Xylanase Production on Pretreated Date Seed Powder in Solid State Fermentation by *Penicillium citrinum*

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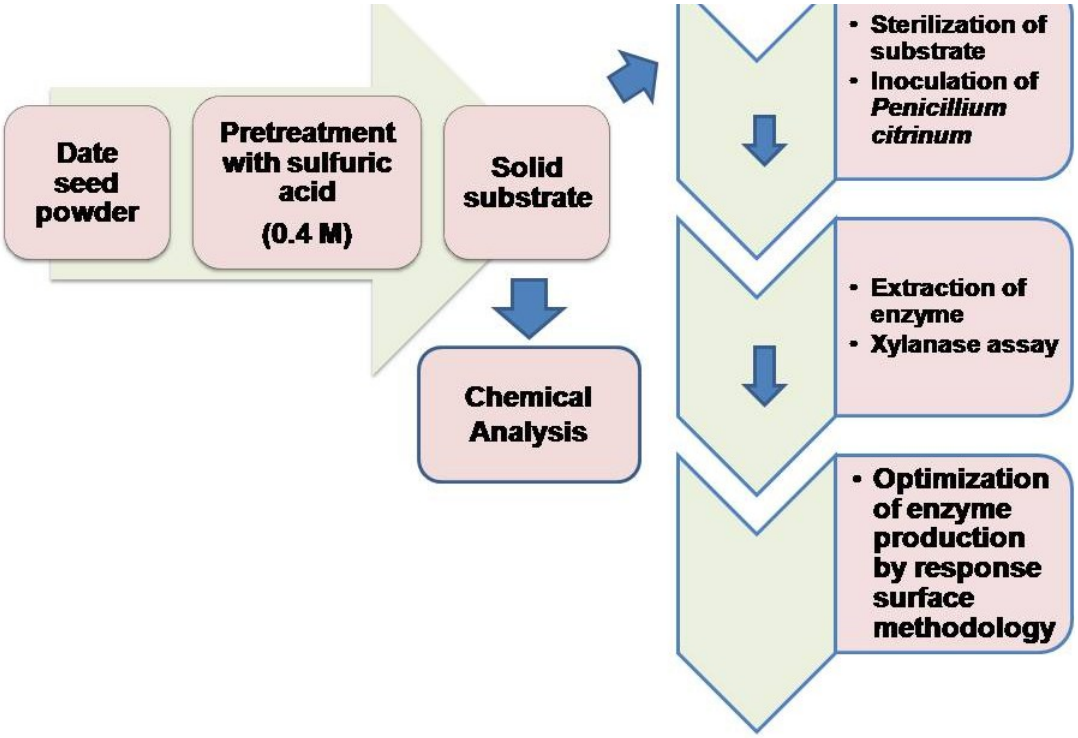
GRAPHICAL ABSTRACT



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Xylanase has been used for bioconversion processes and has been applied in several industrial processes. The increased production cost of enzymes remains the bottleneck for commercial production of lignocellulosic enzymes. The application of agricultural waste as a culture medium is a major strategy to improve xylanase production and reduce production costs. In this study, date seed powder was pretreated with sulfuric acid, and the cellulose, hemicellulose, and lignin contents were 24.4±0.12%, 43.1±1.3%, and 23.5±0.3%, respectively. The moisture content of the date seed powder was 7.49±0.12%. The pretreated date seed powder was used as the substrate for xylanase production by *Penicillium citrinum* solid-state fermentation. The moisture content, pH, and inoculum were optimized for xylan production, and the optimum conditions were 45% moisture content, pH 5.5, and 3% inoculum concentration. The maximum xylanase production was found to be 605.3 U/g.

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Keywords: Date seed powder; Cellulose; Culture medium; Solid-state fermentation; Fungus; Xylanase

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INTRODUCTION

Phoenix dactylifera L., commonly called date palm, is a widely cultivated plant throughout the world, especially in semiarid and arid regions (Al-Farsi and Lee 2008). This is one of the major fruit crops in Arabian countries and African countries, and the environment facilitates the growth of this plant (FAO 2020). Date palm seeds are considered waste, and date processing industries generate millions of tons of date seeds (Al-Shahib and Marshall 2003); the estimated date production was 8.52 million tons, and the date seed constituted 10% of the total weight. Hence, the estimated production of date seed was 852,000 tons in 2018. Seeds are rich in several nutrients and are used as dietary fiber supplements. The material contains significant quantities of proteins (5 to 6%), fats (10 to 12%), and fibers (75 to 80%) (Besbes *et al.* 2004). In date seeds, hemicelluloses and cellulose are composed of approximately 20% and 50% total carbohydrates, respectively (Bouaziz *et al.* 2010). The date seeds presented significant amounts of various fiber fractions, especially neutral detergent fibers, acid detergent fibers, cellulose, hemicelluloses, and lignin. Neutral detergent fiber is a mixture of cellulose, hemicellulose, and lignin that contributes 10 to 75% of the material. Acid detergent fiber is a mixture of lignin and cellulose and contributes 39 to 57% of the total. The lignin content of date seeds is approximately 7 to 11% (Alkhoori *et al.* 2022). Because of the relatively high fiber content of date seeds, they are disposed of in several countries and are also used as

ingredients in the preparation of animal feed. In addition, it was used for the preparation of animal feed for goats, broiler chickens, cattle, and fish. In animal feed, the supplemented date seeds improved plasma estrogen and testosterone levels and improved animal growth. In addition to these nutrient factors, date seeds are rich in phenolic compounds and antioxidant molecules (Suresh *et al.* 2013).

