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RSM Analysis of the Effects of the Oxygen Transfer Coefficient and Inoculum Size on the Xylitol Production by *Candida guilliermondii*

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Abstract

Biotechnology production of xylitol is an excellent alternative to the industrial chemical process for the production of this polyalcohol. In this work the behavior of *Candida guilliermondii* yeast was studied when crucial process variables were modified. The $K_L a$ (between 18 and 40/h) and the initial cell mass (between 4 and 10 g) were considered as control variables. A response surface methodology was applied to the experimental design to study the resulting effect when the control variables were modified. A regression model was developed and used to determine an optimal value that was further validated experimentally. The optimal values determined for $K_L a$ and X_0 were 32.85/h and 9.86 g, respectively, leading to maximum values for productivity (1.628 g/h) and xylitol yield (0.708 g/g).

Index Entries: Xylitol; response surface methodology analysis; xylose.

Introduction

Xylitol has been the subject of several studies owing to its important physicochemical properties, sweetening power, and anticariogenicity, among other advantages over other synthetic and natural sweeteners *(1)*.

The industrial production of xylitol basically follows a chemical route, which involves many different expensive steps, making unfavorable the competition of xylitol with other sweeteners as sorbitol or sucrose (the cost of production of xylitol is approx 10 times higher than the one for producing sucrose or sorbitol) *(1,2)*. For several reasons, the biotechnological process may be considered a viable alternative when compared with the industrial chemical route for the production of xylitol. It does not demand previous purification of xylose and specific microorganisms can

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be used to act directly in the conversion of xylose to xylitol, requiring only simple purification steps and, consequently, a reduction of the production costs.

The conversion of xylose to xylitol by *Candida guilliermondii* occurs through a sequence of oxidation–reduction enzymatic reactions. First, xylose is reduced to xylitol by nicotinamide adenine dinucleotide phosphate (NADPH)-linked xylose reductase; then, xylitol can be excreted from the cell or oxidized to xylulose by NAD^+-linked xylitol dehydrogenase. The activity of these enzymes depends on their respective coenzyme concentration: NADPH is regenerated through the pentose-phospate route and NAD^+ is regenerated in the respiratory chain, being directly influenced by the availability of oxygen. Thus, a high concentration of oxygen leads to a higher reoxidation rate of NADH, favoring the actuation of the xylitol dehydrogenase and, consequently, cell growth. However, a condition of unavailability of oxygen would halt the metabolism of the yeast, causing its death. On the other hand, a low concentration of oxygen unbalances the redox reaction and generates the accumulation of xylitol *(2–4)*.

The productivity of the bioconversion of xylitol from xylose using *C. guilliermondii* may be considered low, owing to the long times involved. Hence, studies aiming at the increase of productivity are necessary for this bioprocess to become an industrial reality. Such studies can be developed using experimental design statistical techniques for model analysis and determination of optimal conditions.

The optimization of the xylitol biological production is very important to the process scale-up. The classical method of optimization involves varying the level of one parameter at a time over a certain range, whereas holding the other test variables constant. This strategy is generally time consuming and requires a large number of experiments to be carried out *(5)*. Experimental designs have been employed for the optimization of the cultivation process as they offer the possibility of studying several variables with a reduced number of experiments *(6–8)*.

This work aimed the study of the kinetics of the production of xylitol by *C. guilliermondii* yeast. Experiments were planned and carried out in bioreactor, varying the aeration rates and the initial cell mass. The experimental conditions that lead to maximum productivity and yield were also determined.

Methods

Microorganism

The yeast *C. guilliermondii* IM/UFRJ 50088 was obtained from The Institute of Microbiology of The Federal University of Rio de Janeiro, Brazil. The strain was maintained at 4°C on xylose agar in a medium with the same composition, as follows.

Media Composition

Growth medium: 20 g/L D-xylose, 1.25 g/L urea, 1.1 g/L KH_2PO_4, 1.5 g/L yeast extract. Cultivation medium: 50 g/L D-xylose, 1.25 g/L urea, 1.1 g/L KH_2PO_4, 0.5 g/L yeast extract. Salt and citric acid solution (40 mL/L) were added to both growth and cultivation media *(9)*. The initial pH was adjusted to 6 with HCl or NaOH. D-xylose was sterilized separately from other components to prevent damage to the nutritional qualities of the medium. The sterilization condition, in both cases, was 0.5 atm/15 min.

