Impactos das Tecnologias nas Ciências Agrárias e Multidisciplinar

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APRESENTAÇÃO

A obra “Impactos das Tecnologias nas Ciências Agrárias e Multidisciplinar” aborda uma série de livros de publicação da Atena Editora, em seu II volume, apresenta, em seus 16 capítulos, os novos conhecimentos tecnológicos para Ciências Agrárias nas áreas de Ciência e Tecnologia de Alimentos e Zootecnia.

As Ciências Agrárias englobam, atualmente, alguns dos campos mais promissores em termos de pesquisas tecnológicas nas áreas de Agronomia, Engenharia Florestal, Engenharia de Pesca, Medicina Veterinária, Zootecnia, Engenharia Agropecuária e Ciências de Alimentos que visam o aumento produtivo e melhorias no manejo e preservação dos recursos naturais. Além disso, a crescente demanda por alimentos aliada à necessidade de preservação e reaproveitamento de recursos naturais, colocam esses campos do conhecimento entre os mais importantes no âmbito das pesquisas científicas atuais, gerando uma crescente demanda por profissionais atuantes nessas áreas.

As tecnologias das Ciências Agrárias estão sempre sendo atualizadas e, a recomendação de uma determinada tecnologia hoje, possivelmente, não servirá para as futuras gerações. Portanto, estamos em constantes mudanças para permitir os avanços na Ciências Agrárias. E, cabe a nós pesquisadores buscarmos essa evolução tecnológica, para garantir a demanda crescente por alimentos em conjunto com a sustentabilidade socioambiental.

Este volume dedicado à Ciência de Alimentos e Zootecnia traz artigos alinhados com a qualidade e a produção sustentável de alimentos, ao tratar de temas como a caracterização físico-química e microbiológica de chás verde e vermelho, a elaboração de empanado de surubim-caparari, a preservação de Lactobacillus acidophilus utilizando Xantana pruni como agente encapsulante, o desempenho produtivo de frangos de corte e de suínos, o consumo de energia elétrica em unidade de produção de leite, o manejo dos resíduos sólidos e o uso da integração lavoura-pecuária-floresta para pecuaristas da região da Amazônia.

Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos nas Ciências Agrárias, os agradecimentos dos Organizadores e da Atena Editora.

Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias para a área de Agronomia e, assim, garantir incremento quantitativos e qualitativos na produção de alimentos para as futuras gerações de forma sustentável.

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SUMÁRIO

CAPÍTULO 7

OBTAINING BIOCATALYSTS BY CELL PERMEABILIZATION OF *SACCHAROMYCES FRAGILIS* IZ 275 WITH LACTOSE HYDROLYSIS CAPACITY

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RESUMO: Permeabilização foi usada para transformar células de microrganismos em biocatalisadores com alta atividade enzimática. As células de levedura de *Saccharomyces fragilis* IZ 275 foram permeabilizadas com etanol, como agente permeabilizante. Alta atividade foi observada em células permeabilizadas comparadas com células não permeabilizadas, que apresentou nenhuma atividade enzimática. O Delineamento Box-Behnken (DBB) com três variáveis em três níveis compreendendo 15 corridas foi utilizado para desenvolver o modelo estatístico de segunda ordem para a otimização das condições da permeabilização celular para uma alta atividade da beta-galactosidase. A metodologia de superfície de resposta foi utilizada para modelar o efeito da concentração de etanol, temperatura e tempo de tratamento. As condições de operação ótimas foram observadas em 35 % de concentração de etanol, temperatura de 15 ºC e tempo de tratamento de 20 minutos. Um valor máximo da atividade da beta-galactosidase foi de 10.59 μmol ONPG. min^{-1} que foi 100 % superior à atividade catalítica das leveduras não permeabilizadas.

