Use of Response Surface Methodology to Predict Optimal Conditions of *Kluyveromyces lactis* Permeabilization by a Physical Method

J. Teles de Faria, a M. Lopes Moraes, b A. Del Borghi, c A. Converti, c,*
F. M. Lopes Passos, d L. A. Minim, a A. P. de F. C. Vanzela, a and F. Coelho Sampaiob

aDepartment of Food Technology, Federal University of Viçosa, Av. P. H. Rolfs s/n, 36571-000 Viçosa, Minas Gerais, Brazil
bDepartment of Pharmacy, Federal University of Jequitinhonha and Mucuri, Rua da Glória, 187, 39100-000 Diamantina, Minas Gerais, Brazil
cDepartment of Chemical and Process Engineering, University of Genoa, via Opera Pia 15, 16145 Genoa, Italy
dDepartment of Microbiology, Federal University of Viçosa, Av. P. H. Rolfs s/n, 36571-000 Viçosa, Minas Gerais, Brazil

**Kluyveromyces lactis** is an important commercial source of *β*-galactosidase, an enzyme of major concern for dairy industry. Among the methods for *β*-galactosidase recovery, cell permeabilization is the best alternative, but it is influenced by a number of operating conditions that need to be optimized. Aim of this study was to determine the best experimental conditions for yeast cells permeabilization by physical disruption with glass beads. Influence of glass beads and dry biomass amounts and process time was investigated varying these independent variables according to a central composite rotatable experimental design. A third order polynomial equation was fitted to the experimental data of permeabilized cells *β*-galactosidase activity, and the Response Surface Methodology employed for optimization. Optimum permeabilization conditions (0.90 g glass beads, 2.00 g dry biomass and 8.0 min of treatment) ensured a maximum *β*-galactosidase activity (6,434 mmol L–1 oNP min–1 g–1) in reasonable agreement with the yield reproduced under the same conditions (5,585.40 mmol L–1 oNP min–1 g–1).

**Key words:**
Cheese whey, *Kluyveromyces lactis*, yeast, *β*-galactosidase, glass beads

**Introduction**

The enzyme *β*-galactosidase (*β*-D-galactohydrolase, EC 3.2.1.23) is a glycosidase, also known as lactase, which is present in a wide variety of sources including plants, animals, and microorganisms.1–3 Strains of the yeast *Kluyveromyces lactis* are among the well-known microorganisms able to assimilate lactose due to their *β*-galactosidase activity, which cleaves lactose to glucose and galactose, thereby enabling them to be used as carbon/energy sources.

The yeast *K. lactis* is an important commercial source of *β*-galactosidase, and its enzyme is one of the most used in the dairy industry to obtain lactose-free milk products for lactose intolerant people.4–8 It is also used for whey hydrolysis to obtain glucose and galactose.9 The production of galacto-oligosaccharides (prebiotics) from lactose by reverse hydrolysis or by the kinetically-controlled synthesis through a transglycosidase reaction has also become of interest.10–12

Most of the studies on *β*-galactosidase from *K. lactis* have been performed with either free11 or immobilized enzymes13 extracted from cultures, or with whole washed,14 permeabilized,15 or immobilized cells.16 Among the methodologies employed for *β*-galactosidase exploitation, cell permeabilization appears to be one of the most promising alternatives to the processes presently used. In fact, the process alleviates the permeability barrier of cell wall, permitting the low molecular mass compounds to pass freely across the cell wall, including substrate and product. Moreover, the use of permeabilized cells allows evaluating the activity of an enzyme *in vivo*, leaving the interactions with other biomolecules notoriously influencing the enzyme activity unaltered.

Cell permeabilization or disruption can be performed either by physical processes employing glass beads15 or a pressure cell17 or by chemical ones such as treatments with organic solvents.1,18 In all cases, it is important to define the action (permeabilization or disruption) as well as to establish the variables or conditions to achieve maximum *β*-galactosidase activity at a laboratory scale.
Cell permeabilization is influenced by a number of experimental conditions that need to be optimized. However, the classical method of optimization in which the level of one parameter is varied at a time over a certain range, while the other variables are held constant, is generally time-consuming and requires a large number of experiments to be carried out. Furthermore, the optimization studies done according to such a traditional protocol do not reflect the interaction effects among the independent variables and do not depict the net effect of the various factors on the enzyme activity. These restrictions can be overcome by the use of statistical experimental factorial designs, and the experimental response is normally fitted by the so-called Response Surface Methodology (RSM).

