Optimization of β-1,4-endoxylanase production by a new *Aspergillus niger* strain growing on wheat straw and application in xylooligosaccharides production

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Abstract

Plant biomass constitutes the main resource of renewable carbon in the planet and its valorization has traditionally been focused on the use of cellulose, although hemicellulose is the second most abundant group of polysaccharides on earth. Enzymes involved in its degradation are usually glycosyl hydrolases and filamentous fungi are good producers of these enzymes. In this study, a new strain of *Aspergillus niger* was utilized for hemicellulase production under solid state fermentation using wheat straw as a single carbon source. Physicochemical parameters for production of an endoxylanase were optimized by using one factor at a time approach and response surface methodology (RSM). Maximum xylanase yield after RSM optimization was increased 3-fold. The enzyme was purified by ultrafiltration and ion-exchange chromatography1.41-fold, with 6.2 % yield. Highest xylanase activity was observed at 50 °C and pH 6. A high pH and thermal stability were found, greater than 90% residual activity between pH 3.0-9.0 and between 30-40°C, after 24 h of incubation, presenting half-lives of 30 min at 50 and 60°C. Enzyme was mostly active for wheat arabinoxylan, and displayed the following kinetic parameters K_m of 26.06 mg·ml^{-1} and V_{max} of 5,647 U·mg^{-1}min^{-1}.Wheat straw xylan hydrolysis with the purified β-1,4 endoxylanase showed that it was able to release xylooligosaccharides, making it suitable for different applications in food technology.

Keywords: Fungi, enzymes, hemicellulose, wastes, hydrolysis

1. INTRODUCTION

Each year, transformation of the animal and plant feedstock, and its intermediate and terminal products, yields 140 billion tons of agricultural and industrial residues. Just 3% of the 13 billion tons/year of plant-biomass residues are utilized in producing goods (Morganti, 2013). The current management system is highly depending on various fossil energy sources like oil, coal and natural gas (Sarkar, Ghosh, Bannerjee, & Aikat, 2012). The development of technologies for renewable energy production is one of the greatest challenges in the twenty-first century, due to the serious problems on energy production and use (Hussein, 2015). Much alternative energy can alter the fossil fuels needs in the future, like biomass, solar, wind and hydrothermal energy. Between these energy sources, the biomass is one of the carbon-based sustainable energy most consumed in the world(Faraco, 2013).

Underutilization of agriculture products yields residues, frequently rich in nutrients, can provide an excellent habitat for the development of microorganisms. Lignocellulose, the main renewable resource in nature and the most abundant carbohydrate in biosphere, is the central component of plant biomass. It consists of three principal constituents: i) cellulose, a homopolysaccharide composed of units of D-glucopyranose linked by β-1,4 O-glycosidic bonds, constituting long linear chains which interact giving rise to microfibrils (Klemm, Philipp, Heinze, Heinze, & Wagenknecht, 1998),ii) hemicellulose, which groups a series of polysaccharides with heterogeneous structures, that differ from each other in their backbone and branches (Scheller & Ulvskov, 2010), and iii) lignin, an amorphous and complex heteropolymer, highly branched and composed by phenylpropane units, which reinforce plant cell wall structure (A. Pandey, Soccol, & Larroche, 2008).

Respect to hemicellulose, xylan is the most abundant non-cellulosic plant polysaccharide, being the main component in the secondary cell wall of dicotyledons and cereal grains (Ebringerová, 2005). It is composed of a backbone of β-1,4-linked D-xylopyranosyl units, frequently acetylated and highly branched by short side chains of arabinose or glucuronic acid. The abundance of each of these substituents depends largely on the nature of the plant biomass. Usually full catabolic processes are achieved in Nature by the synergistic action of different microorganisms found in biomass rich environments (Hu et al., 2007).However, during last years the studies about the enzymatic microbial system involved in the process have increase, by the economical relevance of exploiting this heteropolysaccharide on different biotechnological applications. Due to its complexity, xylan hydrolysis requires the action of different enzymes for is degradation but, among them, two types of glycosidases play a key role in the process: i) endo-β-1,4-xylanases, which hydrolyze the polysaccharide by attacking internal links in the main chain, releasing oligosaccharides with different polymerization degrees and ii) β-xylosidases, which complete the degradation process by converting the xylooligosaccharides into xylose (Biely, 1985).

Although xylanases can be produced by several organisms (including crustaceans, protozoans, snails, insects, bacterial or fungi), the filamentous fungi are usually chosen for these studies by the secretion of high enzyme levels to the culture (facilitating its purification and further characterization), and their robustness against environmental factors (Polizeli, 2005). Fungi from genus *Aspergillus, Trichoderma, Rhizopus* and *Penicillium* are very well known producers of xylanase activities. Specially, *Aspergillus* and *Trichoderma* species have been studied extensively for their ability to secrete high-levels of enzymes in solid-state fermentation(Chen, 2013). *Aspergillus* genus is usually employed to produce xylanolytic enzyme cocktail in industrial plants for applications like paper manufacturing, bread-making, animal feed, juice and wine industries(Loureiro, Romanini, & Tubio, 2016; Takahashi, Kawabata, & Murakami, 2013).However, since the enzymatic system secreted by fungi depends of the strain and the culture conditions, it is interesting to study new strains to produce efficient and robust enzymes.

Solid-state fermentation of lignocellulosic biomass has successfully been applied for biofuels, biotransformation, biological detoxification, and bioremediation, in solving an energy crisis and environmental pollution, and the application in environmental protection by biotransformation of crops and waste products to enhance their nutritional value. These biotransformation processes for crops and waste can be optimized to achieve a suitable high yield. The classical methods for optimization focus on changing one variable, while maintaining the others into fixed levels. This single-dimensional approach is an incomplete approach to produce optimized conditions since it does not consider interactions between factors. The response surface method (RSM), on the contrary, is a strong model for establishing optimal process conditions under complex interactions between independent variables (Montgomery, 2017).

The objective of this study was to optimize fermentation conditions, using one factor at a time (OFAT) and response surface methodology(RSM) approaches of a new *Aspergillus niger* BG strain growing on wheat straw to produce high levels of β-1,4-endoxylanase. In addition, this enzyme was purified, its physico-chemical and kinetics properties were studied, and a process to generate xylooligosaccharides by hydrolysis of wheat straw xylan was designed.

2. MATERIALS AND METHODS

2.1. Microbial strain and growth conditions

A. niger BG strain was isolated from olive tree decomposing soil from Akbou area, Bejaia, located in North-east of Algeria (Azzouz, et al., 2020). It was cultivated using solid-state fermentation (SSF) in a 500 mL flask containing10 g wheat straw moistened at 70%with Mandels medium (Mandels & Weber, 1969) and pH adjusted to 5.0. A spore suspension (10^7 spores/g of substrate) of *A. niger* BG strain was prepared from 7 days grown PDA agar plates in 1% (v/v) Tween 80. The spore count was performed in a Malassez counting chamber (Marienfeld, Germany) (Ang et al., 2015).This spore suspension was seeded in a 500 mL flask and incubated at 28°C for 7 days. Thereafter, the enzymatic extracts were harvested by crushing the contents of the flasks in 100 mL of distilled water with a glass rod and then shaking on an orbital shaker at 100 rpm for 10 min at room temperature. The filtrate was centrifuged at $10,000 \times g$ for 10 min at 4 °C. The clear supernatant was used as crude enzyme with endoxylanase activity and stored at 4 °C until its use. For optimization studies, the composition of the culture medium was varied according to the experimental data, while the inoculums size and source of carbon (wheat straw) were constant.

2.2. Enzyme assay

Endoxylanase activity was measured by the method of Bailey et al., (1992)with slight modification. The liberation of reducing sugars from beechwood xylan was estimated by the dinitro-salicylic-acid method (Miller, 1959), using D-xylose as a standard. Reaction mixtures contained 100 µL of properly diluted enzyme with 900 µL of 2% (w/v) beechwood xylan (Sigma-Aldrich), dissolved in 50 mM citrate buffer (pH 5), and they were incubated for 5 min at 50 °C. Reactions were terminated by the addition of 1.5 mL 3,5-dinitrosalicylic acid reagent (DNS), placed in a boiling water bath for 5 min and cooled in ice water. The absorbance was read at 540 nm. One unit of activity has been defined as the quantity of enzyme needed to release 1.0 µmol of xylose per minute (min) at pH 5 and at 50 °C. For each assay in this study, triplicate measurements were conducted. The concentration of the protein was measured by the method of Bradford (Bradford, 1976), using bovine serum albumin (BSA) as a standard.

2.3. Determination of optimal physicochemical parameters using classical one-factor-at-a-time approach

The production of endoxylanase from wheat straw under SSF was optimized by classical OFAT approach. In this method, one parameter is changed while maintaining the others to predefined levels (Banu, Ali, Rahman, & Konneh, 2020).This was used to study the effects of physicochemical parameters as culture time (1 to 7 days), incubation temperature (20 to 40 ℃), moistening agent level (30 to 95%) and initial pH value (2 to 9). All experiments were run in triplicates and data obtained were subjected to one-way analyses of variance (ANOVA) carried out using XLSTAT software (version 2009.1.02), determining the Fisher's least significant difference in the mean variable. A p-value < 0.05 between the variables was considered significant.

