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Xylanase Production by *Aspergillus niger* LPB 326 in Solid-State Fermentation Using Statistical Experimental Designs

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Summary

Xylanase was produced by *Aspergillus niger* LPB 326 cultivated on lignocellulosic substrate composed by sugarcane bagasse and soybean meal in solid-state fermentation. The effects of various variables were observed and optimized by applying statistical experimental designs. The best xylanase activity was obtained in a medium containing 10 g of sugarcane bagasse and soybean meal in the ratio of 65 and 35 %, respectively, moistened to 85 % of initial water content with a nutrient salt solution composed of (in g/L): $CuSO_4$ 0.4, KH_2PO_4 1.5 and $CoSO_4$ 0.0012, and incubated for 4 days at 30 °C. Under these optimized conditions, a xylanase activity of 3099 IU/g of dry matter was obtained.

Key words: xylanase, *Aspergillus niger*, solid-state fermentation, statistical designs

Introduction

Solid-state cultivation offers advantages over liquid cultivation, especially for fungal cultures. Solid-state fermentation (SSF) has considerable economical potential in producing products for the food, feed, pharmaceutical and agricultural industries (*1*). Due to the nature of the substrates, aeration tends to require lower pressures than are needed for liquid cultivations, and vigorous agitation is not required. The metabolites thus obtained are more concentrated and purification procedures are less costly (*2–4*). Additionally, these processes are of special economic interest for the countries with abundance of biomass and agroindustrial residues, as these can be used as cheap raw materials (*5*). Several reports have shown xylanase induction by lignocelluloses such as wheat bran, rice straw, corncobs, and sugarcane bagasse (*6–10*).

Plant cell wall polysaccharides are the most abundant organic compounds found in nature. Hemicelluloses are polysaccharides more heterogeneous than cellulose and are the second most abundant organic structure in the plant cell wall. The major hemicellulose polymer in cereals and hardwood is xylan, representing up to 30–35 % of the total dry mass (*11*). Xylan consists of a β-1,4-linked D-xylose backbone and can be substituted with different side-groups such as L-arabinose, D-galactose, acetyl, feruloyl, *p*-coumaroyl and glucuronic acid residues (*12*).

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Filamentous fungi have been widely used to produce hydrolytic enzymes for industrial applications, including xylanases, whose levels in fungi are generally much higher than those in yeast and bacteria. On an industrial scale, xylanases are produced mainly by *Aspergillus* and *Trichoderma* spp. (*13*).

Xylanases are glycosidases (*O*-glycoside hydrolases, EC 3.2.1.x) which catalyze the hydrolysis of 1,4- -D-xylosidic linkages in xylan (*14*). A complete cleavage of glycoside linkages in the heteroxylan backbone requires the interaction of a number of the main-chain- and side--chain-cleaving enzyme activities. Endo- -1,4-xylanase (-1,4-D-xylan xylanohydrolase, EC 3.2.1.8) and xylan- --1,4-xylosidase (-1,4-D-xylan xylohydrolase, EC 3.2.1.37), or exoxylanase, are enzymes capable of cleaving the main-chain glycosyl groups (*15*).

Applications of xylanases (*15,16*) can be found in the food and beverage industries (bakery goods, coffee, starch, plant oil and juice manufacture), feedstock improvement (increasing animal feed digestibility), and the quality improvement of lignocellulosic residues (traditionally, the application of xylanases in conjunction with cellulolytic enzymes has been mainly considered for the bioconversion of lignocellulosic materials to produce fuel and other chemicals). Often, the use of xylanases permits such processes to be run with fewer chemicals, under less harsh conditions and with less disturbing side reactions. Thus, the use of xylanases is beneficial for society as well as for the environment (*17,18*).

The cost of an enzyme is one of the main factors determining the economics of a process. Reducing the costs of enzyme production by optimizing the fermentation medium and the process is the goal of basic research for industrial applications (*19*). Recently, statistical designs for optimization have been successfully employed in enzyme production (*20*). The response surface methodology (RSM) is an empirical statistical technique used to find the optimum conditions of a process response variable when the mechanism underlying the process is either not well understood or is too complicated to allow the exact model to be formulated from theory. It evaluates the relation existing between a group of controlled experimental factors and the observed results of one or more selected variables (*21*).