PALAVRAS-CHAVE: agente permeabilizante, beta-galactosidase, biotecnologia microbiana, condições ótimas

ABSTRACT: Permeabilization was used to transform cells of microorganisms into biocatalysts with an enhanced enzyme activity. Yeast cells of *Saccharomyces fragilis* IZ 275 were permeabilized with ethanol, as permeabilizing agent. A high degree of activity was observed in permeabilized cells comparing with not permeabilized cells, which showed no enzymatic activity. A three-variable, three level Box-Behnken Design (BBD) comprising 15 experimental runs was used to develop a second degree statistical model for the optimization of the cells permeabilization conditions for high activity beta-galactosidase. Response surface methodology was used to model the effect of concentration of ethanol, temperature and treatment time. The optimum operating conditions were observed at 35 % concentration of ethanol, temperature of 15 ºC and treatment time of 20 minutes. A maximum value of beta-galactosidase activity was 10.59 μmol ONPG. min^{-1} and was ca. 100 % higher than the catalytic

activity of yeast not permeabilized.
KEYWORDS: permeabilizing agent, beta-galactosidase, microbial biotechnology, optimal conditions

1 I INTRODUCTION

Saccharomyces fragilis is described as a homothallic, hemiascomycetous yeast and production of several enzymes among them beta-galactosidase (Llorente et al., 2000; Dagbagli & Goksungur 2008). The major common feature of *S. fragilis* is the capacity to assimilate lactose and to use this sugar as a carbon source. The long history of safe association with food products helped *S. fragilis* achieve GRAS (Generally Regarded As Safe). This designation means that there are few restrictions on application and largely enhances their potential in the biotechnology sector (Fukuhara 2006; Schaffrath & Breunig 2000). *S. fragilis*, has been more widely adopted by industry, mainly because it possesses traits that are desirable for biotechnology applications. These include the capacity to assimilate key sugars, namely lactose in an extremely rapid growth rate (Fonseca et al., 2008).

The enzyme beta-galactosidase is one among other enzymes with industrial potential used in the hydrolysis of lactose in milk and cheese whey, generating food with low levels of lactose, whose result is a better solubility and digestibility of milk and dairy products, making them ideal for consumers intolerant to this sugar (Husain 2010). To detect enzyme activity, ONPG (o-nitrophenyl-β-D-galactopyranoside) has been used, which in turn has the disadvantage of being unable to enter into intact cells. Therefore, the cells have to be permeabilized before determination of enzyme activity (Kippert 1995).

When it is obtained from the *Saccharomyces fragilis*, beta-galactosidase is an intracellular enzyme and it is necessary the use of techniques to obtain it (Coelho, *et al*., 2008; Panesar *et al*., 2007).

Cell permeabilization techniques are often useful for many applications relating to enzyme technology. For example, permeabilization procedures are usually rapid and do not destroy cellular enzymes. Thus, the total amount of an enzyme associated with a cell can be assayed after permeabilization. A number of permeabilization methods for yeast have been developed, such as use of detergents, organic solvents, and desiccation (Becker, et al., 1996). Cell permeabilization is influenced by several operating conditions that need to be optimized. However, the traditional optimization method in which the level of one parameter is varied at a time over a certain range, while keeping the other variables constant, is generally time consuming, requiring a large number of tests (Sen & Swaminathan, 1997), and does not reflect the interaction effects among the variables and, consequently, does not depict the net effect of the various factors on the enzyme activity (Dagbagli & Goksungur, 2008). These drawbacks can be

overcome by using statistical experimental factorial designs, and the experimental data of responses are usually fitted to second order polynomial functions by the response surface methodology (RSM).

Therefore, in this work a Box-Behnken Design combined with response surface methodology was used to identify the optimal operating conditions to permeabilize *S. fragilis* IZ 275 cells using ethanol as a solvent and varying its concentration, temperature and permeabilization time as the independent variables.

2 I MATERIALS AND METHODS

2.1. Microorganism Maintenance and Inoculum

Saccharomyces fragilis IZ 275 from the Dairy Science and Technology, Graduation and Research Center, University Pythagoras Unopar was used in this study. The stock culture was maintained at - 20 °C on Potato Dextrose Agar (PDA), pH 5.5, at 30 ºC for 10 days. For the inoculum, cells were transferred and grown at 35 °C, pH 5.5 for 24 h on malt extract (15 $g.L^{-1}$).