2.4. Determination of optimal physicochemical parameters with response surface model using Box–Behnken design

Box–Behnken design (BBD) is used for optimization purposes since it allows to estimate the main effects of some factors simultaneously(Wahid & Nadir, 2013). Parameters and levels used for this optimization were selected by classic OFAT (Table 1).A polynomial model based on 27 experiments with 3 replicates was built (Table 2) using Design-Expert 11®software (Version11.0.5.0.USA).The number of experiments (N) required was defined according to equation Eq (1):

$$N = 2k \cdot (k-1) + C_0 \tag{1}$$

Where k and C_0 are the numbers of factors and central points respectively.

The trial data is then fitted through the response surface model (RSM) to discover the relationship between the controllable factors and the response factors (Xie et al., 2020)**.** Regression analysis of the data to fit a second-order polynomial equation (quadratic model) was carried out according to the following general Eq 2, which was used to predict the optimum conditions of the extraction process.

$$R = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j + \varepsilon \tag{2}$$

Where R represents the response surfaces*, β_0* is the constant term (intercept), *β_i, β_{ii}* and *β_{ij}* are the coefficients of linear, quadratic, and interaction, respectively, while X_i, X^2 and $X_i X_j$ are linear variables, quadratic variables,

and term for interaction of the variables respectively, and ε is the residual associated to the experiments (the prediction error, represents the difference between measured R values and predicted R and quantifies the random variability in our design of the experiment. The surface plots were plotted by varying the values of two factors and keeping the values of other factors constant at zero level. The second-order polynomial model that RSM developed consists of linear, quadratic, and interaction models (Eq. 2) (Srivastava et al., 2018). The determination coefficient (R^2) is selected to calculate the goodness of fitting (Eq.3). The closer the value is to 1, the better the model fits.

$$R^2 = 1 - \left[\sum_{i=1}^{n} (R_i - \hat{R}_i)^2 \div \sum_{i=1}^{n} (R_i - \overline{R})^2 \right]$$ (3)

Where n, R_i, $\hat{R}_i$, and $\overline{R}$ are the number of measures, the i-th observation value, the i-th predict value, the

number of trials, and the average of the response factors respectively.

The model prediction ability can be enhanced by omitting it, when the influence of the corresponding item is significant and vice versa. The prediction determination coefficient (R^2predict) is used to calculate the prediction ability of the model as given in Eq. (4).

$$R^2_{predict} = 1 - \left(1 - R^2\right) \left[\left(n - 1\right) \div \left(n - \left(k + 1\right)\right) \right]$$ (4)

Where n is the number of observations and k is the number of independent variables in the regression equation. The closer R^2 predict is to 1, the better the prediction ability of the mode is. The model is simplified by rounding off the insignificant items in the model and the new RSM model is obtained, then, the new model will have better prediction ability (Xie et al., 2020).

2.5. Purification of β-1,4-endoxylanase

Five hundred mL of crude extract, recovered after 96 h culture of *A. niger* BG strain produced in the SSF culture using the optimal production conditions obtained after optimization, were centrifuged for 15 min at $10,000 \times g$ to remove mycelia and spore cells. The supernatant was sequentially filtered, using 0.8, 0.45 and 0.22 μm filter membranes (Merck-Millipore). Then, filtrated supernatant was concentrated and dialyzed against 10 mM sodium phosphate buffer pH 6.0 by ultrafiltration, using a 3-kDa cut-off membrane. All purification steps were performed using an AKTA purifier system (GE Healthcare). Initially, this crude extract was applied to a 5 mL-HiTrap QFF (GE-Healthcare) cartridge, pre-equilibrated with the protein dialysis buffer, at a flow rate of 3 mL/min. Elution was carried out by applying a linear gradient of 1 M NaCl, prepared in the same buffer, from 0 to 50% in 45min. Protein profile was followed by the absorbance at 280 nm. Fractions with endoxylanase activity were pooled, dialyzed in the previous mentioned buffer and concentrated by ultrafiltration using 3-kDa cut-off membrane Amicon Ultra-15 centrifugal devices (Merck-Millipore). Finally, the samples were applied into a Mono Q 5/50 GL (GE Healthcare),equilibrated in the same buffer, at a flow rate of 1 mL/min and elution was carried out by applying a linear gradient of 1 M NaCl (0 to 10% for 35 min). The fractions with the purified protein were pooled, concentrated by ultrafiltration, as previously mentioned, and stored at 4 °C.

2.6. Characterization of β-1,4-endoxylanase
2.6.1. Polyacrylamide gel electrophoresis, mass spectrometry and NH₂ -terminal amino-acid sequencing

The homogeneity and molecular mass of the purified enzyme were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE),following the method of Laemmli (1970), using 12% polyacrylamide gel and Precision Plus protein dual-color standards (Bio-Rad). Proteins were stained with Coomassie brilliant blue R-250 (Sigma-Aldrich).

The Native-PAGE (non-reducing conditions) of the purified xylanase was performed using 12% resolving gel. Xylanase activity was detected according to the protocol described by Yan et al., (2008) with slight modifications. Once the electrophoresis was finished, the gel was washed two times (each for 10 min) with 20 mL of 10 mM phosphate buffer, pH 6.0. The treated gel was then pre-incubated in 20 mL of the buffer containing 10 mM xylanase substrate (EnzChek™ Ultra Xylanase Assay Kit, Invitrogen ™), followed by incubation at 10 min at room temperature. Finally, the detection of xylanase activity was visualized by fluorescence under ultraviolet light by use of the Gel Doc XR +system (Bio-Rad). The protein identification was determined by matrix-assisted laser desorption ionization-time of the flight mass spectrometry (MALDI-TOF), with an Autoflex III instrument (Bruker Daltonics), and the peptide fingerprinting was compared with those available in the NCBI database, with the MASCOT search engine (Matrix Science).

2.6.2. Effect of pH and temperature on enzyme activity and stability

The effect of pH on endoxylanase activity was investigated at pH values ranging from 2.0 to 9.0 at 50 °C for 5 min using beechwood xylan as substrate. The optimum temperature was measured by starting the reaction at different temperatures (30–90 °C) and the relative activity was calculated considering the maximum activity as 100%. The pH stability test was carried out by incubating the enzyme in different pH buffers (2.0 to 9.0) for 30 min, 2, 4, and 24 h at 25 °C followed by the activity assay. The thermostability of the enzyme was assessed by incubating the enzyme at temperatures of 30–80°C for 30 min, 1, 2, 3, 4, 5, 6, and 24 h, before measuring residual activities. Initial activity was determined as 100%.

2.6.3. Substrate specificity and kinetic parameters determination

Substrate specificity was investigated by incubating the purified enzyme with beechwood xylan, wheat arabinoxylan (WARX), avicel, and starch, detecting release of reducing sugars by DNS method. The relative activity was calculated using beechwood xylan as a reference.

Kinetic parameters (K_m and V_{max}) of xylanase towards WARX were determined using substrate concentrations from 2 to 50mg/ml. The data were fitted to a Michaelis−Menten equation using Graph-pad PRISM 7 software (version 7.00).

2.7. Wheat straw xylan hydrolysis using β-1,4-endoxylanase

The hydrolysis products released by enzymatic hydrolysis of wheat straw xylan kindly provided by Andromaco Laboratories (Spain) were analyzed by thin-layer chromatography (TLC). In a 1.5-mL tube, 500 μL of substrate (20mg/ mL) was mixed with 0.03U of pure enzyme. The mixtures were incubated at 40 °C for 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h. The samples were centrifuged at 14,000 × g for 5 min, and the supernatant was used to analyze the hydrolysis products by TLC analysis. For every sample, 5 μL of hydrolysis product were spotted onto TLC plates (POLYGRAM® SIL G/UV$_{254}$. Germany) and the plates were developed with the solvent system butanol/acetic acid/water (3:1:1, v/v/v). The TLC plates were air dried for 15-30 min and visualized by spraying with a solution methanol/sulfuric acid (95:5, v/v), followed by heating at 110 °C for 10 min. A xylooligosaccharides solution (xylose, xylobiose, xylotriose, xylotetraose, and xylopentaose) was used as standard.

3. Results and discussion
3.1. Endoxylanase optimization using the classical OFAT method

In the last years, interest on endoxylanases have increased since they are essential enzymes to convert xylan in sugars that could be used in different biotechnological applications, as prebiotics, animal food or fuels (Ebringerová, Hromádková, & Heinze, 2005).As previously mentioned, *Aspergillus* species are one of the most studied organisms because they are a promising source of robust cellulases and xylanases. After a previous screening, using 62 fungal strains isolated from lignocellulose wastes, a new *A. niger* BG strain was selected by its ability to secrete high levels of enzymes involved in the degradation of plant cell polysaccharides (Azzouz, Bettache, Djinni, et al., 2020).In this work, the capabilities of this strain to produce an interesting endoxylanase, using SSF conditions and wheat straw as a single carbon source, was analyzed. Initially, the maximal activity was obtained after 7 days of incubation at 28 °C (314.47 ± 0.6 U/mL), when wheat straw was moistened with Mandels solution (70%) at pH 5 and inoculated with 10^7spores/g suspension. To increase yield, operating conditions, such as pH, temperature, moisture agent level, and incubation time were varied. Figure 1 shows the variations over time of endoxylanase activity in the different tests carried out by using classical OFAT approach, as explained below.

