DEVELOPMENT OF BIOPROCESS SCALE-UP STRATEGY FOR PIGMENT PRODUCTION IN BENCH-SCALE BIOREACTORS

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ABSTRACT

The search for sustainable alternatives to mineral and synthetic pigments is becoming essential, with increasing awareness of public health and environmental preservation. Microbial-origin biopigments, such as pigments produced by the fungus *Fusarium* sp., can be a sustainable alternative. However, there have been limited studies on the scale-up production of the pigment. This work studied the scale-up criterion of a constant oxygen mass transfer coefficient (k_La) between a 1 L and 10 L bioreactor aiming a high production of the pigment by the fungus *Fusarium* sp. It was concluded that maintaining a constant kLa was able to supply the oxygen demand of the microorganism. Furthermore, the production of the biopigment increased by approximately 10% using the calculated scale-up parameters.

Keywords: Bioprocess. Biopigment. Scale-up. Bioreactor. *Fusarium*.

1 INTRODUCTION

From the foods we consume to the clothes we wear, from the medicines we take to the cosmetics we apply, dyes and pigments have played a fundamental role in our daily lives. However, with increasing awareness of public health and environmental preservation, interest in natural and sustainable alternatives is also on the rise. While synthetic dyes and pigments offer a broad spectrum of colors and shades, they are often linked to negative health and environmental impacts. This growing demand for healthier and more eco-friendly solutions has led to the search for alternatives that could supplement or even replace these compounds[1,2]. In this scenario, microbial-origin pigments emerge as a promising alternative. Moreover, compared to plant and animal sources, the production of microbial color agents through the fermentation process could be more efficient and economical, generally resulting in biodegradable compounds[3].

The high diversity, environmental compatibility, low toxicity and feasibility of lab-scale production are some of the main characteristics that make microbial-origin color agents interesting for biotechnological applications[4]. Bacteria, fungi and microalgae as sources of color agents in the industry are already a reality in the market. Examples of color agents produced on a large scale include β-carotene (*Mucor circinelloides*)[5], canthaxanthin (*Haematococcus lacustres*)[6], astaxanthin (*Xanthophyllomyces dendrorhous*), Arpinkred™ (*Penicillium oxalicum*), and lycopene (*Fusarium sporotrichioides*)[7]. In this context, the fungus species *Fusarium* sp. also attracts attention as a microbial source of pigment, resulting from the biosynthesis of a polyketide-type secondary metabolite. To date, there have been limited studies on the production of this metabolite in large-scale bioreactors. The necessity for validating bioprocesses concerning the fermentation of microorganisms for pigment production is clear. Based on this, there are several strategies and methods for scaling up bioprocesses, depending mainly on the type of fermentation and the type of bioreactor. The constancy of a process parameter between the smaller and the bigger scales is the most suitable method for fermentation processes in stirred-tank reactors (STR). It is possible to work with the constancy of power input per unit volume of medium (P/V), the volumetric oxygen transfer coefficient (k_La), the Impeller tip speed (v_{tip}), the Mixing time (t_m), and the impeller pumping capacity (FL/V)[8]. Since the present project aims to scale up an aerobic fermentation process in a low-viscosity aqueous system, it was decided to perform the scale-up using the volumetric oxygen transfer coefficient (k_La) as the reference criterion.

2 MATERIAL & METHODS

The scale-up criterion of a constant oxygen mass transfer coefficient (k_La) was applied for the production of a pigment by *Fusarium* sp. from 1.4 L scale bioreactors (Multifors 2, Infors HT) to 13 L scale fermenters (Labfors 5, Infors HT). The specifications of each bioreactor are described in Table 1. *Fusarium* sp. was cultivated in both bioreactors at 28ºC using a sustainable medium that is currently under confidentiality due to a technology patenting process.

The systems were each equipped with automated controllers to manage agitation speed, temperature, pH, and dissolved oxygen (DO). An on-line computer monitoring and control of the fermentation process were conducted using a fermentation software program, eve®, from Infors HT, Switzerland, installed in each system.

