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Statistical Optimization of Medium Components of Xylanase Production by Irpex lacteus BAFC 1168 strain F under liquid fermentation

G. V. Díaz[1], E. M. Giorgio[1], M. I. Fonseca[1], L. L. Villalba[1], P. D. Zapata[1]

[1]Laboratorio de Biotecnología Molecular, Instituto de Biotecnología de Misiones (InBioMis), Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones. RutaNacional12, km 7.5.Posadas, Misiones, Argentina.

ABSTRACT

The enhanced xylanase production of white rot fungus *Irpex lacteus* BAFC 1168 strain F using a liquid fermentation culture was investigated. The media nutrients were screened by a Plackett-Burman design and from the analysis of variance, carboxy-methylcellulose, urea and peptone were found to be the most positive and significant nutrient components. Hence, these three variables were selected for further optimization using a five-level central composite design in response surface methodology. The validation of the model experiment was conducted reaching 8.29 U/mL of xylanase activity when the composition medium was 1.6 g/L of CMC, 0.84 g/L of urea and 2 g/L of peptone. Optimum temperature and pH conditions on xylanase activity were also determined using a central composite design. It was reached a maximum xylanase activity of 8.36 U/mL when temperature and pH were 42.8°C and 5.6, respectively.

Indexing terms/Keywords

Xylanases, Irpex lacteus, white rot fungi, response surface methodology (RSM).

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1. INTRODUCTION

Hemicellulolytic microorganisms play a significant role in nature by recycling hemicellulose, one of the main components of plant polysaccharides. Xylanases (1,4-β-D-xylan-xylanhidrolases, EC 3.2.1.8.) are hemicellulolytic enzymes which randomly cleave the 1,4-β-D-xylosidic linkages in xylan, the major constituent of hemicellulose [1, 2].Xylanases derived from microorganisms have immense potential applications in textile, food, feed, paper pulp and biofuel industries due to their wide distribution, high specificity, negligible substrate loss and side product generation [3-5]. The application of xylanases in industrial and environmental technologies requires significant amounts of these enzymes at low cost [6, 7]. Therefore, the optimization of the fermentation medium culture and the search for fungi with outstanding enzyme activity are the keys of basic research for an efficient technology of enzyme production [6-8]. Although there is an extensive literature about optimization of xylanases by Ascomycetes [7, 9-11] there are still relatively little data concerning to white rot Basidiomycetes.

In this context, Misiones rainforest (Northeast of Argentina) provides an idyllic substrate for white rot fungi (WRF) due it has one of the highest biodiversity attribute of Argentina [12], representing an untapped source of new productive genotypes, interesting from both a basic and an applied viewpoint. The aim of this work was to optimize xylanase production of a white rot fungus native from Misiones, *I. lacteus* BAFC 1168 strain F, using statistically designed experiments. We focused our attention on developing a low-cost medium that would allow enhanced xylanase activity, using firstly a Plackett-Burman design (PBD) [13] to identify the significant medium components and then a central composite design (CCD) [14] to optimize these variables.

2. MATERIALS AND METHODS

2.1 Microorganism

The microorganism used in this study was the white rot fungus *I. lacteus* BAFC 1168 strain F and was provided by the Culture Collection of the Faculty of Forestry Sciences, National University of Misiones, Argentina.This strain was maintained and periodically sub-cultured on malt extract agar (MEA) medium (20 g/L agar, 12.7 g/L malt extract) at 4 ± 1°C. *I. lacteus* BAFC 1168 strain F was grown on MEA petri plates for 5 – 7 days at 29 ± 1°C. One agar plug (36 mm^2) from these petri plates covered with mycelium was inoculated into culture mediums corresponding to PBD and CCD experiments.

2.2. Culture conditions

Xylanase production was carried out in 250 mL-Erlenmeyer flasks containing 30 mL media as according to PBD and CCD treatments. The pH was adjusted to 4.8 before autoclaving at 121°C for 20 min. The basic medium used in Plackett-Burman experiments was composed of (g/L) 2.0 $NaNO_3$, 1.0 KH_2PO_4, 0.5 KCl, 0.5 $MgSO_4.7H_2O$, 0.01 $FeSO_4.H_2O$ and basic medium used in central composite design was composed of (g/L) 2.0 $NaNO_3$, 1.0 KH_2PO_4, 0.5 KCl, 0.5 $MgSO_4.7H_2O$, 0.01 $FeSO_4.H_2O$, 10.0 xylose and 1.0 $(NH_4)_2SO_4$. The inoculated flasks were incubated at 29°C for 12 days in static conditions. The broth was centrifuged at 10,000×*g* for 10 min at 4°C, and the supernatants were used for xylanase determination.