The aim of this work is to enhance xylanase production with *Aspergillus niger* LPB 326, which has proved to be a potential strain during previous screening studies, using statistical experimental designs based on the optimization of some physical and chemical parameters involved in solid-state fermentation processes.

Materials and Methods

Microorganism

The fungal strain *Aspergillus niger* LPB 326 was obtained from the Biotechnological Processes Laboratory culture and selected among 20 strains (isolated in our laboratory) through a first test using the xylan agar plate screening method according to Whitaker *et al.* (*22*). Afterwards, it was submitted to a quantitative test in solid-state fermentation with the best xylanase-producing strains previously selected. The strain was cultivated on potato dextrose agar at 28 °C for 5 days. This culture time was chosen according to a sporulation curve which was carried out with this strain during 10 days, showing the best time to stop the culture (higher quantity of viable spores) and start the fermentation. Spores were suspended by adding 40 mL of Tween 80 solution (0.1 %) under agitation with a magnetic stirrer and counted in a Neubauer chamber.

Raw material characterization and influence of substrate proportions

The sugarcane bagasse (SB) was generously assigned by COCAMAR – Cooperativa Agroindustrial (Cianorte, Paraná, Brazil) and the soyben meal (SM) was granted by Cooperativa Agrária Mista Entre Rios Ltda (Guarapuava, Paraná, Brazil). The lignocellulosic material was used without any chemical (acid, alkali) or physical (milling, sieving) pretreatment. Physicochemical analyses of the raw material (ash, moisture content and protein) were performed according to the standard methods of AOAC (*23*). Total sugar was measured by the Somogyi-Nelson method (*24*). Compositional data of SB and SM are given in Table 1.

Table 1. Physical and chemical characteristics of sugarcane bagasse and soybean meal. Analyses were carried out in triplicate (mean±standard deviation)

w(parameters)/%	Sugarcane bagasse	Soybean meal
Ash	1.71±0.28	5.06±0.04
Moisture	10.75±0.22	12.75±0.33
Crude protein	1.18±0.10	43.10±1.13
Total sugar	12.90±0.14	35.40±0.70

The influence of sugarcane bagasse (SB) and soybean meal (SM) ratio on xylanase activity was investigated. SB and SM ratio (SB:SM) tested under solid-state fermentation was: (1) 1:0, (2) 3:1, (3) 1.86:1, and (4) 1:1.

Water absorption tests were also performed with the best SB:SM ratio in order to know the maximum capacity of liquid retention.

Initial conditions of solid-state fermentation

Initial experiments were performed by mixing in an Erlenmeyer flask (250 mL), 10 g of substrate (65 % of SB and 35 % of SM), 10 mL of a mineral salt solution, buffer (pH=6.5) and enough distilled water to adjust the moisture content (80 %). The mineral salt solution was composed of (in g/L): K_2HPO_4 0.23, $MgSO_4 \cdot 7H_2O$ 0.05, $CaCl_2$ 0.005, $NaNO_3$ 0.05, $FeSO_4 \cdot 7H_2O$ 0.009, $ZnSO_4$ 0.002 and $MnSO_4$ 0.012.

The solid medium was autoclaved (15 min at 121 °C) and then inoculated with a spore suspension of 10^6 spores/g of dry matter (used for all experiments). After mixing, the flasks were incubated at 28 °C for four days. A previous kinetic study showed that the highest xylanase activity was achieved within four days of the culture. The initial temperature for the improvement of SSF conditions was fixed at 30 °C, according to a prelimi-

nary investigation which showed that the best xylanase production was obtained at this temperature.

Enzyme extraction

After incubation, 3 g of the homogenized fermented substrate were suspended in 30 mL of NaCl solution (0.9 %), containing Tween 80 (0.1 %) and stirred at 120 rpm in a shaker for 5 min. Solids were filtered under vacuum through Whatman no. 1 filter paper and the filtrate was used as the enzymatic crude extract.

Enzyme assay

Xylanase activity was assayed using 1 % birchwood xylan (Sigma, USA) in 0.05 M citrate buffer (pH=5.3), according to the method of Bailey *et al.* (*25*). The release of reducing sugars was determined using the 3,5-dinitrosalicylic acid (DNS) method (*26*) with a xylose standard curve. One unit (IU) of enzyme activity was defined as the amount of enzyme required to liberate 1 mol of reducing sugars per minute. The results of these analyses were expressed as units per gram of dry matter (IU/g). Cellulase activity was assayed according to the method of Ghose (*27*), using carboxymethylcellulose (CMC 2 %) and Whatman no. 1 filter paper as substrates for cellulase and endo-β-1,4-glucanase, respectively, which were expressed as units/mL.