Dates are mainly comprised of dietary fibers and fermentable sugars. They can be used as the major source of nutrients and carbon sources for the production of several enzymes through microbial production. The microbial fermentation process is a useful technology for the production of several value-added products, including enzymes, carboxylic acids, biofuels, single-cell proteins, and amino acids. Date seed powder has been used as the substrate for the production of exopolysaccharides *via* the bacterium *Bacillus subtilis*. The culture conditions were optimized, and improved exopolysaccharide production was achieved (Yousef *et al.* 2020). Date seed waste was mixed with peapod extract and used as a substrate to produce endoglucanase in solid-state fermentation (SSF) *via Lactobacillus casei*. Furthermore, the enzyme production strategy was optimized, and a maximum of 17.2 U/mL enzyme was reported cellulase *via Cellulomonas uda* (Swathy *et al.* 2020), and improved enzyme production was achieved *via* a statistical approach.

Date fruit waste is rich in soluble sugar (70%), as well as fatty acids, proteins, minerals, and vitamins. Hence, date fruit waste, including date seed can be utilized as a substrate for the production of enzymes (Bahkali *et al.* 2023). The valorization of date seed waste is essential for reducing its environmental impact. It generates greenhouse gas upon landfilling and contributes to climate change. The utilization of date seed powder is helpful for improving the sustainability of date processing industries and improving the circular economy. Landfilling date seed waste can be expensive, so the use of alternate methods to convert date seed powder into value-added products may reduce the production cost of enzymes (Al-Mardeai *et al*. 2023).

Agroindustrial residues are generally considered suitable for bioconversion into useful products through SSF. The organic content of agricultural waste is high and is applied as useful substrates to produce novel industrially and ecologically important products through SSF. Agricultural wastes are used for the production of biofuels, bioremediation agents, biocontrol agents, and commercial enzymes (Chilakamarry *et al.* 2022). During date harvesting, packing, transporting, and syrup extraction, enormous amount of waste is generated. In addition, poor quality dates are discarded or used for the preparation of animal feed. However, significant amounts of low quality dates and separated date seeds were not utilized and directly discarded to the environment (Khorshidian *et al.* 2024). In this regard, the present study aimed to utilize date seed powder for the production of xylanase in SSF.

EXPERIMENTAL

Date Seeds

Date seeds (*Phoenix dactylifera* L.) (GharsSouf cultivar) were separated from the ripe date fruits (2 kg). The seeds were initially soaked in tap water and subsequently washed with demineralized water for the removal of flesh from the seeds. The samples were dried (sun dried) for two days and subsequently oven-dried at 80 °C for 24 h. The samples were ground using a mixer grinder and sieved through a 200-µm sieve. The larger particles were

further ground *via* a heavy-duty grinder and sieved to achieve a particle size of less than 200 μm.

Chemical Analysis of Date Seeds

Removal of extractives content

The extractives (E) of date seeds include several low-molecular-weight carbohydrates, salt fats, and waxes. To remove them from the substrate, 20 ± 1 g of date seed powder was placed in a Soxhlet apparatus under reflux for 4 h with 300 mL of a toluene/ethanol (1:2) mixture. The sample was dried in an oven at 120 °C for 24 h. The extracted sample was cooled to room temperature and weighed. It was further placed in a vacuum desiccator and weighed.

Analysis of Insoluble and Acid Soluble Lignin

The seed powder (5 g) was placed in a 500-mL Erlenmeyer flask and mixed with 25 mL of (v/w) 72% sulfuric acid. The mixture was subsequently placed in a water bath for 60 min at 20 ± 1 °C. Then, 275 mL of double distilled water was added, and the temperature of the water bath was increased to 100 °C and refluxed for 6 h. The mixture was filtered *via* a preweighed (m_1) fritted glass filter with 16to40 μm porosity. The insoluble fractions were separated from the acid soluble fraction. The insoluble fraction obtained on the filter was gently collected, suspended in hot water for 4 h and subsequently dried for 12 h at 110 °C. The mixture was cooled to room temperature, and the weight m_2 was obtained (m_2 = insoluble matter + filter). The insoluble lignin content was calculated *via* the following Eq. 1, and the percentage dry mass was obtained,

$$\text{Insoluble lignin, \%} = \frac{(m_2 - m_1)}{m_0} \times (100 - E(\%)) \quad (1)$$

where E is extractives. The acid-soluble fraction obtained after filtration was subjected to spectrophotometer analysis at 280 nm. The soluble lignin content was calculated *via* the following Eq. 2:

$$\text{Soluble lignin, \%} = \frac{\left(\frac{\text{Absorbance at 280 nm} \times \text{Dilution factor}}{20} \times 100\right)}{1000 \times \text{weight of sample}} \quad (2)$$

Analysis of Holocellulose

The amount of holocellulose (hemicelluloses and cellulose) was tested by mixing 5 g of the free extractive sample (m_0) with 95 mL of hot water. The mixture was placed in a water bath at 70 °C, 2.6 mL of 25% of sodium chlorite solution was added every 60 min, and 0.5 mL of glacial acetic acid was added until 8 h of treatment. The solution was subsequently filtered to differentiate the insoluble fraction from the soluble fraction *via* a preweighed (m_1) crucible filter. The insoluble fraction was further dried at 110 °C overnight. It was cooled *via* vacuum desiccators and weighed (m_2). The amount of holocellulose was calculated using Eq. 3, and the result was expressed as % dry weight:

$$\text{Holocellulose content (HOL), \%} = \frac{(m_2 - m_1)}{m_0} \times (100 - E(\%)) \quad (3)$$

