CATRINA (2023), 29(1):47-56

Sustainable and Eco-Friendly Production of Xylanase and Other Enzymes from Tomato Paste through Solid-State Fermentation Using *Bacillus safensis* MABS6

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ABSTRACT

This study aimed to establish a sustainable and eco-friendly approach for the production of enzymes, including xylanase, exo-polygalacturonase (exo-PG), cellulase (CMCase), and α-amylase, using tomato pomace through solid-state fermentation (SSF) with *Bacillus safensis*. Kinetic studies revealed that xylanase and exo-PG exhibited their peak activities early in the fermentation process, reaching approximately 110 IU/mL and 70 IU/mL, respectively. In contrast, CMCase and α-amylase activities remained relatively constant, maintaining average levels of 19.8 IU/mL and 22.4 IU/mL throughout the fermentation process. Further investigations in a plate-type bioreactor unveiled the significant impact of aeration on enzyme activities. Aeration positively enhanced the activities of xylanase and CMCase, while it had an inverse effect on exo-PG and α-amylase activities. The focus of our study was to establish the optimal conditions for xylanase production, given its versatility and industrial value. The highest productivity was achieved at a pH of 5 and a temperature of 50 °C. Additionally, the presence of Mg+2 ions positively influenced enzymatic activity, whereas the presence of Hg^{+2} and Cu^{+2} ions acted as strong inhibitors. Furthermore, our results demonstrated the remarkable resilience and stability of xylanase across a wide pH range (pH 3-12) and temperatures (30 °C to 60 °C). Overall, these findings contribute to the development of greener enzyme production strategies and provide valuable insights into their widespread applicability across various industrial sectors.

Keywords: *Bacillus safensis* MABS6; Enzymes; Solid-state fermentation; Tomato paste; Xylanase production;

INTRODUCTION

Solid-state fermentation (SSF) offers several advantages over submerged culture fermentation, including cost reduction and smaller reactor sizes (Mussatto *et al*., 2012). Various agro-industrial leftovers such as wheat bran, soy bran, sugar cane bagasse, and citrus peels can be used as substrates in SSF (Mao *et al*., 2020; Li *et al*., 2020; Ejaz & Sohail, 2021; Giese *et al*., 2008; Ruiz *et al*., 2012). Notably, solid substrates like maize cobs, rice bran, rice husk, sunflower head, grape pomace, and wheat bran have been effectively employed for the generation of xylanolytic enzymes through SSF (Singh *et al*., 2008; Ghoshal *et al*., 2012).

Xylanases, also known as endoxylanases, are used in a variety of industrial processes, including bleaching kraft pulp in the pulp and paper industry (Nagar & Gupta, 2021), clarifying juices (Kaushal *et al*., 2021), extracting coffee (Acosta-Fernández *et al*., 2020), plant oils, and starch (Qiao *et al*., 2019), bioconverting lignocellulosic waste into their constituent sugars (Rana, 2022), improving the digestibility of animal feed (Cowieson *et al*., 2010), and many more. Xylanases represent a significant component of hemicellulases in the commercial sector and possess a global market value of around 200 million dollars (Guerrand, 2018).

Xylanases have gained recognition as crucial industrial enzymes due to their significant potential in various biotechnological applications across multiple sectors (Golgeri *et al*., 2022). Xylanases that exhibit favorable pH tolerance and thermotolerance have shown promise for use in numerous industrial processes (Tiwari *et al*., 2022). The utilization of xylanase enzymes is prevalent in the paper and pulp industry, particularly in the bleaching of kraft pulp (Immerzeel & Fiskari, 2023). To meet industrial standards, two approaches are commonly employed for obtaining xylanase: harnessing natural microbial diversity or utilizing genetic engineering methods to enhance xylanase synthesis in host cells (Yin *et al*., 2010; Khandeparker *et al*., 2017). Various microbiological sources of xylanase, including yeast, fungi, actinobacteria, and bacteria, have been documented in the literature (Battan *et al*., 2006). Numerous studies have reported the synthesis of xylanase using microbial systems through submerged fermentation (SmF). However, the solid-state fermentation (SSF) approach is preferred over SmF due to its numerous benefits in xylanase production, primarily attributed to the utilization of cost-effective substrates (Hiremath & Patil, 2011; Kamble & Jadhav, 2012).