2.3. Enzyme activity assay

Xylanase activity assay was performed according to Bailey et al. [15]. The reaction mixture consisting in 0.1 mL of a 1% (w/v) suspension of beechwood xylan (Sigma-Aldrich, USA) in 50 mM sodium acetate buffer pH 4.8 and 0.1 mL of enzyme dilution (in 50 mM sodium acetate buffer pH 4.8) was incubated at 50°C during 60 min. Released reducing sugars were determined by 3,5-dinitrosalicylic acid reagent (DNS) method [16]. Absorbance was measured at 540 nm in a Shimadzu UV- 3600 spectrophotometer. The enzyme activities were expressed as international units (U), defined as the amount of enzyme needed to produce 1µmol of reducing sugar per min at 50˚C.

2.4. Screening of media components using a PBD

A 20-runs PBD was used to select significant medium components affecting xylanase production by *I. lacteus* BAFC 1168 strain F. A total of thirteen variables were screened including six carbon sources: lactose (X_1), glucose (X_2), xylose (X_3), xylan (X_4), carboxy-methylcellulose (CMC, X_5) and crystalline cellulose (CC, X_6) at 0 and 10 g/L as (-) and (+) level, respectively; three nitrogen sources: urea (X_8), peptone (X_9), $(NH_4)_2SO_4$ (X_{10}) at 0 and 1 g/L as (-) and (+) level , and a surfactant Tween 20 (X_{12}) at 0 and 1 mL/L as (-) and (+) level, respectively. Three dummy variables (X_7, X_{11} and X_{13}) were also included to estimate interactive effects not detected in the analysis of the design [17]. The main effect was calculated as the difference between the average of measurements at the high level settings (+) and the average of measurements at low level setting (-) of each factor. Experimental PBD was based on the first order model:

$$Y = \beta_0 + \sum \beta_i X_i \quad \text{Eq. (1)}$$

where Y is the response (xylanase production, U/mL), β_0 is the model intercept, and β_i is the linear coefficient and X_i is the level of independent variable. All experiments were carried out in duplicate and the average of xylanase activities were considered as the response. Experimental results were analyzed using the statistical software package Statgraphics Centurion XVI.I.

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2.5. Optimization of significant variables using response surface methodology (RSM)

Once the critical factors were identified through the PBD, a CCD was used to investigate the optimum concentration and to study the interactions of the three most positive significant factors, namely CMC, urea and peptone. Each variable was studied at five different levels: [-α (-1.68) and +α (+1.68), axial points]; (-1 and +1, factorial points), and the central coded value of zero (Table 1).

Table 1.Range of variables at different levels for the central composite design for CMC, urea and peptone.

Coded value	Independent variables X_i (g/L)		
x_i	CMC (i =1)	Urea (i= 2)	Peptone (i = 3)
-1.68	1.60	0.32	0.32
-1	5	1.00	1.00
0	10	2.00	2.00
1	15	3.00	3.00
1.68	18.40	3.68	3.68

A total of 20 experimental runs performed in duplicate including 6 central points were used to optimize the chosen key factors for achieving maximum xylanase production. The average from replicated values of xylanase activity was taken as dependent variable or response *Y*. For the response surfaces, the experimental data were fitted to a second order polynomial equation of the form:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad \text{Eq. (2)}$$

where Y is the measured response (xylanase production, U/mL), β_0 is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient, X_i and X_j are coded independent variables, subscripts i and j takes values from 1 to the number of variables.

Statgraphics Centurion XVI.I software was used in the experimentaldesign, quadratic model building and data analysis. The optimal levels of the variables were obtained by solving the regression equation and by analyzing the response surface plots using the same software.

