m/s in agreement with other researchers' findings [11-13].

a)



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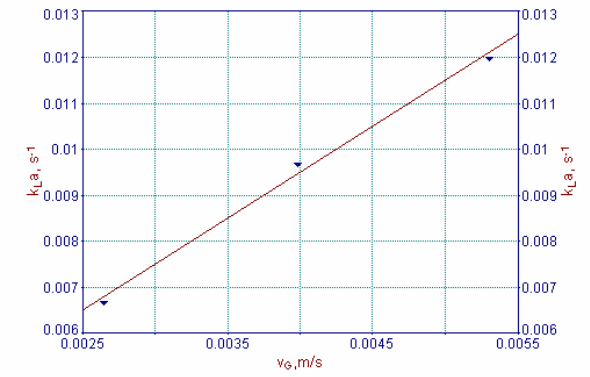

Fig.7. Influence of (a) specific power input and (b) superficial air velocity on volumetric coefficient of oxygen transfer

The k_La variation is presented in the Fig. 7 function of the specific power input and the superficial air velocity for 1,5 atm pressure and 37 ^oC.

For a better characterization of bioreactor performance from the point of view of oxygen transfer, the term of oxygen transfer efficiency, E_{O2}, [14] was introduced and defined as:

$$E_{O2} = \frac{k_L a}{P_g / V} \tag{9}$$

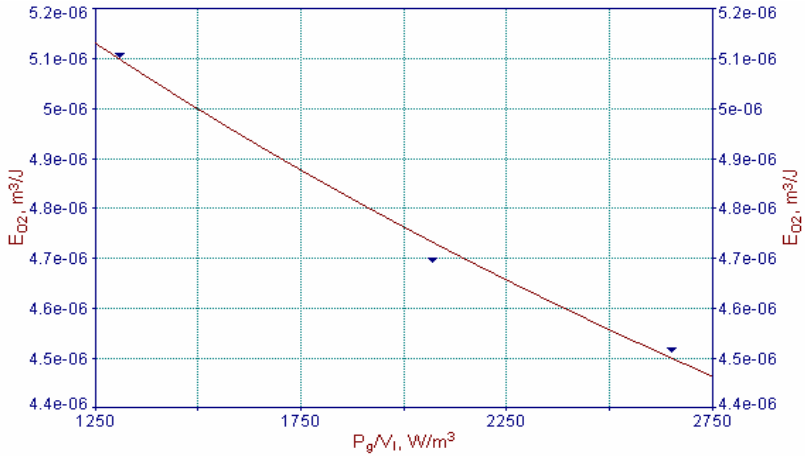

Fig. 8. Influence of specific power input on oxygen transfer efficiency

At specific power consumption over 1000 W/m^3, the oxygen transfer efficiency is drastically reduced.

4. Conclusions

A characterization of O_2 transfer capability in a mechanically stirred aerobic bioreactor for carotenoid pigments formation was experimentally performed.

The dependence of k_La on the specific power input and superficial air velocity for different agitation speeds and gas flow rates demonstrated a limited efficiency of the aerobic bioreactor from the point of view of oxygen transfer capability. This finding is demonstrated by the low values of the exponents correlating k_La with the mentioned parameters in the relationship (6). Consequently, it is recommended to apply higher aeration parameters (n = 300 min^{-1}; D_{VG} = 40 L/min) for the studied bioprocess.

At the same time, at the specific power consumption over 1000 W/m^3, the oxygen transfer efficiency is drastically reduced.

Acknowledgments:

The work was financially supported by the project POSDRU/89/1.5/S/52432 from 1.04.2010 - Institutional organization of a postdoctoral school of national interest "Applied biotechnology with impact in the Romanian economy"; the project was cofunded by the EU Social Fund in the framework of the Sectoral Operational Programme 2007-2013 for Human Resources Development.

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