The primary objective of this study was to evaluate the production of various hydrolytic enzymes with industrial applications using tomato pomace as a solid fermentation medium. Tomato pomace is an abundantly available waste material that possesses a comprehensive chemical composition, including proteins, lipids, carbohydrates, amino acids, carotenoids, and minerals. *Bacillus safensis* MABS6

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was chosen as the organism of interest due to its remarkable performance in synthesizing enzymes through solid-state fermentation (SSF) on different substrates (Alshawi, 2023). Xylanase, CMCase (cellulase), α-amylase, and exo-PG (pectinase) production were investigated in this study. Therefore, this research aimed to assess the potential of tomato pomace as a solid fermentation medium for the production of industrially relevant hydrolytic enzymes. The study also investigated the impact of aeration on enzyme synthesis using a laboratory-scale SSF bioreactor and thoroughly examined the conditions influencing xylanase activity, including pH, temperature, stability, and metal ion interactions.

MATERIALS AND METHODS

Propagation of xylanase-producing strain

Bacillus safensis MABS6, which was previously isolated and tested for its xylanase production ability (Alshawi, 2023), was cultured on a specific medium using the method described by Alshawi (2023). After the incubation period, the broth containing the inoculum was ready to be used.

Preparation of tomato pomace

Fresh tomatoes were obtained from a nearby market and processed to mimic the properties of agro-industrial waste. Following tomato pressing, the different tomato components (peel, seeds, and pulp) underwent a thorough washing with tap water. They were subsequently dried in an oven at 60ºC for 24 hours. The dried components were then stored at room temperature until they were required for subsequent use. Before utilization, the solid material underwent a milling and screening process to exclude particles smaller than 1 mm and larger than 3 mm.

Solid-State Fermentation: Enzymatic Catalysis and Extract

Bacillus safensis MABS6 was utilized in solid-state fermentations conducted on tomato pomace using both 250 mL conical flasks and a plate-type solid-state fermentation (SSF) bioreactor. In the first setup, 5 grams of solid material were dried, ground, and autoclaved. After sterilization, the solid material was inoculated with a freshly propagated bacterial culture (3%). The moisture content of the tomato pomace was adjusted to 70% w/w using a nutrient solution composed of 2.4 grams of urea and 9.8 grams of ammonium sulfate ($(NH_4)_2SO_4$) per liter. The nutrient solution included 2 grams of sulfuric acid (H_2SO_4), 5.0 grams of potassium dihydrogen phosphate (KH_2PO_4), 0.001 grams of heptahydrate $FeSO_4.7H_2O$, 0.0008 grams of $ZnSO_4.7H_2O$, 0.004 grams of $MgSO_4.7H_2O$, and 0.001 grams of $CuSO_4.5H_2O$. The pH of the nutrient solution was adjusted to 5 using diluted H_2SO_4 (Zehra *et al*., 2020), and pH values were regularly monitored using a conventional pH-meter with temperature adjustment.

To extract the fermented solid contents from multiple Erlenmeyer flasks (incubated at 28 ºC for 5 days), each flask was treated with 40 mL of Tween 80 (0.01%) at 24-hour intervals. This extraction process was performed using a rotary shaker operating at 150 rpm for 30 minutes at 4 ºC. After extraction, the resulting suspension was centrifuged at 20,000 rpm for 10 minutes at 4 ºC to separate the supernatant, referred to as the enzymatic extract, which was collected for further analysis. All experimental techniques were conducted in triplicate for robustness and uniformity. To ensure the characterization and stability of xylanase, the enzyme extracts were lyophilized and stored at 4 ºC until needed.

Bioprocessing: the plate-type SSF bioreactor

A single-use stirred tank bioreactor was assembled using five sterile 250 mL Roux flasks connected together to allow aeration. The reactor was connected to a filtered-air source, which introduced sterile air into the first flask and expelled it from the fifth flask. The air flow was measured using a rotameter. Subsequently, the air passed through a glass column for humidification and was sterilized using a cellulose filter with a pore size of 0.45 µm. The humidification system consisted of a glass column filled with 3 mm diameter glass beads, which prolonged the retention time of air inside the water. The bioreactor was placed inside a controlled environment chamber to regulate the ambient temperature at 28 ºC.