Experimental design

Statistical experiments and analyses were carried out using the software STATISTICA 7.1 (StatSoft, Tulsa, OK, USA).

pH and moisture

A 3^{2-0} full factorial design leading to 9 sets of experiments, performed in duplicate, was used to find the optimal conditions of pH and initial moisture variables, and also to show the influence of these variables on xylanase production. Uncoded variables are given in Table 2. The results were adjusted to the following quadratic model (Eq. 1):

$$y=b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{13}x_1x_3+ +b_{23}x_2x_3+b_{11}x_1^2+b_{22}x_2^2+b_{33}x_3^2 \qquad /1/$$

Effect of chemical composition

The Plackett-Burman design is a statistical approach chosen for this optimization study. It allows the investigation of up to *N*-1 variables with *N* experiments. Plackett-Burman designs are efficient for screening purposes when it is not known which components should be present in the medium (*28*). All the experiments were carried out according to a design matrix, which is based on the number of variables to be studied. A Plackett--Burman design with 11 factors (one way), resulting in 12 runs, performed in duplicate, was used to determine the most significant salts in xylanase production. The tested salts and respective concentrations (in g/L) were: (1) KH_2PO_4 0.23, (2) $MgSO_4$ 0.07, (3) $CaCl_2$ 0.04, (4) $NaNO_3$ 0.37, (5) $FeSO_4$ 0.005, (6) $MnSO_4$ 0.012, (7) $ZnSO_4$ 0.002, (8) K_2HPO_4 0.23, (9) KCl 0.12, (10) $CuSO_4$ 0.05, and (11) $CoSO_4$ 0.0005.

Table 2. pH, moisture levels and xylanase activities

Runs	pH (X_1)	*w*(moisture)/% (X_2)	Activity/(IU/g)
1	4	79	1267
2	4	82	1699
3	4	85	1843
4	4.5	79	1303
5	4.5	82	1587
6	4.5	85	1797
7	5	79	1493
8	5	82	1605
9	5	85	1720
10	4	79	1296
11	4	82	1669
12	4	85	1947
13	4.5	79	1353
14	4.5	82	1633
15	4.5	85	1756
16	5	79	1320
17	5	82	1712
18	5	85	1743

The choice of the salts for the experiment was made according to literature references (*19*,*29*,*30*), considering the salts which were used during the first tests in SSF carried out for the screening of the strain (item 2.4). All the values (replicates) were used in statistical evaluations.

Concentration of the salts

In order to find the best concentration of the salts selected in the previous experiment (effect of chemical composition), a 3^{3-1} fractional factorial design was used, resulting in 9 sets of experiments performed in duplicate. The selected salts and the concentrations (uncoded) used in this experiment are described in Table 3.

Table 3. Uncoded values of the concentration of salts

Salts	*γ*/(g/L)		
	–1	0	+1
KH_2PO_4	1	1.5	2
$CuSO_4$	0.2	0.4	0.6
$CoSO_4$	0.0004	0.0012	0.002

Results and Discussion

Influence of substrate ratio and water absorption

The results of xylanase activity varied markedly with the substrate proportions tested, in a range of 20–1937 IU/g (Fig. 1). Therefore, the choice of a suitable C:N ratio was of great importance for xylanase production.

The lowest value was obtained when only sugarcane bagasse was used for fermentation, and the highest activity was achieved with a mixture of SB and SM in a

