Bioprocess development for production of a process-apt xylanase with multifaceted application potential for a range of industrial processes

Sunny Sharma¹ · Vishal Sharma¹ · Parushi Nargotra¹ · Bijender Kumar Bajaj¹

Received: 7 January 2020 / Accepted: 14 March 2020 / Published online: 21 March 2020
© Springer Nature Switzerland AG 2020

Abstract

Discovery of new/novel enzymes with process-apt features has been a continuous practice. A wide range pH-stable and thermostable xylanase was reported hitherto from a newly isolated fungal strain *Aspergillus terreus* S9, from mushroom compost. This is the first ever report where-in a pH-stable and thermostable xylanase is being reported from a strain of *A. terreus*. Solid state fermentation was executed on agro-industrial residues for xylanase production. Maximum xylanase production was supported by wheat bran (40 U/ml), however, substantial xylanase yield was observed on other residues like corn cob powder (38.4 U/ml), cotton cake (38 U/ml), malt (37.6 U/ml) and almond hulls (35.2 U/ml). Almond hulls are being reported for the first time as the substrate for xylanase production. Plackett–Burman design was used to earmark the most effective process variables (wheat bran, medium pH, and incubation time), and the same were optimized by design of experiments (DoE) based on response surface methodology to achieve a yield enhancement of 1.82-fold. *A. terreus* S9 xylanase exhibited an excellent and brisk clarification of mosambi and orange juices. DoE based optimization of process parameters (enzyme dose, treatment time and incubation temperature) resulted in 1.23-fold and 1.28-fold enhanced orange and mosambi juice clarification, respectively. Furthermore, admirable dough raising capacity and pulp brightening ability of *A. terreus* S9 xylanase shows its promising potential for bread making, and eco-friendly biobleaching processes, respectively. The xylanase must be in-depth investigated to decipher the molecular mechanisms of diverse applications.

Keywords Solid state fermentation · *Aspergillus terreus* S9 · Agroindustrial residues · Response surface methodology · Fruit juice clarification · Bakery · Biobleaching

1 Introduction

Xylanases due to their vast application potential in various industries such as food, feed, dairy, bakery, pulp and paper, biofuel, among others, have become the focus of intense research [22, 28, 29, 32]. Considering the hostile industrial process conditions like extremes of pH, elevated temperature, and presence of various enzyme inhibitors, the xylanases intended for process applications must be robust enough to withstand such an environment [8, 29]. Among various sources of xylanolytic enzymes, the microbial

xylanases are preferred as such enzymes may possess process-desired properties, and their bulk production may be executed in an economic and cost-effective manner [36]. Filamentous fungi are considered as the most potent producers of xylanases, and offer several advantages like high xylanase yield and extracellular secretion of enzyme. This last feature eliminates the need for cell disruption. Besides, filamentous fungi present easy cultivation, excellent fermentation properties, ability to efficiently utilize cheap agro-residues as substrates for growth and enzyme production, easy downstream processing, and amenability

✉ Bijender Kumar Bajaj, bajajbijenderk@gmail.com | ¹School of Biotechnology, University of Jammu, Jammu 180006, India.

to genetic manipulation, among others [29]. The microbes may be manipulated by biotechnological interventions for obtaining tailor-made enzymes which are equipped with industrially desired characteristics [38]. However, exploitation of immense microbial diversity for procuring process apt enzymes is still a vital area of research [4, 15].

High production cost is one of the major bottlenecks that limits the wide range industrial application of xylanases. Considering that substrates/medium components constitute the major chunk of enzyme production cost, usage of low-cost agro-industrial residues as substrates to support the growth of process organism may help lowering down the enzyme production cost [28, 29]. Additionally, valorization of agro-industrial residues may offer distinctive economic, environmental, and strategic advantages [28]. Although submerged fermentation (SmF) has widely been used for microbial enzyme production, the solid state fermentation (SSF) has gained substantive interest due to its advantages [20], such as simplicity of media, machinery, control system, downstream processing or process scale-up, lower capital/operational cost, and energy and space requirement, increased enzyme yield, among others [4, 12, 14, 31]. Process optimization has always been instrumental for industrial production. Design of Experiments (DoE) based optimization has got huge impetus due to serious limitations of conventional one variable at a time (OVAT) approach [30, 31]. Plackett–Burman design (PB) and response surface methodology (RSM) are the most frequently used tools for earmarking the most effective process variables, and their optimization, respectively [31].

Application potential of xylanases standalone, or in combination with other enzymes like pectinases and cellulases, has been investigated for liquefaction of fruits and vegetables, clarification of fruit juices, reducing juice viscosity, and for enhancing juice yield [11, 18, 27]. In bread making, the xylanase application may enhance dough rheological characteristics (extensibility, flexibility, stability), bread quality (crumb structure, texture, porosity), and shelf life [8, 37]. Xylanases may help developing environmentally-friendly pulp bleaching process in pulp and paper industry with reduced chlorine-usage and toxic discharges [36]. Furthermore, the xylanase application may improve the fibrillation and drainage properties of the pulp, and the strength of the paper.

A newly isolated *A. terreus* S9 has previously been studied for production of process apt thermostable, and broad range pH stable xylanase under submerged fermentation [26], and purification and characterization of xylanase [29]. The current study was planned to develop an optimized SSF bioprocess for production of xylanase from *Aspergillus terreus* S9. The most effective process variables were earmarked by Plackett–Burman design, and the same

were optimized using response surface methodology. The *A. terreus* S9 xylanase was assessed for its multifaceted application potential for fruit juice clarification (orange juice, mosambi juice, pineapple juice), bread making, and biobleaching of paper pulp.

2 Materials and methods

2.1 Chemicals, medium components and process microorganism

All the chemicals, media and media components used were of analytical grade obtained from Sigma-Aldrich Chemicals Ltd, St. Louis MO, USA; HiMedia Laboratories Ltd, Mumbai, India; Qualigens Fine Chemicals Ltd, Mumbai, India; and Merck and Co. Inc., White House Station, NJ, USA. The process organism *Aspergillus terreus* S9 was a new isolate from mushroom compost at temperature 47–50 °C, moisture 68–70%, pH 7.8, which produced thermostable and a wide range pH stable xylanase. Submerged fermentation was DoE-optimized for xylanase production from *A. terreus* S9 [26], and the enzyme was purified and characterized [29]. In the current study, xylanase production from *A. terreus* S9 was investigated under SSF, and application potential of xylanase was assessed for fruit juice clarification, bread making, and for biobleaching of paper pulp.

2.2 Xylanase production from *Aspergillus terreus* S9 under SSF, and assay of activity

Different agricultural residues such as rice husk, almond hulls, wheat bran, malt, maize bran, cotton cake, corn cob powder, wood waste and saw dust, or oat spelt xylan (control) were used as substrates for xylanase production in a shake flask of 500 ml capacity. The substrate (5.0 g) was mixed with 5.0 ml of mineral salt solution, MSS (g/l; NH_4NO_3 : 5, CaCl_2 :0.1, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$:1, K_2HPO_4 :0.5, NaCl :1 and MnSO_4 :0.1), and inoculated with 2.0 ml of spore suspension (2×10^6 spores/ml), and the contents were well mixed, and incubated at 30 °C for 5 days under static conditions with occasional manual shaking (2–4 times a day). The moisture content was maintained 75% (w/w). Enzyme extraction was executed by adding 100 ml of distilled water in the flask, and shaking at 30 °C for 1 h. The contents were centrifuged ($10,000 \times g$ for 10 min, Eppendorf Centrifuge 5804R), and the supernatant obtained was considered as equivalent to crude enzyme (xylanase). Xylanase activity was analyzed by using oat spelt xylan (0.5%, w/v) as the substrate following the 3,5-dinitrosalicylic acid method [26], and the liberated reducing sugars were quantified spectrophotometrically at 575 nm (UV–VIS 1800, Shimadzu, Japan) using xylose as standard. One unit

(International Unit) of xylanase activity was defined as the amount of enzyme required to liberate one micromole of xylose equivalent per min under assay conditions.