To investigate the impact of aeration on xylanase synthesis, two experimental setups were examined: aeration without humidification. The average air flow rate for the conducted trials was determined to be 120 L/h. Temperature and humidity probes from Hygropalm, a product of Rotronic Instrument Corporation based in New York, USA, were installed in the air phase above the solid substrate beds on the first and fifth flasks to monitor and evaluate temperature and humidity changes during the fermentation process. During the fermentation process, 10 grams of infected tomato pomace were added to each flask. Subsequently, the pomace was moistened to 70% (w/w) as mentioned earlier. The height of the bed in each flask was measured to be 1.3 cm. The highest levels of enzyme activity were observed during the initial five days of fermentation. Upon completion of the fermentation, the fermented solids from each flask were extracted using 80 mL of Tween 80 (0.01%) and then subjected to centrifugation. The resulting supernatant was used to quantify xylanase activity. All tests were conducted in triplicate (Leite *et al*., 2021).

Quantifying Enzymatic Activity

The enzymatic activity of xylanase, exo-polygalacturonase (Exo-PG), α-amylase, and carboxymethyl cellulase (CMC-ase) in the various extracts were measured. For the xylanase activity, 0.1 mL of enzymatic extract was mixed with 0.9 mL of xylan suspension (consisting of 0.5% w/w Birchwood xylan in a 0.1 M sodium acetate buffer with a pH of 5.0), and incubated at 50 ºC for 10 minutes. The evaluation of CMCase activity was conducted as described for xylanase, except the carboxymethyl-cellulose was used as the substrate (Botella *et al*., 2007; Díaz *et al*., 2010). The activity of α-amylase was measured by combining

0.9 mL of soluble starch (0.5% w/v in 0.25 M acetate buffer, pH 5.0) with 0.1 mL of enzymatic extract. The evaluation of Exo-polygalacturonase (Exo-PG) activity included the combination of 0.2 mL of enzymatic extract with 0.8 mL of a pectin solution containing 0.5% pectin in a 0.2 M acetate buffer at pH 5.0. The incubation period for the latter two enzymes was conducted at 45 ºC for 10 minutes (Warren *et al*., 2015).

The measurement of reducing groups produced by the different enzymes was performed using the 3,5-dinitro salicylic acid (DNS) technique (Nisa *et al*., 2021). The choice of standard for enzyme analysis depended on the specific kind of enzyme being studied. D-xylose, D-glucose, or D-galacturonic acid were used as standards and their measurements were compared to the respective standard curve. Enzymatic activity was quantified in international units (IU), where 1 IU was defined as the quantity of enzyme that could generate 1 µmol of reducing sugars per minute under the predefined conditions of the enzyme assay. The enzyme activities that were measured were then quantified and reported as international units per milliliter of extract (IU/mL). Initially, the research dug into the impact of temperature, pH, incubation duration, and the presence of various cations on xylanase activity. Following this, a further part of the investigation examined the influence of pH and temperature on the stability of xylanase activity during the storage period.

Factors influencing xylanase activity

The enzymatic extract obtained through Solid State Fermentation (SSF) for 5 days, as described previously, was subjected to lyophilization. To reconstitute the extract, 0.1 g of the lyophilized product was suspended in 50 mL of deionized water. The resulting solution served as the initial enzyme solution for studying various parameters that affect xylanase activity and stability. Enzyme activity quantification in these tests was performed using a recognized standard procedure (Long *et al*., 2019).

The impact of pH on xylanase activity was investigated following the method described by Díaz *et al*. (2007) with slight modifications. The buffers used in this study included sodium acetate (pH 3.0 to 5.0), citrate-phosphate (pH 5.0 to 7.0), tris-HCl (pH 7.0 to 8.5), and glycine-NaOH (pH 8.5 to 11.0).

The temperature effects on enzyme activity were examined within a range of 25 to 80 °C. The standard temperature used in the conventional test was initially set at 50 ºC, but it was modified to encompass the broader temperature range. Furthermore, the impact of incubation duration was investigated by exposing the crude enzyme solution to a temperature of 50 °C for 6 hours without the presence of substrate. At regular intervals, samples were extracted, rapidly chilled using an ice bath, and then analyzed for remaining xylanase activity using standardized protocols. This approach allowed for a comprehensive evaluation of the influence of incubation time on xylanase activity. To thoroughly evaluate the influence of various cations on xylanase activity, a mixture of 100 µL of the crude enzyme solution and 20 µL of a 0.1 M sodium acetate buffer solution was incubated at 50 ºC for 5 minutes. The experimental procedure involved using a saline solution (0.1 M sodium acetate buffer, pH 5.0) modified by the addition of one of the following salts at a concentration of 0.1 M: CaCl2, FeSO4, CuCl2, $(NH_4)_2SO_4$, $HgCl_2$, $MnSO_4$, $MgSO_4$, KCl, K_2SO_4, $BaCl_2$