2.2. Enzyme Extract

Saccharomyces fragilis IZ 275 was used as inoculum for beta-galactosidase production using cheese whey based media. Cheese whey obtained from a local dairy cooperative was deproteinized by heating at 90 °C (pH 4.6) for 30 minutes. Then it was filtered through Whatman nº 1 filter paper to remove coagulated protein and adjusted to pH 5.0. It was then pasteurized under 65 °C for 30 minutes. The pasteurized cheese whey was inoculated with 10% inoculum (at Optical Density, OD_{670nm} = 0.6) of *Saccharomyces fragilis* IZ 275 strain and incubated at 35 °C, 150 rpm for 24 hours. At the end of the fermentation, cells were harvested by centrifugation at 5000 rpm for 20 minutes. The pellets were washed twice with 0.1 M phosphate buffer (pH 6.8) solution and stored in 0.1 M phosphate buffer. Since beta-galactosidase from *S. fragilis* IZ 275 is an intracellular enzyme, the cells were permeabilized with ethanol according to the experimental design (Table 1). Then the permeabilized cells represented the enzyme extract.

2.3. Cell permeabilization

Cells were collected by centrifugation (5000 rpm for 5 min at 5 °C) and washed twice with distilled water. Cell permeabilization was performed in 50 mL Erlenmeyer flasks each containing 5 mL of the reacting suspension consisting of ethanol according to the experimental design (Table 1), fresh biomass of *S. fragilis* IZ 275 contained in 5 mL and completed with 0.1 M potassium phosphate buffer (pH 6.8). Flasks were incubated on an orbital shaker at 150 rpm at a temperature and for a time depending on the above

design. The cells was harvested by centrifugation at 5000 rpm, 10 minutes and washed twice with the buffer for further analysis. The final pellet was resuspended in 1 mL of buffer, and the enzyme activity of permeabilized cells determined as described later.

2.4. Beta-galactosidase activity

For *S. fragilis* IZ 275, beta-galactosidase activity of permeabilized yeast cells was determined using the chromogenic substrate ONPG (o-nitrophenyl-β-galactopyranoside, as previously described by Inchaurrondo, Yautorno & Voget (1994). A 50 μL sample of permeabilized cell suspension was mixed with 2 mL of 1.25 mM ONPG in buffer and incubated for 5 min at 37 °C. The reaction was discontinued by adding 0.5 mL of 1 M sodium carbonate. Enzymatic activity was measured spectrophotometrically at 420 nm. One unit of beta-galactosidase activity is defined as the hydrolysis of 1 μmol ONPG per minute under the conditions of the assay. All activity tests were performed in triplicate and expressed as mean values.

2.5. Experimental design

The permeabilization of *S. fragillis* IZ 275 cells for high activity of beta-galactosidase was verified using a factorial design and analysis by the response surface method. Controlled conditions for ethanol concentration, temperature and incubation time were tested according to 3-factor 3-level (-1; 0; +1) Box-Behnken Design (Montgomery, 2005) with three replicates at the central point summarizing 15 experimental runs (Table 1). The model generated by the software, based on the experimental runs, can give a robust prediction of experiments performed only within the range of study. Based on this, preliminary experiments were performed to obtain an in-depth knowledge of the process, enabling the selection of the best range for developing a design and eventually arriving at the global optimum for high activity of beta-galactosidase through permeabilization of the *S. fragillis* IZ 275 cell wall by ethanol.

2.6. Storage stability

The ability of permeabilized cells (treated with ethanol) to retain beta-galactosidase activity during storage was studied. A suspension of 1 g of cells (wet wt.) in 20 ml potassium buffer (pH 7.0) was stored at 4 ºC. At regular intervals a predetermined volume of suspension was separated by centrifugation and beta-galactosidase activity was determined in the separated cells.

3 I RESULTS AND DISCUSSION

The optimization of the cell permeabilization conditions was carried out to find the optimal values of independent variables (ethanol concentration, temperature and

treatment time), which would give maximum beta-galactosidase activity. Based on the Box-Benhken Design (BBD), the experimental levels of beta-galactosidase activity under each set of condition were determined and compared with the corresponding predicted levels suggested by the statistical program, Statistic 6.0 (Table 1). The maximum experimental value for beta-galactosidase activity was 10.59 μmol ONPG. min^{-1}, while the value of predicted response was 10.30 μmol ONPG.min^{-1}. Approximately 97 % of validity was achieved, indicating the model exerted an adequate prediction on the enzyme activity. The close correlation between the experimental and predicted data indicates the appropriateness of the experimental design. The maximum beta-galactosidase activity (10.59 μmol ONPG.min^{-1}) was achieved in the following condition, ethanol concentration 35 % (v/v), temperature 15 ºC and time 20 minutes (Table 1).