3.1.1. Effect of the incubation period

The incubation period is an important parameter when considering enzymatic production. Fig.1a shows the impact of the incubation period on the xylanase production when wheat straw was used by *A. niger* BG strain as a substrate of fermentation. The endoxylanase production increased during the first days of incubation, reaching its maximal levels at 3 days-old cultures (330.49 ± 0.6 U/mL) and remaining practically stable during the rest of the studied period. The stabilization on enzymatic production could be related to the reduction of the nutrients in the fermentation medium along time, which impacts in the fungal physiology (Nochur, Roberts, & Demain, 1993). This is an adequate result, since shorter incubation time for enzyme production reduces the possibility of contamination and benefits its economic development for industrial xylanase production (Fadel, et al., 2014). It is necessary to remark that maximal production of this activity has been reported for *Geobacillus thermodenitrificans* X1, *Rhizopus oryzae* UC2 and *Aspergillus fumigatus* MS16 at 4,5 and 6 old-days cultures, by using raw oil palm frond leaves, wheat straw, and banana peels as carbon sources respectively (Verma et al., 2020; Ezeilo et al., 2019 and Zehra et al. 2020). However, maximum xylanase production was observed after 2 days of incubation with strains *A. fumigatus* F-993 and *Aspergillus flavus*, using white corn flour, wheat bran, and pearl millet stover under SSF, respectively (Fadel et al., 2014; Gautam, Kumar, & Dutt, 2015).

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3.1.2. Effect of incubation temperature

The maximum endoxylanase activity (355.16± 0.3 U/mL) was found at 36 °C. At lower temperature, the production of xylanase decreased owing to lower transfer of substrates in the cell membrane, while higher temperature could reduce growth due to the denaturation of enzymes, which results in the higher preservation energy for cellular growth and lower metabolites generation(Pal & Khanum, 2010).Irfan et al. (2014), and Gautam et al., (2015), registered the maximum xylanase activity at 30 °C by *Aspergillus foetidus,* and *A. flavus* ARC-12 respectively. The maximum xylanase production was also reported at 35 °C and 25°C by *A. fumigatus* MS16 (Zehra et al., 2020). These variations could be related to the diversity of the microorganisms and their environmental conditions. Temperature is one of the vital factors, which heavily influences the SSF process, affecting different parameters as cell growth, and production of enzymes and metabolites (Mandal & Ghosh, 2017).

3.1.3. Effect of moisture agent level

The importance of moisture agent level in the SSF medium and its influence on enzyme production and microbial growth can also be attributed to the effect of moisture on the physical properties of solid substrate used for fermentation (Bakri et al., 2008).The increased moisture agent level from SSF had a significant effect on endoxylanase secretion. Fig 1c shows that xylanase production reaches maximum activity at 95% moisture agent level (462.49± 0.2 U/mL).

The moisture level was very different in other fungal growth in SSF conditions. Behnam et al*.,* (2016), found that a moisture level of 71.8% was optimum for maximum xylanase production by strain *R. oryzae.* Also, Mandal and Ghosh (2017), found that cellulase production by strain *A. niger* using banana peel as substrate was maximum with a moisture ratio 3:1(w/v). More porous substrates can enhance the gaseous transfer, and still retain the right amount of moisture agent level supporting the stability of the extracellular enzyme and nutrient solubility (Bharti et al., 2018). Conversely, Ezeilo et al., (2019), Zehra et al., (2020), and Pandey et al., (2016), achieved maximum xylanase production at 40%, 45%, and 50% moisture agent level respectively, using strains of *R. oryzae*UC2*, A. fumigatus* MS16 and *R. oryzae SN5*, and were obtained with raw oil palm frond leaves, banana peels, and wheat bran as carbon sources under SSF respectively.

3.1.4. Effect of pH

The pH in a SSF medium is a key variable that directly influences the stability of inter-membrane transportation, microbial growth, and ultimately the production enzyme. In particular, this is very important for extracellular enzymes, since its release into the medium depends on the mechanism of membrane transport, which is regulated by the concentration of the hydrogen in the medium (Bibi et al., 2014). To examine the optimum initial medium pH for xylanase production, experiments were implemented at different pH values ranging from 2 to 10. Fig 1d shows that xylanase production was quite affected by the initial pH value in the *A. nig*er BG strain cultures growing in wheat straw under SSF conditions. The maximum endoxylanase activity was achieved at pH 9.5 (494.56 ± 0.54 U/ml).

Most of the fungus exhibit the highest xylanase activity level in acidic environment, in medium at pH 4.5 (Irfan et al., 2014) or 6.5 (Carmona et al., 2005). However, the optimization of xylanase production was also reported by Verma et al., (2020)at pH 7–8 in *G. thermodenitrificans* X1 and in more alkaline conditions (pH 8-9) for *R. oryzae* UC2 (Ezeilo et al., 2019),*Aspergillus fischeri* (Senthilkumar et al., 2005)*,* and *Trichoderma* SG2 (Nanjundaswamy & Okeke, 2020). In summary, a classical approach to optimize xylanase production by *A. niger* BG strain on wheat straw indicates that the production was enhanced by the short incubation time (three days), alkaline pH (9.5), high humidification level (95%), and mesophilic temperature (36°C). Under these conditions, *A. niger* BG strain produced high amounts of xylanase with a maximum activity of 494.56 ± 0.54 U/ml.

3.2. Box-Behnken analysis of xylanase production-based RSM design

After classical optimization of xylanase production, a statistical procedure was achieved by RSM using Box-Behnken Design (BBD). Four factors, incubation temperature (X_1), moisture agent level (X_2), pH (X_3) and, incubation time (X_4), were found to have a greater influence on xylanase production by *A. niger* BG strain. All factors were kept constant individually at the optimum level that they obtained in the OFAT trial. Afterward, the BBD design was used to optimize the level of these factors to study their interaction. Experimental conditions and results for xylanase production are presented in Table 2.

3.2.1. Model performance and fitting using RSM

The significance of the model was estimated by analysis of variance (ANOVA). Table 3 shows the ANOVA results for the quadratic model. The model *F*-value of 12.83 implies that the model is significant (*p*-value <0.001), with only a 0.01% chance that an *F*-value this large could occur due to noise. Within the model, X_1

(incubation temperature), X_2 (moisture agent level), X_2X_4 (moisture agent level vs. incubation time), X_3^2

(moisture agent level^2), and X_4^2 (incubation time^2) are significant model terms (table 3) with a p-values less than 0.05. As for factors X$_3$(pH), X$_4$(incubation time), X$_1$X$_2$ (incubation temperature vs. moisture agent level), X$_1$X$_3$ (incubation temperature vs. pH), X$_1$X$_4$ (incubation temperature vs. incubation time), X$_2$X$_3$(moisture agent level vs. pH),X$_3$X$_4$ (pH vs. incubation time), X_1^2 (incubation temperature^2), and X_2^2 (moisture agent level^2), indicates that the factors and interaction between influencing factors are insignificant since the p-values are more than 5%.Lack-of-Fit with F-value of 3.36 implies this is not significant relative to the pure error. There is a 25,11% chance that a Lack-of-Fit F-value this large could occur due to noise and non-significant lack of fit is good.

The calculation of the determination coefficient value (R^2) is 0.9374, indicating the acceptability of the model in estimating the predicted values from the experimental values. The model is adequate in explaining most of the variability in the trial results since the R^2 value is greater than 0.75 (Wan et al., 2009). The Adjusted R² value (0.8643) validates the proposed model. By measuring the signal to noise ratio (Adeq Precision),the precision of the model can be indicated, which should be greater than 4 (Shojaei & Shojaei, 2017). In this report the value was 12.433, which shows a high level of precision and an appropriate response ratio. The Adequate Precision and the coefficient of variation (CV%; 19.92%) (Table 3), suggested that the model was reliable and reproducible by previous reports comparing the average prediction error at design points with the range of predicted values (Cao et al., 2008; Pathania et al., 2017).

Fig. 2 shows the results of the predicted values of xylanase production vs. the actual values of the response surface method. It demonstrates a good correlation with the actual and predicted values of xylanase production and linear distribution is indicative of a good-fitted model. This figure shows the model is fairly realistic although it shows that the discrepancy is very small between the actual and predicted values (He et al., 2018), and the R^{2}value is 93.74%, which indicated the correct precision between the model and the data. So this model can be utilized to sail the design space. Hence, based on the statistical properties, it may be concluded that the model is adequate to establish the principal impacts of the factors (Banu et al., 2020).

The final model of the second-order polynomial quadratic regression equation was developed for xylanase production and is presented by the final equation in terms of coded factors (Eq.5)

$$R = +379.67 - 197.83X_1 - 293.67X_2 - 9.50X_3 + 58.83X_4 + 59.50X_1X_2 + 31.75X_1X_3 - 18.75X_1X_4$$

$$- 6.00X_2X_3 + 145.00X_2X_4 + 79.75X_3X_4 + 70.79X_1^2 - 79.71X_2^2 + 135.54X_3^2 + 299.79X_4^2 \quad (5)$$

Where R; Xylanase production (U/ml), X$_1$; incubation temperature, X$_2$; moisture agent level X$_3$; pH and, X$_4$; incubation time. The negative sign in front of the design terms indicates an antagonistic effect, and the positive sign indicates a synergistic effect. The development-based model with coded factors is a desirable one since it can help to determine the more significant factors that will have an impact on the response (Montgomery, 2017).

