TECHNOLOGICAL STUDY OF AN AEROBIC BIOPROCESS FOR A BACTERIAL PHARMACEUTICAL PREPARATION

Camelia UNGUREANU[1], Ovidiu MUNTEAN[2], Ana Aurelia CHIRVASE[3], Iosif NAGY[4], Adrian ONU[5], Aurora SĂLĂGEANU[6]

The aim of the paper is identifying a kinetic model for an immunomodulator bioproduction using Pseudomonas aeruginosa and the O_2 transfer characterization of the production bioreactor; volumetric Oxygen Transfer Rate coefficient have been evaluated.

Scopul lucrării este identificarea unui model cinetic pentru bioproducţia unui imunomodulator utilizând Pseudomonas aeruginosa şi caracterizarea transferului de oxigen pentru productia în bioreactor; a fost determinat coeficientul volumetric de transfer al oxigenului.

Keywords: microbial immunomodulator, kinetic model, maximum specific growth rate

1. Introduction

Now, in Romania, there is compulsory the introduction of EU quality standards for the pharmaceuticals production. This means the manufacturers are to comply with the GMP (Good Manufacturing Practices) to assess the GMP correspondence for manufacturing area, equipments and all operational activity.

In this European and national context, the NIRD "Cantacuzino" has important achievements in bio-pharmaceutical preparations research and development being the unique manufacturer of vaccines and immunomodulators for human use in Romania [1].

[1] PhD-student Eng., Dept. of Bioengineering and Biotechnology, University Politehnica of Bucharest, Romania
[2] Prof. PhD.Eng., Dept. of Bioengineering and Biotechnology, University Politehnica of Bucharest, Romania
[3] PhD.Eng. Dept. of Bioengineering and Biotechnology, University Politehnica of Bucharest, Romania
[4] PhD. Eng. Dept. of Bioengineering and Biotechnology, University Politehnica of Bucharest, Romania
[5] PhD. National Institute of Research and Development for Microbiology and Immunology "Cantacuzino", Romania
[6] PhD. National Institute of Research and Development for Microbiology and Immunology "Cantacuzino", Romania

In order to assure the recommended quality of the therapeutic biopharmaceuticals the manufacture technologies were optimized to be in line with the EU GMP guidelines (EU Directive 2003/94/EC).

In case of a bacterial immunomodulator preparation the first step of this process was the optimization of the aerobic bioprocess with *Pseudomonas aeruginosa sp*.[2]. Consequently the study of the growth kinetic model and an evolution of the volumetric mass transfer coefficient k_La_v have been done function of the experimental conditions.

2. Experimental part

The bacterium growth and associated immunomodulator bioproduct formation were studied in a Bioengineering aerobic bioreactor of 100 L volume with 42 L cultivation medium. 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 cellular growth was estimated by Optical Density measurement at λ=570 nm and dried weight is determined by the usual drying procedure at 105^{o}C. The substrate consumption was measured by Aminic nitrogen consuming (chemical titration with NaOH in formaldehyde presence).

The cultivation medium contained mainly peptone and meat extract. To be in line with the EU GMP norms these substrates prepared in the past by the manufacturer itself were replaced by ORGANOTECH (microbiological media producer accepted by these guidelines) products.

Main controlled parameters were: temperature:37^{0}C; impeller speed:250 rpm; air flow rate:15 L/min; pH:7. 3.

3. Results and discussion

The evolution of the optical density (growth estimator), aminic nitrogen (as substrate consumption) and dissolved oxygen is represented in the Fig. 2 for 3 discontinuous bioprocesses.