Analysis of Cellulose and Hemicelluloses

The holocellulose obtained after the initial fractionation experiment was further separated into hemicelluloses and cellulose. Briefly, 2 g of holocellulose sample (m_0) was mixed with 10 mL of 17.5% sodium hydroxide. Then, 5 mL of 17.5% sodium hydroxide

was added continuously every 5 min until the volume reached 25 mL. Then, 40 mL of double distilled water was added to the mixture and stirred continuously for 60 min. The solid fraction was considered cellulose, and it was separated from the mixture by filtration using a preweighed (m_1) fritted glass filter with 40to100 µm porosity. The cellulose fraction was washed continuously with 8.3% sodium hydroxide and subsequently washed with double distilled water. Then, the mixture was soaked for 3 min in 30 mL of 10% acetic acid and washed with water continuously to reach a neutral pH. Then, m_2 of the set filter/cellulose was calculated by drying the material at 110 °C for 12 h. The amount of cellulose was evaluated, and the result was expressed as a percentage.

$$\text{Cellulose content (CEL)}, \% = \left(\frac{(m_2 - m_1)}{m_0}\right) \times \text{HOL (\%)} \quad (4)$$

The amount of hemicellulose was determined based on the differences between the levels of cellulose and holocellulose, and the result was expressed as a percentage:

$$\text{Hemicellulose (HEM)}, \% = \text{HOL (\%)} - \text{CEL (\%)} \quad (5)$$

Isolation of Cellulose from the Date Seed Powder

The date seed powder (25 g) was soaked in 95% ethanol (400 mL) for 24 h at 4 °C under constant stirring. The date seed powder was subsequently retained, mixed with double distilled water and heated for 2 h. The powder was subsequently filtered through Whatman no. 4 filter paper. The solid residue was recovered and boiled with 1 N sodium hydroxide for 2 h. The residue was subsequently filtered with Whatman number 4 filter paper, the filtrate consisted of hemicelluloses, and the solid residue was the source of cellulose. The sample was neutralized with double distilled water, and the cellulose was dried at room temperature. The yield of cellulose was calculated using Eq. 6:

$$\text{Cellulose yield}, \% = \frac{\text{Recovered mass after drying}}{\text{Weight of date seed (g)}} \times 100 \quad (6)$$

Culture of *Penicillium citrinum* MTCC 9620 and Inoculum Preparation

The fungal strain *Penicillium citrinum* MTCC 9620 was obtained from Microbial Type Culture Collection, Pune, India. It was cultured in potato dextrose broth medium and incubated at 28 °C for three days. The pH of the culture medium was adjusted to 6.5 before sterilization. After three days, the spore suspension was harvested, and the content was adjusted with 0.01% (w/v) polysorbate 80 (Tween 80) solution with continuous stirring for 60 min. The spores were counted *via* a microscope equipped with a Neubauer chamber.

Solid-State Fermentation (SSF)

The pretreated date seed powder was used as a substrate for xylanase in the SSF. The substrate pretreatment was performed using 4% sulfuric acid. The pretreated date seed powder was used to improve enzyme production. About 5 g medium was moistened with 2.5 mL of phosphate buffer (pH 6.0, 0.1 M). The pretreated material was inoculated with 2×10^8 spores. The mixture was stirred manually for 10 min to ensure complete mixing. The Erlenmeyer flasks were incubated at 30 °C for five days, and an enzyme assay was performed every 24 h.

Xylanase Assay

The fermented solid substrate was used as the source of enzyme. To the fermented solid, 50 mL of 0.05 M citrate buffer (pH 5.5) was added, and the mixture was incubated on a rotary shaker. Then, the mixture was centrifuged at 5000 rpm for 10 min. The resulting supernatant was used as the source of the enzyme. Xylanase activity was determined by detecting the release of reducing sugars from xylan *via* the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). Xylose was prepared at various concentrations and used for the preparation of standards. To determine enzyme activity, 0.2 mL of enzyme mixture was incubated with 1% beech wood xylan (prepared in citrate buffer, pH 5.5) for 10 min at 37 °C. Then, 0.5 mL of DNA mixture was added, mixed, and placed in a boiling water bath. The mixture was cooled, and 5 mL of double distilled water was added. The optical density of each sample was read at 540 nm *via* a UV–visible spectrophotometer. One unit of xylanase (U) was defined as the amount of enzyme that released 1 µmol of xylose/min under standard assay conditions.

Effects of Bioprocess Conditions on Xylanase Production by *Penicillium citrinum*

The pretreated date seed powder was used as the source of carbon and energy. The effects of the fermentation period, incubation temperature, initial medium pH, moisture content, and inoculum concentration of the fungal suspension on xylanase production were assayed *via* a one-way approach. The pH of the solid substrate was adjusted with buffers at 1.0-unit increments before autoclaving (pH 4.5 to 6.5). The moisture content of the culture medium was maintained between 30% and 60% in increments of 5%. The incubation temperature ranged from 20 to 50 ℃. To determine the optimum inoculum concentration, different fungal inoculum concentrations ranging from 1% to 5% (v/w) were used. The culture was incubated at 30 ℃ for three days, and crude xylanase was extracted from the fermented medium. After incubation period, 50 mL of citrate buffer (0.05 M, pH 5.5) was added, and the Erlenmeyer flask was kept on a rotary shaker for 30 min at 150 rpm. Then it was centrifuged at 5000 rpm for 10 min and the supernatant was used as the crude enzyme.