3.2.2. Analysis of the interactions between influencing factors

The interaction of significant parameters and the effects on response were investigated by interaction plots and three-dimensional response surfaces plots (Fig. 3a-b) based on the regression analysis of BBD. The variation in response of xylanase production and the interaction between X$_2$X$_4$ (moisture agent level and incubation time) having significant p-value (0.0251) proved the presence of interactions between moisture agent level and incubation time (Fig.3a), and was found to be more favorable for enhanced xylanase production compared to the results of the other interactions. Fig. 3b depicts the effect of X$_2$X$_4$ on the xylanase activity when the incubation temperature and the initial pH were fixed at level 0. The xylanase activity increase significantly (p < 0.05) by increasing the incubation time and the moisture agent level. The xylanase production mainly depends on the moisture agent level, as its quadratic and linear effects were highly significant (p < 0.0001), confirming the single-factor experiment results (Table 3), and the influence in the metabolic rate of the interaction between these two parameters.

Similarly, Behnam et al., (2019), reported that the interaction term of moisture agent level and incubation time, was significant for xylanase production from *Mucor indicus* and were insignificant for *Mucor hiemalis* and *R. Oryzae* xylanase and cellulose production through SSF on wheat bran. According to studies conducted by de Almeida Antunes Ferraz et al*.,* (2020),and Azzouz et al. (2020a;2020b) an interaction between the moisture agent level and an incubation time has proved to be insignificant on the biomass and xylanase production by strains *Penicillium roqueforti* ATCC 10110, *Trichoderma afroharzianum*, and *A. niger* BG strain using yellow mombin residue and wheat bran under solid-state fermentation respectively. As a result, the moisture agent level and cultivation time affect xylanase production by *A. niger*. The optimum moisture agent level will depend on the microorganism requirement, type of substrates and end products, and short incubation times provide conditions for the economical production of enzymes.

3.2.3. Validation of the model

According to the database generated with software Design Expert 11.6.0, the optimum values variables were determined. Validation of the statistical model and the regression equation was performed from the optimum conditions proposed by the model. The model validation was tried by running the 02 trial run created with the numerical optimization team in the model (Table 4). According to the 02 trial run the actual and predicted xylanase production was not significantly different and therefore it is concluded that the actual model was good predicted the xylanase production. Hence the values of the four variables were found as 35°C(incubation temperature, X1), 96% (moisture agent level, X2), 11 (medium pH, X3), and 96 h(incubation time, X4), yielding a maximum predicted xylanase activity of 1344 U/ml which was found to have been closed at the actual activity of 1413.88± 0.2 U/mL. Many researchers are using RSM modeling strategy for improving the xylanase production of several fungi with wheat straw as the substrate. These fungi include *Aspergillus candidus* (Garai & Kumar, 2013), *A. fumigatus* ABK9 (Das et al., 2013). Therefore, it may be concluded that BBD based RSM can significantly enhance the xylanase production and demonstrated a xylanase activity of 1413.88 ± 0.2 U/mL. The specific activity of the xylanase enzyme on RSM optimized condition was registered as 516.01 U/mg of protein. The effect of the optimization method is shown by the increased enzyme production and also by the decrease in enzyme production cost, which is mostly based on the use of inexpensive and easily acceded complex substrates like wheat straw and bran (Allala et al., 2020).

3.3. Purification and physicochemical properties of *A. niger* BG endoxylanase

Crude proteins with maximum activity (1413.88 U/ml) were collected for xylanase purification under the procedure described in materials and methods. After two anionic-exchange chromatographic steps, a fraction with xylanase activity was purified with a yield of 6.2%. During the process, the specific activity was increased from 516.06 to 728.25U/mg (1.41-fold purification) (Table 5).

The homogeneity of the protein was analyzed by SDS-PAGE, showing a unique protein band with a molecular mass of approximately 20 kDa (Fig. 4a). This was also corroborated by the zymogram of native xylanase on PAGE, showing a unique activity band (Fig.4b). MALDI-TOF analysis of the purified xylanase revealed several peptides that were sequenced and compared with NCBI protein database. Xylanase protein showed 94 % amino acid identity to an endoxylanase from *A. niger* (BAO02695.1) with a theoretical molecular mass of 22.671kDa.The higher molecular mass of protein obtained by SDS-PAGE may be caused by glycosylation of the fungal protein, a conserved posttranslational modification that is found in all eukaryotes, very important for protein stability and secretion (Deshpandeet al., 2008). This experimental molecular mass is close to those reported in other *A. niger* xylanases, as those from *A. niger* strain DSM 195, *A. niger* strain BCC14405, IBT-90, CGMCC1067, and *A. niger* strain IBT-90, which are also glycoprotein with higher experimental molecular mass (35.5; 21 and 20 kDa) than those corresponding to their sequences (Deng et al., 2006; Do et al., 2013; Korona et al., 2006).

The optimum temperature for purified xylanase was 50 °C, as shown in Fig 5a.It is the same optimum temperature reported for endoxylanase from *A. fumigatus* R1 (Deshmukh et al., 2016) and *Aspergillus nidulans* (Maitan-Alfenas et al*.,* 2016). However, it was higher than those reported from *A. oryzae* LC1 and *A. niger* XZ-3S, which had an optimal temperature around 30 and 40 °C, respectively(Bhardwaj et al., 2020; Fu et al., 2012). In addition, the endoxylanase for the new *A. niger* BG strain, was very stable at 30 and 40 ºC, since it maintained more than 90% of its activity after 24h of incubation, and more than 60% of activity remained at 50 and 60°C after 30 min of incubation (Fig. 5a).In this sense, endoxylanases stable at 40 °C are necessaries for xylooligosaccharides production, as well as in baking industry (Numan & Bhosle, 2006). Furthermore, the stability of the endoxylanase from *A. niger* BG strain at 30, 40, and 50 °C, as well as its stability at acidic conditions, suggest that this enzyme presents suitable properties to be utilized for lignocellulosic biomass treatments (Brienzo et al., 2015). At higher temperatures, such as 50 and 60 °C, 60% enzyme activity was conserved in the first 30 min, but after 1 h the xylanase activity was partially lost, probably due to protein denaturation. Previous studies reported xylanase thermostability from *Aspergillus* strains, indicates that they are generally stable at temperatures 35-45 °C (Fu et al., 2012), and 40–60 °C (Deshmukh et al., 2016).

The effect of pH value (2–9) on the activity and stability of purified endoxylanase is shown in Fig. 5b. The results indicated that the highest xylanase activity is observed at pH 6, being very active between 3-7. It showed a broad range of pH stability, from 3 to 9 after 24 h incubation at 25°C, suggesting its ability to acts at both acidic and basic pH(Fig. 5b). Some studies indicate that xylanase produced by *Aspergillus* have optimum pH at the acid region, usually at pH 4.8, 5.0, 5.5, and 6.0, as i.e. *Aspergillus terreus, A. oryzae* LC1, *A. fumigates* FC2-2 or *A. nidulans,* respectively (Narra et al*.,* 2014; Yang et al., 2015; Maitan-Alfenas et al*.,* 2016; Bhardwaj et al*.,* 2020).The optimum pH in the acid range permits the application of xylanase in the wine industry, mostly in reactors where maceration and fermentation occur concurrently, but pH stability is more important for industrial applications. In the current study, the purified endoxylanase showed stability in a pH range 3-9, similar to that

reported in the main xylanase of *A. oryzae* LC1, expressed in prokaryotic system *E. coli* (Bhardwaj et al., 2020), and higher than those described for other *A. niger* strain (pH 3.0-7.0) (Karaoglan et al., 2014).

3.4. Substrate specificity and kinetic parameters of *A. niger* BG endoxylanase

The action of purified endoxylanase towards various substrates is shown in Figure 5c. The results show that the enzyme was specific to xylan-containing substrates, being the highest activity obtained on wheat arabinoxylan (165.70%), and taking as 100% the activity on beechwood xylan, the standard substrate used in this work. Both xylans have high xylose content, but outstanding differences can be noticed. Beechwood xylan has few ramifications, containing approximately 94% xylose whereas wheat arabinoxylan is highly branched with arabinose residues (Nieto-Domínguez et al., 2019). In addition, this enzyme acts, although with minor efficiency, on Avicel (43.9%), which is microcrystalline cellulose. The results suggest that the new *A. niger* endoxylanase is a versatil enzyme able to acts efficiency on linear and ramified xylan but also on polysaccharides with β-1,4-glucose bonds. However, *A. niger* endoxylanase is not active on starch, a homogenous polysaccharide consisting of numerous glucose units joined by α-1,4-glycosidic bonds, which indicates the enzyme selectively acts on □-linkage.

Kinetic parameters of purified xylanase were determined according to the Michaelis–Menten plots using various concentrations of wheat arabinoxylan substrate. K_m and V_{max} values of the enzyme were found to be 26.06mg/mL and 5647U/mg x min, respectively. The small K_m value shows that the purified xylanase has a high affinity for the substrate, which is a significant characteristic for industrial processes. The V_{max} value (5647 U/mg) in the present study is relatively high compared to the V_{max} of xylanases reported for other fungal strains (Table 6).