To determine the k_La in the 1.4L and 13L bioreactor systems, the dynamic gassing-out method was used[8,9]. Oxygen was removed from the medium by purging it with inert nitrogen gas. Subsequently, the liquid medium was reoxygenated and dissolved oxygen (DO) through time was monitored once the air supply and stirring were resumed (Figure 1).

Table 1 Bioreactors specifications.

Specification		Multifors 2 (1.4 L)	Labfors 5 (13 L)
Working volume	V (L)	1	10
Impeller type	-	Rushton	Rushton
Number of impellers	N_e	2	2
Impeller diameter	D_i (mm)	38	70
Number of blades	-	6	6
Tank height	H_R (mm)	222	470
Tank diameter	D_T (mm)	90	200
Liquid height	H_L (mm)	160	355
Culture medium density	ρ (kg.m^{-3})	1037	1037

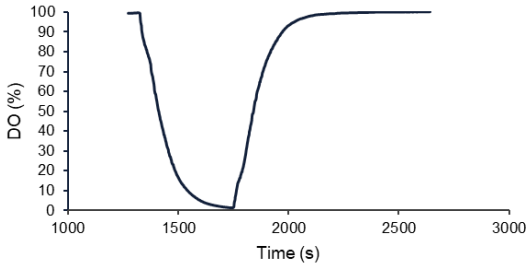

Figure 1 Dynamic gassing-out method for determining the volumetric oxygen mass transfer coefficient (k_La) in the 1 L fermenter system.

The oxygen concentration in the liquid medium during this period can be described by Equation 1:

$$\frac{dC}{dt} = k_L a\,(C^* - C_L) \tag{1}$$

where k_L is the oxygen transfer coefficient for the liquid film (cm.h^{-1}), a is the gas-liquid interfacial area per unit volume of liquid (cm^2.cm^{-3}), and C^* and C represent the saturation and local dissolved oxygen concentrations in the liquid medium (mmol.L^{-1}), respectively. Assuming k_La and C^* remain constant throughout the process, integrating the above equation yields Equation 2:

$$ln\left(\frac{C^*-C_1}{C^*-C_2}\right) = k_L a\,(t_2 - t_1) \tag{2}$$

where C_1 and C_2 represent the local dissolved oxygen concentrations in the liquid medium (mmol.L^{-1}) at times t_1 and t_2, respectively. Equation 2 can also be reorganized as Equation 3:

$$C_2 = C^* - (C^*-C_1)e^{-kLa(t-t_1)} \tag{3}$$

The Microsoft Excel add-in program Solver was used for a what-if analysis, considering the minimum error that could be achieved between the experimental C_2 values and those calculated through altering the k_La value in Equation 3. This resulted in the best approximated value of the real k_La of the liquid medium.

To determine the stirring speed (Ni) and aeration rate needed (Q) to achieve the same k_La value in the 13 L bioreactor as in the 1.4 L bioreactor the following equations were used:

$$k_L a = A\left(\frac{P_g}{V}\right)^B (v_s)^C \tag{4}$$

$$P_g = 0.72\left(\frac{(P_0)^2 N_i (D_i)^3}{Q^{0.56}}\right)^{0.45} \tag{5}$$

$$P_0 = 5\rho N_e (N_i)^3 (D_i)^5 \tag{6}$$

$$v_s = \frac{Q}{\pi\frac{D_T^2}{4}} \tag{7}$$

where P_g is the gassed power, v_s is the gas superficial velocity, V is the working volume, P_0 is the impeller power number, D_i is the impeller diameter, ρ is the density of the culture medium, N_e is the number of impellers, and D_T is the diameter of the tank. Coefficients A, B and C were selected according to the tank geometry and the type of the broth used, being A = 0.0206, B = 0.4 and C = 0.5. The Microsoft Excel add-in program Solver was used for a what-if analysis altering the values of N_i and Q with the objective of achieving a k_La of 33.23 h^{-1} in the 13 L bioreactor, which was the k_La value calculated for the 1.4 L bioreactor. A Q ≤ than 4.5 L/min was stablished as a restriction due to equipment limitation.