Central Composite Design and Response Surface Methodology

The effects of the moisture content of the medium, pH, and inoculum concentration on xylanase production were studied *via* CCD with 20 experimental runs. The selected variables were analyzed at three different levels. The experiments included 8 axial points and 6 replicates of the central points, and the variables were analyzed at three different levels (−1, 0, +1). The coded values of the experimental variables and their levels are illustrated in Table 1. The statistical software Design-Expert (Version 8.0.1, State-Ease, Minneapolis, MN, USA) was used to perform the experiments and analyze the response surface graphs. The experimental results were fitted with a second-order polynomial equation, and the individual, quadratic and interactive effects were analyzed *via*Eq.7,

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (7)$$

where Y is the predicted enzyme activity, β_0 is an offset term, β_1 has a linear effect, β_{11} has a square effect, β_{12} has an interactive effect, and A, B, and C are independent variables.

Table 1. Coded Variables and Their Levels for Xylanase Production in Solid-State Fermentation

Factor	Name	Units	Low Actual	High Actual	Mean
A	Moisture	%	30	60	45
B	pH		4.5	6.5	5.5
C	Inoculum	%	1	5	3

Statistical Analysis

The SSF bioprocess conditions were optimized by RSM and CCD using design expert software (version 8.0.1, Stat Ease Inc., Minneapolis, MN, USA). A total of 20 experiments were performed. The designed model, statistical results, and equations were validated using ANOVA ($p<0.05$) at 95% confidence level. The optimum response (enzyme yield, Y) was predicted, and triplicate experiments were performed to validate the experimental design.

RESULTS AND DISCUSSION

Characterization of Date Seed Powder

The chemical composition of the date seed powder was analyzed. The moisture content was 7.49±0.12%, and the observed result was similar to that of a previous report (Hamada *et al.* 2002).The cellulose content of the date seeds was 24.4±0.12%, and the hemicellulose content was 43.1±1.3%. The lignin content was significantly lower (23.5±0.3%) than the cellulose and hemicellulose contents. Date seed powder is composed of 0.86% ash, 5.25% protein, and 8.06% fat (Jahan *et al.* 2023). The water-holding capacity of date seed powder ranges from 5.96 to 6.87 g/g dry matter, the soluble dietary fiber content ranges from 2.8 to 3.5%, and the insoluble dietary fiber content ranges from 82.1 to 84.4% (Gökşen *et al.* 2018). Elnajjar *et al*. (2018) determined the proximate composition of date seed powder and reported 1 to 2% moisture, 20 to 30% carbohydrate, 1 to 5% volatile matter, 210 to 20% protein, and 2 to 5% ash contents. On the basis of the present study and previous reports, palm seed powder is rich in high energy density and is a potential source for the production of enzymes and energy.

Effect of Pretreatment on Xylanase Yield

Biomass pretreatment was used to reduce cellulose crystallinity. The sulfuric acid pretreatment method increased the maximum availability of cellulose to microorganisms (Sen *et al.* 2016). The date seed powder was ground into fine particles between 0.1 and 1.5 mm to increase the efficiency of acid pretreatment. Jadhav and Dey (2025) used 3% potassium hydroxide and autoclaving procedure to hydrolyze the water hyacinth biomass. The xylose yield was 0.253 g/g of water hyacinth at 2% biomass concentration after 20 min of treatment. The combination of hydrothermal and dilute acid (0.5% (v/v) sulfuric acid) pretreatment method increased the soluble fraction of carbohydrates from date press cake. The reaction time, and temperature ranges were 60 to 90, and 80 to 140 °C, respectively (Oladzad *et al*. 2024). As described in Fig. 1, increased production of xylanase (73.2 ± 1.2 U/g) was observed in the biomass treated with 0.4 M H_2SO_4compared with the 0.1 and 0.2 M H_2SO_4. In the 0.6 M H_2SO_4 treatment, xylanase production slowly decreased with *Penicillium citrinum*. However, the untreated control had a value of only 29.1 ± 0.8

U/g, and this result revealed the importance of pretreatment with biomass. The optimum acid and alkali concentrations are essential for the effective removal of lignin from the biomass and biomass conversion process. The increased concentration of acid hydrolyzes and dissolves cellulose and other sugars which reduced the availability of substrate for enzyme production (Wang *et al*. 2024). The enzyme activity observed in this study was consistent with previous results (Bhardwaj *et al.* 2021; Chaudhary *et al.* 2021). Alkaline-peroxide-pretreated sugarcane bagasse showed 850 U/gds xylanase (Lin *et al.* 2021). *P. purpurogenum* was cultured using alkali-pretreated corn cobs and produced 1097 U/gds xylanase. The mixture of pretreated wheat bran and sugarcane bagasse was used, and the xylanase yield was 10 U/gds. *Trichoderma reesei* NCIM 1186 and *P. citrinum* NCIM 768 were grown in steam-pretreated wheat bran, and the yield was 6.71 FPU/gds (Camassola and Dillon 2007; Lodha *et al.* 2020; Sunkar *et al.* 2020).