Standard Run	Variables			Response	
	X_1	X_2	X_3	Beta-galactosidase Activity (μmol ONPG.min^{-1})	
			Experimental	Predictive	
1	-1	-1	0	6,79	6,98
2	1	-1	0	10,59	10,30
3	-1	1	0	7,15	7,44
4	1	1	0	9,83	9,64
5	-1	0	-1	7,02	7,15
6	1	0	-1	9,74	10,35
7	-1	0	1	8,33	7,71
8	1	0	1	10,16	10,03
9	0	-1	-1	9,74	9,41
10	0	1	-1	9,21	8,79
11	0	-1	1	8,59	9,01
12	0	1	1	9,11	9,43
13	0	0	0	10,26	9,68
14	0	0	0	9,51	9,68
15	0	0	0	9,28	9,68

	Coded value of variables		
	X_1	X_2	X_3
Levels	Ethanol Concentration (%)	Temperature (ºC)	Time (min)
- 1	29	15	15
0	32	20	20
1	35	25	25

Table 1 - Beta-galactosidase activity of permeabilized *Saccharomyces fragilis* IZ 275 cells.

Based on the results for obtaining high activity of beta-galactosidase from permeabilized cells of *S. fragillis* IZ 275, the effect of linear (L) terms of ethanol concentration was significant ($p < 0.05$), indicating the establishment of the

concentration for the highest design limit (35 %). The linear (L) and quadratic (Q) terms of the temperature and time were not significant ($p > 0.05$), indicating that established temperature and time for the lowest design limit, 15 ºC and 15 minutes, respectively proved to be sufficient for the process.

The predicted levels of beta-galactosidase activity is shown in Table 1 along with experimental data. The significance of the variables for the beta-galactosidase activity was assessed by carrying out analysis of variance (ANOVA) with results shown in Tables 2 and 3.

The coefficient of determination R^2 of the model was 0.89648 (Table 2), which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value of 0.89648 means that 89.6 % of the variability was explained by the model, which is acceptable for biological system and only 10.4 % was as a result of chance. The coefficient of variation (C.V.) obtained was 12.95 %. The Coefficient of Variation (C.V.) indicates the degree of precision with which the treatments were carried out. A low value of C.V. suggests a high reliability of the experiment (Mason *et al*., 1989).

Source	Response value
R-squared	0.896
Adjusted R-squared	0.710
Standard deviation	1.209
C.V. %	12.95

Table 2 - Statistical information for ANOVA.

Results obtained from ANOVA is presented in Table 3. Values of ‘‘Prob. > F’’ less than 0.05 indicate the model terms are significant. Values greater than 0.10 indicate the model terms are not significant. The “Lack of Fit” F-value of 2.02396 implies that there is insignificant lack of fit. The “Lack of Fit” (Prob > F) value of 0.347587 implies that there is only 34.75 % chance that the “Lack of Fit” F-value could occur due to noise.

Sources	Sum of squares	df	Mean squares	F value	p-value [Prob > F]
X_1 – Ethanol concentration	15.20761	1	15.20761	57.90435	0.016835*
X_2 – Temperature	0.02101	1	0.02101	0.08001	0.803876
X_3 - Time	0.02880	1	0.02880	0.10966	0.772010
X_1X_2	0.31360	1	0.31360	1.19406	0.388577
X_1X_3	0.19803	1	0.19803	0.75400	0.476757
X_2X_3	0.27562	1	0.27562	1.04947	0.413359
X^2_1	1.92296	1	1.92296	7.32186	0.113744
X^2_2	0.51004	1	0.51004	1.94203	0.298112
X^2_3	0.08216	1	0.08216	0.31282	0.632231
Lack of Fit	1.59467	3	0.53156	2.02396	0.347587
Pure Error	0.52527	2	0.26263		
Total SS	20.47769	14			

Table 3 - Analysis of variance (ANOVA) for quadratic model of beta-galactosidase activity of permeabilized *S. fragillis* IZ 275.

* Significant

In order to optimize variables that influence beta-galactosidase activity from permeabilized cells of *S. fragillis* IZ 275, response surface plots were generated from the regression model. The three-dimensional response surfaces for beta-galactosidase activity: ethanol concentration, temperature and time were plotted (Figure 1).