2.3 Screening of significant variables for xylanase production under SSF by *A. terreus* S9

Plackett–Burman design (Design-Expert 6.0 Stat-Ease, Inc., Minneapolis, USA) was used for screening of eight process variables for their influence on xylanase production. The process variables screened at their respective lower and higher levels (indicated in parentheses) were: wheat bran (0.1–2% w/v), yeast extract (0.1–0.5% w/v), soybean meal (0.5–2% w/v), KH_2PO_4 (0.05–2% w/v), MgSO_4 (0.01–0.1% w/v), Tween-80 (0.02–0.05% w/v), incubation time (24–120 h) and medium pH (6–9). A set of 12 experimental trials was generated, and experiments were performed, and the resultant response i.e. xylanase yield for each run was analyzed.

2.4 Optimization of significant variables for xylanase production using RSM

The three most significant variables i.e. wheat bran, pH and incubation time, identified by PB design, that influenced the xylanase production from *A. terreus* S9 under SSF, were optimized by response surface methodology (Design-Expert 6.0 Stat-Ease, Inc., Minneapolis, USA). These variables were used at five coded levels ($-\alpha$, -1 , 0 , $+1$, $+\alpha$), and other variables were maintained at constant level. A set of 20 experimental runs was generated by the software, and the experiments were performed in triplicates. The data was analyzed by multiple regression analysis, and a polynomial equation was derived. Statistical analysis of the model was done for evaluation of analysis of variance (ANOVA). The analysis included the Fisher's F-test (overall model significance), its associated probability p (F), correlation coefficient (R), and determination coefficient (R^2).

Optimum level of the variables (wheat bran, pH and incubation time) was predicted based upon point prediction tool of design expert software, and experiments were conducted to validate the model. Regression equation and 3-D surface plots were examined.

2.5 Application potential of *A. terreus* S9 xylanase for fruit juice clarification

The application potential of *A. terreus* S9 xylanase was investigated for clarification of fresh fruit juices (orange juice, mosambi juice, and pineapple juice) procured from local vendors on the University campus. Either of the fruit juice (20.0 ml) was subjected to *A. terreus* S9 xylanase treatment (2.0 ml, 12.5 U/ml) over a varying time interval

(24–96 h). The fruit juice without enzyme treatment was considered as control. The xylanase-treated and untreated (control) fruit juices were examined for transmittance at 650 nm, and the clarification efficacy was calculated using the following formula, and expressed as % clarification:

$$\% \text{ Clarification} = \frac{T_t - T_c}{T_c} \times 100$$

where T_t = Transmittance of test; T_c = Transmittance of control.

2.6 DoE based optimization of fruit juice clarification by *A. terreus* S9 xylanase

The process variables for clarification of orange and mosambi juices were optimized using RSM design. Three process variables selected for optimization of fruit juice clarification at lower and higher values (given in parentheses) were: enzyme dose (25–50 U/ml), time of treatment (24–36 h) and temperature (30–50 °C). A set of 20 experiments was generated by RSM design, and the experiments were performed in triplicates. Multiple regression analysis was used to analyze the data, and a polynomial equation was derived. Statistical analysis of the model was done for evaluation of analysis of variance (ANOVA). The 3-D response surface plots generated by the software, and regression equation were examined to understand the interactive effects of different variables on fruit juice clarification. The optimal level of variables was determined based on point prediction tool of design expert software, and the experiments were conducted using the optimum values of variables for validation of the process model.

2.7 Application of xylanase for bread making, and biobleaching of paper pulp

The *A. terreus* S9 xylanase was evaluated for its application potential in bread making. The dough prepared for bread consisted of wheat flour (100 g), water (40 g), *A. terreus* S9 xylanase (2.4 ml, total 30 U), and baker's yeast (3.0 g). The wheat flour and the baker's yeast were procured from local market, and the xylanase was produced *in-house* in the laboratory from *A. terreus* S9. The dough contents were mixed well, kneaded manually, and incubated at 30 °C. A control was set up simultaneously which consisted of all the dough ingredients except *A. terreus* S9 xylanase. The control and experimental tests were incubated at 37 °C for 12 h, and then observed visually for the dough rise.

The xylanase from *A. terreus* S9 was evaluated for its biobleaching potential of paper pulp. The paper pulp was kindly supplied by M/S Ballarpur Industries, Yamuna Nagar, Haryana, through Prof. Jitender K. Sharma, Head, Department of Biotechnology, Kurukshetra University,

Kurukshetra. Appropriately diluted *A. terreus* S9 xylanase or distilled water (control) was used to prepare the paper pulp suspensions at a consistency of 10% (w/v), and the same were incubated inside sealed polyethylene bags at 60 °C for 2 h. The pulp was filtered through Whatman filter paper No. 41, and washed with 200 ml of distilled water. The brightness parameters were observed, and Kappa number was determined.

The Kappa number was determined [9] by following the steps approved by the Pulp Properties Committee of the Process and Product Quality Division of Technical Association of the Pulp and Paper Industry (TAPPI). Sample was conditioned prior to weighing for 20 min, and then 1.0 g of pulp specimen which consumes approximately 50% of the potassium permanganate solution was weighed. Similarly, another 1.0 g of pulp specimen was weighed for setting up a control. The pulp specimen was then disintegrated in 500 ml or less quantity of distilled water with special attention to avoid extensive cutting of the fibers, and the disintegrated test specimen was transferred to another reaction beaker (2.0 l). The beaker used for disintegration was rinsed with distilled water, and the washings were added to the 2.0 l reaction beaker to raise the total volume to 795 ml. The reaction mixture was maintained at a constant temperature of 25 °C in the water bath during the entire reaction. The suspension was continuously stirred and vortexed. One ml of potassium permanganate solution (0.1 N) and 100 ml of the sulfuric acid solution were taken in a 250 ml beaker. The mixture was brought to 25 °C and added to the disintegrated test specimen, and then 5.0 ml of distilled water was added. The washings from beaker were added to raise the volume of reaction mixture to a total of 1000 ml. The reaction was stopped after 10 min by adding 20.0 ml of the potassium iodide solution. The contents were well mixed, and the free iodine was titrated with the sodium thiosulfate solution, using starch (a few drops) as an indicator. The blank was processed in the same way but omitting the pulp.

The Kappa number is defined as the volume (ml) of 0.1 N potassium permanganate solution consumed by one gram of moisture-free pulp under the specified conditions. The results are corrected to 50% consumption of the permanganate added.

Kappa number was calculated by using the following formula:

$$K = p \times f / W$$

$$p = (b - a)N / 0.1$$

where K = Kappa number; f = Correction factor to 50% permanganate consumption, dependent on the value of p; W = weight of moisture free pulp; p = 0.1 N permanganate

consumed by test specimen, ml; b = amount of thiosulphate consumed in blank; a = amount of thiosulphate consumed by test specimen; N = Normality of thiosulphate (0.2 N).