For the validation of the calculated parameters, the parameters Q = 4 L.min^{-1} and Ni = 365 rpm were applied at a 10 L process in the 13 L bioreactor. The experimental kLa was calculated by the dynamic gassing-out method. The extraction of the pigment was performed by collecting samples of 3 mL daily from the culture medium and subjecting them to four extractions with ethyl acetate

(1:1), vortexing it for 1 min. The solvent was recovered by centrifugation at 3200 rpm for 5 min, and a 2 mL aliquot of each extraction was added to a pool of extracts, whose absorbance was measured (Abs500) in a UV-Vis spectrophotometer.

3 RESULTS & DISCUSSION

The k_La calculated through the dynamic gassing-out method of the 1.4 L bioreactor was equal to 33.23 h^{-1}. The estimated Q and N_i were 3.95 $L.min^{-1}$ and 364.65 rpm, respectively, to achieve the same k_La at the 10 L scale. The validation was carried out using rounded values of Q = 4 $L.min^{-1}$ and N_i = 365 rpm due to easier set up in different bioreactors. Figure 2 shows the DO through time in the dynamic gassing-out method for determining the k_La in the 10 L scale bioreactor.

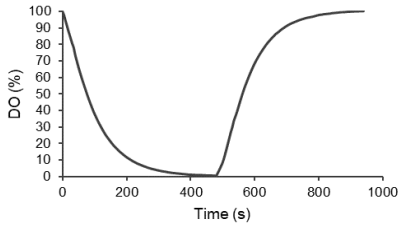

Figure 2 Dynamic gassing-out method for determining the volumetric oxygen mass transfer coefficient (k_La) in the 10 L fermenter system.

The estimated k_La obtained through the experimental data in the 10 L working volume bioreactor was 38.64 h^{-1}. Figure 3a and 3b show the Abs500 of the 1 L bioreactor and 10 L bioreactor, respectively.

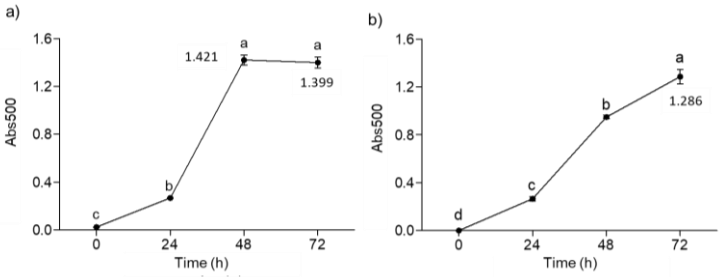

Figure 3 Pigment absorbance kinetics after 72h of *Fusarium sp*. fermentation in a 10 L (a) and 1 L (b) bioreactor.

In the 1 L fermentation it was able to conclude that the bioprocess needed a minimum k_La of 33.23 h^{-1}. Therefore, the k_La of 38,64 h^{-1} obtained at the 10 L scale process was able to supply the oxygen demanded by the microorganism. The literature suggests that a constant k_La is a useful scale-up criterion for processes involving aeration, being the most used in this type of processes[8]. Furthermore, using the calculated parameters of Q = 3.95 $L.min^{-1}$, N_i = 364.65 rpm and k_La = 38.64 h^{-1} the pigment production achieved the minimum Abs500 criterion of 1.286 obtained in the 1 L process (Figure 3b), reaching 1.421 at the 48h mark, which represents approximately 10% increase in pigment production. Therefore, the scale-up from 1 L to 10 L bench-scale bioreactor using the constancy of k_La as criterion was successful.

4 CONCLUSION

The experimental data showed that the k_La obtained in the 10 L process with the calculated parameters using the constancy of k_La as a criterion was able to supply the oxygen demand of the microorganism. Furthermore, the production of the biopigment increased by approximately 10% using the calculated scale-up parameters. Finally, the scale-up from 1 L to 10 L bench-scale bioreactor was successful.

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