2.6. Optimum temperature and pH

To select the optimum conditions of temperature and pH for xylanase activity, supernatants from CCD run N°13 was used in a new five level CCD. A total of twelve experimental runs, including 4 central points, were performed to study the effects of temperature and pH at different levels on xylanase activity.For experimental design and data analysis were used the Statgraphics Centurion XVI.I software.The temperatures used were 29, 35, 50, 65 and 72°C and the following buffers were used: citrate-phosphate 0.1 M for pH 3.39 and 3.8, sodium acetate 0.1 M for pH 4.8 and phosphate acetate 0.1 M for pH 5.8 and 6.2.

3. RESULTS AND DISCUSSION

3.1. Screening of media components using a PBD

The experimental matrix of the design and the responses obtained for each trial is shown in Table 2, revealing a wide variation according to the process conditions used (from 0.00 to 0.87 U/mL).Among these, the highest xylanase activity was obtained in experiment trial N°18, followed by the trials 9, 10, and 6. The multiple regression analysis revealed a R^2 of 0.93, indicating that the model represents the data adequately.

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Table 2. Experimental design and results for the Plackett-Burman design.

Runs	Coded levels													Xylanase Activity (U/mL)
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	
1	+	-	-	-	-	+	-	+	-	+	+	+	+	0
2	-	+	+	-	+	+	-	-	-	-	+	-	+	0
3	-	+	+	-	-	-	-	+	-	+	-	+	+	0
4	-	-	-	-	+	-	+	-	+	+	+	+	-	0.373
5	-	-	-	-	-	-	-	-	-	-	-	-	-	0
6	+	+	+	-	-	+	+	-	+	+	-	-	-	0.319
7	-	-	+	+	-	+	+	-	-	-	-	+	-	0
8	-	+	-	+	+	+	+	-	-	+	+	-	+	0.208
9	-	+	-	+	-	+	+	+	+	-	-	+	+	0.617
10	+	+	-	-	+	+	-	+	+	-	-	-	-	0.759
11	+	-	+	+	+	+	-	-	+	+	-	+	+	0.503
12	+	+	+	+	-	-	+	+	-	+	+	-	-	0
13	+	-	+	-	+	+	+	+	-	-	+	+	-	0
14	-	-	-	+	-	+	-	+	+	+	+	-	-	0.464
15	+	-	+	+	-	-	-	-	+	-	+	-	+	0.179
16	+	+	-	-	-	-	+	-	+	-	+	+	+	0.254
17	-	+	+	+	+	-	-	+	+	-	+	+	-	0.640
18	-	-	+	-	+	-	+	+	+	+	-	-	+	0.876
19	+	+	-	+	+	-	-	-	-	+	-	+	-	0
20	+	-	-	+	+	-	+	+	-	-	-	-	+	0

Table 3 details the analysis of variance for xylanase production and shows the rank of significance of the factors. P values less than 0.05 (> 95% confidence level) indicate that the model terms were statistically significant.

Table 3. Results of the regression analysis of the Plackett-Burman design for xylanase production.

Variable	Effect	F-Ratio	p-value	Ranking
X_1	-58.20	14.68	0.0007	4
X_2	20.03	1.74	0.1989	8
X_3	-7.99	0.28	0.6034	10
X_4	1.45	0.01	0.9244	13
X_5	76.22	25.27	0.0000	3
X_6	27.43	3.26	0.0825	6
X_7	5.16	0.12	0.7369	11
X_8	75.96	25.00	0.0000	2
X_9	238.79	247.04	0.0000	1
X_{10}	14.79	0.95	0.3393	9
X_{11}	-47.78	9.89	0.0041	5
X_{12}	-20.93	1.90	0.1801	7
X_{13}	4.10	0.07	0.7893	12

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X_1: Lactose; X_2: Glucose; X_3: Xylose; X_4: Xylan; X_5: carboxymethylcellulose (CMC); X_6: cellulose crystalline (CC); X_7: false variable I (FVI); X_8: peptone; X_9: urea; X_{10}: $(NH_4)_2SO_4$; X_{11}: false variable II (FVII); X_{12}: tween 20; X_{13}: false variable III (FVIII).

Pareto chart illustrates the effects of the experimental factors on xylanase production (Fig. 1).