3 Results and discussion

3.1 Xylanase production from *A. terreus* S9 using various agroindustrial residues under SSF

Solid state fermentation was executed using a variety of agro-industrial residues as the substrates (rice husk, almond hulls, wheat bran, malt, maize bran, cotton cake, corn cob powder, wood waste, and saw dust) for xylanase production from *A. terreus* S9 (Fig. 1). Control was set up in which pure xylan was used as the substrate for xylanase production *albeit* under same conditions. Maximum xylanase production was supported by wheat bran (40 U/ml), however, substantial xylanase yield was observed on other residues like corn cob powder (38.4 U/ml), cotton cake (38 U/ml), malt (37.6 U/ml) and almond hulls (35.2 U/ml) (Fig. 1). Other substrates though were utilized by *Aspergillus terreus* S9 for growth but xylanase yield was relatively low. The current study is the first ever report of usage of almond hull as the substrate for SSF based xylanase production. *A. terreus* S9 not only successfully utilized almond hull as substrate for growth but sustained considerable xylanase production (35.2 U/ml) under SSF (Fig. 1).

The agro-residues constitute good quality substrates for xylanase production as they not only serve as source of carbon and energy, but also act as inducers for enzyme production. Various physical attributes of the substrates viz. crystalline or amorphous nature, degree of hydration, porosity, the accessible surface area and particle dimensions are very imperative for sustaining adequate growth of fungi, and enzyme production under SSF [31]. The degree of hydration of various agro-residues varies, and

Fig. 1 Xylanase production by *Aspergillus terreus* S9 using various agroindustrial residues as substrates under solid state fermentation

might be responsible for differential growth and enzyme yield on different substrates. Among various substrates, wheat bran due to its loose texture provides sufficient surface area, and ample nutrients like carbohydrates, proteins, lipids, vitamins and minerals to meet the growth and metabolic demand of the process organism. Thus, wheat bran is regarded as the universal substrate that supports adequate growth and enzyme production by the process organism [6, 39]. Wheat bran alone or in combination with other solid substrates of agricultural origin has been used for fungal xylanase production [6, 13]. However wheat straw [19], sorghum straw [1], corncob [20] and de-oiled *Jatropha curcas* seed cake [25] have also been reported as efficient substrates for SSF based fungal xylanase production.

SSF was executed for xylanase production by cultivating *Aspergillus niger* on spent coffee waste as the sole carbon source. Various process parameters were optimized using a Box-Behnken design to achieve 1.39-fold increased xylanase yield [23]. Similarly, spent mushroom substrate of *Pleurotus florida* was employed as the substrate for SSF based production of lignocellulolytic enzymes such as cellulases and xylanases using species of *Trichoderma* and *Aspergillus* [21]. Thus, utilization of agricultural residues as substrates not only contributes towards cost-effective enzyme production but also promises valorization of wastes, and discrete environmental benefits.

3.2 Screening of effective variables, and their optimization for xylanase production

Eight process variables were screened for their influence on xylanase production by *A. terreus* S9 under SSF through a set of 12 experimental trials generated by Plackett–Burman design. The variable response (xylanase yield 7.8 U/ml to 42 U/ml) in the 12 experimental trials indicated the different effect of the process variables on the enzyme production. ANOVA was used for the analysis of statistically significance of variables. Regression analysis was performed on the results and a first order polynomial Eq. (1) was derived representing xylanase production as a function of the independent variables.

$$Y(\text{xylanase yield}) = +24.28 + 5.15A - 0.16B + 3.82C + 0.19D - 0.32E - 0.30F + 7.10G + 0.34H \dots \dots \quad (1)$$

Y: response i.e. xylanase yield; A: wheat bran; B: yeast extract; C: pH; D: soybean meal; E: Tween 80; F: KH_2PO_4 ; G: incubation time; H: MgSO_4 .

The analysis of variance (F-test) showed that the model was well adjusted to the experimental data, and the model F-value of 141.83 implied the significance of the model.

There was only a 0.09% chance that a model F-value this large could occur due to noise. Values of Prob > F less than 0.05 indicated that the model terms were significant while values greater than 0.10 indicated the non-significance of the model terms. In this case, the variables A (wheat bran), C (pH) and G (incubation time) were the significant model terms. The determination coefficient ($R^2 = 0.9974$) showing the goodness of fit of the model indicated that the sample variation of more than 99.74% was attributable to the variables, and only less than 0.26% of the total variation could not be explained by the model. Furthermore, the Pred R-Squared of 0.9578 was in reasonable agreement with the Adj R-Squared of 0.9903. Adeq Precision measures the signal to noise ratio, and the ratio of 38.657 indicated an adequate signal. A lower value of the coefficient of variation (CV 4.06) verified the precision and reliability of the experiments. ANOVA analysis showed that incubation time was the most significant factor (p value of 0.0001) for xylanase production, and was followed by wheat bran (p value 0.0004), and pH (p value 0.0009). Thus, three variables (A: wheat bran, C: pH, G: incubation time), among a total of eight screened, were found to have significant positive influence on xylanase production, and were selected further for process optimization based on RSM.

PB design is the most widely employed statistical tool for screening of multivariate systems including the identification of significant process variables for enzyme/xylanase production from various microorganisms [1, 7]. Ammonium sulfate, glucose and peptone were reported to have significant effect on xylanase production, among a total of 12 process variables screened by Plackett–Burman design [7]. Sorghum straw was earmarked as a good substrate for SSF based production of xylanase from *A. tubingensis* FDHN1 [1]. Sharma and Bajaj [28] screened a total of ten process variables based on Plackett–Burman design, and reported that four variables, i.e. wheat bran, incubation time, pH, and CaCl_2 were the most effective ones for xylanase production from *A. terreus* S9 under SmF.

Three significant variables (wheat bran, pH, and incubation time) selected on the basis of PB-design which influenced the xylanase production from *A. terreus* S9 maximally, were optimized using the central composite design (CCD) of RSM design. A total of 20 experimental trials were performed as designed by the tool (Table 1) and the response obtained was analyzed by ANOVA (Table 2). The close proximity of the experimental and predicted values showed the high precision of the experiments conducted with negligible chance of error. The model F-value of 465.13 implied that the model was significant, and there was only a 0.01% chance that a model F-value this large could occur due to noise. Values of Prob > F less than 0.05 indicated the significance of the model terms. In this case A (wheat bran), B (pH), C (incubation time), A^2 , B^2 , C^2 , AC

Table 1 Experimental and predicted response (xylanase activity U/ml) for xylanase production from *A. terreus* S9 under SSF using RSM-designed experiments

Experimental variables*				Response (U/ml)	
Run order	A	B	C	Experimental	Predicted
1	1.05	7.5	8.73	9.2	9.74
2	0.1	6	120	19.8	18.65
3	0.55	7.5	72	36	38.78
4	2	6	120	55	55.05
5	0.1	6	24	3.9	3.56
6	1.05	7.5	72	47.8	46.95
7	2	9	24	15	15.8
8	2.65	7.5	72	42.5	42.72
9	2	6	24	11.8	10.82
10	1.05	4.98	72	27	28.27
11	1.05	7.5	72	47.5	46.95
12	1.05	10.02	72	45	44.22
13	0.1	9	24	8	7.6
14	1.05	7.5	72	47.6	46.95
15	1.05	7.5	72	47.5	46.95
16	1.05	7.5	152.73	52.5	52.66
17	1.05	7.5	72	47.2	46.95
18	0.1	9	120	32	32.63
19	1.05	7.5	72	46.9	46.95
20	2	9	120	70	69.99

*A: Wheat bran; B: pH; C: incubation time

(wheat bran and incubation time) and BC (pH and incubation time) were the significant model terms. Values greater than 0.1 indicate the model terms are not significant. The determination coefficient ($R^2 = 0.9976$) signifying the goodness of fit of the model implied that the sample variation of more than 99.76% was attributable to the variables,

and only less than 0.24% of the total variation could not be explained by the model. The Pred R-Squared of 0.9869 was in reasonable agreement with the Adj R-Squared of 0.9955. Adeq Precision of 74.838 indicated an adequate signal. The low value of coefficient of variation (CV 3.52) further substantiated the reliability and precision of experiments.

