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Newly Isolated *Penicillium* sp. for Cellulolytic Enzyme Production in Soybean Hull Residue

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HIGHLIGHTS

- Xylanase and avicelase produced by Penicillium sp.
- Temperature and agitation in the extraction process influence enzyme activity. Avicelase was more stable at thermal deactivation than xylanase.
- The using alternative substrates is a way to reduce costs production of cellulases.

Abstract: (1) Background: The aim of this study was to evaluate the production and partial characterization of xylanase and avicelase by a newly isolated *Penicillium* sp. in solid-state fermentation, using soybean hulls as substrate. (2) Methods: Temperature, time, number of spores, and substrate moisture on xylanase and avicelase bioproduction were evaluated, maximizing activity with 30°C, $1x10^6$ spores/g substrate, 14 and 7 days of fermentation with 70 and 76% substrate moisture contents, for xylanase and avicelase, respectively. (3) Results: Different solvents, temperatures, and agitation in the enzymatic extraction were evaluated, obtaining higher activities, 430.77 and 26.77 U/g for xylanase and avicelase using 30 min extraction and 0.05 M citrate buffer solution (pH 4.5), respectively at 60°C and 175 rpm and 50°C and 125 rpm. The optimum pH and temperature for enzymatic activity determination were 5.3 and 50°C. Enzyme extract stability was evaluated, obtaining higher stability with pH between 4.5 and 5.5, higher temperature of up to 40°C. The kinetic thermal denaturation (K_d), half-life time, D-value, and Z-value were similar for both enzymes. The xylanase Ed value (89.1 kJ/mol) was slightly lower than the avicelase one (96.7 kJ/mol), indicating higher thermostability for avicelase. (4) Conclusion: In this way, the production of cellulases using alternative substrates is a way to reduce production costs, since they represent about 10% of the world demand of enzymes, with application in animal feed processing, food production and

breweries, textile processing, detergent and laundry production, pulp manufacturing and the production of biofuels.

Keywords: Xylanase; avicelase; solid-state fermentation; partial characterization; enzymatic thermostability.

INTRODUCTION

Cellulases are a complex system consisting of three enzymes that act synergistically to hydrolyze the cellulose system, breaking down the β (1 → 4) cellulose glycosidic links [1,2]. These enzymes are produced by various microorganisms such as *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma* species [3].

Xylanases, also referred to as endo-1,4-β-xylanase and β-1,4-D-xylan hydrolases (EC 3.2.1.8), are the main enzymes involved in xylan degradation, reducing the polymerization degree of the substrate. The sites selected for xylan substrate hydrolysis depend on its nature, chain length, branching degree and number of substituents [4,5]. Xylanase induction in filamentous fungi is a complex phenomenon, and the response level varies for each microorganism. A substrate that induces xylanase production in one species might be a production inhibitor on another species [6]. Xylanases are of great interest, since xylan is the most abundant renewable source of carbon, after cellulose, it is found in wood and agricultural waste and is the main polysaccharide component of hemicellulose [7,8].

There are two types of exoglucanases, 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91) or avicelase, and 1,4-β-D-glucan glucohydrolase (EC 3.2.1.74) that remove respectively cellobiose units and glucose units, both acting from the non-reducing ends of oligosaccharides produced by endoglucanase action. The term "Avicelase" is commonly regarded as synonymous with exoglucanase or cellobiohydrolase [9]. Since exoglucanase is normally found to be synthesized along with two other cellulases types, it is also important to estimate the relative ability of a particular cellulase-producing strain on exoglucanase release and the parameters affecting the synthesis [10].

Avicelases are found to have potential applications in agricultural waste materials bioconversion in to useful products such as fuels and animal feed. Among cellulases, the exoglucanases appear to catalyze most bond-cleavages in the saccharification of crystalline cellulose and is one of the most abundant components in cellulase preparation, especially fungus-derived commercial enzymes. Although a number of microorganisms were reported to produce cellulases, in comparison to that of endoglucanase and beta-glucosidase, reports on exoglucanase or avicelase production are remarkably scarce [11].

The cellulolytic enzyme market is the third largest in the world in production volume due to its numerous applications such as textiles, paper recycling, extraction and juices preparation, and on the use of biodegradable detergents enzymatic agents as an additive in animal feed. Cellulases production may prove to be the first in production volume for new procedures with lower energy and maintenance costs and a selection of economically and technologically viable microorganisms; it remains a current research topic, with several recent studies [2,12-14].

Thus, this study aimed at evaluating the production and partial characterization of xylanase and avicelase from a new isolated from *Penicillium* sp., in solid-state fermentation using soybean hulls residue.

MATERIAL AND METHODS

Bioproduction

The microorganism used in this study was isolated, qualitatively selected, and identified as *Penicillium* sp. by Bortoli and coauthors [15].

Initially, assays were carried out in order to evaluate time and temperature influence on cellulolytic enzymes bioproduction by the isolated fungus, with the enzyme activity analyzed at 3 days intervals for up to 21 days at temperatures ranging from 20 to 35°C, using soybean hulls as a substrate with 70% humidity and a 10^7 spores/g substrate inoculum. The substrate mixture was sterilized in a vertical downward autoclave (Phoenix, model AV75) at 121ºC and 1atm for 15min. A spores concentration was inoculated in to the mixture and later incubated in a germination chamber (Tecnal, Piracicaba, Brazil; model TE401).The extraction conditions were 50°C and 100 rpm for 30 min, using citrate buffer (0.05 M and pH 4.8) [16].