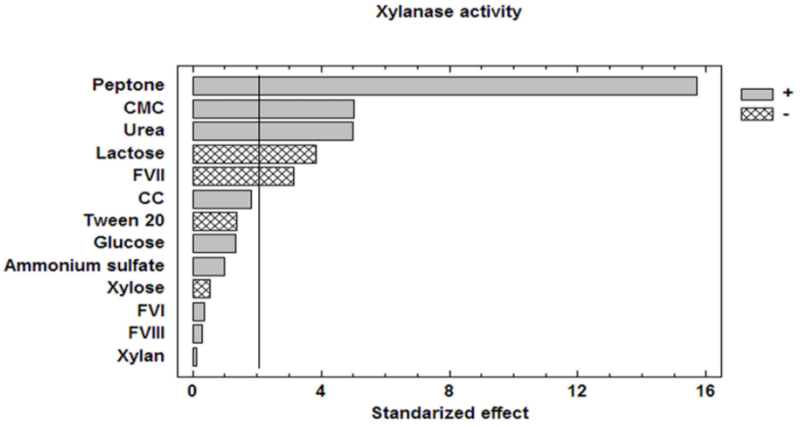

Figure 1. Pareto chart for xylanase production by *I. lacteus* BAFC 1168 strain F. CMC: carboxymethylcellulose; CC: cellulose crystalline; FVI, FVII and FVIII: dummy variables. Bars extending beyond the vertical line correnspond to effects statistically significant at 95% confidence level.

Two of the three nitrogen sources, peptone and urea, had significant positive effects on xylanase production, indicating an increase of enzyme activity when the variables levels were the higher. Regarding to carbon sources, in this study, CMC showed statistical significant and positive effect on xylanase production by *I. lacteus* BAFC 1168 strain F. In contrast, lactose and FVII had significant and negative effect on enzyme production; therefore these variables were no longer taken into account, while the positive factors were included in the next CCD optimization. The other variables, CC, Tween 20, glucose, ammonium sulfate, xylose and xylan had no significant effects on xylanase production.

3.2. Optimization of significant variables using RSM

Different concentrations of the selected nutrients, CMC, urea and peptone were tested to optimize them using a RSM. A rotary CCD design with 20 experiments was carried out and the responses are given in Table 4.

Table 4.Experimental design and results of the central composite design for CMC, urea and peptone.

Run	CMC	Urea	Peptone	Xylanase Activity U/mL)
1	0	0	0	0.942
2	0	0	0	0.921
3	-1	-1	-1	0.604
4	0	0	-1.68	0.088
5	1	-1	1	1.288
6	-1	1	1	0.598
7	1	1	-1	0.244
8	0	0	0	1.057
9	0	1.68	0	0.215
10	1	-1	-1	0.00
11	0	0	0	0.885
12	1.68	0	0	1.224
13	-1.68	0	0	7.041
14	-1	-1	1	1.588
15	0	0	0	0.797
16	1	1	1	1.296
17	-1	1	-1	0.096
18	0	0	0	0.928
19	0	-1.68	0	1.508
20	0	0	1.68	1.412

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It can be seen considerable variation in xylanase production depending upon the medium composition, where the maximal xylanase activity reached was 7.04 U/mL in the experiment N° 13 with 1.6 g/L of CMC, 2 g/L of urea and 2 g/L of peptone.

To explain the effect of the levels of CMC, urea and peptone on xylanase production, the results obtained from the CCD were fitted to a second order polynomial equation [Eq. (3)]:

$$Y = 964.175 – 710.179(CMC) – 245.196\ (urea) + 461.984\ (peptone) + 875.06\ (CMC)^2$$

$$+ 217.498\ (CMC)\ (urea) + 106.505\ (CMC)\ (peptone) – 286.327(urea)^2$$

$$– 84.76\ (urea)\ (peptone) – 341.192\ (peptone)^2 \qquad \text{Eq. (3)}$$

where Y is the predicted response of xylanase production.

The statistical significance of the regression model and the analysis of variance for xylanase production are shown in Table 5.

Table 5.Results of the regression analysis of the central composite design for CMC, urea and peptone on xylanase production.