3.5. Xylooligosaccharides production

Wheat straw xylan was hydrolysed by purified β-1,4-endoxylanase, and the xylooligosaccharides products were determined by TLC. The relative migration of hydrolyzed product indicated that β-1-4-endoxylanase released xylopentaose, xylotetraose and xylotriose after 5 min (Fig 6), whereas xylobiose appears after 4 h of incubation time. According to the results, the reducing sugars are as a function of hydrolysis time, when increasing the incubation time, the concentrations of xylooligosaccharidesare enhanced. Previous studies have indicated that endoxylanase is a key step in the conversion of xylans into shorter oligosaccharides (xylopentaose, xylotetraose, xylotriose, and xylobiose) (Long et al., 2020; Verma et al., 2020). Furthermore, enzymatic production of xylooligosaccharides is favored over chemical methods (Tan et al., 2008). The ability to produce oligosaccharides is a reasonable and reliable alternative for large amounts production on an industrial scale such as those needed in aquaculture and poultry industry (Aachary & Prapulla, 2011), because they have been qualified as anti-diabetic, anti-hypercholesterolemic, or anti-apoptotic molecules(Kim et al., 2006).The application of enzymatic produced xylooligosaccharides present a promoting green alternative, and have been studied as a promoting prebiotic constituent(Nieto-Domínguez et al., 2017), because they are not degraded by the human digestive enzymes, and fermented into the gastrointestinal tract (Amorim, Silvério, Prather, & Rodrigues, 2019). Accordingly, the β-1,4-endoxylanase has a high yield of xylooligosaccharides production and is a potentially suitable candidate for the enzymatic treatment of biomass.

4. CONCLUSION

The production of xylanase by *A. niger* BG strain using wheat straw was statistically optimized using classical optimization OFAT approach and RSM. The purified enzyme showed high stability at a large range of pH (3-9) and temperature (30-60°C), also showed maximum specificity towards wheat arabinoxylan. It was successfully used to produce xylooligosacharides from wheat straw xylan. These results suggest that it could be of interest for biotechnological processes.

AUTHORS' CONTRIBUTIONS

Zahra Azzouz: Conceptualization, Methodology, Validation, Writing - original draft. Azzeddine Bettache and Nawel Boucherba: Methodology, Writing - review & editing, Investigation. Laura I. de Eugenio: Methodology, Validation, Writing - review & editing, Formal analysis, Investigation. Maria Jesus Martinez: Resources, Writing - review & editing, Supervision. Said Benallaoua: Writing-review & editing, Supervision, Project administration.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCE

Aachary, A. A., & Prapulla, S. G. (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. *Comprehensive Reviews in Food Science and Food Safety*, *10*(1), 2–16. https://doi.org/10.1111/j.1541-4337.2010.00135.x

Abdel-Sater, M. A., & El-Said, A. H. M. (2001). Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. *International Biodeterioration and Biodegradation*, *47*(1), 15–21. https://doi.org/10.1016/S0964-8305(00)00113-X

Allala, F., Bouacem, K., Boucherba, N., Mechri, S., Kriaa, M., Arkoub-Djoudi, W., … Bouanane-Darenfed, A. (2020). α-Amylase production by *Tepidimonas fonticaldi* strain HB23: statistical optimization and compatibility study for use in detergent formulations. *Environmental Science and Pollution Research*, 1–9. https://doi.org/10.1007/s11356-020-10142-2

Amorim, C., Silvério, S. C., Prather, K. L. J., & Rodrigues, L. R. (2019). From lignocellulosic residues to market: Production and commercial potential of xylooligosaccharides. *Biotechnology Advances*, *37*(7), 107397. https://doi.org/10.1016/j.biotechadv.2019.05.003

Ang, S. K., Yahya, A., Aziz, S. A., & Salleh, M. (2015). Isolation , Screening , and Identification of Potential Cellulolytic and Xylanolytic Producers for Biodegradation of Untreated Oil Palm Trunk and Its Application in Saccharification of Lemongrass Leaves. *Preparative Biochemistry and Biotechnology*, *45*(3), 279–305. https://doi.org/10.1080/10826068.2014.923443

Azzouz, Z., Bettache, A., Boucherba, N., Amghar, Z., & Benallaoua, S. (2020). Optimization of xylanase production by newly isolated strain *Trichoderma afroharzianum* isolate az 12 in solid state fermentation using response surface methodology. *CELLULOSE CHEMISTRY AND TECHNOLOGY*, *54*(5–6), 451–462. https://doi.org/doi.org/10.35812/CelluloseChemTechnol.2020.54.46

Azzouz, Z., Bettache, A., Djinni, I., Boucherba, N., & Benallaoua, S. (2020). Biotechnological production and statistical optimization of fungal xylanase by b ioconversion of the lignocellulosic biomass residues in solid-state fermentation. *Biomass Conversion and Biorefinery*, 1–13. https://doi.org/10.1007/s13399-020-01018-z

Bailey, M. J., Biely, P., & Kaisa, P. (1992). Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology*, *23*, 257–270. https://doi.org/doi:10.1016/0168-1656(92)90074-j

Bakri, Y., Al-jazairi, M., & Al-kayat, G. (2008). Xylanase Production by a Newly Isolated *Aspergillus niger* SS7 in Submerged Culture. *Polish Journal of Microbiology*, *57*(3), 249–251.

Banu, A., Ali, M. Y., Rahman, M. A., & Konneh, M. (2020). Stability of micro dry wire EDM: OFAT and DOE method. *International Journal of Advanced Manufacturing Technology*, *106*(9–10), 4247–4261. https://doi.org/10.1007/s00170-020-04923-9

Behnam, S., Karimi, K., & Khanahmadi, M. (2019). Cellulase Production Under Solid-State Fermentation by Ethanolic *Zygomycetes* Fungi: Application of Response Surface Methodology. *Nutrition and Food Sciences Research*, *6*(1), 27–34. https://doi.org/10.29252/nfsr.6.1.27

Behnam, S., Karimi, K., Khanahmadi, M., Agriculture, I., Resources, N., & Salimian, Z. (2016). Optimization of xylanase production by *Mucor indicus , Mucor hiemalis, and Rhizopus oryzae* through solid state fermentation. *Biological Journal of Microorganism*, *4*(16), 1–10.

Bhardwaj, N., Verma, V. K., Chaturvedi, V., & Verma, P. (2020). Cloning, expression and characterization of a thermo-alkali-stable xylanase from *Aspergillus oryzae* LC1 in *Escherichia coli* BL21(DE3). *Protein Expression and Purification*, *168*(December 2019), 105551. https://doi.org/10.1016/j.pep.2019.105551

Bharti, A. K., Kumar, A., Kumar, A., & Dutt, D. (2018). Exploitation of Parthenium hysterophorous biomass as low-cost substrate for cellulase and xylanase production under solid-state fermentation using *Talaromyces stipitatus* MTCC 12687. *Journal of Radiation Research and Applied Sciences*, (2017), 1–10. https://doi.org/10.1016/j.jrras.2018.01.003

Bibi, Z., Ansari, A., Zohra, R. R., Aman, A., & Ul Qader, S. A. (2014). Production of xylan degrading endo-1, 4-β-xylanase from thermophilic *Geobacillus stearothermophilus* KIBGE-IB29 . *Journal of Radiation Research and Applied Sciences*, *7*(4), 478–485. https://doi.org/10.1016/j.jrras.2014.08.001

Biely, P. (1985). Microbial xylanolytic systems. *Trends in Biotechnology*, *3*(11), 286–290. https://doi.org/10.1016/0167-7799(85)90004-6

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, *254*, 248–254.

Brienzo, M., Tyhoda, L., Benjamin, Y., & Görgens, J. (2015). Relationship between physicochemical properties and enzymatic hydrolysis of sugarcane bagasse varieties for bioethanol production. *New Biotechnology*, *32*(2), 253–262. https://doi.org/10.1016/j.nbt.2014.12.007

Cao, Y., Meng, D.-J., Lu, J., & Long, J. (2008). Statistical optimization of xylanase production by *Aspergillus niger* AN-13 under submerged fermentation using response surface methodology. *African Journal of Biotechnology*, *7*(5), 631–638. Retrieved from http://www.academicjournals.org/AJB

Carmona, E. C., Fialho, M. B., Buchgnani, É. B., Coelho, G. D., Brocheto-braga, M. R., & At, J. (2005). Production , purification and characterization of a minor form of xylanase from *Aspergillus versicolor*. *Process Biochemistry*, *40*, 359–364. https://doi.org/10.1016/j.procbio.2004.01.010

Chen, H. (2013). *Modern Solid State Fermentation: Theory and Practice* (Springer D). New York London: Springer Dordrecht Heidelberg. https://doi.org/10.1007/978-94-007-6043-1

Christakopoulos, P., Nerinckx, W., Kekos, D., Macris, B., & Claeyssens, M. (1996). Purification and characterization of two low molecular

mass alkaline xylanases from *Fusarium oxysporum* F3. *Journal of Biotechnology*, *51*(2), 181–189. https://doi.org/10.1016/0168-1656(96)01619-7

Das, A., Paul, T., Halder, S. K., Jana, A., Maity, C., Das Mohapatra, P. K., … Mondal, K. C. (2013). Production of cellulolytic enzymes by Aspergillus fumigatus ABK9 in wheat bran-rice straw mixed substrate and use of cocktail enzymes for deinking of waste office paper pulp. *Bioresource Technology*, *128*, 290–296. https://doi.org/10.1016/j.biortech.2012.10.080

de Almeida Antunes Ferraz, J. L., Oliveira Souza, L., Gustavo de Araújo Fernandes, A., Luiz Ferreira Oliveira, M., de Oliveira, J. R., & Franco, M. (2020). Optimization of the solid-state fermentation conditions and characterization of xylanase produced by *Penicillium roqueforti* ATCC 10110 using yellow mombin residue (Spondias mombin L.). *Chemical Engineering Communications*, *207*(1), 31–42. https://doi.org/10.1080/00986445.2019.1572000