Figure 1a shows the effects of temperature and ethanol concentration on beta-galactosidase activity. Permeabilization with low concentration of ethanol and low temperature of process showed the lowest enzyme activity. The beta-galactosidase activity was higher in the temperature range of 15 – 25 ºC and concentration of ethanol up to ca. 31 %. Panesar et al. (2007), showed that the optimum process conditions for cell permeabilization were 50 % (v/v) ethanol concentration, 25 ºC temperature and treatment time of 15 min. Figure 1b depicts the response surface plot as a function of time versus ethanol concentration. Change of time does not significantly affect the curvature of the surface. From a graphical representation, there is a dependence of beta-galactosidase activity on the concentration of permeabilizing agent (ethanol 32 – 35 %). A maximum permeabilization of 2.816 $mmol.L^{-1}$ $ONP.min^{-1}.g^{-1}$ was obtained by treating cells with 75 % (v/v) of ethanol at 20 °C for 15 min (De Faria et al., 2013). Figure 1c shows high permeabilization effectiveness within the range of time and temperature studied, while below and above these ranges, a significant decrease of activity can be noticed. This confirms that the range these variables were chosen properly and sufficient for the process.

In this study, it was also clear that the effect of ethanol concentration on beta-galactosidase activity was more important than the temperature and time. In a preliminary study by our group showed that within the tested time and temperature range at different concentrations of ethanol 50, 75 and 100 % there was a high activity

of beta-galactosidase with no significant difference.

Trawczyńska & Wójcik (2015) defined the optimum operating conditions for yeast cell permeabilization at 53 % of ethanol concentration, temperature of 14.8 ºC and treatment time of 40 min. The use of the whole yeast cells as biocatalysts is a very promising alternative and has gained a lot of interest in recent years (Yu et al., 2007).

Whole cells of *S. fragilis* IZ 275 exhibited no beta-galactosidase activity. The mechanism of enzyme release has not been fully studied. However, cell wall lysis is not believed to be the mode of enzyme solubilization. Perhaps the solvent extracts a lipid component from the yeast cell membrane, allowing leakage of intracellular or periplasmic protein. A similar procedure utilizing lower concentrations of solvent (< 20 %) has been reported for the measurement of intracellular enzyme in situ (Wendorff & Amundson 1971). In this case, no intracellular enzyme leaks out, rather small molecular weight substrate molecules diffuse into the cell.