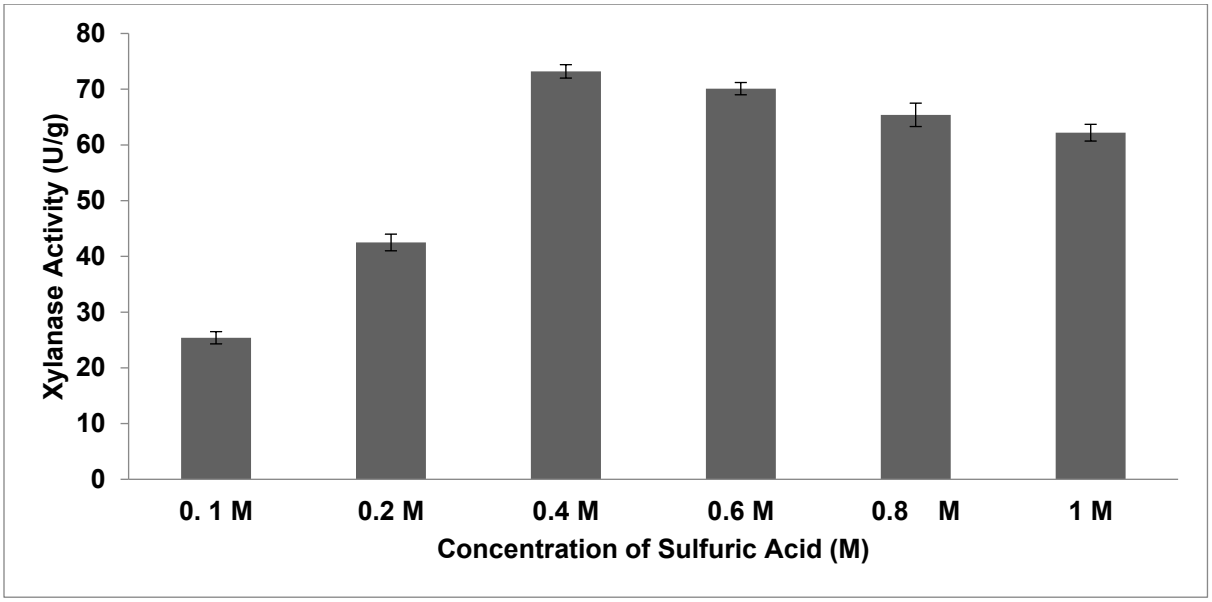

Fig. 1. Pretreatment of date seed powder with various concentrations of sulfuric acid

Effect of Fermentation Period on Xylanase Production

Xylanase production was monitored for 120 h, and after 24 h of incubation, the enzyme activity was 4.2 ± 0.13 U/g. It increased after 48 h (35.2 ± 1.2 U/g) of fermentation, and enzyme production was more or less similar after 120 h of incubation. After 120 h of incubation, the xylanase activity was 96.5 ± 1.4 U/g. During the fermentation period tested, no apparent loss of xylanase activity was observed (Fig. 2). The increased production of xylanase using pretreated date seed powder could be significantly related to the availability of monosaccharides released during the process of acid hydrolysis. Moreover, increased production may also be related to the release of oligosaccharides, which are considered the true inducers of xylanase production (Rastegari 2018; Najjarzadeh *et al.* 2020). In *Aspergillus flavus* and *A. fumigatus* F-993, optimum xylanase production was achieved within two days of incubation in wheat bran, white corn flour, and pearl millet stover under SSF (Fadel *et al.* 2014; Gautam *et al.* 2015; Ezeilo *et al.* 2020).

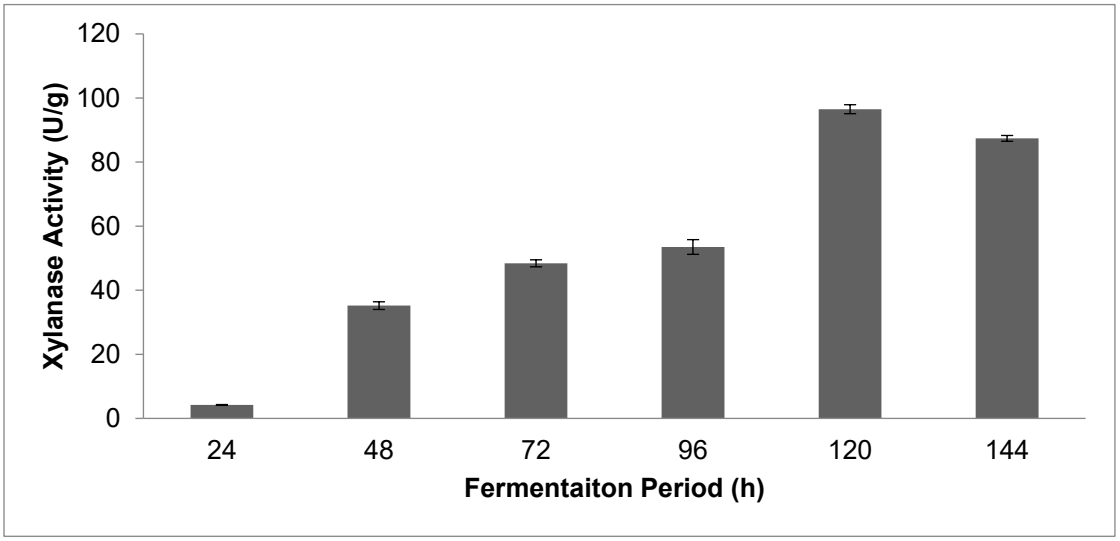

Fig. 2. Effect of fermentation period on xylanase activity by *P. citrinum* in solid-state fermentation using date seed waste as a substrate.