To identify the most suitable operating conditions for *K. lactis* permeabilization by physical disruption with glass beads, a central composite rotatable design was used in this work, varying time, glass beads amount and biomass amount as the independent variables. The resulting β-galactosidase activity of permeabilized cells was worked out as a response by RSM.

**Materials and methods**

**Microorganism and maintenance**

The strain *Kluyveromyces lactis* CBS2359 belonging to the Collection of the Federal University of Viçosa (UFV) was used in this study. The stock culture was maintained at –80 °C on YPD (10 g L⁻¹ yeast extract; 20 g L⁻¹ peptone and 20 g L⁻¹ D-glucose) containing 40 % glycerol. Before each experiment, cells were transferred and grown for 48 h at 30 °C on Petri dishes containing Sabouraud agar (5 g L⁻¹ casein peptone; 5 g L⁻¹ meat peptone, 20 g L⁻¹ D-glucose and 15 g L⁻¹ agar-agar).

**Preparation of culture medium**

Lyophilized cheese whey was supplied by Conaprole Industry (Montevideo, Uruguay). The preparation contained *w* = 70 % lactose, *w* = 12 % proteins, *w* = 2 % fats, *w* = 6 % salts and 4 % moisture. To obtain a lactose content consistent with that of whey, 5.75 g of lyophilized cheese whey were dissolved in 100 mL distilled water. The preparation was heated at 100 °C for 10 minutes to dissolve the crystallized lactose and centrifuged for 5 minutes at 2,000 rpm (Centrifuge series 600, Beckman, Fullerton, CA, USA) to remove the precipitate. Reconstituted whey was sterilized by autoclaving at 121 °C for 15 minutes, centrifuged (2,000 rpm, 5 minutes) under sterile conditions to remove the precipitate, and the supernatant used as culture medium.

**Growth conditions**

Loops full of cells were transferred from plates to 500 mL Erlenmeyer flasks containing 200 mL of whey with approximately 5.0 g L⁻¹ lactose. The flasks were maintained at 30 °C under agitation (150 rpm) for 24 h. Cells from the preceding culture were harvested by centrifugation (2,000 rpm, 5 minutes) and washed twice with 0.1 mol L⁻¹ potassium phosphate buffer, pH 7.0. The final pellet was resuspended in 5 mL of 0.1 mol L⁻¹ potassium phosphate, pH 7.0.

**Cell permeabilization**

Physical permeabilization was performed at room temperature using glass beads with 425–600 μm diameter (Sigma, St. Louis, MO, USA) by vigorously stirring at 3,000 rpm in a vortex, model AP-56 (Phoenix, Araraquara, SP Brazil). It was carried out in 15 mL Falcon tubes containing 5 mL of permeabilization mixture. During this procedure, suspension was kept at 4 °C to prevent overheating, whereas time of agitation, glass beads and biomass amounts were varied. The supernatant was removed by centrifugation (2,000 rpm, 5 minutes) and stored. Cells were washed twice with 0.1 mol L⁻¹ potassium phosphate buffer, pH 7.0, and re-suspended in 3 mL of the same washing buffer. Enzyme activity was determined either in the supernatant or in permeabilized cells.

**Experimental design and statistical analysis**

Experimental results were collected from experiments conducted according to a central composite rotatable design (CCRD) comprising three independent variables (treatment time, glass beads amount and biomass amount) at five levels (–1, 0, +1, +2). The points having one factor and axial distance α were chosen to be 1.682 (2¹/₄, where *n* is the number of variables, i.e. *n* = 3) to make this design orthogonal. To estimate the experimental error as well as check the suitability of the proposed model, the central point was repeated 5 times (all factors at level 0), while the remaining runs were carried out in triplicate. The coded and corresponding uncoded values of the independent variables are given in Table 1. Therefore, the total number of tested conditions, corresponding to 2ⁿ + 2 × *n* + central point runs, was 19, while that of experiments was 47.

The lower and upper borders of variable ranges were chosen according to Numanoğlu and Sungur, as well as previous results (unpublished).
The coded levels of each factor were obtained by the equation:

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} \quad i = 1, 2, 3$$

where $x_i$ and $X_i$ are the coded and actual values of independent variable $i$, $X_0$ the actual value of independent variable at central point, and $\Delta X_i$ the step change of $X_i$ corresponding to a unit variation of the coded value.