The polynomial Eq. (2) obtained after multiple regression analysis for xylanase production under SSF is as follows:

$$Y = +46.95 + 11.15A + 4.74B + 17.32C - 8.13A^2 - 3.78B^2 - 8.28C^2 + 0.24AB + 7.29AC + 2.49BC \dots \dots \quad (2)$$

Y: response i.e. xylanase yield; A: wheat bran; B: pH; C: incubation time; A^2 , B^2 , C^2 : squared terms; AB, AC, BC: interactive terms.

When considered in linear terms, incubation time had the maximum positive influence on the response (i.e. xylanase yield), and was followed by wheat bran and pH. However, with respect to squared terms, all the variables had negative effect on xylanase production. In interactive terms, the effect of all the variables was positively significant for xylanase production by *A. terreus* S9 (Eq. 2). The interactive effect of wheat bran (A) and incubation time (C) had the maximum positive impact on the response, and was followed by that of pH (B) and incubation time (C), and that of wheat bran (A) and pH (B).

The response surface plots showing the interactive effects of various variables that have significant influence on xylanase production are presented in Fig. 2a, b. The variation in response (xylanase production), when the values of individual variables changes from a reference point keeping the other variables constant is depicted in perturbation plot (Fig. 2c). Since A (wheat bran), B (pH), C (incubation time), A^2 , B^2 , C^2 , AC (wheat bran and

Table 2 ANOVA analysis for xylanase production from *A. terreus* S9 using CCD tool of RSM design under solid state fermentation

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	6594.98	9	732.78	465.13	< 0.0001	Significant
A	1151.05	1	1151.05	730.63	< 0.0001	Significant
B	307.2	1	307.2	195	< 0.0001	Significant
C	3623.46	1	3623.46	2300.01	< 0.0001	Significant
A^2	546.53	1	546.53	346.91	< 0.0001	Significant
B^2	207.78	1	207.78	131.89	< 0.0001	Significant
C^2	715.32	1	715.32	454.05	< 0.0001	Significant
AB	0.45	1	0.45	0.29	0.6042	–
AC	424.86	1	424.86	269.68	< 0.0001	Significant
BC	49.5	1	49.5	31.42	0.0002	Significant
Residual	15.75	10	1.58			
Cor total	6610.74	19				

A, wheat bran; B, pH; C, incubation time

Fig. 2 Response surface plots showing interaction between different variables for xylanase production by *Aspergillus terreus* S9 viz. incubation time and wheat bran (a), incubation time and pH (b); and perturbation plot (c)

incubation time) and BC (pH and incubation time) were significant model terms, it suggested that wheat bran, pH and incubation time were the critical variables for xylanase production, both individually and in interactive terms. The incubation time was the most vital variable influencing the xylanase production, and was followed by wheat bran and pH (regression Eq. 2). Furthermore, the interaction AC (wheat bran and incubation time) was more favorable for enhanced xylanase production as compared to the interaction BC (pH and incubation time). The cross product term coefficients, AC ($p = 0.0001$) and BC ($p = 0.0002$) having significant p values revealed the presence of strong interactions among wheat bran, incubation time and pH. Thus, it could be inferred that right kind of balance between various variables which either influence the nutrients availability/medium composition, and/or the microenvironment for growth of the process organism, is absolutely important for successful development of a bioprocess.

Validation of the statistical model was done by using the point prediction tool of the design expert software wherein an optimum level of all three variables was predicted, and the experiments were executed. The optimum level of three variables was i.e. wheat bran 2.52% w/v, pH 8.85, and incubation time 148.8 h. The closer proximity of the predicted response (74.4 U/ml) and the actual experimental response (72.8 U/ml) validated the model. RSM-based optimization resulted in 1.82-fold increased xylanase yield as compared to that under unoptimized conditions. Hence, DoE based statistical optimization of process variables is a powerful tool as it showed substantial enhancement in xylanase production from *A. terreus* S9 under SSF.

RSM based optimization of variables e.g. solid substrates, moisture content, moistening medium, temperature and incubation time resulted in 5.4-fold increase in xylanase production from *Aspergillus niger* DFR-5 under SSF [17]. Optimization of upstream and downstream process parameter for xylanase production from *A. tubingensis*

FDHN1 under solid-state fermentation using low-cost agro-residues as substrates resulted in 6.66-fold enhanced xylanase yield [1]. RSM based optimization of variables e.g. particle size (mm), liquid to solid ratio (w/v), and avicel (g) resulted in two-fold increase in cellulase production from *Penicillium oxalicum* EU2106 under SSF [33]. Optimization of variables like wheat bran, incubation time, pH, and CaCl_2 using response surface methodology resulted in 1.92-fold increased xylanase yield from *A. terreus* S9 under SmF [28]. Similarly, process variables like temperature, initial moisture content, and inoculum (log number of spores), were optimized using a Box-Behnken design for xylanase production from *Aspergillus niger* under SSF on spent coffee waste to achieve 1.39-fold increased yield [23].

3.3 Application of *A. terreus* S9 xylanase for fruit juice clarification

The turbidity and viscosity of fruit juices is due to the presence of various polysaccharide components such as cellulose, hemicelluloses, starch and pectin. The yield and quality of the fruit juices can be enhanced by using appropriate carbohydrate degrading enzymes [3]. The application of xylanases in the fruit juice industries may be significant for degrading the xylan component present in the fresh fruit juices [11]. Xylanase from *A. terreus* S9 was examined for the clarification of fruit juices such as orange juice, mosambi juice and pineapple juice over varying time intervals. The results demonstrated that the maximum clarification was observed for orange juice (73.33%), and it was followed by mosambi juice (44%) and pineapple juice (42.43%). Orange juice showed maximum clarification after 48 h, while mosambi juice and pineapple juice showed that after 24 h of xylanase treatment. Thereafter, enzyme clarification level remained more or less constant.

The maximum clarification of the orange juice may be due to the fact that the raw orange juice contained higher hemicellulosic content compared to other fruit juices. Xylanases from various microorganisms have been studied for their fruit juice clarification potential. Ahmed et al. [3] reported xylanase mediated clarification of orange juice (64%) after 72 h, while Elegbede and Lateef [11] documented higher level of clarification (74.22%) after 24 h. Similarly, Adiguzel et al. [2] reported that treatment of different fruit juices (orange, pomegranate, apricot, peach, apple, grape, and kiwi) with *Pediococcus acidilactici* GC25 xylanase improved the clarification, increased the reducing sugar amount, and decreased the turbidity. Xylanase produced from *Aspergillus niger* under optimized SSF conditions on spent coffee waste, was purified and examined for its efficacy for clarification/enrichment of various fruit juices like strawberry, blueberry, and raspberry [23].

Various process parameters like effect of enzyme dosage, temperature and time of enzyme treatment were optimized to get maximum efficacy. Thus, application of xylanases can potentially play a crucial role in the fruit juice industry for enhancing clarification, quality and juice yield.