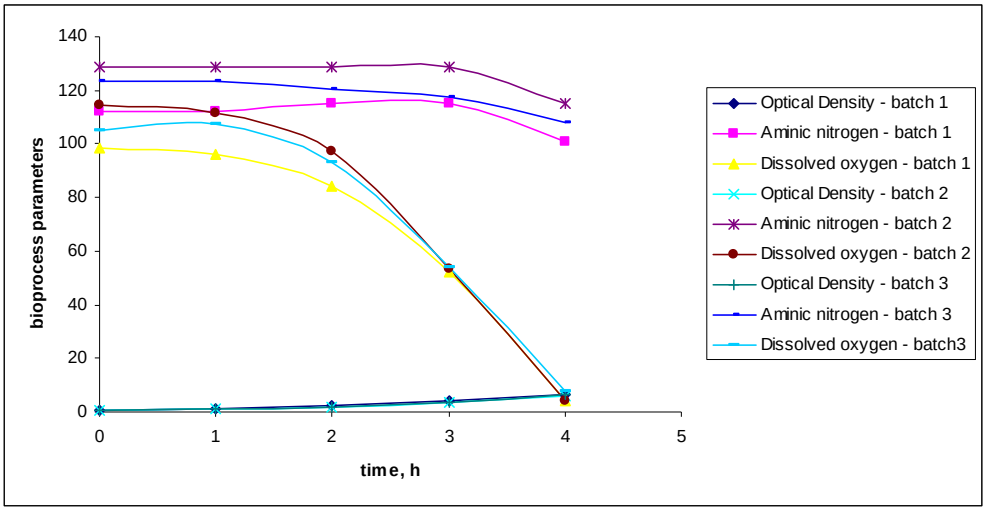

Fig. 1. The evolution of optical density, aminic nitrogen and dissolved oxygen

Cellular concentration (C_x) expressed as dried weight [g/L] is calculated on the basis of the experimental determination of the relationship $C_x = f$ (Optical Density). The linear correlation is represented in the Fig. 2, and the value of the regression coefficient demonstrates that the equation $y = 0.3282x$ is appropriate to be used in the future to estimate directly the concentration values by measuring the Optical Density.

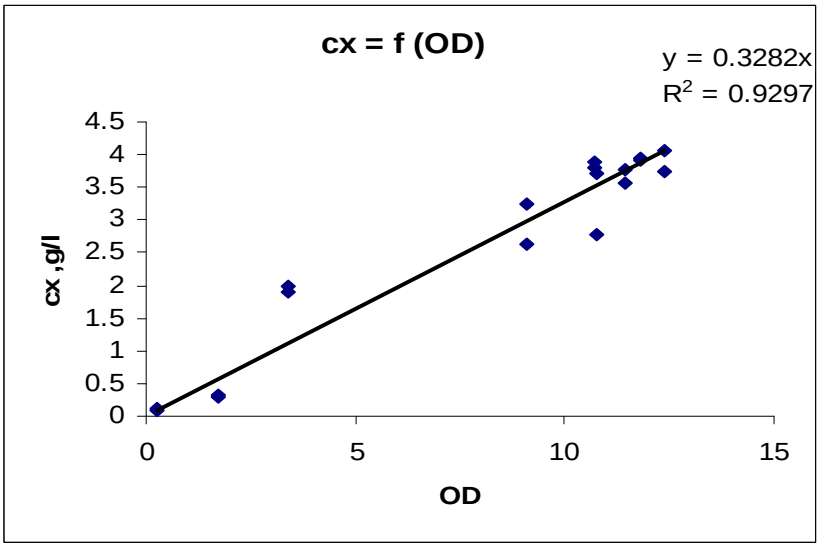

Fig. 2. Cellular concentration in function of the optical density

To express the kinetic growth and to calculate μ_m an exponential model is proposed:

$$\frac{dC_X}{dt} = \mu_m C_X \quad (1)$$

After the integration and linearization the equation (1) becomes:

$$\ln\frac{C_X}{C_{Xo}} = \mu_m t \quad (2)$$

Can estimate a value of μ_m=0,8 h^{-1} which can be considered an appropriate value for a bacterium like *Pseudomonas* [3]. The linear regression representation is presented in the Fig. 3.