The experimental data of $\beta$-galactosidase activity, selected as the response ($Y$) and expressed in mmol L$^{-1}$ oNP min$^{-1}$ g$^{-1}$, were then analyzed by Response Surface Methodology (RSM) with the aim of finding a quadratic model able to successfully fit them:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + 
\beta_11 x_1^2 + \beta_22 x_2^2 + \beta_33 x_3^2 
+ \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3$$

where $\beta_0$, $\beta_1$, ..., $\beta_{123}$ represent the coefficient estimates with $\beta_0$ having the role of a scaling constant. The first term in the equation is the intercept, the second to fourth terms are the individual linear effects of each independent variable, the fifth to seventh are quadratic ones, while the others refer to the interaction effects between the independent variables two by two, as well as all together.

The Student’s $t$-test permitted us to check the statistical significance of the regression coefficients, while the Fisher’s test for analysis of variance (ANOVA), obtained by PROC GLM (General Linear Models) procedure in “SAS” software (version 9.0, SAS Institute Inc., Cary, NC, USA), was used in the regression analysis of experimental data to evaluate the statistical significance of the model. The “Design Expert” software (trial version 7.0.0, Stat-Ease, Minneapolis, MN, USA) was employed for graphical optimization procedure. The model for the response was expressed in terms of coded variables, without taking into account the statistically non-significant terms.

### Analytical methods

The optical density at 600 nm (OD$_{600}$) was determined by a spectrophotometer, series 600 (Beckman, Fullerton, CA, EUA), and then related to cell concentration, $X$ (g dry mass L$^{-1}$), through a calibration curve (OD$_{600} = 4.2194 X$). $\beta$-galactosidase activity was determined as previously described, with modifications. Yeast cells were recovered by centrifugation (2,000 rpm, 5 minutes) after permeabilization or disruption. Samples (0.1 mL) of the supernatant or the permeabilized cells suspension were mixed with 2.0 mL of 0.1 mol L$^{-1}$ potassium phosphate buffer containing 0.1 mmol L$^{-1}$ MgSO$_4$ (pH 7.2) and 0.5 mL of 4 mg mL$^{-1}$ o-nitrophenyl-$\beta$-D-galactopyranoside solution (ONPG, Sigma, St. Louis, MO, USA). After incubation for 7 minutes at room temperature, the reaction was stopped by addition of 1.0 mL of the previous mixture to 0.5 mL of 1.0 mol L$^{-1}$ of Na$_2$CO$_3$, and the absorbance determined at 420 nm. $\beta$-galactosidase activity was expressed in mmol L$^{-1}$ of o-nitrophenol (oNP) released per minute according to a standard curve. To normalize the effect of cell concentration, the enzyme activity was also expressed as grams of cell mass when needed. All the activity tests were performed in triplicate and expressed as mean values.

### Results and discussion

Table 2 lists the results of $\beta$-galactosidase ($\beta$-gal) activity in Kluyveromyces lactis cells permeabilized by physical disruption varying the amount of glass beads and dry biomass, as well as treatment time according to the Central Composite Rotatable Design (CCRD) presented in Table 1.

One can see from these results that all three independent variables influenced the selected response. The maximum $\beta$-gal activity (6,434 mmol L$^{-1}$ oNP min$^{-1}$ g$^{-1}$) was obtained with 0.90 g of glass beads, 2.00 g of dry biomass and after 8.0 minutes of treatment, whereas permeabilization with 0.60 g of glass beads and 2.00 g of dry biomass and after 8.0 minutes of treatment yielded the lowest $\beta$-gal activity (163.1 mmol L$^{-1}$ oNP min$^{-1}$ g$^{-1}$) (Table 2). To confirm the efficiency of the proposed cell permeabilization procedure, $\beta$-gal activity was also assayed in the supernatants collected from the first centrifugation, where it was found to always be lower than 1.0 mmol L$^{-1}$ oNP min$^{-1}$ g$^{-1}$ (data not shown). This result was indicative of the expected alteration of cell wall, in the absence of complete disruption.

The experimental $\beta$-gal activity of permeabilized cells recovered from experiments carried out according to the CCRD were then submitted to...
multiple regression analysis by the third order polynomial eq. (2), which provided the regression coefficients and standard errors listed in Table 3, together with the Student’s t-test and the p-value estimated to check the statistical significance.