3.4 DoE based optimization of orange and mosambi juice clarification by *A. terreus* S9 xylanase

DoE was used to predict the optimal level of processing conditions for effective fruit juice clarification of two fruit juices i.e. orange juice and mosambi juice using *A. terreus* S9 xylanase. Three process variables i.e. dose of *A. terreus* S9 xylanase, time period and temperature of treatment, were optimized using DoE. Xylanase dose and temperature are the key factors that influence the enzyme mediated fruit juice clarification. The enzyme concentration or dosage is critical for the process as low dose may not be adequate for completion of reaction/process, while higher dose may results in enzyme wastage, and cost increment. Temperature has a positive effect on flux enhancement in fruit juice clarification processes [5]. The rate of clarification increases with increase in temperature, as the catalytic activity of enzyme gets enhanced with rise in temperature, however, very high temperature may interfere with native structural confirmation of enzyme and may alter enzyme–substrate binding [26] and decrease the reaction rate, and hence the clarification effect [5]. Similarly, incubation time period is an important parameter for accomplishing higher fruit juice clarification, and needs to be properly adjusted as less incubation time may leave the juice clarification far from completion, and longer incubation time may lead to undesired interactions between juice components, and reduce overall productivity of the process [26].

For optimization of three process variables (enzyme dose, time and temperature) for orange juice clarification, 20 experimental runs were performed and the corresponding responses (clarification %) were fed into the design matrix as shown in the Table 3. Different values of the response in different experimental runs showed the crucial role of the process variables at various levels. The maximum clarification (80.77%) was observed in the run order 17 (enzyme dose 50 IU/ml; time 36 h; temperature 50 °C) while the minimum clarification (20.09%) was observed in the run order 6 (enzyme dose 25 IU/ml; time 24 h; temperature 50 °C). The experimental design was tested by using the Fisher's statistical test for analysis of variance (ANOVA) and the results are shown in Table 4. In this case, the model terms i.e. enzyme dose (A), time (B), temperature (C), A^2 , enzyme dose and time (AB), and time and temperature (BC) were significant.

Table 3 Experimental and predicted response for orange juice clarification from *Aspergillus terreus* S9 xylanase using response surface methodology

Run order	Experimental variables*			(% Clarification)	
	A	B	C	Experimental	Predicted
1	37.5	40.09	40	46.68	47.92
2	16.48	30	40	25.05	22.93
3	37.5	30	40	43.5	40.58
4	50	36	30	74.8	74.31
5	37.5	30	40	41.45	40.58
6	25	24	50	20.09	20.02
7	37.5	30	40	45	40.58
8	25	36	30	21.09	20.21
9	37.5	19.91	40	22.5	22.05
10	37.5	30	40	39.09	40.58
11	37.5	30	40	32.5	40.58
12	37.5	30	40	42.05	40.58
13	50	24	30	52.54	50.25
14	58.52	30	40	70.31	73.22
15	37.5	30	56.82	35.09	35.37
16	25	24	30	39.23	41.58
17	50	36	50	80.77	77.87
18	25	36	50	25	26.73
19	50	24	50	25.4	25.72
20	37.5	30	23.18	50	50.51

*A: enzyme dose; B: time; C: temperature

The regression Eq. (3) generated for the quadratic model by the design of expert for clarification in terms of coded factors was suggested as follows:

$$Y = +40.58 + 14.95A + 7.69B - 4.50C + 2.65A^2 - 1.98B^2 + 0.84C^2 + 11.36AB - 0.74AC + 7.02BC \dots \dots \quad (3)$$

Y: response i.e. clarification; A: Enzyme dose, B: Time, C: Temperature; A², B², C²: squared terms; AB, AC and BC: interactive effects of the variables on the response.

The model F-value of 46.70 implied that the model was significant. There was only a 0.01% chance that a model F-value this large could occur due to noise. In this case A, B, C, A², AB and BC were the significant model terms (Table 4). The determination coefficient (R² = 0.9768) signifying the goodness of fit of the model implied that the sample variation of more than 97.68% was attributable to the variables, and only less than 2.32% of the total variation could not be explained by the model. The Pred R-Squared of 0.9231 was in reasonable agreement with the Adj R-Squared of 0.9558. Adeq Preciso' ratio of 22.114 indicated an adequate signal.

The response surface plots showing the interactive effects of various variables on response (% clarification of orange juice) are presented in Fig. 3a, b. Variation in response (clarification), when the values of individual variable change from a reference point keeping the other variables constant is depicted in perturbation plot (Fig. 3c). Since A, B, C, A², AB and BC are significant model terms, it suggested that the variables (enzyme dose, time, and temperature) individually and in interactive terms have a substantial influence on clarification. The clarification level may be markedly affected by even little change in the respective values of variables i.e. enzyme dose, temperature and time. The enzyme dose and time were positively significant while the temperature was negatively significant (regression Eq. 3; Fig. 3). The orange juice clarification was influenced more by

Table 4 ANOVA analysis for orange juice clarification from *Aspergillus terreus* S9 xylanase based on response surface methodology designed experiments

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	5752.54	9	639.17	46.7	< 0.0001	Significant
A	3053.78	1	3053.78	223.13	< 0.0001	Significant
B	808.3	1	808.3	59.06	< 0.0001	Significant
C	276.73	1	276.73	20.22	0.0011	Significant
A ²	101.3	1	101.3	7.4	0.0215	Significant
B ²	56.31	1	56.31	4.11	0.0700	
C ²	10.07	1	10.07	0.74	0.4112	
AB	1031.94	1	1031.94	75.4	< 0.0001	Significant
AC	4.41	1	4.41	0.32	0.5828	
BC	394.24	1	394.24	28.81	0.0003	Significant
Residual	136.86	10	13.69			
Lack of Fit	38.37	5	7.67	0.39	0.8379	Not significant
Pure error	98.49	5	19.7			
Cor total	5889.4	19

Fig. 3 Response surface plots showing interaction between different variables for clarification of orange juice using *Aspergillus terreus* S9 xylanase

the enzyme dose than the incubation time. Furthermore, the interactive effect of enzyme dose and time (AB) was more pronounced on juice clarification than that of time and temperature (BC), and both the interactions (AB and BC) were positively significant. The cross product term coefficients, AB ($p \leq 0.0001$) and BC ($p = 0.0003$) having significant p values revealed the presence of strong interactions among variables for clarification of orange juice. Thus successful clarification of juices requires the necessary balance between enzyme dosage and external environmental factors that influence the characteristic composition of the juices.

The model was validated by performing the experiment using the values of variables predicted by the point prediction tool of RSM. The predicted values of variables were: enzyme dose (57.84 U/ml), time (39.60 h) and temperature (56.27 °C). A close proximity of the predicted response (92.61% clarification) and the experimental response (90% clarification) validated the model. The optimization resulted in 1.23-fold increased clarification of orange juice as compared to that under unoptimized conditions.

Similarly, mosambi juice clarification was DoE-optimized for the same three variables i.e. enzyme dose,

treatment time and temperature. The predicted and experimental values were examined, and ANOVA was done. The model F-value of 314.66 implied the significance of the model. There was only a 0.01% chance that a model F-value this large could occur due to noise. ANOVA results indicated that model terms i.e. A (enzyme dose), B (time), C (temperature), A^2 , B^2 , C^2 , AC (enzyme dose and temperature) and BC (time and temperature) were significant (Table 5). The regression Eq. (4) generated for the quadratic model by the design software for clarification in coded terms was as follows:

$$Y = +33.64 + 7.84A + 2.24B + 2.12C + 2.40A^2 - 1.55B^2 - 0.98C^2 - 0.26AB + 0.94AC + 0.61BC \dots \dots \quad (4)$$

Y: response that is clarification; A: enzyme dose; B: time; C: temperature; A^2 , B^2 , C^2 : squared terms; AB, AC and BC: interactive terms of variables.