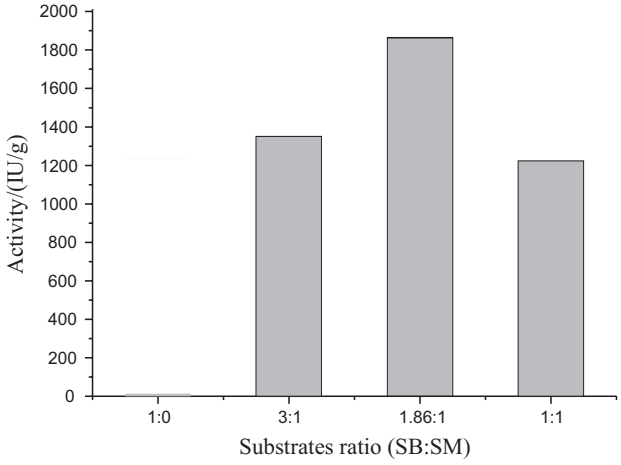

Fig. 1. Influence of the substrate (sugarcane bagasse (SB) and soybean meal (SM)) composition on xylanase activity (IU/g of dry matter)

ratio of 1.86:1. This shows that the amount of nitrogen present in the sugarcane bagasse is too low to support good growth and enzyme production, and that the nitrogen source and its quantity affect xylanase production, as it was also stated by Park *et al.* (*19*), working with rice straw. According to these results, the SB:SM ratio of 1.86:1 was chosen for the optimization studies. The water absorption tests showed that the maximum capacity of liquid retention (without free water) for this ratio was 85 %.

Study of pH and moisture

The experimental design and the results of the 3^{2-0} full factorial design are shown in Table 2.

In this work, the levels of pH studied showed no significant effect on the production of xylanase at $p \leq 0.05$. However, the initial moisture of the medium influenced xylanase production showing a tendency to increase with an increase in moisture content, according to contour plot in Fig. 2. This result corroborates the studies undertaken by Souza *et al.* (*20*), where the initial moisture and bagasse concentration had the most important effects on xylanase production. Generally, fungi prefer low pH levels for good growth. In this work the natural pH of the substrates, which was around 6.0 (without adjustment or buffering), was the best for development of the fungi and xylanase production. Nevertheless, the initial moisture could not be increased in the following experiments due to the presence of free water in moisture adjustments above 85 %. The critical importance of moisture level in SSF media and its influence on the biosynthesis and secretion of enzymes can be attributed to the interference of moisture with the physical properties of the solid particles. An increase in moisture level must not wet through the substrate (leaving free water); besides, it is believed to reduce the porosity of some substrates, thus limiting oxygen transfer. Otherwise, low moisture content causes reduction in the solubility of nutrients of the substrate and low degree of swelling (*31*). The highest xylanase activity achieved was 1947 IU/g.

Regression analysis was performed to fit the response function with the experimental data. Table 4 shows the analysis of variance of pH and moisture factors, fitted according to the quadratic model with two-way interactions (linear×linear). Analysis of variance (F-test) showed that the second-order (quadratic) model fitted well to the experimental data.

Table 4. Analysis of variance (ANOVA) for pH and moisture factors

	SS	df	MS	F	p
pH L+Q	7067.8	2	3533.9	1.04035	0.392228
Moisture L+Q	668165.7	2	334082.8	98.35187	0.000001*
pH×moisture	41659.6	1	41659.6	12.26432	0.006702*
Lack of fit	1372.4	3	457.5	0.13468	0.936888
Pure error	30571.3	9	3396.8		
Total SS	748836.8	17			

*$p \leq 0.05$; $R^2=0.96$
SS – sum of squares; df – degrees of freedom; MS – mean square

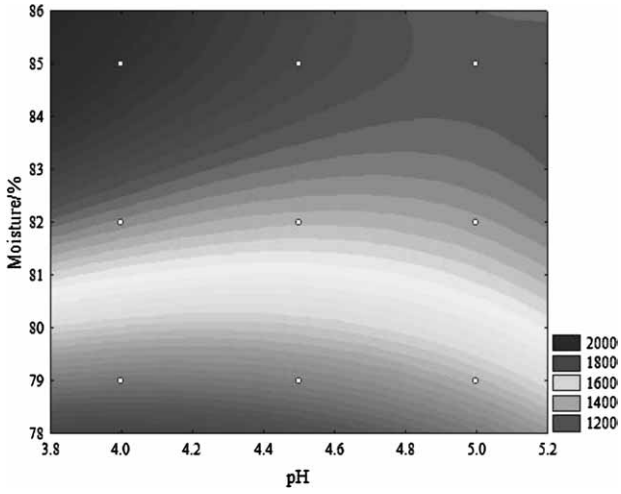

Fig. 2. Contour plot showing the optimum regions and values for pH and moisture in xylanase production

The statistical significance of the second-order model equation was checked and the determination coefficient (R^2) of the model was calculated to be 0.96, indicating that 96 % of the variability in the response could be explained by the model. The lack-of-fit tests did not result in a significant p-value, indicating that the model is sufficiently accurate to predict the responses of the factors within the ranges studied.