Effect of Spore Concentration on Xylanase Production

To determine the effect of spore concentration on xylanase production, date seed powder was inoculated with fungal spore suspensions at various concentrations (0.5×10^7 spores/g to 3×10^7 spores/g) in the SSF. Xylanase production was initially low at 0.5×10^7 spores/g and increased significantly at 2×10^7 spores/g (94.3 ± 0.7 U/g) (Fig. 3). Furthermore, the increase in inoculum level decreased enzyme production, which might be attributed to less spore germination due to very high spore density in the solid medium. As described previously by Gillot *et al*. (2016), *P. camemberti* spore germination was associated with self-regulated quorum sensing, and an inoculum concentration of1×10^6 spores/mL was reported as the optimum concentration. In the present study, an inoculum size of 2×10^7 spores/g was found to be optimal for xylanase production.

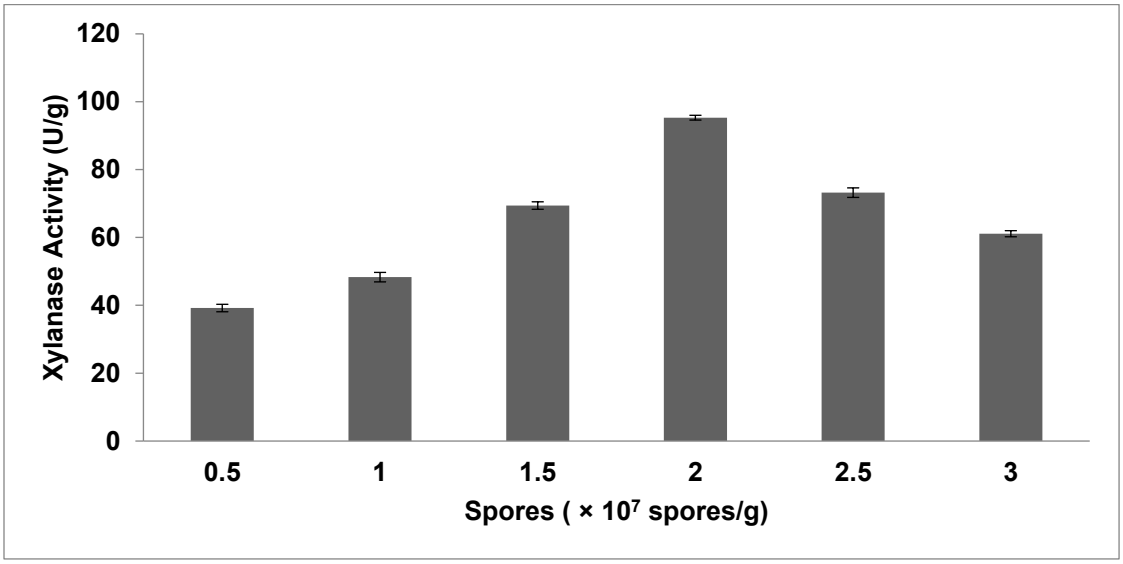

Fig. 3. Effect of the fungal spore suspension on xylase activity. The fungal spores were treated at various concentrations, and enzyme activity was assayed.

Effect of Moisture on Xylanase Production

The impact of the moisture content of the date seed powder on xylanase production by the fungal strain was determined. The results are shown in Fig. 4, and xylanase activity reached a maximum at 55% moisture content. The excess water content reduces the growth rate of fungal spores, and the reduction in water content is not enough to transport heat and air from the solid medium, resulting in reduced xylanase activity. An optimum moisture content is required for fungal growth and enzyme production. The reduced moisture level poorly supports the growth of fungal spores, whereas the increased moisture content affects the transport of metabolic products in SSF (Pokorny *et al.* 1997; Luo *et al.* 2022; Cai and Yang 2023).

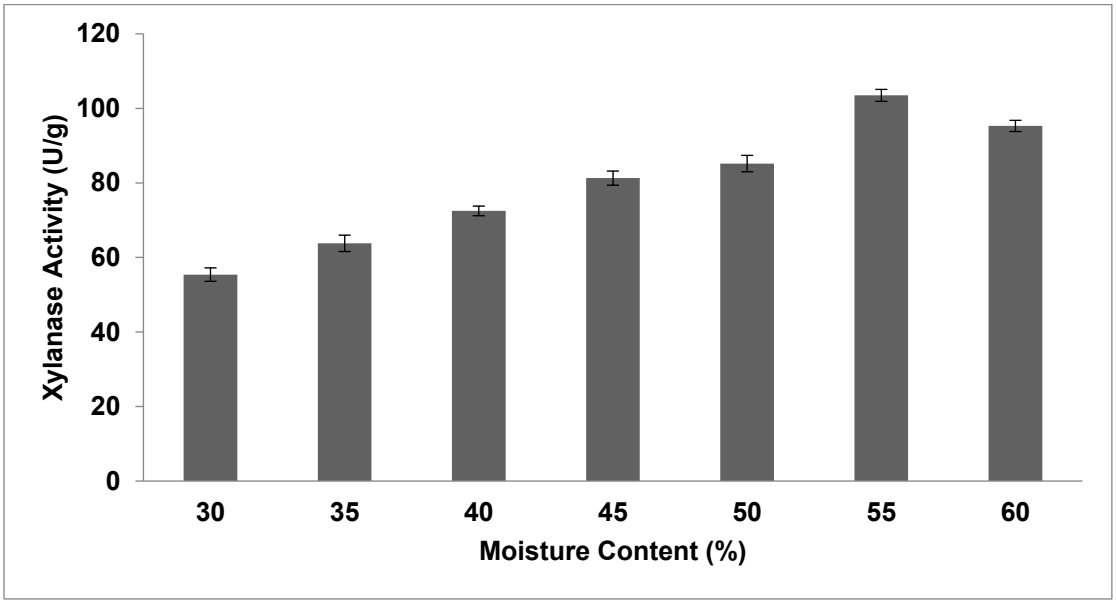

Fig. 4. Effect of moisture content on xylanase production by *P. citrinum* in solid-state fermentation using date seed waste as a substrate