The response surface plots showing the interactive effects of various variables on clarification of mosambi juice are presented in Fig. 4a, b. Variation in response (clarification), when the values of individual variable change

Table 5 ANOVA analysis for mosambi juice clarification from *Aspergillus terreus* S9 xylanase based on response surface methodology designed experiments

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	1123.9	9	124.88	314.66	< 0.0001	Significant
A	838.53	1	838.53	2112.85	< 0.0001	Significant
B	68.39	1	68.39	172.33	< 0.0001	Significant
C	61.14	1	61.14	154.07	< 0.0001	Significant
A ²	82.68	1	82.68	208.32	< 0.0001	Significant
B ²	34.41	1	34.41	86.7	< 0.0001	Significant
C ²	13.83	1	13.83	34.84	0.0002	Significant
AB	0.53	1	0.53	1.32	0.2767	
AC	7.05	1	7.05	17.76	0.0018	Significant
BC	2.94	1	2.94	7.41	0.0215	Significant
Residual	3.97	10	0.4			
Lack of Fit	3.04	5	0.61	3.27	0.1097	Not significant
Pure error	0.93	5	0.19			
Cor total	1127.87	19

Fig. 4 Response surface plots showing interaction between different variables for clarification of mosambi juice using *Aspergillus terreus* S9 xylanase

from a reference point keeping the other variables constant is depicted in perturbation plot (Fig. 4c). Since A, B, C, A^2 , B^2 , C^2 , AC and BC were significant model terms, it suggested that enzyme dose, temperature and time were crucial for clarification of mosambi juice, both as a single component as well as in interactive terms. Thus, even minor variations in the level of enzyme dose, temperature and time, may influence the juice clarification efficacy. The variables i.e. enzyme dose, time and temperature were positively significant (regression Eq. 4; Fig. 4). Furthermore, clarification (%) of mosambi juice was influenced maximally by enzyme dose, and was followed by that by time and temperature. The interaction of enzyme dose and temperature (AC) had the higher influence on clarification as compared to that between time and temperature (BC) as depicted in the regression Eq. 4 and Fig. 4, and both the interactions were positively significant.

The model was validated by performing the experiment at the values of variables predicted by the point prediction tool of software i.e. enzyme dose (57.50 U/ml), time period (39.55 h) and temperature (56.46 °C) of incubation. The predicted response of 56.19% was quite close to the actual experimental response of 54.25%, proving the validity of the model. The optimization of process variables resulted in 1.28-fold increased clarification of mosambi juice.

Clarification of fruit juices requires the carbohydrate degrading enzymes for increasing yield and quality characteristics of juices. Several reports are available involving the usage of pectinases and cellulases for the clarification of fruit juices but rare reports are available on clarification of fruit juices using xylanases alone, and/or in conjunction with other enzymes. Dhiman et al. [10] optimized the critical factors for clarification of citrus fruit juice by employing xylanase from *Bacillus stearothermophilus* using central composite design of response surface methodology and reported an increased xylanase yield of 1.19-fold over conventional method. However, Pal and Khanum [18]

employed central composite rotatable design (CCRD) for optimization of variables with the constraint of minimum pectinase and cellulase, and maximum xylanase and incubation time, for enhanced juice yield and clarity. The closeness of observed response (90.2% yield and 80.9% clarity) to the predicted one (89.6% yield and 80.3% clarity) specified the validity of developed model. Rosmine et al. [24] used xylanase from *Streptomyces* sp. strain ER1 (KY449279) for achieving enhanced clarification of orange, mosambi and pineapple juices to the level of 20.87%, 23.64% and 27.89%, respectively. Thus, xylanases may have promising potential in fruit juice industry.

A xylanase produced from *Aspergillus niger* was purified, and investigated for its fruit juice clarification potential of various fruits like strawberry, blueberry, and raspberry pulp [23]. Similar to the current study, enzyme dosage, temperature and time of enzyme treatment, were earmarked as the most vital variables which influenced the clarification of fruit juices. Thus, fruit juice industry may use xylanases for enhancing clarification, quality and juice yield.

3.5 Application of *A. terreus* S9 xylanase in bread making and biobleaching of paper pulp

Xylanase owing to its potential of conferring beneficial properties to the bread may be exploited in the bread making industry for enhancing bread quality. Application of *A. terreus* S9 xylanase displayed amazing capability of dough raising in comparison to control; thus showing its application potential in bread making (Fig. 5I). Xylanases have been used either standalone or in combination with other enzymes for improving the characteristics of bakery products [16, 27]. The dough rise due to *A. terreus* S9 xylanase application is attributed to the cleavage of arabinoxylans resulting in release of water, consequently softening the dough. The monomers and oligomers released may

Fig. 5 Application of *Aspergillus terreus* S9 xylanase for bread making (I, C-control i.e. without enzyme, X-with application of xylanase), and biobleaching of pulp and paper industry (II, A-pulp without enzyme treatment, B-pulp with enzyme treatment)

affect the water balance, and modify the protein-starch interactions which may result in decreased resistance of dough to gas flow, and hence the improved dough-rise. Furthermore, use of xylanases decrease the dough firmness, create more uniform and finer crumbs, and increase the volume by forming the water soluble arabinoxylan from insoluble arabinoxylan, and even play role in reducing the staling rate of the bread [27].

Similar results were obtained by Elegbede and Lateef [11] who have shown that crude xylanase from *Aspergillus flavus* SD4A and *Aspergillus fumigatus* L2 strain enhanced the dough volume to 2.2 and 1.87-fold, respectively. Yegin et al. [37] reported that a novel xylanase from an extremophile *Aureobasidium pullulans* NRRL Y-2311-1 was more effective for decreasing the bread firmness, and for providing remarkable improvement (30%) in bread specific volume, as compared to the commercial counterparts. Similarly, Cunha et al. [8] employed a recombinant xylanase (r-XynBS27) from *Streptomyces* S27 as an additive in the bread making process, and reported decreased firmness, stiffness and consistency, and an improved specific volume and reducing sugar content. Thus, xylanases may have immense potential for application in improving the quality characteristics of bread in a bakery industry.

Application of xylanases in pulp bleaching may play an imperative role in developing eco-friendly biobleaching process which may results in reduced usage of toxic chlorine compounds, and hence decrease the hazardous discharges from paper industry. In the current study, the *A. terreus* S9 xylanase treated pulp showed increased brightness as compared to the untreated pulp (Fig. 5II). Furthermore, the Kappa number (measure of residual lignin in the pulp) of 11.8 of xylanase treated pulp confirmed the bleaching potential of *A. terreus* S9 xylanase. Xylanases have been used in paper industry even for the improvement of the paper quality [36]. Application potential of xylanases from diverse microbial sources has been explored for biobleaching of pulp in pulp and paper industries. Due to hot and alkaline nature of the pulp, the xylanases intended for the pulp and paper industry must be alkalistable and thermostable. Xylanases facilitate the release of xylan and lignin (brown color of paper is due to the presence of polyphenolic compounds) by acting on β -1,4- linked xylosyl residues, which results in not only enhanced whiteness and brightness of the paper [35] but improved water retention and pulp fibrillation, thus, enhancing the paper quality.