De Souza, C. G. M., Girardo, N. S., Ferreira Costa, M. A., & Peralta, R. M. (1999). Influence of growth conditions on the production of xylanolytic enzymes by *Aspergillus flavus*. *Journal of Basic Microbiology*, *39*(3), 155–160. https://doi.org/10.1002/(SICI)1521-4028(199906)39:3<155::AID-JOBM155>3.0.CO;2-I

De Souza Moreira, L. R., De Carvalho Campos, M., De Siqueira, P. H. V. M., Silva, L. P., Ricart, C. A. O., Martins, P. A., … Filho, E. X. F. (2013). Two β-xylanases from *Aspergillus terreus*: Characterization and influence of phenolic compounds on xylanase activity. *Fungal Genetics and Biology*, *60*, 46–52. https://doi.org/10.1016/j.fgb.2013.07.006

Deng, P., Li, D., Cao, Y., Lu, W., & Wang, C. (2006). Cloning of a gene encoding an acidophilic endo-β-1,4-xylanase obtained from *Aspergillus niger* CGMCC1067 and constitutive expression in *Pichia pastoris*. *Enzyme and Microbial Technology*, *39*(5), 1096–1102. https://doi.org/10.1016/j.enzmictec.2006.02.014

Deshmukh, R. A., Jagtap, S., Mandal, M. K., & Mandal, S. K. (2016). Purification, biochemical characterization and structural modelling of alkali-stable β-1,4-xylan xylanohydrolase from *Aspergillus fumigatus* R1 isolated from soil. *BMC Biotechnology*, *16*(1), 1–12. https://doi.org/10.1186/s12896-016-0242-4

Deshpande, N., Wilkins, M. R., Packer, N., & Nevalainen, H. (2008). Protein glycosylation pathways in filamentous fungi. *Glycobiology*, *18*(8), 626–637. https://doi.org/10.1093/glycob/cwn044

Do, T. T., Quyen, D. T., Nguyen, T. N., & Nguyen, V. T. (2013). Molecular characterization of a glycosyl hydrolase family 10 xylanase from *Aspergillus niger*. *Protein Expression and Purification*, *92*(2), 196–202. https://doi.org/10.1016/j.pep.2013.09.011

Driss, D., Bhiri, F., Elleuch, L., Bouly, N., Stals, I., Miled, N., … Chaabouni, S. E. (2011). Purification and properties of an extracellular acidophilic endo-1,4-β-xylanase, naturally deleted in the "thumb", from *Penicillium occitanis* Pol6. *Process Biochemistry*, *46*(6), 1299–1306. https://doi.org/10.1016/j.procbio.2011.02.022

Ebringerová, A., Hromádková, Z., & Heinze, T. (2005). Hemicellulose. In *Polysaccharides I* (Vol. 186, pp. 1–67). Berlin/Heidelberg: Springer-Verlag. https://doi.org/10.1007/b136816

Ezeilo, U. R., Wahab, R. A., & Mahat, N. A. (2019). Optimization studies on cellulase and xylanase production by *Rhizopus oryzae* UC2 using raw oil palm frond leaves as substrate under solid state fermentation. *Renewable Energy*, 1–12. https://doi.org/10.1016/j.renene.2019.11.149

Fadel, M., Keera, A. A., Abdel-Aziz, S. M., & Kahil, T. (2014). Clean production of xylanase from white corn flour by *Aspergillus fumigates* F-993 under solid state fermentation. *World Applied Sciences Journal*, *29*(3), 326–336. https://doi.org/10.5829/idosi.wasj.2014.29.03.13848

Faraco, V. (2013). *Lignocellulose Conversion: Enzymatic and Microbial Tools for Bioethanol Production* (Springer S). London. https://doi.org/DOI 10.1007/978-3-642-37861-4

Fu, G., Wang, Y., Wang, D., & Zhou, C. (2012). Cloning, Expression, and Characterization of an GHF 11 Xylanase from *Aspergillus niger* XZ-3S. *Indian Journal of Microbiology*, *52*(4), 682–688. https://doi.org/10.1007/s12088-012-0314-7

Furniss, C. S., Williamson, G., & Kroon, P. A. (2005). The substrate specificity and susceptibility to wheat inhibitor proteins of *Penicillium funiculosum* xylanases from a commercial enzyme preparation. *Journal of the Science of Food and Agriculture*, *85*(4), 574–582. https://doi.org/10.1002/jsfa.1984

Garai, D., & Kumar, V. (2013). A Box – Behnken design approach for the production of xylanase by *Aspergillus candidus* under solid state fermentation and its application in saccharification of agro residues and Parthenium hysterophorus L . *Industrial Crops & Products*, *44*, 352–363. https://doi.org/10.1016/j.indcrop.2012.10.027

Gautam, A., Kumar, A., & Dutt, D. (2015). Production of Cellulase-Free Xylanase by *Aspergillus flavus* ARC- 12 Using Pearl Millet Stover as the Substrate Under Solid-State Fermentation. *Journal of Advanced Enzymes Research*, *1*, 1–9.

Goyal, M., Kalra, K. L., Sareen, V. K., & Soni, G. (2008). Xylanase production with xylan rich lignocellulosic wastes by a local soil isolate of *Trichoderma viride*. *Brazilian Journal of Microbiology*, *36*, 535–541. https://doi.org/10.1590/S1517-838220080003000025

Handa, C. L., Couto, U. R., Vicensoti, A. H., Georgetti, S. R., & Ida, E. I. (2014). Optimisation of soy flour fermentation parameters to produce b -glucosidase for bioconversion into aglycones. *Food Chemistry*, *152*, 56–65. https://doi.org/10.1016/j.foodchem.2013.11.101

He, X., Song, C., Li, Y., Wang, N., Xu, L., Han, X., & Wei, D. (2018). Ecotoxicology and Environmental Safety E ffi cient degradation of Azo dyes by a newly isolated fungus *Trichoderma tomentosum* under non-sterile conditions. *Ecotoxicology and Environmental Safety*, *150*, 232–239. https://doi.org/10.1016/j.ecoenv.2017.12.043

Hu, K., Wu, K., Pan, R., Liu, B., Mycosyst, J. C.-C. J., & 2007, U. (2007). Solid-state mixed fermentation increases activities of xylanase and cellulase. *Chin J Mycosyst*, *26*, 273–8.

Hussein, A. K. (2015). Applications of nanotechnology in renewable energies - A comprehensive overview and understanding. *Renewable and Sustainable Energy Reviews*, *42*, 460–476. https://doi.org/10.1016/j.rser.2014.10.027

Irfan, M., Nadeem, M., & Syed, Q. (2014a). One-factor-at-a-time (OFAT) optimization of xylanase production from *Trichoderma viride* -IR05 in solid-state fermentation. *Journal of Radiation Research and Applied Sciences*, *7*(3), 317–326. https://doi.org/10.1016/j.jrras.2014.04.004

Kalogeris, E., Christakopoulos, P., Kekos, D., & Macris, B. J. (1998). Studies on the solid-state production of thermostable endoxylanases from Thermoascus aurantiacus : Characterization of two isozymes. *Journal of Biotechnology*, *60*, 155–163.

Karaoglan, M., Yildiz, H., & Inan, M. (2014). Screening of signal sequences for extracellular production of *Aspergillus niger* xylanase in *Pichia pastoris*. *Biochemical Engineering Journal*, *92*, 16–21. https://doi.org/10.1016/j.bej.2014.07.005

Kim, K. H., Kim, Y. W., Kim, H. B., Lee, B. J., & Lee, D. S. (2006). Anti-apoptotic activity of laminarin polysaccharides and their enzymatically hydrolyzed oligosaccharides from *Laminaria japonica*. *Biotechnology Letters*, *28*(6), 439–446. https://doi.org/10.1007/s10529-005-6177-9

Kimura, T., Ito, J., Kawano, A., Makino, T., Kondo, H., Karita, S., … Ohmiya, K. (2000). Purification, characterization, and molecular cloning of acidophilic xylanase from *Penicillium* sp.40. *Bioscience, Biotechnology and Biochemistry*, *64*(6), 1230–1237. https://doi.org/10.1271/bbb.64.1230

Klemm, D., Philipp, B., Heinze, T., Heinze, U., & Wagenknecht, W. (1998). General considerations on structure and reactivity of cellulose. *Comprehensive Cellulose Chemistry*, *1*, 9–165.

Korona, B., Korona, D., & Bielecki, S. (2006). Efficient expression and secretion of two co-produced xylanases from *Aspergillus niger* in

Pichia pastoris directed by their native signal peptides and the Saccharomyces cerevisiae α-mating factor. *Enzyme and Microbial Technology*, *39*(4), 683–689. https://doi.org/10.1016/j.enzmictec.2005.12.003

Krisana, A., Rutchadaporn, S., Jarupan, G., Lily, E., Sutipa, T., & Kanyawim, K. (2005). Endo-1,4-β-xylanase B from *Aspergillus cf. niger* BCC14405 Isolated in Thailand: Purification, Characterization and Gene Isolation. *Journal of Biochemistry and Molecular Biology*, *38*(1), 17–23. https://doi.org/10.5483/BMBRep.2005.38.1.017

Laemmli, U. . (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature Publishing Group*, *227*, 726–734. Retrieved from http://www.mendeley.com/research/discreteness-conductance-chnge-n-bimolecular-lipid-membrane-presence-certin-antibiotics/

Li, L., Tian, H., Cheng, Y., Jiang, Z., & Yang, S. (2006). Purification and characterization of a thermostable cellulase-free xylanase from the newly isolated *Paecilomyces themophila*. *Enzyme and Microbial Technology*, *38*(6), 780–787. https://doi.org/10.1016/j.enzmictec.2005.08.007

Li, X. L., Zhang, Z. Q., Dean, J. F., Eriksson, K. E., & Ljungdahl, L. G. (1993). Purification and characterization of a new xylanase (APX-II) from the fungus *Aureobasidium pullulans* Y-2311-1. *Applied and Environmental Microbiology*, *59*(10), 3212–3218.