Effect of Temperature on Xylanase production

Fungal fermentation is highly sensitive to culture temperature, and the optimum temperature is required for better fungal growth and metabolite production. In SSF, temperature is a major factor affecting microbial growth and the biosynthesis of enzymes (Mandal and Ghosh 2018). As shown in Fig. 5, the incubation temperature significantly influenced xylanase production. When the culture was incubated at 30 °C, xylanase activity reached its maximum (Fig. 5). The decreased production of xylanase at lower incubation temperatures may be due to the low level of nutrient transfer in the cell membrane; however, increased temperatures could significantly reduce the microbial growth rate due to the denaturation of intracellular enzymes. The optimum temperature for maximum xylanase activity was 30 °C (Irfan *et al.* 2014; Gautam *et al.* 2015). Moreover, the optimum temperature was reported to be 35 °C for *A. fumigatus* MS16 based on laboratory process conditions (Zehra *et al.* 2020).

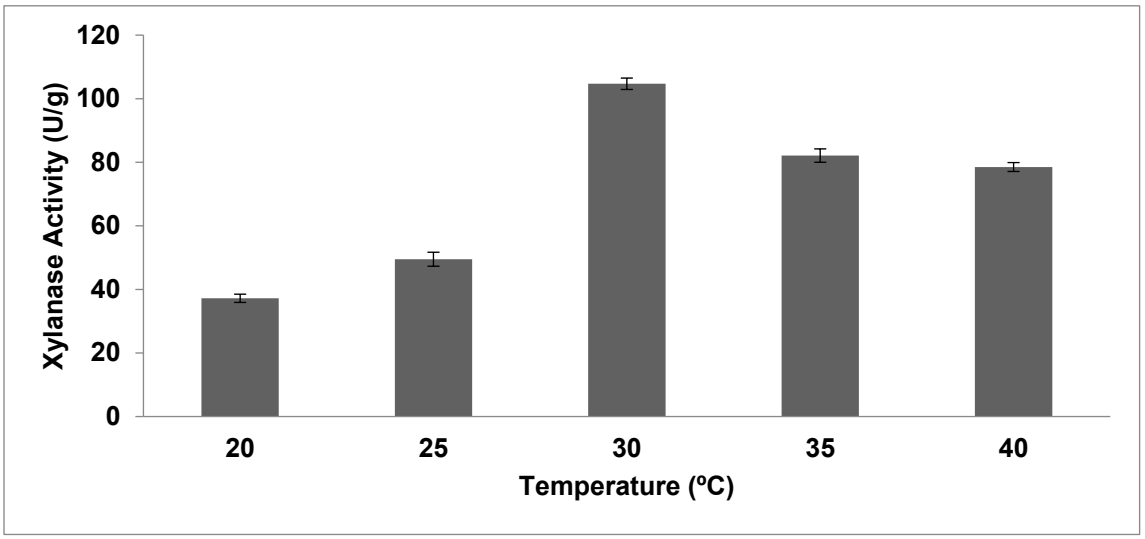

Fig. 5. Effect of temperature on the enzyme activity of *P. citrinum* in solid-state fermentation using date seed waste as a substrate

Optimization of Xylanase Production *by Penicillium citrinum*

Three variables (pH, moisture, and inoculum) were chosen for optimization of xylanase production using CCD and RSM.ANOVA made it possible to test the significance of the quadratic model built with the experimental results. The model F - value was 177.8, indicating that the designed CCD model was statistically significant. The coefficient value was obtained on the basis of the central composite design. Table 2 shows the 20 experimental trials with multiple combinations of the three variables along with the final responses. As described in Table 2, significant variation (enzyme activity) was found, depending on the composition of the selected variables. The maximum production of xylanase was determined to be 605.3 U/g at run number 14, and the corresponding moisture content was 45%, the pH was 5.5, and the inoculum concentration was 3%. The analysis of variance results revealed that the fitted second-order polynomial variation of the designed model was 7.95%, indicating good reliability in the experiments performed. In this case, C, AB, A^2, B^2, and C^2 are significant model terms.

The goodness of fit of the model was checked by analyzing the determination coefficient (R^2) of the model. The adjusted R squared value was 0.988, the predicted R squared value was 0.96, and the adequate precision value was 34.1. The "Pred R-Squared" of 0.96 is in reasonable agreement with the "Adj R-Squared" of 0.988.The R^2 value was near 1.0, indicating very close agreement between the theoretical values predicted by the quadratic model and the experimental results, and the selected quadratic model was significant at the 5% level. Table 3 shows the analysis of variance results, F values, and corresponding p values of the interactive effects of the variables. In this study, a validation experiment was performed to evaluate the response predicted by the designed model. Additional experiments were performed, and the experimental value (613.5 U/g) was very close to the predicted response Y (607.5 U/g), which validated the experimental design. The amount of xylanase yield varied based on the solid medium, strain, pretreatment method of biomass and culture conditions. In agave bagasse medium, xylanase production was registered as 29,000 U/kg by *Penicillium citrinum* (Valle-Pérez *et al*. 2021) and the yield was 4260 U/g in water hyacinth medium inoculated with *Penicillium crustosum* (Espinoza-Abundis *et al*. 2023) in SSF.