Variable	Sum of Squares	Df	Mean Square	F value	p-value
A	6,88789E6	1	6,88789E6	1017,73	0,0000
B	821063,	1	821063,	121,32	0,0001
C	2,91476E6	1	2,91476E6	430,67	0,0000
AA	1,10351E7	1	1,10351E7	1630,50	0,0000
AB	378441,	1	378441,	55,92	0,0007
AC	90746,5	1	90746,5	13,41	0,0146
BB	1,18148E6	1	1,18148E6	174,57	0,0000
BC	57474,1	1	57474,1	8,49	0,0332
CC	1,67764E6	1	1,67764E6	247,88	0,0000
LOF	1,48978E7	5	2,97957E6	440,25	0,0000
Pure error	33839,6	5	6767,92		
Total	4,14268E7	19			

A: carboxymethylcellulose (CMC); B: urea; C: peptone; LOF: lack of fit; df: degree of freedom

Linear, quadratic and cross terms were all statistically significant and the relationship between the response and the experimental data is illustrated in Fig. 2. Fig. 2A describes the effects of CMC and urea on xylanase production when peptone was fixed at its middle level (2 g/L); it can be observed that xylanase production was major when CMC and urea concentrations were the minor, 1.6 g/L and 0.32 g/L, respectively. Fig. 2B demonstrates the effects of CMC and peptone on xylanase production when the urea concentration was fixed at its middle level (2 g/L); similarly, the maximum xylanase yield was reached at the lowest CMC concentration (1.6 g/L); however, xylanase production increased when peptone levels were the highest (3-3.68 g/L). From Fig. 2C it can be concluded that xylanase production improved when urea and peptone levels were decreasing and increasing, respectively, while CMC concentration was fixed at its middle level (10 g/L).

CMC revealed to be the main positive medium component on xylanase production while the nitrogen sources, urea and peptone, were also statistically significant on the enzyme activity. The multiple regression analysis revealed a R^2 of 0.64, indicating that the model represents the data. Nevertheless, the statistically significant lack of fit (LOF) from ANOVA analysis indicated either that the model was not fitting all the design points well or the responses were highly repetitive around the center point (where the pure error was derived from).

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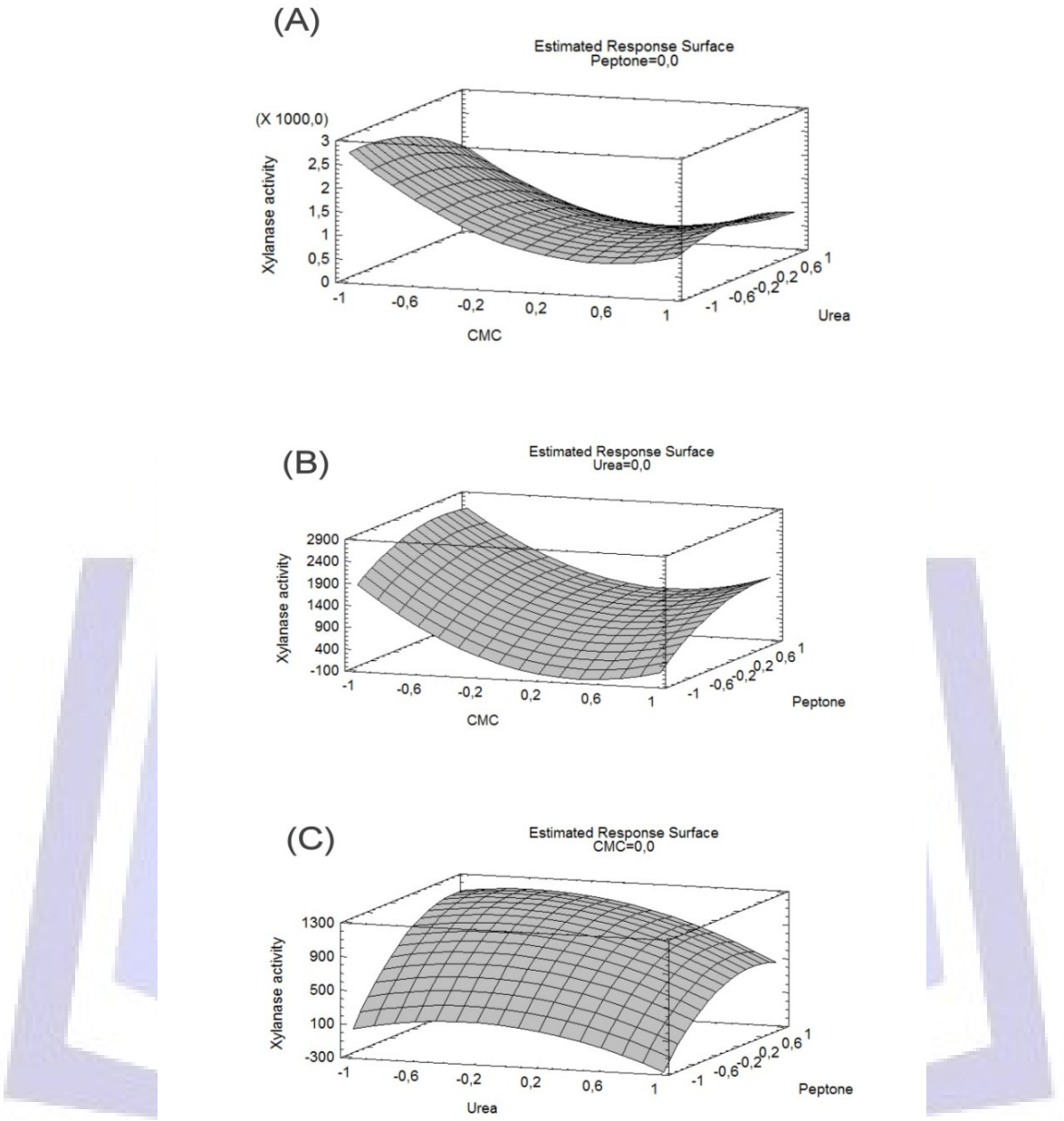