Chemical composition and concentration of salts

The chemical composition based on the presence or absence of some salts greatly influenced xylanase production, with activities reaching values above 3000 IU/g of dry matter. The salts are related directly to the microorganism metabolism, stimulating or inhibiting enzyme production. Statistical analyses were made to identify those medium variables (factors) that had a significant effect, either positively or negatively (*32*), on xylanase production. In Fig. 3 (Pareto chart) the salts that were

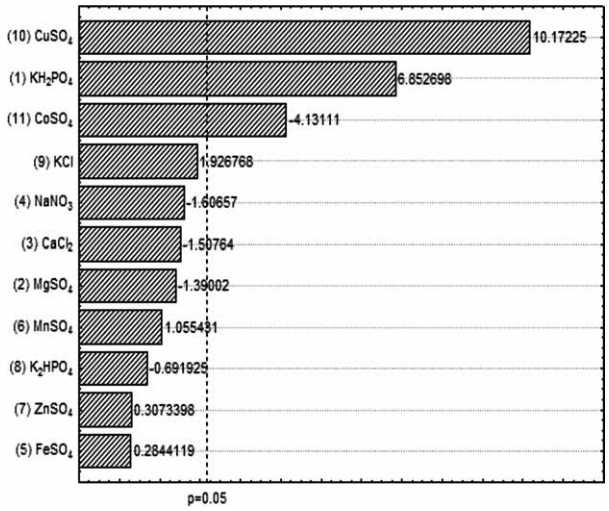

Fig. 3. Pareto chart of standardized effects of different salts showing the significance at p≤0.05 in xylanase production

significant for xylanase activity at p≤0.05, with a coefficient of determination (R^2) of 0.94, are shown. Three salts ($CuSO_4$, KH_2PO_4 and $CoSO_4$) were selected for the optimization of the concentration.

The concentration (in g/L) of $CuSO_4$, KH_2PO_4 and $CoSO_4$ that best induced xylanase production (3004 IU/g of DM) was found to be 0.4, 1.5 and 0.0012, respectively. These optimized values for each salt can be seen in Fig. 4.

Experimental data were fitted to a model with two--way interactions (linear×linear) achieving a coefficient of determination (R^2) of 0.92, indicating that 92 % of the variability in the response could be explained by the model. Statistical data are shown in Table 5. All the concentrations of salts were significant at p≤0.05.

Table 5. Statistical analysis obtained for xylanase production at different levels of salt concentration

	SS	df	MS	F	p
(1) $CuSO_4$ L+Q	346509	2	173254	5.28272	0.030366*
(2) $CoSO_4$ L+Q	2880182	2	1440091	43.91001	0.000023*
(3) KH_2PO_4 L+Q	882264	2	441132	13.45061	0.001977*
1×2	521056	1	521056	15.88758	0.003177*
Lack of fit	19278	1	19278	0.58779	0.462909
Pure error	295168	9	32796		
Total SS	4476229	17			