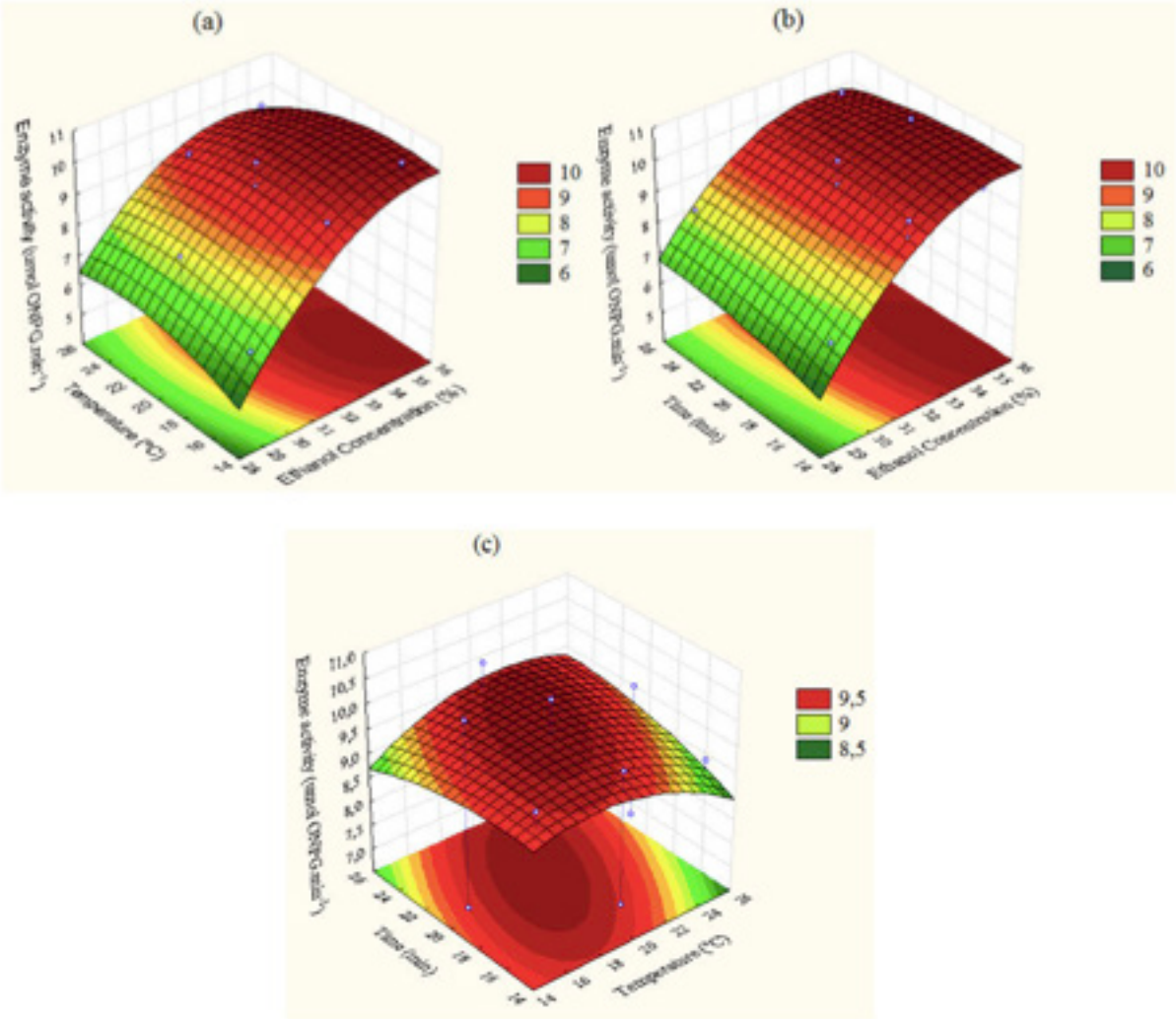

Figure 1. Response surface plot representing the effect of a) temperature and ethanol concentration, b) time and ethanol concentration, c) time and temperature on beta-galactosidase activity (μmol ONPG.min^{-1}) of permeabilized cells.

The validity of the results predicted by the regression model, was confirmed by carrying out repeated experiments under optimal permeabilization conditions (i.e.

ethanol concentration; 35 % - v/v, temperature; 15 ºC and time; 20 minutes). The results obtained from three replications demonstrated that the average of the maximum beta-galactosidase activity (10.59 μmol ONPG.min^{-1}) obtained was close to the predicted value (10.30 μmol ONPG.min^{-1}). The excellent correlation between the predicted and measured values from these experiments indicates validity of response model.

Application of Box-Behnken design in the enzyme activity by *Saccharomyces fragilis* IZ 275, it was presented as progress in predicting conditions for cell permeabilization. The response surface proved to be a powerful tool for bioprocess optimization converting to a mathematical model which predicts the location of the optimum range. The maximum enzyme activity of 10.59 μmol ONPG.min^{-1} was reached at 35 % ethanol concentration (v/v), 15 ºC and 20 minutes. The permeation process can be used for other studies requiring permeabilized cells to obtain other metabolites of interest.

Storage stability

Yeast cells after permeabilization at the optimal conditions have been tested with respect to maintaining enzymatic activity during storage. The cells showed 78 % loss of enzymatic activity when they were stored in phosphate buffer pH 6.8 at 5 ºC, for a period of 9 days. Based on the results it can be seen that there was a significant decrease in enzyme activity after 3 days (72 hours) of storage. The activity observed in the supernatant was very low.

4 I CONCLUSION

Ethanol alcohol can effectively improve the permeability of *Saccharomyces fragilis* IZ 275 cells. Statistical optimization of permeabilization of cell membrane by ethanol has been successfully carried out using RSM based on the 15 factorial Box-Behnken design. The proposed mathematical model with estimated parameters describes well the permeabilization process. The optimum operating conditions for the permeabilization process to achieve maximum enzyme activity were ethanol concentration of 35 %, 15 ºC temperature and process duration of 20 min. Under these conditions of process variables, the predicted value of maximum enzyme activity was found to be 10.59 μmol ONPG.min^{-1} (wet wt.). The fact that ethanol permeabilized *S. fragilis* IZ 275 cells retained enzyme activity for a certain period suggesting that these permeabilized cells could be used as a source of biocatalyst for different applications.