Figure 2. Three-dimensional response surface plots for xylanase production by *I. lacteus* BAFC 1168 strain F showing the interactive effects of medium components. (A) CMC and urea when peptone is fixed on its middle level. (B) CMC and peptone when urea is fixed on its middle level and (C) urea and peptone when CMC is fixed on its middle level.

Despite the maximum predicted xylanase production was lower (5.06 U/mL) than the experimentally obtained (7.04 U/mL); it was decided to carry out the validation of the model and verify the reliability of the predicted result. Three experiments were conducted incubating *I. lacteus* BAFC 1168 strain F under the optimal predicted conditions: 1.6 g/L CMC, 0.84 g/L urea and 2.5 g/L peptone. The average xylanase yield obtained was 8.29 U/mL, much higher than the predicted value. The xylanase activity reached at this point was 9.5 times higher than that obtained with the most effective culture medium in Plackett-Burman experiment.

3.3. Optimum temperature and pH

Optimum temperature and pH for enhancing xylanase activity weredetermined in *I. lacteus* BAFC 1168 strain F supernatants employing a CCD. Center point values of temperature and pH were 50°C and 4.8, respectively. In this CCD, five levels (-1.41, -1, 0, 1, 1.41) of the factors were studied in twelve experiments (Table 6).

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Table 6. Experimental design and results of the central composite design for temperature an pH on xylanase activity. Coded and real values (in parenthesis) of independent variables.

Run	Temperature (°C)	pH	Xylanase activity (U/mL)
1	-1 (35)	1 (5.8)	7.317
2	0 (50)	0 (4.8)	7.032
3	1 (65)	-1 (3.8)	0.00
4	0 (50)	-1.41 (3.39)	0.00
5	0 (50)	1.41 (6.21)	7.139
6	-1.41 (28.78)	0 (4.8)	5.020
7	1 (65)	1 (5.8)	2.961
8	-1 (35)	-1 (3.8)	0.611
9	1.41 (71.21)	0 (4.8)	0.718
10	0 (50)	0 (4.8)	7.062
11	0 (50)	0 (4.8)	7.079
12	0 (50)	0 (4.8)	7.114

The results were then analyzed using the ANOVA (Table 7) and it was observed a statistically significance of all linear, quadratic and interactive effects of temperature and pH on xylanase activity.

Table 7.Results of the regression analysis of the central composite design for temperature and pH on xylanse activity.

Variable	Sum of Squares	df	Mean Square	F value	P-value
A	3,08078E7	1	3,08078E7	18232,66	0,0000
B	9,81559E7	1	9,81559E7	58090,66	0,0000
AA	6,32293E7	1	6,32293E7	37420,41	0,0000
AB	7,19953E6	1	7,19953E6	4260,83	0,0000
BB	4,48712E7	1	4,48712E7	26555,69	0,0000
Blocks	1352,4	1	1352,4	0,80	0,4054
LOF	1,26461E6	11	114965,	68,04	0,0000
Pure error	10138,2	6	1689,7		
Total (corr.)	2,2785E8	23			

A: temperature; B: pH; LOF: lack of fit; df: degree fo freedom.