One can see that the three factors $x_1$, $x_2$, and $x_3$, the interaction among all the factors, and the quadratic term of $x_1$ were statistically significant at 5 % ($p$-value < 0.05) for $\beta$-gal activity of permeabilized cells. The amount of dry biomass ($x_2$) had a negative effect, which means that using it at the highest level (+1.68), corresponding to 4.68 g, would cause a decrease in enzymatic activity. On the other hand, both the amount of glass beads ($x_1$) and the treatment time ($x_3$) exerted positive effects on the response, being the former stronger, as suggested by the higher linear coefficient (844.98). The amount of glass beads also showed a significant positive quadratic effect on $\beta$-gal activity. All interactions among entire factors were significant, as shown by a $p$-value < 0.05 for interactive terms, and led to a decrease in the response, except for the association of $x_1$ with $x_3$.

The results gathered in Table 3 indicate that $x_1$, $x_3$, $x_1^2$ and $x_1 x_3$ showed significant positive effects, whilst $x_2$, $x_1 x_2$ and $x_2 x_3$ and $x_1 x_2 x_3$ had significant negative effects, hence highlighting that $\beta$-gal activity increased as the levels of factors of the former group increased, the opposite taking place with those of the latter above certain values.

The results of analysis of variance (ANOVA) for $\beta$-gal activity are summarized in Table 4. Even though the $F$-test value for lack of fit was statistically significant (27.06), this fact does not invalidate the model for predictive purposes, because: a) the determination coefficient ($r^2$) was 0.8185, indicating that the fitted model explained more than 75 % of the variability in $\beta$-gal activity and that there was a sufficient correlation between experimental data and the model; b) the model was significant according to the $F$-test ($p$-value < 0.0001) (Table 4), which means that the regression was statistically significant at 99 % confidence level; and c) the regression coefficient was significant according to the t-test ($p$-value < 0.05) (Table 3).

Figs. 1 to 3 show the contour plots of $\beta$-gal activity from $K$. lactis cells submitted to different permeabilization conditions.

---

### Table 2 – Design matrix for experiments of $K$. lactis cells permeabilization and average results of $\beta$-galactosidase activity of permeabilized cells

<table>
<thead>
<tr>
<th>Run</th>
<th>Independent variable</th>
<th>$\beta$-gal activity (mmol L$^{-1}$ oNP min$^{-1}$ g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$x_1$ = -1, $x_2$ = -1, $x_3$ = -1</td>
<td>535.5</td>
</tr>
<tr>
<td>2</td>
<td>$x_1$ = -1, $x_2$ = -1, $x_3$ = +1</td>
<td>740.6</td>
</tr>
<tr>
<td>3</td>
<td>$x_1$ = -1, $x_2$ = +1, $x_3$ = -1</td>
<td>1,451</td>
</tr>
<tr>
<td>4</td>
<td>$x_1$ = -1, $x_2$ = +1, $x_3$ = +1</td>
<td>493.5</td>
</tr>
<tr>
<td>5</td>
<td>$x_1$ = +1, $x_2$ = -1, $x_3$ = -1</td>
<td>1,786</td>
</tr>
<tr>
<td>6</td>
<td>$x_1$ = +1, $x_2$ = -1, $x_3$ = +1</td>
<td>6,434</td>
</tr>
<tr>
<td>7</td>
<td>$x_1$ = +1, $x_2$ = +1, $x_3$ = -1</td>
<td>165.3</td>
</tr>
<tr>
<td>8</td>
<td>$x_1$ = +1, $x_2$ = +1, $x_3$ = +1</td>
<td>1,035</td>
</tr>
<tr>
<td>9</td>
<td>$x_1$ = -1.68, $x_2$ = 0, $x_3$ = 0</td>
<td>187.1</td>
</tr>
<tr>
<td>10</td>
<td>$x_1$ = +1.68, $x_2$ = 0, $x_3$ = 0</td>
<td>3,362</td>
</tr>
<tr>
<td>11</td>
<td>$x_1$ = 0, $x_2$ = -1.68, $x_3$ = 0</td>
<td>163.1</td>
</tr>
<tr>
<td>12</td>
<td>$x_1$ = 0, $x_2$ = +1.68, $x_3$ = 0</td>
<td>913.9</td>
</tr>
<tr>
<td>13</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = -1.68</td>
<td>482.6</td>
</tr>
<tr>
<td>14</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = +1.68</td>
<td>824.6</td>
</tr>
<tr>
<td>15</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = 0</td>
<td>380.1</td>
</tr>
<tr>
<td>16</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = 0</td>
<td>693.6</td>
</tr>
<tr>
<td>17</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = 0</td>
<td>302.7</td>
</tr>
<tr>
<td>18</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = 0</td>
<td>1,322</td>
</tr>
<tr>
<td>19</td>
<td>$x_1$ = 0, $x_2$ = 0, $x_3$ = 0</td>
<td>555.7</td>
</tr>
</tbody>
</table>