After defined bioproduction time and temperature, the influence of the number of spores (4.6 to 7.4 Log spores/g wet substrate), and the substrate moisture contents (34-76%) on xylanase and avicelase

production were evaluated using a 2^2 full factorial design (Table 1). The dependent variable was the enzyme activity (U/g). Agitation, temperature, and extraction time were kept constant (100 rpm, 50°C and 30 min, respectively). The volume of added water was measured using mass balance calculation, discounting the initial moisture of the mixture and the spores suspension. Moisture was determined by drying at 105°C until the substrate (soybean hulls) reached a constant weight. The water activity was determined using the AquaLab apparatus (AquaLab, model CX-2), whose operation is based on fast response hygrometers.

Cellulolytic Enzymes Extraction

Different solvents were tested, including distilled water, 1% NaCl, sodium citrate buffer (0.05 M, pH 4.8), sodium acetate buffer (0.1 M pH 5.0), and Tween 80 (0.1% v/v) for cellulolytic enzymes extraction. The extraction was conducted with 50 ml solvent and 3.33g fermented substrate, stirred at 100 rpm, 50°C, for 30 min [16,17]. The enzymatic extract was separated from both the mycelium and the solid medium by filtration process, followed by centrifugation (MPW Med. Instruments; Model 351R) at 4,000 rpm, 4ºC, for 15 min.

The influence of temperature (35.9 to 64.1°C) and agitation (54.5 to 195.5 rpm) on the extraction process was evaluated using a 2^2 full factorial design, with the enzyme activity measurement (U/g) (Table 2). The other variables were kept fixed.

Partial Cellulolytic Extracts Characterization

The pH (3.9 to 6.7) and temperature (36 to 64°C) used for xylanase and avicelase activity determination were evaluated by a 2^2 full factorial design (Table 4). 0.05 M Tris-HCl and 0.01 M NaOH buffer solutions were employed for pH adjustment.

Enzyme extracts were submitted to different temperatures (-20, 4, 25, 40, 50, 60 and 70°C) at pH 4.8 to determine their stability. Stability at different pH values (3.5, 4.5, 5.5 and 6.5) was evaluated at 25°C. The samples were collected periodically to evaluate the enzymatic activity. Thermal and pH stabilities were expressed in residual enzymatic activity (%).

Half-life time ($t_{1/2}$), thermal denaturation rate constant (Kd), and the D value were determined at 25, 40, 50, 60 and 70°C (Table 4).

The influence of temperature on the enzymatic inactivation constant rate (Kd) is in accordance with the Arrhenius equation ($Kd=Ae^{Ed/RT}$), and its linearization {ln (Kd)= ln(A)- Ed/RT}. Ed and A parameters were estimated from the inclination and angular coefficient, where A is pre-exponential Arrhenius constant, Ed is enzymatic denaturation activation energy, R is universal constant of gases ($8{,}314x10^{-3}$ kJ/mol.K) and T is absolute temperature (K).

Half-life ($t_{1/2}$) ($t1_{/2}$= 0,693/Kd) and decimal reduction value (D) {D=ln(10)/Kd}, correspond to the time required for a 50% reduction in initial activity at a given temperature and 10% reduction in the initial value reaction rate. The Z value, specified as the variation in temperature required for D value to undergo one log cycle reduction, was estimated by the angular coefficient inverse of the linear correlation of log (D) versus temperature (°C).

Xylanase and Avicelase Determination

Xylanase activity was determined by the reducing sugar release in a 1% xylan obtained from birchwood (Sigma) in a 0.05 M sodium citrate buffer, pH 5.3, at 50°C [13]. The released reducing sugars were determined by the dinitrosalicylic acid method according to Miller [18] using 5.0 to 0.1 mg/mL xylose concentrations for the standard curve construction. An enzyme activity unit (U) was defined as the amount of enzyme capable of releasing 1 µmol reducing sugar per minute (expressed as xylose equivalent), at 50°C.

Avicelase activity (EC 3.2.1.91) consisted of adding 1 mL crude enzyme extract to 1 mL of 1% microcrystalline cellulose solution (Avicel) in 0.05 M acetate buffer, pH 5.0 and incubated at 50°C for 30 min under periodic agitation [19]. The released reducing sugars were determined by the dinitrosalicylic acid method according to Miller [18] and one unit (U) of activity was defined as 1 µmol of glucose equivalent released per minute under the conditions described above, using a glucose standard curve. Appropriate controls were conducted in parallel with all assays.

The final enzyme activities for xylanase and avicelase were expressed in enzyme production per gram of dry substrate ($U/g = \frac{[\text{enzyme}] \times V_{buffer}}{M}$), where [enzyme] = enzyme activity (U/mL); V_{buffer} = buffer volume in the extraction (mL); M = dry substrate mass (g).

Statistical Analysis

The results of time and temperature influence on enzyme production and of different solvents for enzymes extraction (in triplicate) were statistically processed by analysis of variance (ANOVA) and the differences in average were compared by Tukey test (95% confidence) using Statistica software (Statsoft version 5.0). The results obtained in the factorial design were statistically analyzed according to the experimental design methodology, using the same software.

RESULTS AND DISCUSSION

Bioproduction

The enzymatic activity results as a function of bioproduction time (Fig. 1a) presented maximum activity at 14 and 7 fermentation days respectively for xylanase and avicelase, and such periods were maintained for the other experiments.