Long, L., Sun, L., Lin, Q., Ding, S., & St John, F. J. (2020). Characterization and functional analysis of two novel thermotolerant α-l-arabinofuranosidases belonging to glycoside hydrolase family 51 from *Thielavia terrestris* and family 62 from *Eupenicillium parvum*. *Applied Microbiology and Biotechnology*, *104*(20), 8719–8733. https://doi.org/10.1007/s00253-020-10867-7

Lonsane, B. K., Saucedo-castaneda, G., Ghildyal, N. P., Ramakrishna, M., & Krishnaiah, M. M. (1992). Scale-up strategies for solid state fermentation systems. *Process Biochemistry*, *27*, 259–273. https://doi.org/10.1016/0032-9592(92)85011-P

Loureiro, D. B., Romanini, D., & Tubio, G. (2016). Structural and functional analysis of *Aspergillus niger* xylanase to be employed in polyethylenglycol/salt aqueous two-phase extraction. *Biocatalysis and Agricultural Biotechnology*, *5*, 204–210. https://doi.org/10.1016/j.bcab.2015.12.008

Maitan-Alfenas, G. P., Oliveira, M. B., Nagem, R. A. P., de Vries, R. P., & Guimarães, V. M. (2016). Characterization and biotechnological application of recombinant xylanases from *Aspergillus nidulans*. *International Journal of Biological Macromolecules*, *91*, 60–67. https://doi.org/10.1016/j.ijbiomac.2016.05.065

Mandal, M., & Ghosh, U. (2017). Optimization of SSF parameters by OFAT for biosynthesis of cellulase using isolated *Aspergillus niger*. *Indian Journal of Chemical Technology*, *24*, 623–629.

Mandels, M., & Weber, J. (1969). The Production of Cellulases. *Food Microbiology,23*, 391-414.

Miller, G.-L. (1959). Use of Dinitrosalicyiic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/https://doi.org/10.1021/ac60147a030

Montgomery, D. C. (2017). *Design and Analysis of Experiments. Hoboken, NJ : John Wiley & Sons, Inc* (9 edition). Retrieved from https://lccn.loc.gov/2017002355

Morganti, P. (2013). Saving the environment by nanotechnology and waste raw materials: Use of chitin nanofibrils by EU research projects. *Journal of Applied Cosmetology*, *31*(3–4), 89–96.

Mussatto, S. I., & Teixeira, J. A. (2010). Lignocellulose as raw material in fermentation processes, 897–907.

Nanjundaswamy, A., & Okeke, B. C. (2020). Comprehensive Optimization of Culture Conditions for Production of Biomass-Hydrolyzing Enzymes of *Trichoderma* SG2 in Submerged and Solid-State Fermentation. *Applied Biochemistry and Biotechnology*,1-19, https://doi.org/10.1007/s12010-020-03258-1

Narra, M., Dixit, G., Divecha, J., Kumar, K., Madamwar, D., & Shah, A. R. (2014). Production , purification and characterization of a novel GH 12 family endoglucanase from *Aspergillus terreus* and its application in enzymatic degradation of deligni fi ed rice straw. *International Biodeterioration & Biodegradation*, *88*, 150–161. https://doi.org/10.1016/j.ibiod.2013.12.016

Nieto-Domínguez, M., de Eugenio, L. I., York-Durán, M. J., Rodríguez-Colinas, B., Plou, F. J., Chenoll, E., … Jesús Martínez, M. (2017). Prebiotic effect of xylooligosaccharides produced from birchwood xylan by a novel fungal GH11 xylanase. *Food Chemistry*, *232*, 105–113. https://doi.org/10.1016/j.foodchem.2017.03.149

Nieto-Domínguez, M., Martínez-Fernández, J. A., De Toro, B. F., Méndez-Líter, J. A., Cañada, F. J., Prieto, A., … Martínez, M. J. (2019). Exploiting xylan as sugar donor for the synthesis of an antiproliferative xyloside using an enzyme cascade. *Microbial Cell Factories*, *18*(1), 1–12. https://doi.org/10.1186/s12934-019-1223-9

Nochur, S. V., Roberts, M. F., & Demain, A. L. (1993). True cellulase production by *Clostridium thermocellum* grown on different carbon sources. *Biotechnology Letters*, *15*(6), 641–646. https://doi.org/10.1007/BF00138556

Numan, M. T., & Bhosle, N. B. (2006, April 30). α-L-arabinofuranosidases: The potential applications in biotechnology. *Journal of Industrial Microbiology and Biotechnology*, 33,247–260. https://doi.org/10.1007/s10295-005-0072-1

Okafor, U., Okochi, V., Onyegeme-okerenta, B., & Nwodo-Chinedu, S. (2007). Xylanase production by *Aspergillus niger* ANL 301 using agro - wastes. *African Journal of Biotechnology*, *6*(14), 1710–1714. https://doi.org/10.1001/2014.01/02

Paice, M. G., Jurasek, L., Carpenter, M. R., & Smillie, L. B. (1978). Production, characterization, and partial amino acid sequence of xylanase A from *Schizophyllum commune*. *Applied and Environmental Microbiology*, *36*(6), 602–608.

Pal, A., & Khanum, F. (2010). Bioresource Technology Production and extraction optimization of xylanase from *Aspergillus niger* DFR-5 through solid-state-fermentation. *Bioresource Technology*, *101*(19), 7563–7569. https://doi.org/10.1016/j.biortech.2010.04.033

Pandey, A. K., Edgard, G., & Negi, S. (2016). Optimization of concomitant production of cellulase and xylanase from *Rhizopus oryzae* SN5 through EVOP-factorial design technique and application in Sorghum Stover based bioethanol production. *Renewable Energy*, *98*, 51–56. https://doi.org/10.1016/j.renene.2016.05.071

Pathania, S., Sharma, N., & Handa, S. (2017). Optimization of culture conditions using response surface methodology for synergism production of cellulase , xylanase and pectinase by *Rhizopus delemar* F2 under solid state fermentation. *Journale of Pharmacognosy and Phytochemistry*, *6*(6), 1872–1878.

Polizeli, M. M. L. T. M. (2005). Xylanases from fungi : properties and industrial applications, 577–591. https://doi.org/10.1007/s00253-005-1904-7

Sarkar, N., Ghosh, S. K., Bannerjee, S., & Aikat, K. (2012, January). Bioethanol production from agricultural wastes: An overview. *Renewable Energy*. https://doi.org/10.1016/j.renene.2011.06.045

Scheller, H. V., & Ulvskov, P. (2010). Hemicelluloses. *Annual Review of Plant Biology*, *61*(1), 263–289. https://doi.org/10.1146/annurev-arplant-042809-112315

Senthilkumar, S. R., Ashokkumar, B., Chandra Raj, K., & Gunasekaran, P. (2005). Optimization of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design. *Bioresource Technology*, *96*(12), 1380–1386. https://doi.org/10.1016/j.biortech.2004.11.005

Shah, A. R., & Madamwar, D. (2005). Xylanase production under solid-state fermentation and its characterization by an isolated strain of *Aspergillus foetidus* in India. *World Journal of Microbiology and Biotechnology*, *21*(3), 233–243. https://doi.org/10.1007/s11274-004-3622-1

Shojaei, S., & Shojaei, S. (2017). Experimental design and modeling of removal of Acid Green 25 dye by nanoscale zero-valent iron. *Euro-Mediterranean Journal for Environmental Integration*, *2*(1), 15. https://doi.org/10.1007/s41207-017-0026-9

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Srivastava, A., Singh, V., Haque, S., Pandey, S., Mishra, M., Jawed, A., … Tripathi, C. K. M. (2018). Response Surface Methodology-Genetic Algorithm Based Medium Optimization, Purification, and Characterization of Cholesterol Oxidase from *Streptomyces rimosus*. *Scientific Reports*, *8*(1), 1–13. https://doi.org/10.1038/s41598-018-29241-9

Takahashi, Y., Kawabata, H., & Murakami, S. (2013). Analysis of functional xylanases in xylan degradation by *Aspergillus niger* E-1 and characterization of the GH family 10 xylanase XynVII. *SpringerPlus*, *2*(1), 1–11. https://doi.org/https://doi.org/10.1186/2193-1801-2-447

Tan, S. S., Li, D. Y., Jiang, Z. Q., Zhu, Y. P., Shi, B., & Li, L. T. (2008). Production of xylobiose from the autohydrolysis explosion liquor of corncob using *Thermotoga maritima* xylanase B (XynB) immobilized on nickel-chelated Eupergit C. *Bioresource Technology*, *99*(1), 200–204. https://doi.org/10.1016/j.biortech.2006.12.005

Verma, R., Bhalla, A., & Kumar, S. (2020). Valorization of Lignocellulosic Residues for Cost-Effective Production of Thermo-Alkali-Stable Xylanase by *Geobacillus thermodenitrificans* X1 ofHot Spring. *Waste and Biomass Valorization*, *11*(3), 1205–1215. https://doi.org/10.1007/s12649-018-0402-y