### Table 3 – Estimated regression coefficients for $K$. lactis cells permeabilization

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>794.72</td>
<td>117.21</td>
<td>6.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_1$</td>
<td>844.98</td>
<td>106.28</td>
<td>7.95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_2$</td>
<td>-372.65</td>
<td>106.28</td>
<td>-3.51</td>
<td>0.0010</td>
</tr>
<tr>
<td>$x_3$</td>
<td>391.06</td>
<td>106.28</td>
<td>3.68</td>
<td>0.0006</td>
</tr>
<tr>
<td>$x_1^2$</td>
<td>492.74</td>
<td>104.28</td>
<td>4.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_1 x_2$</td>
<td>-961.13</td>
<td>138.86</td>
<td>-6.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_1 x_3$</td>
<td>783.76</td>
<td>138.86</td>
<td>5.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_2 x_3$</td>
<td>-617.57</td>
<td>138.86</td>
<td>-4.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_1 x_2 x_3$</td>
<td>-326.95</td>
<td>138.86</td>
<td>-2.35</td>
<td>0.0277</td>
</tr>
</tbody>
</table>

### Table 4 – Results of analysis of variance (ANOVA) for $K$. lactis cells permeabilization performed according to the CCRD

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.0017 · 10^8</td>
<td>8</td>
<td>1.2521 · 10^7</td>
<td>27.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>2.2212 · 10^7</td>
<td>48</td>
<td>4.6275 · 10^5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>1.6905 · 10^6</td>
<td>6</td>
<td>2.8175 · 10^5</td>
<td>22.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pure error</td>
<td>5.3071 · 10^6</td>
<td>42</td>
<td>1.2636 · 10^5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.2238 · 10^8</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, DF = degrees of freedom, MS = mean square.
To better visualize the combined effects of the three independent variables on the enzymatic activity, the treatment time was maintained constant at three levels (−1, 0 and +1). Performing the permeabilization treatment for 3 minutes (Fig. 1a), the maximum enzymatic activity was obtained at the highest level of dry biomass with the lowest level of glass beads, or vice versa, whereas the minimum value of the response was found at the lowest levels of both factors. Thus, a low permeabilization time combined with high and low amounts of glass beads and dry biomass, respectively, resulted in an increased β-gal activity of K. lactis permeabilized cells. On the other hand, when the permeabilization time was progressively increased to 5.5 minutes (Fig. 1b) and to 8 minutes (Fig. 1c), the higher the level of glass beads and the lower that of biomass, the higher the β-gal activity.

To confirm the maximum β-gal activity obtained experimentally at the optimum levels of treatment time and amounts of glass beads and dry biomass (Table 3), the above third order polynomial model (eq. 2) was then used to predict values of the response either under these optimal conditions or slightly varying them. For this purpose, we conferred to the independent variables either the same importance or weight by means of the “Design expert” software and assumed maximization of the enzyme activity as the main criterion for constraints optimization. The results of such a confirmation attempt listed in Table 5 show that the estimated β-gal activity ranged between 5,523 and 5,585 mmol L⁻¹ oNP min⁻¹ g⁻¹, i.e. it was 13–14% lower than the maximum experimental value (6,434 mmol L⁻¹ oNP min⁻¹ g⁻¹) obtained under optimum conditions (run 6, Table 3), which confirms the reproducibility of results.