Figure 6a shows the effects of pH and moisture on xylanase production by *P. citrinum*. When the pH and moisture content were low, xylanase production was negligible. However, xylanase production increased at higher moisture levels, and increased enzyme production was achieved at pH 6.0. Figure 6b shows that the effects of inoculum and moisture on xylanase production and inoculum concentration effectively improved xylanase production compared with the moisture content of the medium.

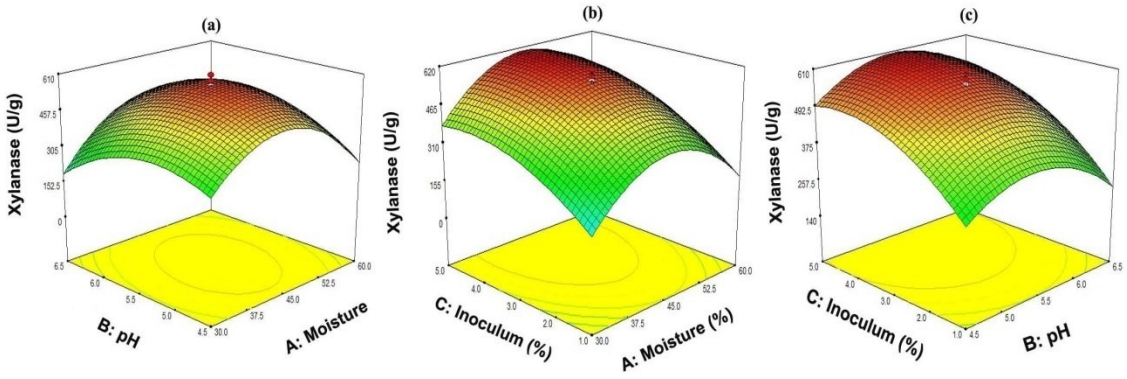

Fig. 6. Response surface graph of selected variables and their interactive effects on xylanase production by *P. citrinum* in solid-state fermentation using date seed waste as a substrate

Table 2. Central Composite Design Matrix with Experimental Results for Xylanase Biosynthesis by *P. citrinum*

Runs	Moisture (%)	pH	Inoculum (%)	Xylanase Activity (U/g)
1	30	4.5	5	304
2	30	4.5	1	116.8
3	45	5.5	3	579.2
4	30	6.5	5	216
5	45	3.8	3	307.4
6	45	5.5	0	140.1
7	60	6.5	1	98.3
8	45	5.5	3	561.3
9	45	5.5	6.3	554.8
10	19.7	5.5	3	4.9
11	45	7.1	3	218.3
12	60	4.5	5	255
13	45	5.5	3	549.4
14	45	5.5	3	605.3
15	45	5.5	3	562.1
16	60	6.5	5	377
17	60	4.5	1	56
18	45	5.5	3	548.6
19	30	6.5	1	1.1
20	70.2	5.5	3	11.4

Figure 6c shows the effects of inoculum and pH on xylanase production, and improved xylanase production was achieved at higher inoculum levels than the pH of the substrate. Desai and Iyer (2022) optimized xylanase production by *Aspergillus niger* DX-

23 in SSF using a corn cob waste substrate. The response surface methodology-optimized medium reached 306.12 ± 7.4 U/g under SSF conditions. Pal and Khanum (2010) optimized xylanase production in solid-state fermentation by *Aspergillus niger* DFR-5 using a wheat bran substrate. The optimum moisture contents were 70% and 40°C, and the samples were incubated for 6 days. de Carvalho *et al*. (2023) recently optimized xylanase production in solid-state fermentation by *Aspergillus oryzae*, and the optimum conditions for xylanase production were 100 h, 55% humidity, and 25 °C. The optimized culture medium increased xylanase production by 1.65-fold. Morán-Aguilar *et al*. (2023) used brewery spent grain for the production of xylanase by *Aspergillus niger* CECT 2700. The optimum enzyme production was achieved after 5 days of fermentation, with 80% moisture content.

Table 3. Analysis of Variance for Xylanase Biosynthesis by *P. citrinum*

Source	Sum of Squares	df	Mean Square	F Value	p value Prob > F
Model	931833.5	9	103537.1	177.83	< 0.0001
A-Moisture	1858.889	1	1858.889	3.192733	0.1043
B-pH	2622.471	1	2622.471	4.504223	0.0598
C-Inoculum	182156.5	1	182156.5	312.8627	< 0.0001
AB	16928	1	16928	29.07467	0.0003
AC	714.42	1	714.42	1.227051	0.2939
BC	1441.845	1	1441.845	2.47644	0.1466
A^2	571394	1	571394	981.3974	< 0.0001
B^2	171442.6	1	171442.6	294.4611	< 0.0001
C^2	90305.63	1	90305.63	155.1044	< 0.0001
Residual	5822.25	10	582.225		
Lack of Fit	3504.235	5	700.8469	1.511739	0.3306
Pure Error	2318.015	5	463.603		
Cor Total	937655.7	19			

CONCLUSIONS

1. Date seed powder is a cost-effective culture medium for the production of xylanase in solid-state fermentation. Sulfuric acid pretreatment was found to be effective. The pretreated seed powder presented increased amounts of cellulose, hemicelluloses, and lignin.
2. *Penicillium citrinum* was utilized with pretreated date seed powder for the production of xylanase. A low-cost culture medium was prepared, and the optimum culture conditions were optimized. The optimum culture conditions were 45% moisture content, pH 5.5, and 3% inoculum concentration.

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