The cellulase-free xylanase from *A. flavus* was reported to be more effective than that from *A. niger* with the kappa efficiency of 36.32 and 25.93, respectively [9]. Similarly, the enzymatic pretreatment of recycled paper pulp

with mangrove fungal xylanases resulted in release of high amounts of reducing sugars (70–650 mg/g of pulp) at 50 °C after 60 min [34]. Sridevi et al. [32] employed xylanase from *Trichoderma asperellum* for biobleaching of paper pulp and reported reduction in the Kappa number by 4.2 points, and increased the brightness by 4.0 points. Thus, xylanase usage in pulp and paper industry promotes the development of green technology by reducing the chlorine consumption thereby, making the process more environment-friendly.

4 Conclusions

The xylanase from *Aspergillus terreus* S9 exhibited an excellent application potential for biobleaching of paper pulp, fruit juice clarification, and bread making. The DoE based optimization of parameters for xylanase production using agroresidues as substrates resulted in substantially enhanced xylanase yield (1.82-fold). Similarly, optimization of parameters enhanced the xylanase mediated clarification of orange and mosambi juices by 1.23-fold and 1.28-fold, respectively. Furthermore, *A. terreus* S9 xylanase exhibited commendable application potential for biobleaching of paper pulp, and bread making. The mechanistic insights into the multifaceted application potential of *A. terreus* S9 xylanase for a variety of industrial processes may be investigated in-depth for fully realizing its industrial potential.

Acknowledgements Dr. Bijender Kumar Bajaj (BKB) gratefully acknowledges the Commonwealth Scholarship Commission, UK, for providing Commonwealth Fellowship, and Institute of Advanced Study, Durham University, Durham, UK for providing COFUND-International Senior Research Fellowship, for ‘Research Stays’, respectively, at Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK, and at Department of Biosciences Durham University, UK,. BKB and Sunny Sharma thank Department of Science and Technology (Govt. of India) for financial support in the form of Research Project to Dr BK Bajaj (Ref. SR/SO/BB-66/2007) and Junior Research Fellowship to Sunny Sharma, Mr. Vishal Sharma thankfully acknowledges the Department of Science and Technology (Govt. of India) for providing Inspire Fellowship for doctoral research; Ms Parushi Nargotra thanks the Ministry of Human Resource Development, Govt. of India, for providing Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-Fellowship for Doctoral studies. Authors thank the Director, School of Biotechnology, University of Jammu, Jammu, for necessary laboratory facilities.

Authors’ Contributions Dr. Bijender Kumar Bajaj (BKB) conceptualized the research hypothesis, and planned the experiments; Sunny Sharma designed and performed the experiments; Sunny Sharma, Vishal Sharma, and Parushi Nargotra analyzed and interpreted the data and wrote the draft MS; Dr. Bijender Kumar Bajaj corrected and submitted the MS.

Funding This study was funded by the Department of Science and Technology (Govt. of India) (Grant No. Ref. SR/SO/BB-66/2007).

Compliance with ethical standards

Conflict of interest All Authors declare that there is no Conflict of interest.

References

- Adhyaru DN, Bhatt NS, Modi HA (2015) Optimization of upstream and downstream process parameters for cellulase-poor-thermosolvent-stable xylanase production and extraction by *Aspergillus tubingensis* FDHN1. *Bioresour Bioprocess* 2:3. <https://doi.org/10.1186/s40643-014-0029-1>
- Adiguzel G, Faiz O, Sisecioglu M, Sari B, Baltaci O, Akbulut S, Adiguzel A (2019) A novel endo- β -1, 4-xylanase from *Pediacoccus acidilactici* GC25; purification, characterization and application in clarification of fruit juices. *Int J Biol Macromol* 126:571–578. <https://doi.org/10.1016/j.ijbiomac.2019.02.054>
- Ahmed SA, Saleh SA, Mostafa FA, El Aty AAA, Ammar HA (2016) Characterization and valuable applications of xylanase from endophytic fungus *Aspergillus terreus* KP900973 isolated from *Corchorus olitorius*. *Biocatal Agric Biotechnol* 7:134–144. <https://doi.org/10.1016/j.bcab.2016.05.015>
- Bajaj BK, Singh NP (2010) Production of xylanase from an alkalitolerant *Streptomyces* sp. 7b under solid-state fermentation, its purification, and characterization. *Appl Biochem Biotechnol* 162(6):1804–1818. <https://doi.org/10.1007/s12010-010-8960-x>
- Bhattacharjee C, Saxena VK, Dutta S (2017) Fruit juice processing using membrane technology: a review. *Innov Food Sci Emerg Technol* 43:136–153. <https://doi.org/10.1016/j.ifset.2017.08.002>
- Chapla DK, Divecha J, Madamwar D, Shah A (2010) Utilization of agro-industrial waste for xylanase production by *Aspergillus foetidus* MTCC 4898 under solid state fermentation and its application in saccharification. *Biochem Eng J* 49:361–369. <https://doi.org/10.1016/j.bej.2010.01.012>
- Chaturvedi S, Kohli KU, Rajni S, Khurana SMP (2015) Statistical optimization of medium composition for xylanase production by solid state fermentation using agroresidues. *Am J Microbiol Res* 3:85–92
- Cunha CCDQB, Gama AR, Cintra LC, Bataus LAM, Ulhoa CJ (2018) Improvement of bread making quality by supplementation with a recombinant xylanase produced by *Pichia pastoris*. *PLoS One* 13(2):e0192996
- de Alencar Guimaraes NC, Sorgatto M, de Carvalho Peixoto-Nogueira S, Betini JHA, Zanoelo FF, Marques MR, Moraes MDLT, Giannesi GC (2013) Bioprocess and biotechnology: effect of xylanase from *Aspergillus niger* and *Aspergillus flavus* on pulp biobleaching and enzyme production using agroindustrial residues as substrate. *Springerplus* 2(1):380. <https://doi.org/10.1186/2193-1801-2-380>
- Dhiman SS, Garg G, Sharma J, Mahajan R (2011) Characterization of statistically produced xylanase for enrichment of fruit juice clarification process. *New Biotechnol* 28(6):746–755. <https://doi.org/10.1016/j.nbt.2010.11.004>
- Elegbede JA, Lateef A (2018) Valorization of corn-cob by fungal isolates for production of xylanase in submerged and solid state fermentation media and potential biotechnological applications. *Waste Biomass Valor* 9:1273–1287. <https://doi.org/10.1007/s12649-017-9932-y>
- Ergun SO, Urek RO (2017) Production of ligninolytic enzymes by solid state fermentation using *Pleurotus ostreatus*. *Ann Agrar Sci* 15:273–277. <https://doi.org/10.1016/j.aasci.2017.04.003>
- Jin N, Ma S, Liu Y, Yi X, He R, Xu H, Qiao DR, Cao Y (2012) Thermophilic xylanase production by *Aspergillus niger* in solid state fermentation using wheat straw and corn cob. *Afr J Microbiol Res* 6:2387–2390
- Lizardi-Jiménez MA, Hernández-Martínez R (2017) Solid state fermentation (SSF): diversity of applications to valorize waste and biomass. *3 Biotech* 7:44. <https://doi.org/10.1007/s13205-017-0692-y>
- Nargotra P, Sharma V, Gupta M, Kour S, Bajaj BK (2018) Application of ionic liquid and alkali pretreatment for enhancing saccharification of sunflower stalk biomass for potential biofuel-ethanol production. *Bioresour Technol* 267:560–568. <https://doi.org/10.1016/j.biortech.2018.07.070>
- O'Shea N, Kilcawley KN, Gallagher E (2016) Influence of α -amylase and xylanase on the chemical, physical and volatile compound properties of wheat bread supplemented with wholegrain barley flour. *Eur Food Res Technol* 242(9):1503–1514. <https://doi.org/10.1007/s00217-016-2651-y>
- Pal A, Khanum F (2010) Production and extraction optimization of xylanase from *Aspergillus niger* DFR-5 through solid-state-fermentation. *Bioresour Technol* 101:7563–7569. <https://doi.org/10.1016/j.biortech.2010.04.033>
- Pal A, Khanum F (2011) Efficacy of xylanase purified from *Aspergillus niger* DFR-5 alone and in combination with pectinase and cellulase to improve yield and clarity of pineapple juice. *J Food Sci Technol* 48:560–568. <https://doi.org/10.1007/s13197-010-0175-1>
- Pandya JJ, Gupte A (2012) Production of xylanase under solid-state fermentation by *Aspergillus tubingensis* JP-1 and its application. *Bioprocess Biosyst Eng* 35:769–779. <https://doi.org/10.1007/s00449-011-0657-1>
- Pérez-Rodríguez N, Oliveira F, Pérez-Bibbins B, Belo I, Agrasat AT, Domínguez JM (2014) Optimization of xylanase production by filamentous fungi in solid-state fermentation and scale-up to horizontal tube bioreactor. *Appl Biochem Biotechnol* 173:803–825. <https://doi.org/10.1007/s12010-014-0895-1>
- Rajavat AS, Rai S, Pandiyan K, Kushwaha P, Choudhary P, Kumar M, Chakdar H, Singh A, Karthikeyan N, Bagul SY, Agnihotri A (2020) Sustainable use of the spent mushroom substrate of *Pleurotus florida* for production of lignocellulolytic enzymes. *J Basic Microbiol* 60(2):173–184. <https://doi.org/10.1002/jobm.201900382>
- Raveendran S, Parameswaran B, Ummalyma SB, Abraham A, Mathew AK, Madhavan A, Rebello S, Pandey A (2018) Applications of microbial enzymes in food industry. *Food Technol Biotechnol* 56(1):16–30. <https://doi.org/10.17113/ftb.56.01.18.5491>
- Ravindran R, Williams GA, Jaiswal AK (2019) Spent coffee waste as a potential media component for xylanase production and potential application in juice enrichment. *Foods* 8(11):585. <https://doi.org/10.3390/foods8110585>
- Rosmine E, Sainjan NC, Silvester R, Alikunju A, Varghese SA (2017) Statistical optimisation of xylanase production by estuarine *Streptomyces* sp. and its application in clarification of fruit juice. *J Genet Eng Biotechnol* 15(2):393–401. <https://doi.org/10.1016/j.jgeb.2017.06.001>
- Sadaf A, Khare SK (2014) Production of *Sporotrichum thermophile* xylanase by solid state fermentation utilizing deoiled *Jatropha curcas* seed cake and its application in xylooligosaccharide synthesis. *Bioresour Technol* 153:126–130. <https://doi.org/10.1016/j.biortech.2013.11.058>
- Sharma HP, Patel H, Sugandha (2017) Enzymatic added extraction and clarification of fruit juices—a review. *Crit Rev Food*