Finally, an effort was made to compare the results of this work with those of similar treatments reported in the literature. In the present work, the ratio of glass beads to cell suspension volume varied from 0.09 g mL⁻¹ to 1.10 g mL⁻¹, and the highest values of β-gal activity were observed at the highest ratios. Numanoğlu and Sungur, working on K. lactis ATCC 8583 permeabilization by a physical treatment with glass beads in a vortex, ob-

![Fig. 1 – Contour plots of β-galactosidase activity of K. lactis permeabilized cells obtained at a treatment time of (a) 3.0 min, (b) 5.5 min, (c) 8.0 min](image)

<table>
<thead>
<tr>
<th>Number</th>
<th>Glass beads (g)</th>
<th>Dry biomass (g)</th>
<th>Time (min)</th>
<th>β-gal activity (mmol L⁻¹ oNP min⁻¹ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.90</td>
<td>2.00</td>
<td>8.00</td>
<td>5585.40</td>
</tr>
<tr>
<td>2</td>
<td>0.90</td>
<td>2.00</td>
<td>7.98</td>
<td>5567.46</td>
</tr>
<tr>
<td>3</td>
<td>0.90</td>
<td>2.03</td>
<td>8.00</td>
<td>5522.81</td>
</tr>
</tbody>
</table>
served, using a treatment time of 10 minutes, a progressive increase in enzyme activity (up to approximately 2,500 mmol L\(^{-1}\) min\(^{-1}\) g\(^{-1}\) of cells) when this ratio was increased up to 0.6018 g glass beads mL\(^{-1}\) cell suspension, but no significant influence was observed beyond this threshold. The maximum \(\beta\)-gal activity reported by these authors was less than one half of that obtained in this work under optimum conditions, which may be explained by the fact that their amount of glass beads per gram of dry biomass was much higher (18.36 g g\(^{-1}\) to 321.56 g g\(^{-1}\)) than the one utilized here (maximum ratio of 0.450 g g\(^{-1}\)). Such a comparison suggests that the glass beads/dry biomass ratio is a crucial parameter that needs to be optimized for a successful permeabilization process.

Conclusions

In this study, Kluyveromyces lactis cells were permeabilized by a physical disruption process making use of glass beads to exalt their \(\beta\)-galactosidase activity. The experiments were performed according to a central composite rotatable experimental design to determine the effects of three important independent variables (namely glass beads amount, dry biomass amount, and treatment time) on \(\beta\)-galactosidase activity of permeabilized cells, and the Response Surface Methodology was employed for optimization. Linear, quadratic and interaction effects of these variables were determined, and a statistically significant third order polynomial model was obtained by fitting the experimental data. The maximum \(\beta\)-galactosidase activity predicted by this model (5,585 mmol L\(^{-1}\) oNP min\(^{-1}\) g\(^{-1}\)) under optimum levels of glass beads (0.90 g), dry biomass (2.00 g) and treatment time (8.00 minutes) was only 13 % lower than the experimental one obtained under the same conditions, demonstrating the reproducibility of results. The permeabilization method developed in this work has the potential to decrease the cost of \(\beta\)-galactosidase to be used as catalyst in the food industry, in that it would not require complex and expensive purification steps to reach high enzyme activity. In addition, it would prevent the possible risk of toxicity associated with chemical permeabilization procedures as well as the expensive removal of contaminants, which is absolutely necessary in any food application.

ACKNOWLEDGEMENTS

The authors thank the Brazilian agencies CNPq and FAPEMIG for the financial support.

---

List of symbols and abbreviations

- ANOVA – analysis of variance
- CCRD – central composite rotatable design
- DF – degrees of freedom
- MS – mean square
- oNP – o-nitrophenol
- OD – optical density
- ONPG – o-nitrophenyl-\(\beta\)-D-galactopyranoside
- RSM – Response Surface Methodology
- SS – sum of squares
- YPD – Yeast Extract Peptone Dextrose
- \(n\) – number of variables
- \(r^2\) – determination coefficient
- \(w\) – mass percentage, %
- \(X\) – cell concentration, g dry mass L\(^{-1}\)
- \(x_i\) – coded values of independent variable \(i\)
- \(X_i\) – actual values of independent variable \(i\)
- \(X_0\) – actual value of independent variable \(i\) at central point
- \(X_1\) – amount of glass beads, g
- \(X_2\) – amount of dry biomass, g
- \(X_3\) – treatment time, min
- \(Y\) – \(\beta\)-galactosidase activity, mmol L\(^{-1}\) oNP min\(^{-1}\) g\(^{-1}\)
- \(\alpha\) – axial distance
- \(\beta_{0}, \beta_{11}, ..., \beta_{123}\) – coefficient estimates
- \(\beta\)-gal – \(\beta\)-galactosidase
- \(\Delta X_i\) – step change of \(X_i\)

References