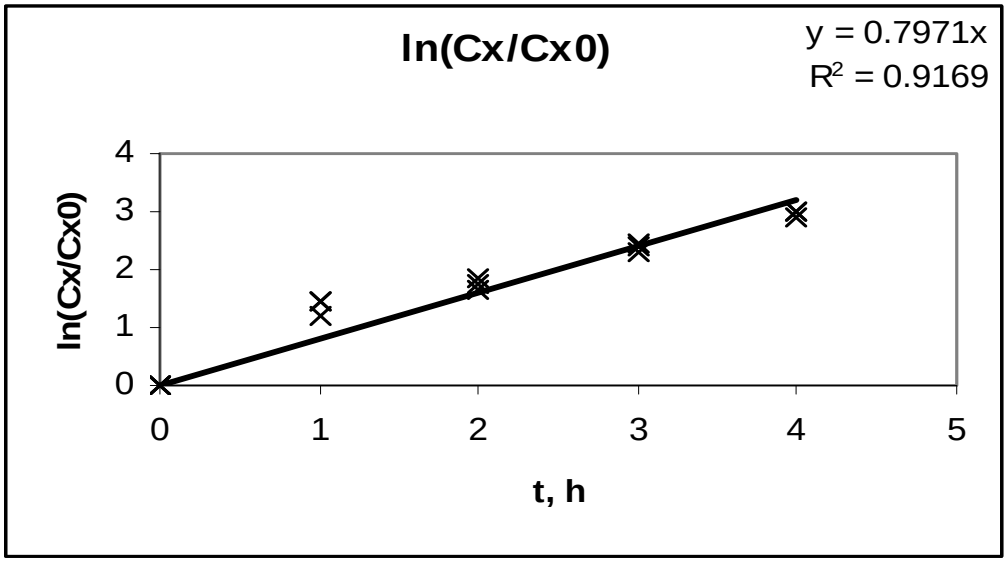

Fig. 3. μ_m estimation

To assess the Oxygen Transfer Rate corresponding to the culture needs an evaluation of volumetric mass transfer coefficient k_La_v is to be done function of the experimental conditions.

The quantification was done by 2 methods:

a) Firstly the empirical relationship to calculate k_La_v for coalescent liquids was used:

$$k_La_v = 2{,}6\cdot 10^{-2}\left(P_g/V\right)^{0.4} v_G^{0.5} \quad (3)$$

where:

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

for Re > 10000, $P = 6\rho_L n^3 d_a^5$ (5)

Re = 2, $37 \cdot 10^5$;
P = 138 W;
P_g = 109 W;
P_g/V = 2592 W/m^3;
v_G = 0, 0020 m/s;

One can estimate with already used relationships (4) a value of P_g/V of 2592 W/m^3 and v_G = 0, 0020 m/s, in these conditions $k_L a_v$ was estimated as 0, 0269 s^{-1}.

b) This value is well compared with the level of $k_L a_v$ = 0, 0202 s^{-1}, obtained from the equation expressing the O_2 balance in the liquid phase [5,6] with the following hypothesis: the complete mixing of liquid and gaseous phases and the resistance of mass transfer in gaseous phase is neglected:

$$\frac{dC_O}{dt} = \frac{k_L a_v}{1-\varepsilon_G}(C_{OS} - C_O) - Y_{OX} v_{RX} \quad (6)$$

k_L value results from the relationship (7): [6]

$$k_L = 0.42\left(\frac{\eta_L g}{\rho_L}\right)^{1/3}\left(\frac{D_A \rho_L}{\eta_L}\right) \quad (7)$$

and a_v from: $a_v = \frac{6\varepsilon_G}{d_b}$ (8)

Volumetric fraction of gas, ε_G is obtained from: [6]

$$\varepsilon_G = 0{,}31\left(\frac{v_G}{\sqrt[4]{\frac{\sigma_L g}{\rho_L}}}\right) + 0{,}45\frac{(n - n_0^*)d_a^2}{D\sqrt{gD}} \quad (9)$$

and bubble diameter (d_b) is calculated from the van Direndonck [6] relationship:

$$d_b = \sqrt{\frac{0.41\sigma_L}{(\rho_L - \rho_G)g}} \quad (10)$$

if $n \geq 2{,}5 n_0^*$.