Inoculum Conditions

Cells were previously activated in a 500-mL conical flask containing 200 mL of working volume of growth medium, which was incubated at 30°C in a rotary shaker at 200 rpm (throw = 5 cm) for 24 h. The inoculum was prepared in 500-mL conical flask with 200 mL of working volume of growth medium, inoculated with 20 mL of activation culture and incubated in similar conditions for 32 h. After cell quantification, the volume required to achieve the inoculum concentration was centrifuged at 8000*g* for 20 min.

Cultivation Process

Experiments were carried out in a batch bioreactor (1.5 L; BIOFLO III, New Brunswick, USA) with 1.2 L working volume, at 30°C and controlled pH of 6.0. For each initial cell mass value (X_0) different oxygen transfer coefficient (K_La) values were evaluated. The pH and temperature were maintained constant throughout the cultivation. The cultivation was brought to an end when xylose in the medium was exhausted.

Analytical Methods

The samples were withdrawn, each at 3 h. The cells were measured for absorbance at 570 nm and a calibration curve, obtained by the dry weight method, was used.

D-xylose and xylitol were measured by high-performance liquid chromatography–Waters using a Shodex SC1011 ion-exchange column for sugars (300 × 8 mm^2) at 75°C and degasified Milli-Q water as the mobile phase at a flow rate of 0.8 mL/min *(7,13)*.

The oxygen transfer coefficient (K_La) was determined through a correlation (Eq. 1) between K_La (/h), agitation (rpm), and air flow (L/min) developed for the system employed *(10)*, the gassing-out method was used, as suggested by Moser *(11)*.

$$K_L a = [0.014 \times \mathrm{Log}(Q) + 0.094] \times N \quad (1)$$

Experimental Design

The K_La and initial cell mass (X_0) were chosen as the most important variables to improve the bioconversion performance. Based on previous studies for the process the others variables were kept constant *(3,12,13)*.

Table 1
Central Composite Design and Experimental Results

Order of experiments	Original variables: Initial cell mass (g)	Original variables: K_La (/h)	Codified variables: X_1	Codified variables: X_2	$Y_{P/S}$ (g/g)	Q_P (g/h)
F1	4	18	–1	–1	0.614	0.523
F2C	7	29	0	0	0.756	1.167
F3C	7	29	0	0	0.761	1.184
F4	4	40	–1	1	0.335	0.731
F5	10	40	1	1	0.514	1.572
F6	10	18	1	–1	0.632	0.442
F7	7	13.4	0	–1.414	0.518	0.391
F8	2.8	29	–1.414	0	0.879	1.428
F9	7	44.5	0	1.414	0.476	1.291
F10	11.2	29	1.414	0	0.639	1.213

C, central point; K_La, volumetric oxygen transfer coefficient; X_1, normalized initial cell mass; X_2, normalized volumetric oxygen transfer coefficient.

The yield of xylitol on xylose consumed ($Y_{P/S}$) and mass productivity (Q_P) are important factors to evaluate the viability to any process. For that reason, these factors were selected to analyze the process and to determine K_La and X_0 conditions that would permit to maximize the bioconversion process within the operational interval experimentally evaluated.

A central composite design was developed. High, intermediate, and low levels of the factors (K_La and X_0) were considered. The experimental design matrix is showed in Table 1 *(14)*. The statistical analysis was performed using the program STATISTICA 6 *(15)*.

Results and Discussion

Table 1 presents the experimental results obtained for each response variable (Q_P and $Y_{P/S}$). The yield of xylitol on xylose consumed ($Y_{P/S}$) is defined as the fraction of xylose that was converted to xylitol (g/g), and mass productivity (Q_P) is defined as xylitol amount produced in time unit (g/h). These values were calculated using the values obtained for xylitol and xylose at the end of each bioconversion.

Modifications in the studied variables (K_La and initial cell mass) induce important changes in the kinetic behavior of the yeast, as pointed out previously. The yeast performance is altered when oxygen supply is high. In this condition it uses substrate only for the cell growth, i.e., the regeneration of NAD^+ is increased in the respiratory chain in which oxygen is used as final electron receptor. In this circumstance, the xylitol dehydrogenase activity is stimulated allowing the enhancement in the oxidation rate of xylitol *(2,3,12)*.