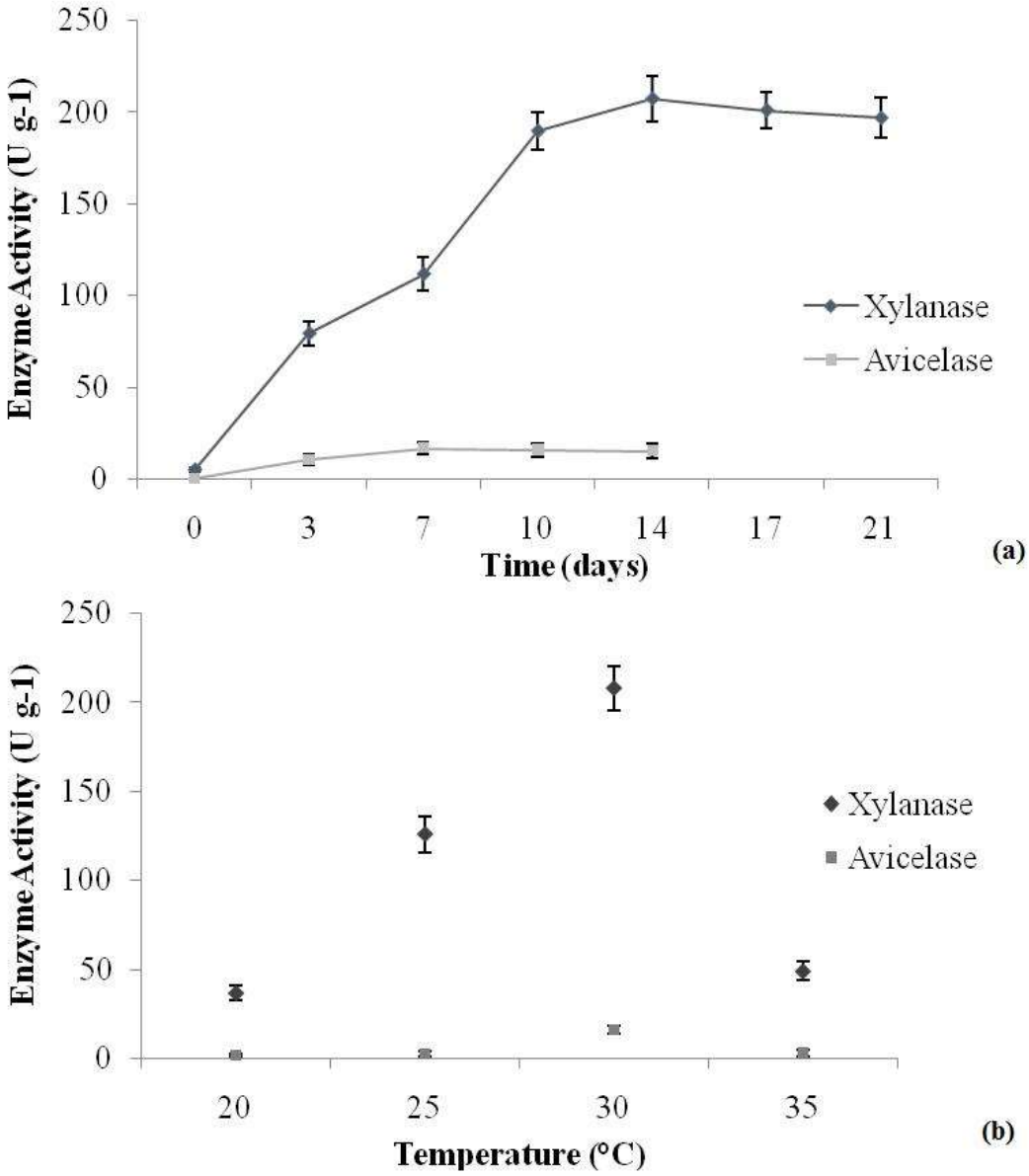

Figure 1. (a) Time and fermentation temperature (b) influence on xylanase and avicelase production.

The optimum fermentation temperature was 30°C (Fig. 1b) for both xylanase and avicelase, and it had the greatest influence on xylanase activity. Similar temperature results were obtained by Kim coauthors [20] and Deswal coauthors [21] with *Penicillium* sp. and *Fomitopsis* sp. However, the required fermentation period was higher than that obtained by Ghoshal and coauthors [22].

After estabilishing ideal temperature and time for bioproduction, the influence of spores concentration and substrate moisture content (soybean hulls) on xylanase and avicelase bioproduction by the isolated *Penicillium* sp. (Table 1) was investigated.

Table 1. 2^2 full factorial design matrix of spore concentration and fermentation moisture (real and coded values) on xylanase and avicelase production.

Assays	Independent Variables		Xylanase (U/g)	Avicelase (U/g)
	X_1	X_2		
1	-1 (5)	-1 (40)	36.76	11.52
2	1 (7)	-1 (40)	35.48	13.43
3	-1 (5)	1 (70)	416.58	21.93
4	1 (7)	1 (70)	435.35	23.73
5	-1.41 (4.6)	0 (55)	128.97	12.65
6	1.41 (7.4)	0 (55)	220.86	10.91
7	0 (6)	-1.41 (34)	21.62	8.85
8	0 (6)	1.41 (76)	220.00	21.99
9	0 (6)	0 (55)	210.00	10.71
10	0 (6)	0 (55)	224.42	11.17
11	0 (6)	0 (55)	222.02	10.85

X_1: Spore concentration (Log); X_2: Humidity (%); Fixed independent variables: 10 g of soybean hull, 30°C. Humidity and water activity correspondence: 34% = 0.9440 (±0.0008); 40% = 0.9488 (±0.0006); 55% = 0.9557 (±0.0004); 70% = 0.9576 (±0.0016); 76% = 0.9586 (±0.0004)

The highest yields of xylanase (416.58 to 435.35 U / g) occurred at 70% moisture, independent of spore concentration (5 - 7 log spores). The highest avicellase production (21.93 to 23.73 U / g) occurred at the highest moisture content (70 and 76%), in the same spore concentration range, showing that the moisture variable has the greatest influence on the bioproduction of these enzymes.