Wahid, Z., & Nadir, N. (2013). Improvement of One Factor at a Time Through Design of Experiments 1, *21*, 56–61. https://doi.org/10.5829/idosi.wasj.2013.21.mae.99919

Wan Omar, W. N. N., Nordin, N., Mohamed, M., & Amin, N. A. S. (2009). A two-step biodiesel production from waste cooking oil: Optimization of pre-treatment step. *Journal of Applied Sciences*, *9*(17), 3098–3103. https://doi.org/10.3923/jas.2009.3098.3103

Xie, Y., Hu, P., Zhu, N., Lei, F., Xing, L., & Xu, L. (2020). Collaborative optimization of ground source heat pump-radiant ceiling air conditioning system based on response surface method and NSGA-II. *Renewable Energy*, *147*, 249–264. https://doi.org/10.1016/j.renene.2019.08.109

Yan, Q. J., Wang, L., Jiang, Z. Q., Yang, S. Q., Zhu, H. F., & Li, L. T. (2008). A xylose-tolerant β-xylosidase from *Paecilomyces thermophila*: Characterization and its co-action with the endogenous xylanase. *Bioresource Technology*, *99*(13), 5402–5410. https://doi.org/10.1016/j.biortech.2007.11.033

Yang, Q., Gao, Y., Huang, Y., Xu, Q., Luo, X. M., Liu, J. L., & Feng, J. X. (2015). Identification of three important amino acid residues of xylanase AfxynA from *Aspergillus fumigatus* for enzyme activity and formation of xylobiose as the major product. *Process Biochemistry*, *50*(4), 571–581. https://doi.org/10.1016/j.procbio.2015.01.021

Zehra, M., Syed, M. N., & Sohail, M. (2020). Banana peels: A promising substrate for the coproduction of pectinase and xylanase from *Aspergillus fumigatus* MS16. *Polish Journal of Microbiology*, *69*(1), 19–26. https://doi.org/10.33073/pjm-2020-002

Zhou, P., Zhu, H., Yan, Q., Katrolia, P., & Jiang, Z. (2011). Purification and properties of a psychrotrophic *Trichoderma* sp. xylanase and its gene sequence. *Applied Biochemistry and Biotechnology*, *164*(6), 944–956. https://doi.org/10.1007/s12010-011-9186-2

Table 1 Independent variables and levels of variation in Box-Behnken design (BBD).

Study Type	Response Surface					
Design Type	Box-Behnken		**Runs**		27	
Design Mode	Quadratic		**No Blocks**			
Factor	**Name**	**Units**	**Type**	**Level (-1)**	**Level (+1)**	**Level (0)**
X_1	Temperature	°C	Numeric	32	40	36
X_2	Moisture	%	Numeric	90	100	95
X_3	pH		Numeric	8	11	9.5
X_4	Time	hour	Numeric	48	96	72
Response	**Name**	**Units**				
R	Xylanase	U/ml	**Analysis**	Polynomial		

Table 2 BBD for 27 runs of experiments

Run	Factor 1 X_1:Temperature °C	Factor 2 X_2: Moisture %	Factor 3 X_3:pH	Factor 4 X_4: Time hour	Response Xylanase Activity U/mL
1	40	95	11	72	334 ± 0.18
2	32	95	8	72	874 ± 0.03
3	40	90	9.5	72	608 ± 0.03
4	36	95	8	48	860 ± 0.01
5	40	95	9.5	48	465 ± 0.01
6	36	95	9.5	72	304 ± 0.1
7	36	95	11	96	1041 ± 0.05
8	40	95	8	72	411 ± 0.21
9	36	90	9.5	96	759 ± 0.03
10	32	95	9.5	48	923 ± 0.06
11	36	90	9.5	48	894 ± 0.46
12	36	95	9.5	72	424 ± 0.05
13	36	95	9.5	72	411 ± 0.05
14	32	100	9.5	72	126 ± 0.25
15	36	100	9.5	48	123 ± 0.05
16	36	95	8	96	870 ± 0.01
17	32	95	11	72	670 ± 0.28
18	36	95	11	48	712 ± 0.33
19	40	95	9.5	96	456 ± 0.05
20	36	90	11	72	743 ± 0.38
21	36	100	11	72	116 ± 0.01
22	40	100	9.5	72	61 ± 0.30
23	36	100	9.5	96	568 ± 0.01
24	32	90	9.5	72	911 ± 0.36
25	36	100	8	72	56 ± 0.02
26	36	90	8	72	659 ± 0.30
27	32	95	9.5	96	989 ± 0.42

Table 3 ANOVA for quadratic model

Source	Sum of squares	DFa	Mean of squares	F-value	Prob> F	
Model	2,309E+06	14	1,650E+05	12.83	< 0.0001	Significant
Linear						
X_1-Temperature	3,881E+05	1	3,881E+05	30.18	0.0001	
X_2.Moisture	1,035E+06	1	1,035E+06	80.47	< 0.0001	
X_3-pH	1083.00	1	1083.00	0.0842	0.7766	
X_4-Time	41536.33	1	41536.33	3.23	0.0975	
Interaction						
X_1X_2	14161.00	1	14161.00	1.10	0.3147	
X_1X_3	4032.25	1	4032.25	0.3135	0.5858	
X_1X_4	1406.25	1	1406.25	0.1093	0.7466	
X_2X_3	144.00	1	144.00	0.0112	0.9175	
X_2X_4	84100.00	1	84100.00	6.54	0.0251	
X_3X_4	25440.25	1	25440.25	1.98	0.1849	
Quadratic						
X_1^2	26727.79	1	26727.79	2.08	0.1750	
X_2^2	33884.90	1	33884.90	2.63	0.1305	
X_3^2	97981.56	1	97981.56	7.62	0.0173	
X_4^2	4,793E+05	1	4,793E+05	37.27	< 0.0001	
Residual	1,543E+05	12	12860.26			
Lack of Fit	1,457E+05	10	14565.05	3.36	0.2511	Not
Pure Error	8672.67	2	4336.33			significant
Cor Total	2,4604E+06	26				

Model Summary Statistics

Std. Dev.	113.40	R²	0.9374
Mean	569.19	Adjusted R²	0.8643
C.V. %	19.92	Adeq Precision	12.4331

[DFa] Degree of Freedom

Table 4 Validation of quadratic model for xylanase production with *A. niger* BG strain optimized by RSM

Solutions number	Temperature (°C)	Moisture level (%)	agent pH	Incubation time (h)	Xylanase Predicted (U/ml)	Xylanase Actual (U/ml)	Desirability
1	35	96	11	96	1344	1413.88±0.2	1.000
2	33	90	9.0	48	911	824.54 ±0.6	1.000

Table 5 Purification steps of xylanase from *A. niger* BG strain

Purification step	Protein(mg/mL)	Activity (U/ml)	Specific activity (U/ mg)	Purification (fold)	Yield (%)
Supernatant	2.74	1413.88	516.01	1.00	100.00
Ultrafiltration	1.95	1065.74	546.53	1.06	75.38
Hitrap QFF	0.52	506.93	974.88	1.89	36.83
Mono Q	0.12	87.39	728.25	1.41	6.20

Table 6 Comparative production data of native xylanase from different fungal strains

Xylanase	Source species	Activities U/mg	pH Optimal	Optimal Temperature (°C)	Substrate	pH Stability	Thermo-Stability (°C)	Molecular Weight (kDa)	Km mg/ml	Vmax U/mg	References
Native	*A. niger* BG strain	728.25	6.0	50	Wheat straw	3.0-9.0	30-60	20	26.06	5647	This study
xyn11B	*A. niger* BCC14405	317	5	55	Birchwood xylan	5.0-10	50	21	8.9	11.10	(Krisana et al., 2005)
xyn11A	*A. pullulans* Y-2311-1	2.240	4.8	54	Birchwood xylan	3.8-5.4	50	25	7.	2.650	(X. L. Li, Zhang, Dean, Eriksson, & Ljungdahl, 1993)
xyn11A	*F. oxysporum* f. sp. Lycopersici F3	331	6	60	Oat spelt xylan	4.0-10	//	21	0.41	//	(Christakopoulos, Nerinckx, Kekos, Macris, & Claeyssens, 1996)
xyn11A	*P. sp.* thermophila J18	1180	7	75-80	Birchwood xylan	6.0-11	75	25.8	1.6	1424	(L. Li, Tian, Cheng, Jiang, & Yang, 2006)
xyn11C	*P. occitanis* P0I6	358	3	45	Oat-spelt xylan	2.0-10	45	22	14.13	806.3	(Driss et al., 2011)
xyn11A	*P. sp.* 40 strain 40	1250	2	50	Oat-spelt xylan	2.0-5.0	30	25	8.3	6100	(Kimura et al., 2000)
xyn11A	*S. Commune Delar*/ATCC 38548	15	5	45-50	Soluble larch xylan	6.0-8.0	//	33	8.37	0.443	(Paice, Jurasek, Carpenter, & Smillie, 1978)
xyn11B	*T. funiculosus* IMI134756	196	3.7-4.7	//	Birchwood xylan	//	//	27.84	22	506	(Furniss, Williamson, & Kroon, 2005)
xyn11A	*T.* sp. SC9	426	6	42.5	Birchwood xylan	3.5-9.0	35	20.5	2.1	//	(Zhou, Zhu, Yan, Katrolia, &

Jiang, 2011)