*p≤0.05; R^2=0.92
SS – sum of squares; df – degrees of freedom; MS – mean square

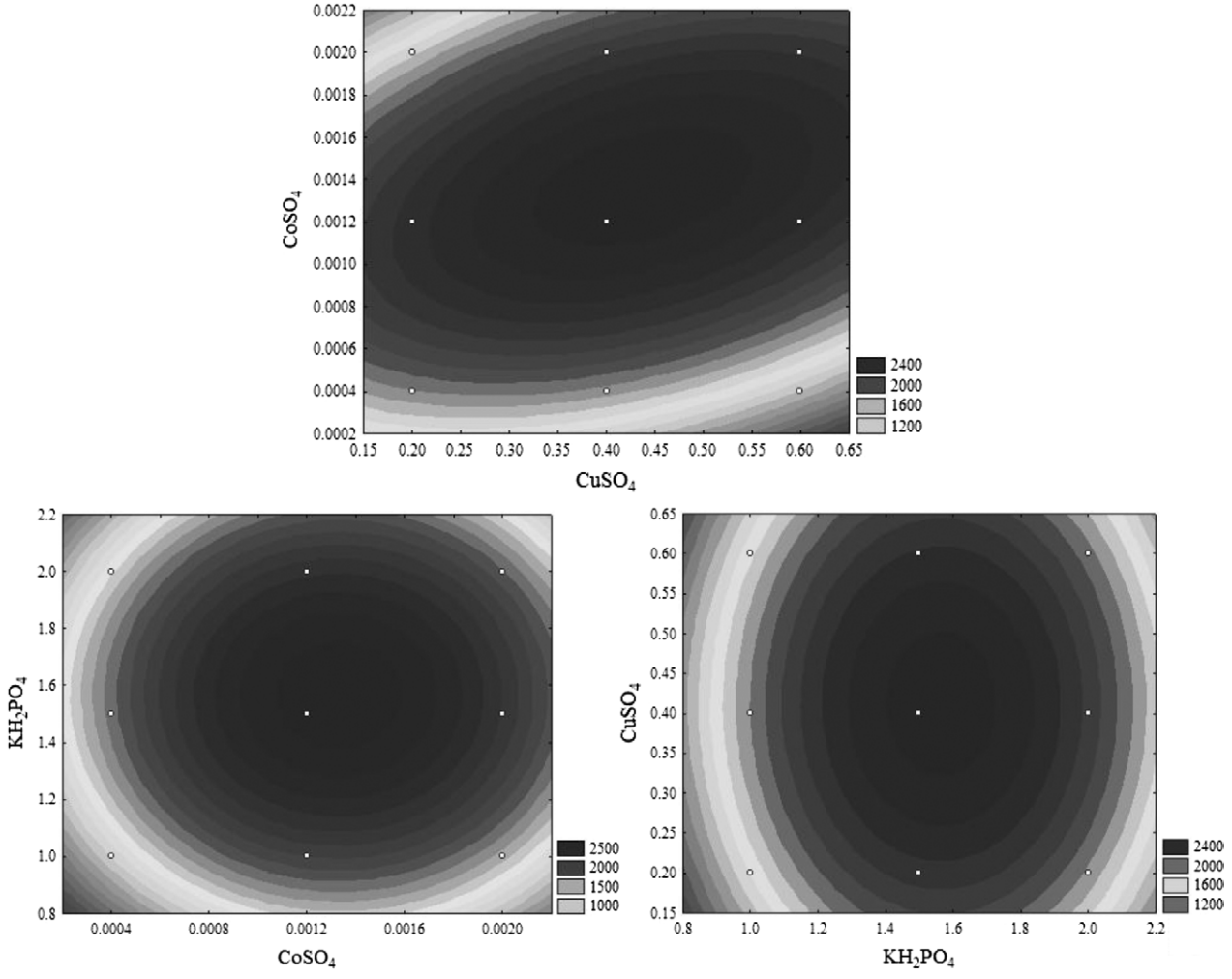

Fig. 4. Contour plots showing the optimum region of each salt (central point) for highest xylanase production

Table 6. Comparison of xylanase production in solid-state fermentation on various lignocelluloses using non-mutant *Aspergillus* strains

Organism	Substrate	*w*(initial moisture)/%	Cultivation conditions	Xylanase activity/(IU/g)	Reference
A. awamori	Sugarcane bagasse	92	30 °C, 60 hours	2500	(*33*)
A. fischeri	Wheat bran	80	30 °C, 3 days	1024	(*34*)
A. foetidus	Untreated corncobs	75	30 °C, 4 days	3065	(*35*)
A. niger	Wheat bran+wheat straw	70	30 °C, 4 days	2500	(*36*)
A. niger LPB 326	Sugarcane bagasse+soybean meal	84	30 °C, 4 days	3099	This work
A. ochraceus	Wheat bran+xylan	60	28 °C, 14 days	724	(*37*)
	Wheat straw	60	28 °C, 14 days	488	

Experimental rechecking was carried out to confirm the optimal conditions of xylanase production, achieving a xylanase activity of 3099 IU/g. Cellulase activities were also assayed resulting in 46 U/mL for filter paper assay and 37 U/mL for carboxymethylcellulose (CMC) assay. Xylanase production obtained by other authors using *Aspergillus* strains (without mutation) in SSF is compared with this work in Table 6.