- Sci Nutr 57(6):1215–1227. <https://doi.org/10.1080/10408398.2014.977434>
27. Sharma K, Thakur A, Goyal A (2019) Xylanases for Food Applications. In: Green Bio-processes. Springer, Singapore, pp 99–118. https://doi.org/10.1007/978-981-13-3263-0_7
 28. Sharma S, Bajaj BK (2018) Xylanase production from a new strain of *Aspergillus terreus* S9 and its application for saccharification of rice straw using combinatorial approach. Environ Prog Sustain Energy 37(3):1210–1219. <https://doi.org/10.1002/ep.12779>
 29. Sharma S, Sharma V, Nargotra P, Bajaj BK (2018) Process desired functional attributes of an endoxylanase of GH10 family from a new strain of *Aspergillus terreus* S9. Int J Biol Macromol 115:663–671. <https://doi.org/10.1016/j.ijbiomac.2018.04.096>
 30. Silva MDFGD, Dionísio AP, Abreu FAPD, Pinto CO, Silva LMAE, de Brito ES, Pontes DF (2018) Optimization of enzymatic treatment to produce yacon juice clarified by microfiltration with high levels of chlorogenic acid and fructooligosaccharides. J Food Process Preserv 42(6):1. <https://doi.org/10.1111/jfpp.13641>
 31. Singh S, Bajaj BK (2016) Bioprocess optimization for production of thermoalkali-stable protease from *Bacillus subtilis* K-1 under solid state fermentation. Prep Biochem Biotechnol 46:717–724. <https://doi.org/10.1080/10826068.2015.1135455>
 32. Sridevi A, Ramanjaneyulu G, Devi PS (2017) Biobleaching of paper pulp with xylanase produced by *Trichoderma asperellum*. 3 Biotech 7(4):266. <https://doi.org/10.1007/s13205-017-0898-z>
 33. Su LH, Zhao S, Jiang SX, Liao XZ, Duan CJ, Feng JX (2017) Cellulase with high β -glucosidase activity by *Penicillium oxalicum* under solid state fermentation and its use in hydrolysis of cassava residue. World J Microbiol Biotechnol 33:37. <https://doi.org/10.1007/s11274-016-2200-7>
 34. Torres JMO, dela Cruz TEE (2013) Production of xylanases by mangrove fungi from the Philippines and their application in enzymatic pretreatment of recycled paper pulps. World J Microbiol Biotechnol 29(4):645–655. <https://doi.org/10.1007/s11274-012-1220-1>
 35. Verma D, Satyanarayana T (2012) Molecular approaches for ameliorating microbial xylanases. Bioresour Technol 117:360–367. <https://doi.org/10.1016/j.biortech.2012.04.034>
 36. Walia A, Guleria S, Mehta P, Chauhan A, Parkash J (2017) Microbial xylanases and their industrial application in pulp and paper biobleaching: a review. 3 Biotech 7(1):11. <https://doi.org/10.1007/s13205-016-0584-6>
 37. Yegin S, Altinel B, Tuluk K (2018) A novel extremophilic xylanase produced on wheat bran from *Aureobasidium pullulans* NRRL Y-2311-1: effects on dough rheology and bread quality. Food Hydrocoll 81:389–397. <https://doi.org/10.1016/j.foodhyd.2018.03.012>
 38. Zhao XQ, Zhang XY, Zhang F, Zhang R, Jiang BJ, Bai FW (2018) Metabolic engineering of fungal strains for efficient production of cellulolytic enzymes. In: Fungal cellulolytic enzymes. Springer, Singapore, pp 27–41. https://doi.org/10.1007/978-981-13-0749-2_2
 39. Zimbardi ALRL, Sehn C, Meleiro LP, Souza FHM, Masui DC, Nozawa MSF, Guimaraes LHS, Jorge JA, Furriel RPM (2013) Optimization of β -glucosidase, β -xylosidase and xylanase production by *Colletotrichum graminicola* under solid-state fermentation and application in raw sugarcane trash saccharification. Int J Mol Sci 14:2875–2902. <https://doi.org/10.3390/ijms14022875>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.