Stability of xylanase activity

To optimize storage conditions, it was crucial to investigate the effects of pH and temperature on xylanase activity. The reconstituted lyophilized extract was mixed with various buffer systems, covering a pH range of 3.0 to 10 (0.01 g of extract in 5 mL buffer). The mixture was then incubated at 25 ℃ for twenty-four hours without any substrate, and the remaining xylanase activity was measured using predetermined parameters.

The thermal stability of the enzyme was assessed in the absence of substrates. The crude enzyme solution was incubated for one hour at six different temperatures, spanning the range of study from 30 to 70 ℃. After incubation, the level of residual xylanase activity was measured following the predetermined parameters.

Statistical Analysis

The collected data was subjected to statistical analysis using GraphPad Prism 9 software. The results are presented as means ± standard error (S.E.).

RESULTS

Biomanufacturing of Enzymes

Bacillus safensis MABS6 was cultivated using tomato pomace that was moistened to a level of 70%. This cultivation resulted in the production of substantial amounts of xylanase exo-PG, CMCase, and α-amylase (Figure 1 A-D). Xylase activity fluctuated during the whole observation period (Figure 1A). A decline in xylanase activity was first detected on the third day, and peaked until the fifth day, decline on the sixth day, followed by a gradual decline until the tenth day. Then the enzyme activity exhibited stable trend, with little variations until the fifteenth day. However, the lowest level of activity was recorded on the 13th day. The exo-PG (Figure 1B), a similar pattern was seen, with the peak activity occurring within a shorter timeframe, namely between the 4th and 6th days, and reaching a maximum level of 70 IU/mLon above mentioned days. The production of CMCase (Figure 1C) and α-amylase (Figure 1D) remained almost unchanged during the course of the fifteen days of fermentation, with respective average levels of 19.79 and 22.43 IU/ml.

Enzyme Synthesis in Plate-Type SSF Reactors

The enzymatic concentration obtained in conical flasks is shown in Table (1), along with the average values determined from the five plates-bioreactor. The measurements were recorded on the fifth day of the culture, signifying the time. The bioreactor data refer to