Conclusion

Statistical tools applied in this work proved to be efficient for optimizing enzyme production in solid-state fermentation providing suitable designs and models for the experiments. The optimum conditions for xylanase production obtained in this work were the following: sugarcane bagasse and soybean meal ratio of 1.86:1, initial moisture content of 85 %, pH of the medium of around 6.0, and the presence of $CuSO_4$, KH_2PO_4 and $CoSO_4$ in the concentrations (in g/L) of 0.4, 1.5 and 0.0012, respectively. The optimized medium increased xylanase production by 1.5-fold. The highest xylanase activity obtained was 3099 IU/g. Other strategies such as strain mutation and purification may even be applied in order to enhance xylanase activities.

References

1. A. Pandey, C.R. Soccol, P. Nigam, V.T. Soccol, L.P.S. Vandenberghe, R. Mohan, Biotechnological potential of agro-industrial residues. II: Cassava bagasse, *Bioresour. Technol. 74* (2000) 81–87.
2. P. Nigam, D. Singh, Solid-state (substrate) fermentation systems and their applications in biotechnology, *J. Basic Microbiol. 34* (1994) 405–414.
3. R. Chatterjee, A. Dutta, R. Banerjee, B.C. Bhattacharyya, Production of tannase by solid-state fermentation, *Bioprocess Biosyst. Eng. 14* (1996) 159–162.
4. S. Kumaran, C.A. Sastry, S. Vikineswary, Laccase, cellulase and xylanase activities during growth of *Pleurotus sajor-caju* on sago hampas, *World J. Microbiol. Biotechnol. 13* (1997) 43–49.
5. L.R. Castilho, R.A. Medronho, T.L.M. Alves, Production and extraction of pectinases obtained by solid state fermentation of agroindustrial residues with *Aspergillus niger*, *Bioresour. Technol. 71* (2000) 45–50.
6. Q.K. Beg, B. Bhushan, M. Kapoor, G.S. Hoondal, Effect of amino acids on production of xylanase and pectinase from *Streptomyces* sp. QG-11-3, *World J. Microbiol. Biotechnol. 16* (2000) 211–213.
7. S. Gupta, R.C. Kuhad, B. Bhushan, G.S. Hoondal, Improved xylanase production from a haloalkalophilic *Staphylococcus* sp. SG-13 using inexpensive agricultural residues, *World J. Microbiol. Biotechnol. 17* (2001) 5–8.
8. S.S. Kesker, High activity xylanase from thermotolerant *Streptomyces* T7: Cultural conditions and enzyme properties, *Biotechnol. Lett. 14* (1992) 481–486.
9. R.C. Kuhad, M. Manchanda, A. Singh, Optimization of xylanase production by hyper xylanolytic mutant and strain of *Fusarium oxysporum*, *Process Biochem. 33* (1998) 641–647.
10. V. Puchart, P. Katapodis, P. Biely, L. Kremnicky, P. Christakopoulos, M. Vrsanska, D. Kekos, B.J. Macris, M.K. Bhat, Production of xylanases, mannanases, and pectinases by the thermophilic fungus *Thermomyces lanuginosus* – Effect of growth, substrates and development of a culture medium using statistically designated experiments, *Enzyme Microb. Technol. 24* (1999) 355–361.
11. J.P. Joseleau, J. Comtat, K. Ruel: Chemical Structure of Xylans and Their Interaction in the Plant Cell Walls. In: *Xylans and Xylanases*, *Progress in Biotechnology, Vol. 7*, J. Visser, G. Beldman, M.A. Kusters-van Someren, A.G.J. Voragen (Eds.), Elsevier, New York, USA (1992) pp. 1–15.
12. K.C.B. Wilkie, S.L. Woo, A heteroxylan and hemicellulosic materials from bamboo leaves, and a reconsideration of the general nature of commonly occurring xylans and other hemicelluloses, *Carbohydr. Res. 57* (1977) 145–162.
13. N. Kulkarni, A. Shendye, M. Rao, Molecular and biotechnological aspects of xylanases, *FEMS Microbiol. Rev. 23* (1999) 411–456.
14. T. Collins, C. Gerday, G. Feller, Xylanases, xylanase families and extremophilic xylanases, *FEMS Microbiol. Rev. 29* (2005) 3–16.
15. M.P. Coughlan, G.P. Hazlewood, β-1,4-D-xylan degrading enzyme systems, biochemistry, molecular biology and applications, *Biotechnol. Appl. Biochem. 17* (1993) 259–289.
16. Q.K. Beg, M. Kapoor, L. Mahajan, G.S. Hoondal, Microbial xylanases and their industrial applications, a review, *Appl. Microbiol. Biotechnol. 56* (2001) 326–338.
17. P. Sá-Pereira, H. Paveia, M. Costa-Ferreira, M. Aires-Barros, A new look at xylanases: An overview of purification strategies, *Mol. Biotechnol. 24* (2003) 257–282.
18. J. Buchert, M. Tenkanen, A. Kantelinen, L. Viikari, Application of xylanases in the pulp and paper industry, *Bioresour. Technol. 50* (1994) 65–72.
19. Y. Park, S. Kang, J. Lee, S. Hong, S. Kim, Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs, *Appl. Microbiol. Biotechnol. 58* (2002) 761–766.
20. M.C.O. Souza, I.C. Roberto, A.M.F. Milagres, Solid-state fermentation for xylanase production by *Thermoascus au-*