When oxygen supply is reduced, the cell growth is limited; xylose is consumed essentially for energy maintenance and xylitol production. The

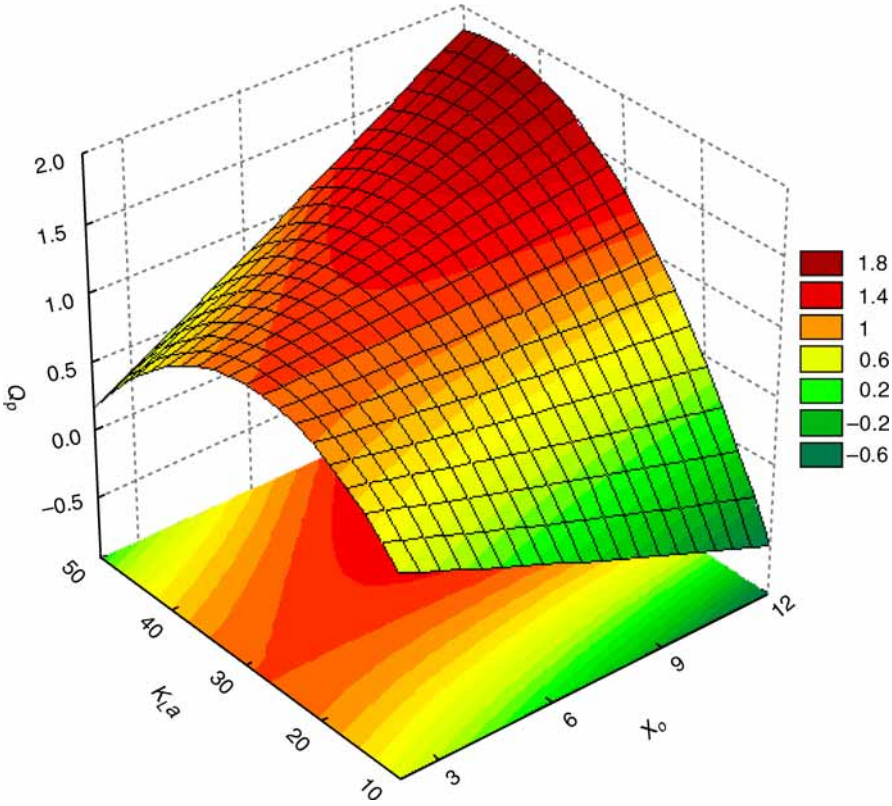

Fig. 1. Response surface for xylitol productivity (Q_P) using K_La and X_0 as controlled variables.

low-cell-growth rate is owing to a decrease of the regeneration rate of NAD^+ in the respiratory chain, allowing the accumulation of xylitol (2). On the other hand, bioconversion times are longer, lowering the productivity values (F1, F6, and F7).

In the bioconversion coded by F2C, F3C, F5, F8, F9, and F10, an equilibrium between cell mass and available oxygen was observed, allowing a low reoxidation of NADH and consequently, an accumulation of xylitol.

For the best productivity value (1.572 g/h) obtained in F5, the product yield was among the lowest one (0.514 g/g). It is possible to conclude that the amount of the dissolved oxygen for cell (in this cultivation) is still high, making the xylitol oxidation fast and facilitating the cell growth. This cannot be considered an appropriate condition to the synthesis of xylitol because the product yield is still low.

Table 1 shows that, for bioconversions with the same K_La and different inoculum levels, the yield coefficients do not present substantial changes. For instance, for a K_La of 29/h and initial cell mass variations between 2.76 and 11.24 g, the productivity and product yield values were approx 1 g/h and 0.7 g/g, respectively. It is possible to conclude that the behavior of the yeast was controlled basically by K_La.