Furthermore, the use of permeabilized cells can help to overcome the problems and costs associated with enzyme extraction and purification from yeast cells and in the development of a low-cost technology for biotechnological application.

REFERENCES

Becker, J. M., Caldwell, G. A., & Zachgo, E. A. (1996). **Biotechnology: a laboratory course**. San Diego: Academic.

Coelho, M. A. S., Salgado, A. M., & Ribeiro, B. D. (2008). **Tecnologia enzimática**. Rio de Janeiro EPUB.

Dagbagli, S., & Goksungur, Y. (2008). **Optimization of b-galactosidase production using *Kluyveromyces lactis* NRRL Y-8279 by response surface methodology**. Electronic Journal of Biotechnology, 11, 11-2.

De Faria, J. T., Rocha, P. F., Converti, A., Passos, F. M. L., Minim, L. A., & Sampaio, F. C. (2013). **Statistical investigation of *Kluyveromyces lactis* cells permeabilization with ethanol by response surface methodology**. Brazilian Journal of Microbiology, 44(4), 1067-74.

Fonseca, G. G., Heinzle, E., Wittmann, C., & Gombert, A. K. (2008). **The yeast *Kluyveromyces marxianus* and its biotechnological potential**. Applied Microbiology and Biotechnology, 79, 339-54.

Fukuhara, H. (2006). ***Kluyveromyces lactis:* a retrospective**. FEMS Yeast Research, 6, 323-4.

Husain, Q. (2010). **β-galactosidase and their potential applications: a review**. Critical Reviews in Biotechnology, 30, 41-62.

Inchaurrondo, V. A., Yautorno, O. M., & Voget, C. E. (1994). **Yeast Growth and b-Galactosidase Production During Aerobic Batch Cultures in Lactose-Limited Synthetic Medium**. Process Biochemistry, 29, 47-54.

Kippert, F. (1995). **A rapid permeabilization procedure for accurate quantitative determination of b-galactosidase activity in yeast cells**. FEMS Microbiology Letters, 128, 201-6.

Llorente, B., Malpertuy, A., Blandin, G., Artiguenave, F., Wincker, P., & Dujon, B. (2000). **Genomic exploration of the hemiascomycetous yeasts: 12. Kluyveromyces marxianus var. marxianus**. FEBS Letters, 487, 71-5.

Mason, L. R., Gunst, R. F., & Hess, J. S. (1989). **Statistical design & analysis of experiments**. New York: Wiley.

Montgomery, D. C. (2005). **Design and analysis of experiments**. 6th. New York: John Wiley & Sons.

Panesar, P. S., Panesar, R.**,** Singh, **R. S., & Bera, M. B. (2007). Permeabilization of Yeast Cells with Organic Solvents for β-galactosidase Activity**. Research Journal of Microbiology, 2(1), 34-41.

Schaffrath, R., & Breunig, K. D. (2000). **Genetics and molecular physiology of the yeast Kluyveromyces lactis**. Fungal Genetics and Biology, 30, 173-90.

Sen, R., & Swaminathan, T. (1997). **Application of response-surface methodology to evaluate the optimum environmental conditions for the enhanced production surfactin**. Applied Microbiology and Biotechnology, 47, 358-63.

Trawczynska, I., & Wójcik, M. (2015). **Optimization of permeabilization process of yeast cells for catalase activity using response surface methodology**. Biotechnology & Biotechnological Equipment, 29(1), 72-7.

Wendorff, W. L., Amundson, C. H. (1971) **Characterization of beta-galactosidase from *Saccharomyces fragilis***. Journal of Milk and Food Technology, 34 (6), 300-306.

Yu, M. A., Wei, Y. M., Zhao, L., Jiang, L., Zhu, X. B. & Qi, W. (2007). **Bioconversion of ethyl 4-chloro-3-oxobutanoate by permeabilized fresh brewer's yeast cells in the presence of allyl bromide**. Journal of Industrial Microbiology and Biotechnology, 34, 151-156.

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