Equations 1 and 2 show the second order coded models that describe xylanase (14 days) and avicelase (7 days) activities as a function of the spores concentration and humidity within the studied ranges. For xylanase, linear spore concentration and linear moisture had a significant ($p < 0.05$) positive influence. The models were validated using analysis of variance. 0.87 and 0.93 correlation coefficients and 1.71 and 3.61 F values for xylanase and avicelase were obtained, respectively.

$$\text{Xylanase (U/g)} = 218.48 + 18.44.X_1 + 132.82.X_2 - 28.10.X_2^2 \quad (1)$$

$$\text{Avicelase (U/g)} = 10.90 + 1.46.X_1^2 + 4.92.X_2 + 3.29.X_2^2 \quad (2)$$

Where: X_1 = Spores concentration (Log); X_2 = Humidity (%).

The definition of optimal conditions for production of avicellase and xylanase, such as time, temperature, spore concentration and humidity, are important due to the characteristic specificity of each microorganism and substrate used. In this way, the literature presents results different from those of the present study, being these ones for each enzyme, microorganism and production condition. Astolfi [23], using the same substrate as the one in the present study, obtained 1130.7 U/g on the sixth fermentation day using *Trichoderma reesei* NRRL 3652 with soybean husk moisture at 70% and 10^6 spores/g. Pirota coauthors [24] using wheat bran as a substrate and *Aspergillus niger* (P6B2 isolate) obtained 1076.9 U/g enzymatic activity for xylanase at 35°C, 10^7 spores/g, and 60% substrate moisture after 72 h fermentation. Mamma and coauthors [25] using orange peel substrates, obtained 77.1 and 37.8 U/g maximum activities with *A. niger* and *Penicillium decumbens*, at 90% humidity, 2.3 x 10^7 spores/g, for 10 cultivation days. The enzymatic activity values for xylanase reported in this work are intermediate compared to other studies found in the literature, though with a maximum activity in 14 days of culture.

Despite there are only a few studies reporting avicelase activity obtained from fungal isolates, the avicelase activities obtained from isolated fungus *Penicillium* sp. were high (23.7 U/g). Pereira and

coauthors [26] reported a 3.6 U/g activity for avicelase obtained from *Myceliophthora thermophila* JCP 1-4 fungus, from 55 to 70°C. Kuo et al., [27] obtained 0.2 U/mL with improved avicelase activity from the isolated *Meyerozyma caribica* cultivated at 25-27°C, for 6 days.

Cellulolytic Enzymes Extraction

Different solvents were tested for the enzymatic extraction process (distilled water; NaCl 1%; sodium citrate buffer (0.05 M, pH 4.5); sodium acetate buffer (0.2 M, pH 5.5) and tween 80, 0.1%, v/v). The higher enzyme activities were achieved using sodium citrate buffer (0.05 M, pH 4.5), 176.54 and 14.22 U/g for xylanase and avicelase, respectively. No statistical differences were observed between sodium acetate and tween 80 buffers for avicelase extraction. But differing statistically from the other solvents for xylanase extraction. Followed by a 0.2 M sodium acetate buffer pH 5.5 and tween 80 0.1% (v/v), in which 129.69 and 116.52 U/g and 15.17 and 15.03 U/g were obtained for xylanase and avicelase activities, respectively. NaCl and distilled water showed the low extraction capacity for these enzymes, 98.83 and 46.65 U/g and 1.91 and 2.65 U/g for xylanase and avicelase, respectively. From the results, 0.05 M sodium citrate buffer, pH 4.5 was defined as the extraction solvent for the subsequent tests. Corroborating with the results obtained in the present study, Dhillon coauthors [16] obtained better FPase, Cmcase, and xylanase extractions with citrate buffer at pH 4.8.

The influence of both temperature and agitation on enzyme extraction process was evaluated through a complete 2^2 factorial design (Table 2). The maximum activities for xylanase and avicelase were 430.77 and 26.77 U/g, at 60ºC and 175rpm and 50ºC and 125rpm, respectively.

Table 2. 2^2 full factorial design matrix of temperature and agitation influence on xylanase and avicelase extraction.

Assays	Independent Variables		Xylanase	Avicelase
	X_1	X_2	(U/g)	(U/g)
1	-1 (40)	-1 (75)	283.63	5.62
2	1 (40)	-1 (175)	280.48	4.03
3	-1 (60)	1 (75)	309.54	3.25
4	1 (60)	1 (175)	430.77	5.44
5	-1.41 (35.9)	0 (125)	350.46	3.8
6	1.41 (64.1)	0 (125)	370.01	4.57
7	0 (50)	-1.41 (54.5)	151.6	0.4
8	0 (50)	1.41 (195.5)	272.62	5.72
9	0 (50)	0 (125)	282.93	26.77
10	0 (50)	0 (125)	267.65	24.15
11	0 (50)	0 (125)	252.36	25.55

X_1= Temperature (ºC); X_2= Agitation (rpm).