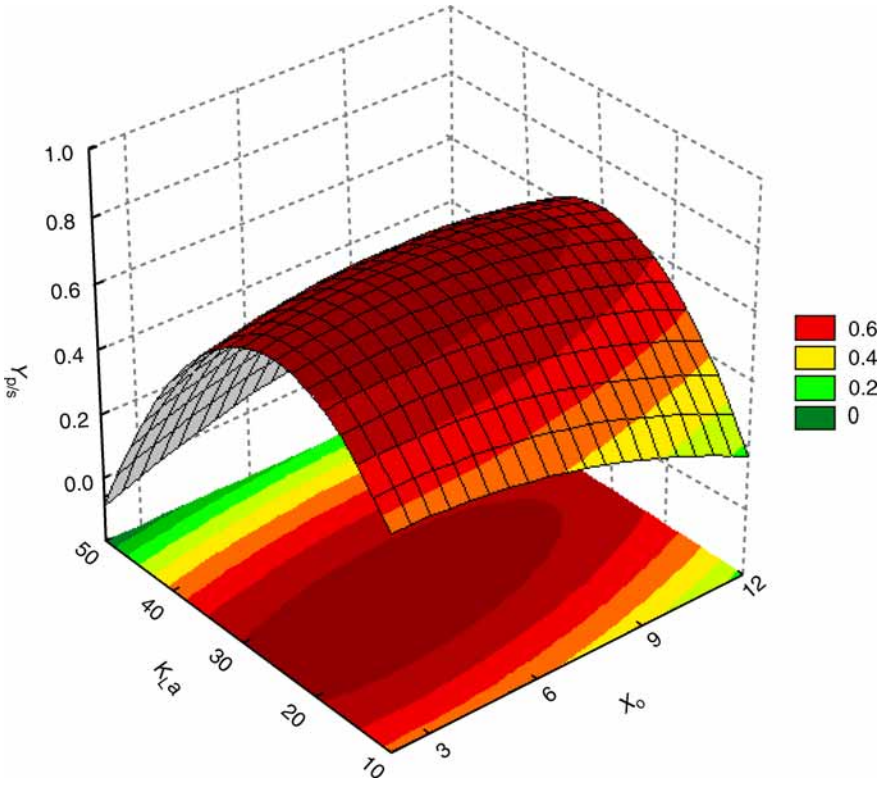

Fig. 2. Response surface for xylitol yield on xylose consumed ($Y_{P/S}$) using K_La and X_0 as controlled variables.

The following models were obtained for the relation between productivity (Eq. 2) and product yield (Eq. 3) with the controlled values:

$$Q_P = 1.183 + 0.057X_2 + 0.326X_1 - 0.236X_1^2 + 0.230X_1X_2 \quad (2)$$

$$Y_{P/S} = 0.759 - 0.018X_2 - 0.026X_2^2 - 0.057X_1 - 0.157X_1^2 + 0.040X_2X_1 \quad (3)$$

where all the coefficients in the equations above present statistical significance (p-level < 0.05). The pure errors were 1.4×10^{-4} and 1.1×10^{-5} for Q_P and $Y_{P/S}$, respectively *(10)*.

When the productivity was the sole response variable considered within the evaluated interval, an ascending profile was found and the maximum value was at the upper extreme point of the interval (Fig. 1).

On the other hand, when the product yield was the only variable considered, an optimal point was determined (0.771 g/g) for K_La of 26.25/h and an initial cell mass of 5.41 g (Fig. 2). However, it was important to consider the optimization of both response variables together through the *desirability* function *(14,15)*. This function allowed the superposition of product yield and productivity response surfaces ($Y_{P/S}$ and Q_P) and permitted the determination of the optimal process point.