rantiacus using response surface methodology, *Appl. Microbiol. Biotechnol. 52* (1999) 768–772.

21. J. Ahmed, H.S. Ramaswamy, M.O. Ngadi, Rheological characteristics of arabic gum in combination with guar and xanthan gum using response surface methodology: Effect of temperature and concentration, *Int. J. Food Prop. 8* (2005) 179–192.
22. J.R. Whitaker, A.G.J. Voragen, D.W.S. Wong: *Handbook of Food Enzymology*, Routledge, USA (2002) pp. 879–916.
23. Official Methods of Analysis of AOAC International, AOAC, Washington, DC, USA (2000).
24. M. Somogyi, Notes on sugar determination, *J. Biol. Chem. 95* (1952) 18–23.
25. M.J. Bailey, P. Biely, K. Poutanen, Interlaboratory testing of methods for assay of xylanase activity, *J. Biotechnol. 23* (1992) 257–271.
26. G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugars, *Anal. Chem. 31* (1959) 426–428.
27. T.K. Ghose, Measurement of cellulase activities, *Pure Appl. Chem. 59* (1987) 257–268.
28. L.P. Ooijkaas, F.J. Weber, R.M. Buitelaar, J. Tramper, A. Rinzema, Defined media and inert supports: Their potential as solid-state fermentation production systems, *Trends Biotechnol. 18* (2000) 356–360.
29. S. Couri, S.C. Terzi, G.A.S. Pinto, S.P. Freitas, A.C.A. Costa, Hydrolytic enzyme production in solid-state fermentation by *Aspergillus niger* 3T5B8, *Process Biochem. 36* (2000) 255–261.
30. D. Haltrich, B. Nidetzky, K.D. Kulbe, W. Steiner, S. Zupancic, Production of fungal xylanases, *Bioresour. Technol. 58* (1996) 137–161.
31. P. Ellaiah, K. Adinarayana, Y. Bhavani, P. Padmaja, A. Srinivasulu, Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species, *Process Biochem. 38* (2002) 615–620.
32. R.L. Plackett, J.P. Burman, The design of optimum multifactorial experiments, *Biometrika, 33* (1946) 305–325.
33. J.L.S. Lemos, M.C.A. Fontes, N.J. Pereira, Xylanase production by *Aspergillus awamori* in solid-state fermentation and influence of different nitrogen sources, *Appl. Biochem. Biotechnol. 91* (2001) 681–689.
34. S.R. Senthilkumar, B. Ashokkumar, R.K. Chandra, P. Gunasekaran, Optimization of medium composition for alkali--stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design, *Bioresour. Technol. 96* (2005) 1380–1386.
35. A.R. Shah, D. Madamwar, Xylanase production under solid-state fermentation and its characterization by an isolated strain of *Aspergillus foetidus* in India, *World J. Microbiol. Biotechnol. 3* (2005) 233–243.
36. F. Deschamps, M.C. Huet, Xylanase production in solid--state fermentation: A study of its properties, *Appl. Microbiol. Biotechnol. 22* (1985) 177–180.
37. S.R. Biswas, A.K. Mishra, G. Nanda, Xylanase and β-xylosidase production by *Aspergillus ochraceus* during growth on lignocelluloses, *Biotechnol. Bioeng. 31* (1988) 613–616.