For xylanase activity, statistically treated results showed a significant positive effect ($p < 0.05$) for temperature (linear and quadratic) and agitation (linear) variables, indicating an increase in temperature and/or agitation on extraction solution would probably increase xylanase activity (Fig. 2). For avicelase, Equation 3 elaborated from the statistical treatment of results, presented the second order coded model as a function of temperature and agitation extraction, within the studied ranges, with 0.99 correlation coefficients and 37.6 F values. The maximum activity was obtained at 50ºC and 125 rpm, referring to the experimental planning central point assays.

$$\text{Avicelase (U/g)} = 25.49 - 10.44.X_1^2 - 11.01.X_2^2 \quad (3)$$

Where: X_1: Temperature (ºC); X_2= Agitation (rpm).

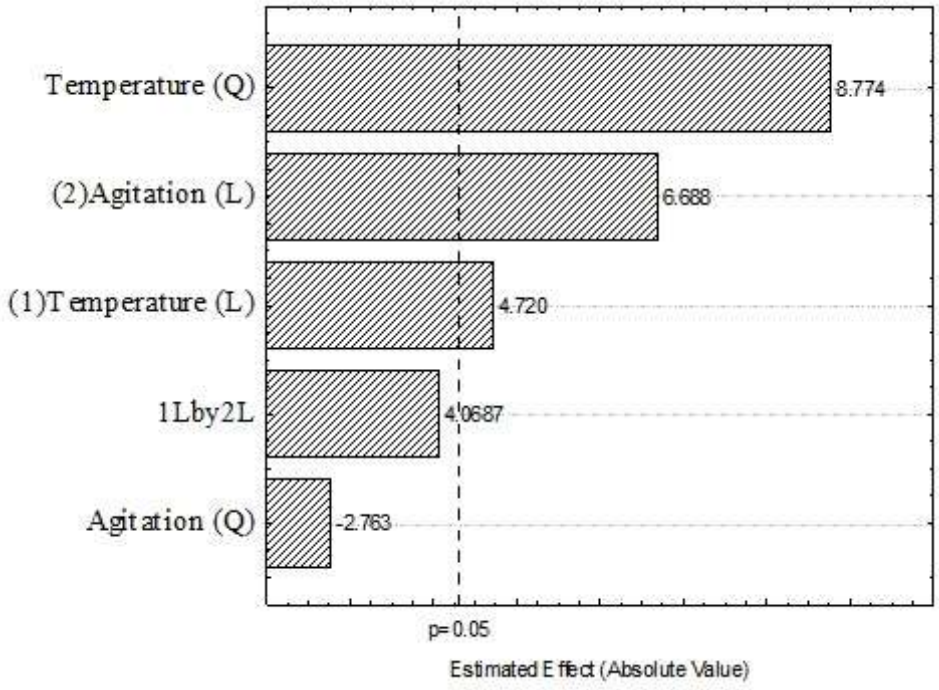

Figure 2. Temperature and agitation estimated effects for xylanase extraction.

Cellulolytic Extracts Partial Characterization

The highest xylanase (463.31 U/g) an;d avicelase (27.31 U/g) activity values were obtained at 50°C and pH 5.3 as per activity determination conditions (Table 3).

Table 3. 2^2 full factorial design matrix of pH and temperature on xylanase and avicelase activity determination.

Assays	Independent Variables		Xylanase (U/g)	Avicelase (U/g)
	X_1	X_2		
1	-1 (4.3)	-1 (40)	158.30	2.6
2	1 (6.3)	-1 (40)	312.22	1.03
3	-1 (4.3)	1 (60)	155.06	3.21
4	1 (6.3)	1 (60)	274.63	2.42
5	-1.41 (3.9)	0 (50)	74.61	1.69
6	1.41 (6.7)	0 (50)	323.95	3.52
7	0 (5.3)	-1.41 (36)	314.64	6.14
8	0 (5.3)	1.41 (64)	104.07	9.85
9	0 (5.3)	0 (50)	463.31	27.31
10	0 (5.3)	0 (50)	425.73	24.46
11	0 (5.3)	0 (50)	444.52	23.45

X_1 = pH; X_2=temperature (°C).

Statistical analysis allowed contour curves construction (Fig. 3), confirming temperature and pH optimization for both enzymes activity, which were similar to the conditions optimized by other researchers using different microorganisms [19,23,27].