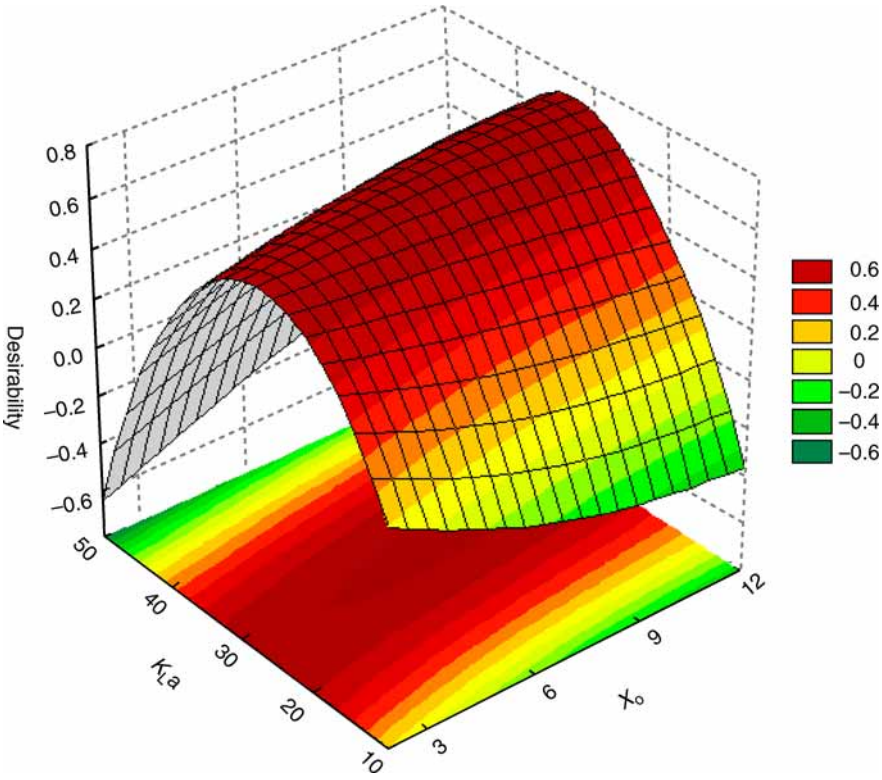

Fig. 3. Response surface for desirability function using K_La and X_0 as controlled variables.

Statistical Optimization

The multiobjective optimization resources of STATISTICA 6 were used to conjugate the response surface of each response variable and the determination of values which render the optimal point for both productivity and product yield. Figure 3 shows the superposition of product yield and productivity response surface. It displays in the dark region the maximum values of desirability function *(14,15)* (function that represents the superposition of two response variables will be maximized) and $Y_{P/S}$ and Q_P condition which lead to this optimal point.

The optimal point (desirability function = 0.748) corresponds to cell mass value of 9.86 g and K_La value of 32.85/h. In these conditions the model predicts productivity of 1.40 ± 0.09 g/h and a product yield of 0.70 ± 0.02 g/g, with a confidence level of 95%.

A new experiment was performed assuming the conditions established by the desirability function (K_La = 32.85/h and X_0 = 9.86 g) with the aim to validate them. The results obtained are showed in the Fig. 4. The values of productivity (1.628 g/h) and product yield (0.708 g/g) achieved in this cultivation were closed to the ones predicted by desirability function.

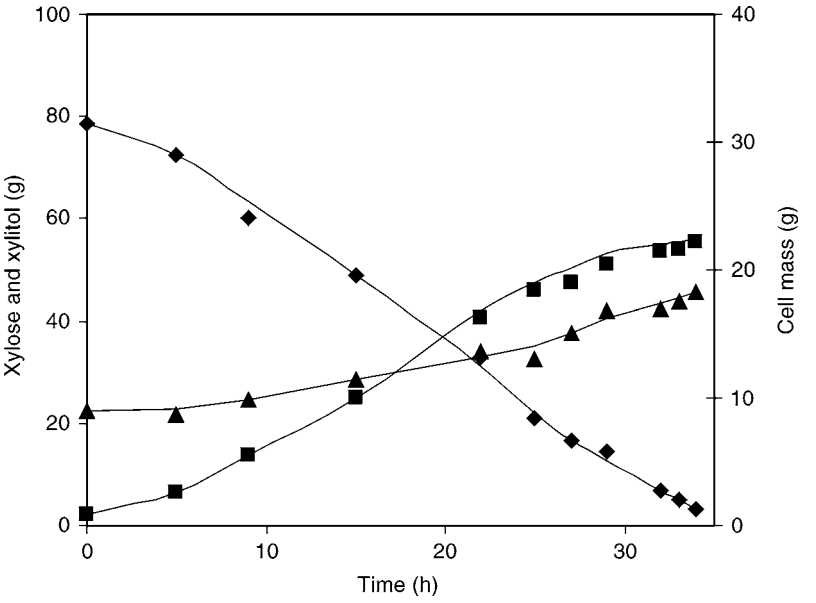

Fig. 4. Kinetics at the optimal condition ($K_L a = 32.85/h$ and $X_0 = 9.86$ g); (-♦-) xylose, (-■-) xylitol, and (-▲-) cell mass.

Conclusion

Experimental verification of optimal point leads to the conclusion that the statistical model can predict efficiently the bioconversion performance by *C. guilliermondii* in the bioreactor at the study conditions. The insertion of statistical tools into the realm of the experimental investigation of the bioconversion of xylose by *C. guilliermondii* allowed a systematic approach, which made possible the optimization of the process.

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