The multiple regression analysis revealed a R^2 of 0.99, indicating that the model represents the data adequately. Nevertheless, the statistically significant lack of fit from ANOVA analysis indicated that the responses were highly repetitive around the center point (where the pure error was derived from). To explain the effect of the temperature and pH on xylanase activity, the results obtained from the CCD were fitted to a second order polynomial equation [Eq. (4)]:

$$Y = 7{,}072.1 - 1{,}387.62\,(\text{temperature}) + 2{,}476.84\,(\text{pH}) - 2{,}222.57(\text{temperature})^2$$

$$- 948.653\,(\text{temperature})(\text{pH}) - 1{,}872.32\,(\text{pH})^2 \qquad \text{(Eq. 4)}$$

where Y is xylanase activity (U/mL). Fig. 3 shows the three dimensional plot resulting from the effects of temperature and pH on xylanase activity; it can be seen that the enzyme activity improved when the levels of the independent variables increased to midrange.

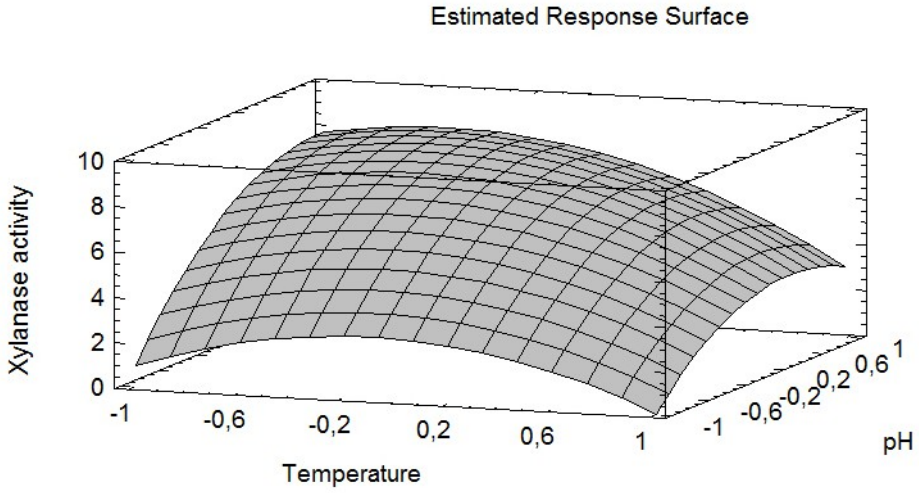

Figure 3.Three-dimensional response surface plot for temperature and pH effects on xylanase activity from supernantants of *I. lacteus* BAFC 1168 strain F.

The model predicted a maximum xylanase activity of 8.37 U/mL at 42.8°C and pH 5.6. To validate the model it was carried out three experiments under these conditions resulting in 8.36 U/mL of xylanases. The excellent correlation between predicted and experimental values justifies the validity of the response model and the existence of an optimum point.

4. DISCUSSION

The effect of carbon and nitrogensources on enzymes production by fungi is of great importance [7]. However, in contrast to lignin-degrading enzymes, the information on hydrolases, specifically on xylanases produced by white rot Basidiomycetes is still scarce [8]. In this paper, the effects of six carbon sources, three nitrogen sources and a surfactant on xylanase production of the white rot fungus *I. lacteus* BAFC 1168 strain F using a PBD were screened and the most significant variables were identified. To our knowledge, many earlier studies on xylanase production were conducted by varying one factor at a time (OFAT); however, this is the first kind of work where many factors affecting xylanase production were studied simultaneously with the white rot fungus *I. lacteus*.