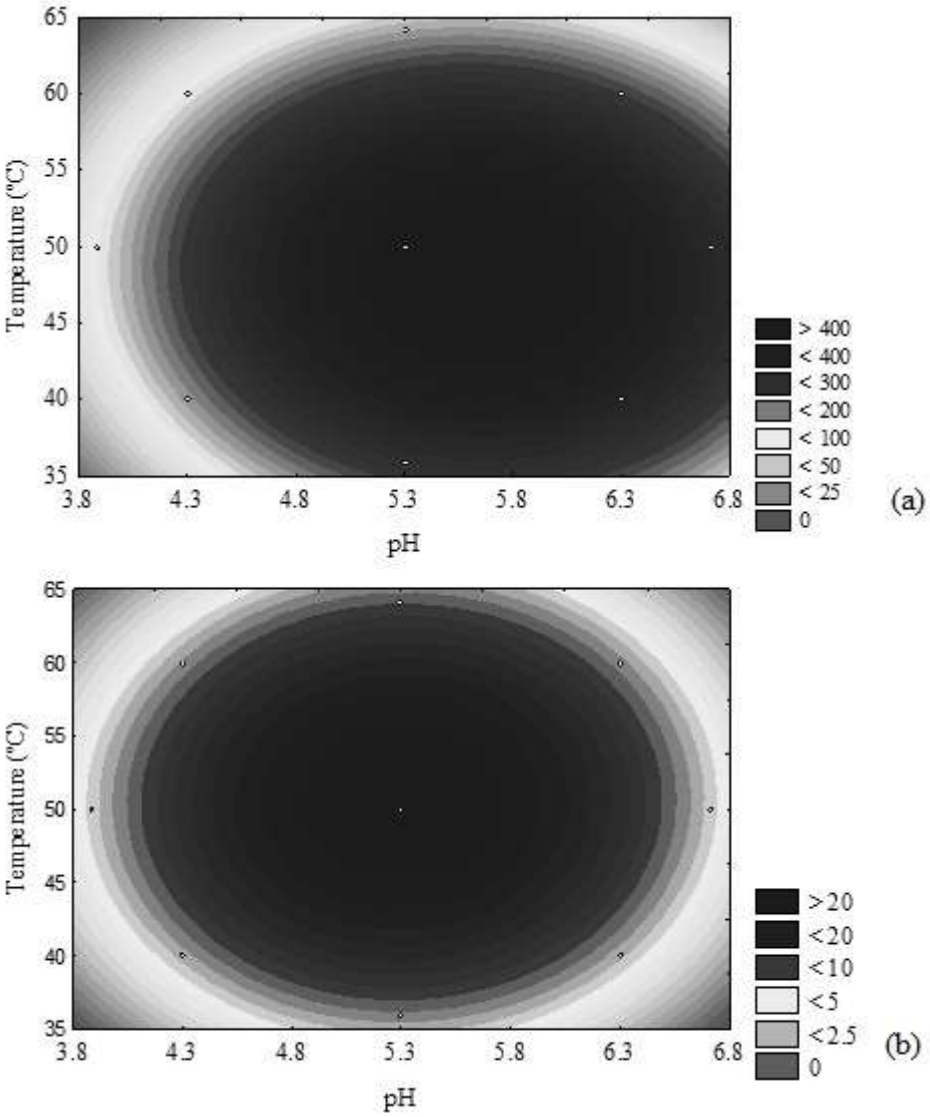

Figure 3. Contour curves indicating temperature and pH influence on xylanase activity (a) and avicelase (b) determination.

In relation to stability at different pH values, xylanase and avicelase showed higher stability between 4.5 and 5.5, that is, about 50% activity reduction after 108 and 144 h, respectively (Fig. 4).