Characteristic rotation speed (n_0^*) is calculated using this expression:

$$n_0^* = 2\left(\frac{\sigma_L g}{\rho_L}\right)^{1/4}\frac{D}{d_a}\left(\frac{h - h_a}{D}\right)^{1/2} \quad (11)$$

In all of the above equations, the following parameters have been used:

- D = 0,4 m;
- H = 0,8 m;
- h = 0,07 m;
- d_a = 0,2 m;
- h_a = 0,1 m
- n = 250 rpm;
- Q_G = 15 l/min.

The height of the liquid -h - has been calculated using the geometrical parameters at the liquid volume of 42 L.

The density, the viscosity, and the superficial tension of the culture medium have been adopted at the corresponding values for water at 37 °C;

Diffusion coefficient of O_2 dissolved in water has been calculated at 37°C by using this relationship: $D_{O_2} = D_{20}\left[1 + 0{,}2\frac{\sqrt{\eta_L}}{\sqrt[3]{\gamma}}(T-20)\right]$ (12)

Using all these relationships (11) (12) the following values were obtained:

- n_0^*=2,3 s^{-1}; bubble diameter is calculated with the relationship (10);
- d_b = 0,0017 m;
- ρ_G = 0,0462 kg/ m^3;
- a_v = 161,4 m^2/m^3;
- k_L = 1,25·10^{-4} m/s.

k_La_v = 0,0202 s^{-1}.

Finally this value (0, 0202 s^{-1}) obtained from the equation expressing the O_2 balance is well compared with the level of k_La_v = 0, 0269 s^{-1} calculated for coalescent liquids.

4. Conclusions

For the studied conditions:

a) An exponential growth model can express the experimental data.
b) A k_La_v level of 0,020-0,027 s^{-1} is determined. This value is lower than the literature recommended level of minimum 100 h^{-1} [7,8] for bacteria cultivation in conditions where the dissolved oxygen does not limit the growth for usual cellular mass concentrations of C_x = 10-20 g/l as d. w.

It means that one can recommend the increase of both: the impeller speed and the air flow rate.

Glossary

- C_O – oxygen concentration, g/l
- C_{OS} – saturation oxygen concentration, g/l
- C_X – cellular concentration, g/l
- D - tank diameter, m

- D_{O2} - diffusion coefficient
- D_{20} - diffusion coefficient at 20 ^{o}C;
- d_a – diameter of the circle described by the stirrer, m
- db - bubble diameter, m
- g – acceleration of gravity, cm/s^2
- h - the height of the liquid, m
- h_a – aerated liquid height, m
- H – height of cylindrical part, m
- k_La_v – volumetric Oxygen Transfer Rate coefficient (G/L interface), s^{-1}
- n - stirrer speed, rpm
- n_0^* - characteristic rotation speed, s^{-1}
- P – power consumed in liquid medium (without air), W
- Pg - specific power consumed in aerated medium, W
- Pg/V - power consumed in volume unit of aerated medium, W/m^3
- Re - Reynolds number, $Re = \frac{wd\rho}{\eta}$
- Q_G – volumetric air flow rate
- t - time, h
- T – temperature, ^{0}C
- v_G - air superficial rate, m/s
- Y_{OX} – oxygen yield related to cells
- γ - specific weight, kgf/m^3
- ε_G – gas hold-up
- ρ_L – liquid density, kg/ m^3
- ρ_G – gas density, kg/ m^3
- η_L – liquid viscosity, cP
- μ_m - maximum specific growth rate, h^{-1}
- λ – wavelength, nm

R E F E R E N C E S

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