The wide variation in the responses according to the process conditions used in the PBD design indicated the strong influence exerted by independent variables on xylanase production and the importance on evaluating the media components for different fungi species [7]. From the analysis of variance, it can be concluded that two of the three nitrogen sources, peptone and urea, had significant positive effects on xylanase production, indicating an increase of enzyme activity when the variables levels were the higher. Among other nitrogen sources, the presence of peptone and urea were described as necessary medium components for xylanase production during the microbial fermentation [7, 18]. Regarding to carbon sources, in this study, CMC showed statistical significant and positive effect on xylanase production by *I. lacteus* BAFC 1168 strain F. Royer and Nakas [19] also demonstrated positive effects of cellulolytic substrates on xylanase production in studies with *Trichoderma longibrachiatum*. In contrast, lactose had significant and negative effect on enzyme production. In other fungi species lactose could not produce any significant production of xylanases [20]. The negative effect of lactose could own to its simple structure, playing a repressive role on xylanases synthesis or expression.Because of this, lactose was not included in the CCD experiment.

When the CCD was carried out, CMC revealed to be the main positive medium component on xylanase production while the nitrogen sources, urea and peptone, were also statistically significant on the enzyme activity. These results were in agreement with others previously reported by Kachlishvili et al. [21], who also described high xylanolytic activities in closely related fungal specimens such as *Pleurotus dryinus, P. tuberregium, Lentinus edodes* and *Funalia trogii* when were cultured with peptone. However, in the literature exists contradictory evidence on the nature and concentration effects of the carbon and nitrogen sources on xylanase production.Although the quadratic equation failed to predict the maximum xylanase production and keeping in mind a similar situation reported by Chaichanan et al. [22], we accomplished the validation of the model obtaining a remarkable xylanase production of 8.29 U/mL under the predicted conditions by the model (1.6 g/L CMC, 0.84 g/L urea and 2.5 g/L peptone).*I. lacteus* BAFC 1168 strain F could secrete an amount of xylanasesmuch higher than the predicted value and 9.5 times higher than inour most effective culture medium in Plackett-Burman experiment. The xylanase activity rechead at this point is similar with than obtained by Salmon et al. [23] using the Basidiomycete *Ganoderma applanatum* LPB MR-56. The ability of *I. lacteus* BAFC 1168 to produce xylanases is much higherthan the capacity of others Basidiomycetes; itcould produce a 36-foldactivity than the maximum xylanase production reported for another strain of *Ganoderma applanatum* by Dinnis et al. [24] and 66-fold higher than the observed in *Phlebia rufa* (0.125 U/mL), 83-fold higher than *Trametes versicolor*(nearly 0.075 U/mL) and more than 100-fold than *Bjerkandera adusta* (less than 0.075 U/mL) [24].

In addition to nutrient sources, xylanase activity is also strongly influenced by cultivation conditions as temperature and pH [1, 25]. Hence, controlling these parameters and optimize them in the crude extract could achieve an optimal enzyme yield

for a best process result and could serve to reduce the cost of enzyme purification. The literature discloses that most of the fungi produce higher xylanase activity when occurring temperatures below 50°C and exhibit optimum pH towards the acidic side, since 4 to 7 [1, 26-28]. In this work,employing a CCD and its corresponding validation, we concluded thatoptimum temperature and pH for xylanase activity in *I. lacteus* BAFC 1168 strain F was 42.8°C and 5.6, respectively. On the other side, there was an excellent correlation between the predicted xylanase activity (8.37 U/mL) and the experimental one (8.36 U/mL) demonstrating the existence of an optimum point. Studies carried out with *I. lacteus* [29] also conducted that the most suitable pH value for xylanase activity was within the acid region nearly pH 6. However, its optimum temperature was nearly 60°C.

Easy and cheap enzyme overproductionis important for efficient biotechnological applications [25]. According to the present results, *I. lacteus* BAFC 1168 strain F have a surprising potential for xylanase production when growing on CMC, peptone and urea and demonstrated the use of nonconventional, statistically based design techniques can serve as efficient tool in process development, where an analysis of the effect and interaction of many experimental factors are required. The enhancement of xylanase activity is a remarkable feature because some biotechnological application as an efficient saccharification process, high xylanase activity is necessary to degrade hemicellulose for increasing yields of fermenting 5 carbon sugars to obtain bioetanol. Nevertheless, it is still necessary to explore other variables to improve these levels of xylanasesand to obtain xylanases and celluloses in the same culture for optimizing the global bioethanol process.

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Author' biography with Photo

Gabriela VerónicaDíaz is graduated in Genetics from Universidad Nacional de Misiones. She is student of PhD in Applied Science in Universidad Nacional de Misiones. Her current interests are on the topics of enzymatic fermentation and biotechnological applications for xylanolytic enzymes.