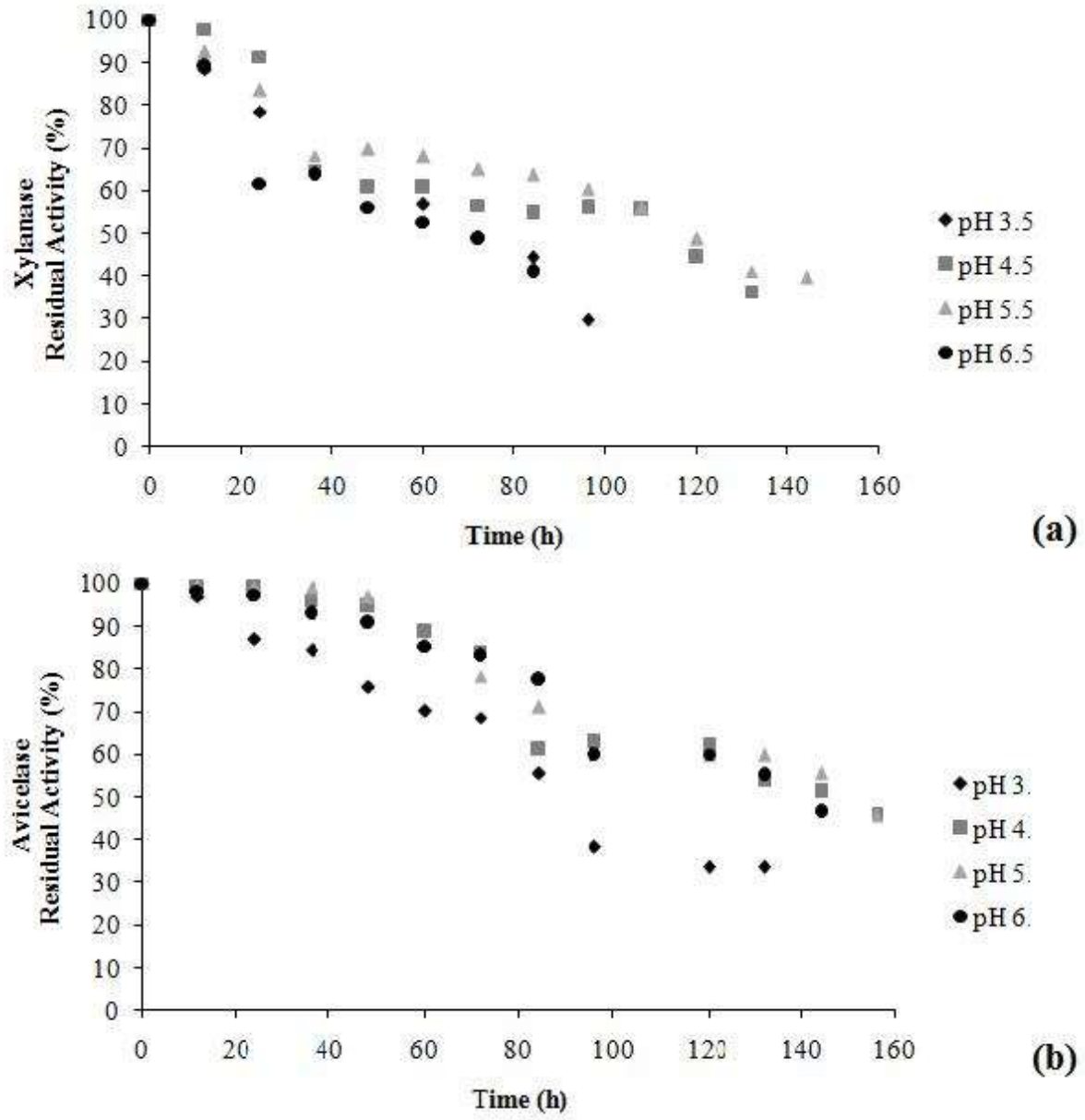

Figure 4. Cellulolytic enzymes xylanase (a) and avicelase (b) stability at different pH levels.

Regarding stability at storage temperature, xylanase maintained approximately 80% residual activity for up to 60 h at 25°C and 70% for up to 40 h storage at 40°C. Xylanase enzyme residual activity reduction (about 50%) was observed after 108 h reaction (Fig. 5a) at both temperatures. This stability time was similar to or even higher than that obtained with other enzymatic extracts presented in the literature [23,28]. Avicelase residual activity reached about 50% at 144 and 96 h, stored at 25 and 40°C, respectively (Fig. 5b), with a linear residual activity reduction as a function of storage time. Drastic activity losses for both enzymes were observed at 50, 60 and 70°C causing enzyme deactivation in a short period of time (Fig. 5a and b).

Both enzymes, at low temperatures, showed a linear residual activity reduction in relation to storage time (Fig. 5). However, xylanase presented higher thermal stability (residual activity over 50% after more than 160 days storage) (Fig. 5c) in comparison to avicelase (50% residual activity reduction after 40 days storage) (Fig. 5d). Storage at -20°C showed a significant difference from that under refrigeration (4°C) for xylanase, but not for avicelase. Most studies found in the literature only evaluated avicelase stability at high temperatures, which presents lower stability [27,29-31] than that obtained in the present study.

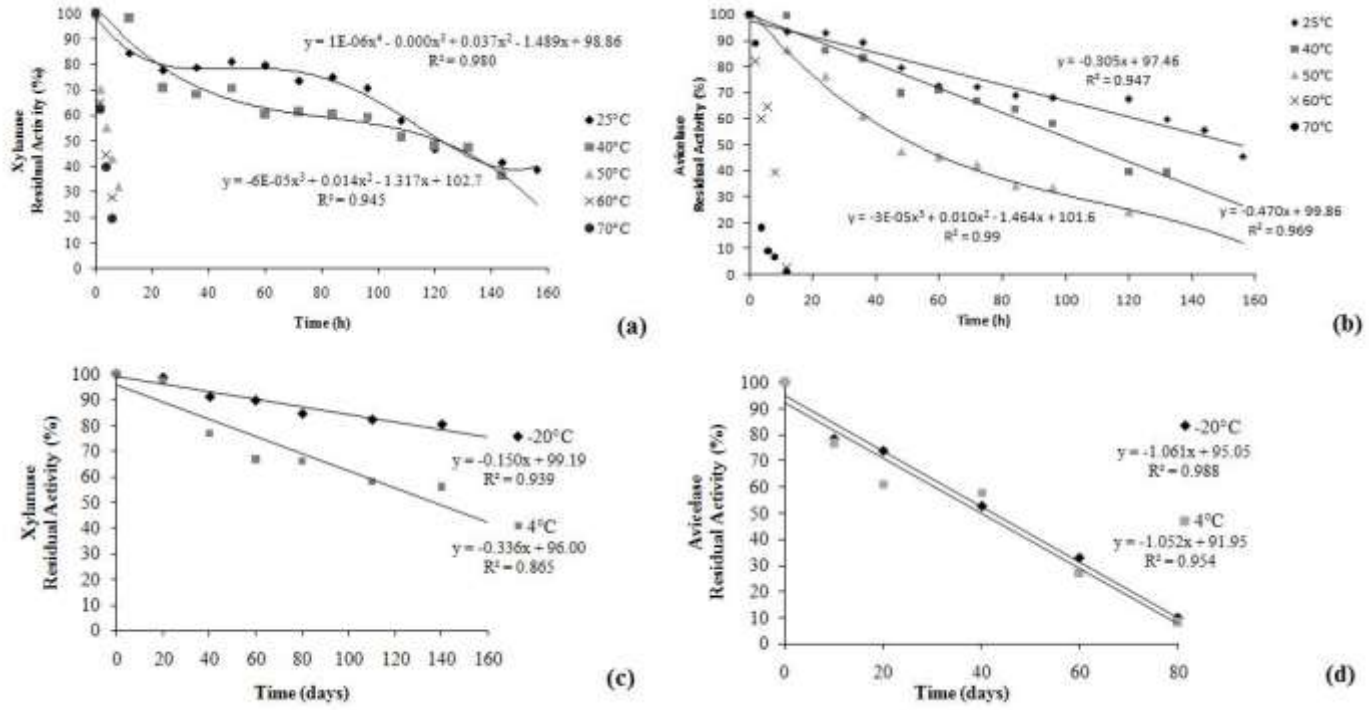

Figure 5. High temperatures enzymatic stability influence on xylanase (a) and avicelase (b) and low temperatures on xylanase (c) and avicelase (d).

Half-life ($t_{1/2}$), kinetic thermal denaturation constant (Kd), and D-value for different enzymes are shown in Table 4. Half-life and decimal reduction (D) times of a biocatalyst correspond to values that are numerically equal to the time required to achieve 50% and 10% decrease in its initial enzymatic activity, respectively.

Table 4. Experimental k_d, $t_{1/2}$, D, Ed and Z values for xylanase and avicelase, as a temperature function.

Temperature (ºC)	K_d (min^{-1})	$t_{1/2}$ (h)	D Value (min)	Ed (KJ/mol/T.K)	Z Value (ºC)
Xylanase					
25	0.005	138.6294	460.517	89.1	22.2
40	0.005	138.6294	460.517		
50	0.138	5.022806	16.6854		
60	0.209	3.316494	11.01715		
70	0.267	2.596057	8.623914		
Avicelase					
25	0.004	173.286	575.646	96.7	20.4
40	0.006	115.524	383.764		
50	0.011	63.013	209.326		
60	0.279	2.484	8.253		
70	0.381	1.819	6.044		

Temperature increase affected the enzymes' thermal deactivation process evaluated with similar inactivation rates (K_d) between xylanase and avicelase at different temperatures. Avicelase was more stable at thermal deactivation than xylanase, with half-life times and D values ranging from 173.29 to 63.01 h and 575.65 to 209.33 min at temperatures from 25 to 50°C. Xylanase showed stability only up to 40ºC, and both showed strong thermal deactivation at higher temperatures. Such high parameters values are desirable during industrial operations since they ensure an enzyme more resistant to deactivation caused by temperature effects [32].

The 22.2 and 20.4 Z values (Table 4) indicated that a change in these temperature ranges would generate one logarithmic cycle reduction on xylanase and avicelase enzymatic activities, respectively.

Driss and coauthors [33] used *Penicillium occitanis* for xylanase enzyme bioproduction, obtaining a 729.6, 420, and 115.5 h for half-life and 0.00095, 0.00165 and 0.00600 for thermal denaturation kinetic

constant (K_d) at 50, 60 and 70°C. Such values were higher than those found in the present study. Zanin and coauthors [34] obtained 0.0492 and 4.1678 for K_d; and 14.1 and 0.17 for $t_{1/2}$, respectively at 60 and 70°C, using *A. niger* on cellobiose substrate. No data were found in the literature regarding deactivation energy and Z value for avicelase produced by *Penicillium* sp.

For enzymatic processes, besides thermal deactivation studies, thermodynamic parameters determination is also needed to elucidate the molecules behavior against enzymatic inactivation under different physiological conditions, as well as the effects of temperature on the enzymatic deactivation rate [35].

Arrhenius linearization was used to represent enzymatic activity variation as a function of enzymatic denaturation kinetic constants (K_d) at different temperatures (1/T). E_d value for xylanase (89.1 kJ/mol) was slightly lower than for avicelase (96.7 kJ/mol), corroborating with the higher thermostability for avicelase. Different authors found variable E_d values for xylanase obtained with enzymatic extracts from different isolates. Driss and coauthors [33] using *P. Occitanis* immobilized to increase digestibility in broiler ration, found an E_d value of 83.02 kJ/mol for immobilized xylanase. Balsan and coauthors [36] found E_d value of 177.6 kJ/mol for enzyme cellulase from *T. reesei* (NS 50013) in lignocellulolytic culture media. Zanin and coauthors [34], using *A. niger* on cellobiosis substrate, obtained 80.6 Kcal/mol values for thermal deactivation energy in free enzyme.

CONCLUSION

This work obtained xylanase and avicelase bioproduction by a new isolated *Penicillium* sp. in solid-state fermentation, using soybean hulls as substrate that presented maximized activity for xylanase and avicelase (435.35 and 23.73 U/g,) with 30°C, 1x106 spores/g substrate, at 14 and 7 fermentation days with 70 and 76% substrate moisture, respectively. The citrate buffer solution (0.05 M, pH 4.5), at 60°C and 175 rpm or 50°C and 125 rpm for xylanase or avicelase, respectively, showed better activity results, 430.77 and 26.77 U/g. The optimum pH and temperature for enzymatic activity determination were 5.3 and 50°C for both enzymes studied. The avicelase was more stable for thermal deactivation than xylanase, presenting the half-life times and D values ranging from 173.29 to 63.01 h and 575.65 to 209.33 min from 25 to 50°C, respectively. However, the half-life times and D values for xylanase ranged from 138.63 to 5.02 h and from 460.52 to 16.68 min, respectively, at the same temperature range. Z values for xylanase and avicelase were 22.2 and 20.4, respectively. Ed value for xylanase (89.1 kJ/mol) was slightly lower than for avicelase (96.7 kJ/mol), indicating higher thermostability for avicelase. Finally, the production of xylanase and avicellase in agroindustrial substrate allows the reduction of the cost of these, generating attention from the industrial point of view, mainly as a resource for the biofuel industry, animal feed, food, textile and detergent.

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