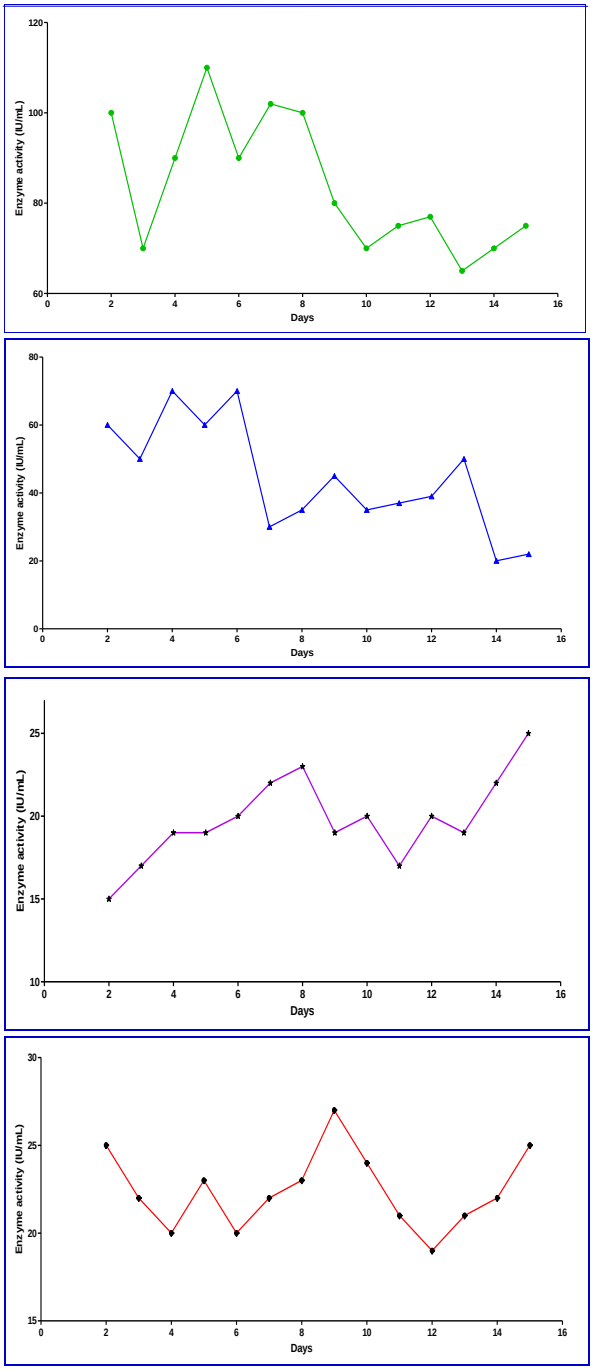

Figure (1): Enzymatic Activities during Batch Fermentations in Conical Flasks. Green line, Xylanase Activity Blue line, Exo-PG Activity; Violet line, CMCase Activity and Red line, α-Amylase Activity.

the average activity observed across the five plates. No statistically significant changes were observed among the plates under the experimental circumstances. Over the course of the five-day fermentation process, the temperature phase exhibited a high degree of stability, ranged from 28 to 29 °C. However, no significant fluctuations were detected between the air inlet and outlet. Significant differences were observed while using dry air. The humidity level at the air inlet was measured to be roughly 56%, however the outflow exhibited a notable rise to 86%. Nevertheless, even in situations when the air was not humidified, the humidity levels inside the solid substrate remained consistently over 50% during the 5-day culture period in all five plates. The observed amount of humidity is deemed enough for supporting the proliferation of the bacterial strain.

As shown by the data presented in Table (1), the introduction of aeration had a significant and beneficial impact on the production of both xylanase and CMCase. In the case of xylanase, the use of water-saturated aeration demonstrated a 1.8-fold improvement in productivity, whilst the use of dry air resulted in a 1.6-fold increase, similar to the values seen in the conical flask configuration without forced aeration. In the context of CMCase, the observed activity exhibited a 1.4-fold augmentation when exposed to air saturated with water, and a 1.5-fold augmentation when exposed to dry air. In contrast, the synthesis of exo-PG and α-amylase was shown to be adversely affected by aeration, resulting in a 50% decrease in their respective activity.

It is worth noting that in the plate-type bioreactor, the fulfillment of these maximums may occur sooner or later, depending on the individual enzyme being considered. Comparsion among different enzyme test was also evaluated which reveals that xylanase enzyme was the most active enzyme tested along a time period of 15 days (Figure 2).

Analysis of factors influencing xylanase activity

pH

The analysis of factors influencing xylanase activity revealed a distinct pH impact profile, as depicted in Figure (3A). Curve fitting analysis demonstrated that xylanase activity reached its peak at pH 5.0. Evaluating the pH values as indicators of complete activity, activity levels of 97% were observed at pH 4.5 and 4. It is worth noting that no enzyme activity was detectable at pH levels exceeding 10.

Temperature and various cations

The influence of temperature on enzyme activities followed a typical pattern, consistent with the findings illustrated in Figure (3B). The graph displayed a gradual decrease in xylanase activity as the temperature exceeded 50 ºC. Moreover, the impact of various cations on xylanase activity was examined and summarized in Table (2). The data indicated that only Mg^{2+} had a stimulating effect on xylanase activity, while Hg^{2+}, Cu^{2+}, and Mn^{2+} exhibited significant inhibitory effects. The inhibitory effect of Fe^{2+}, Ca^{2+}, or Ba^{2+} on enzymatic activity was relatively milder. Notably, the concentration of K^{+} demonstrated a twofold inhibitory effect when doubled.

Investigating the pH stability of xylanase activity

The investigation into the stability of xylanase activity focused on the influence of pH. The study revealed a remarkable stability of xylanase across the examined pH values, as depicted in Figure (4). The enzymatic activity displayed minimal variation throughout the pH range, indicating a consistent level of stability.

Thermal stability of xylanase enzyme

The thermal stability of the xylanase enzyme was evaluated and showed in Figure (5). The results indicated that the xylanase activity remained consistent

Table (1): Enzyme Activities and conditions applied in different bioreactor Systems.

Bioreactor System used	Measured enzymes				Conditions applied			
					Humidity (%)†		Temperature (°C)‡	
	Xylanase (IU/mL)	Exo-PG (IU/mL)	CMCase (IU/mL)	α-Amylase (IU/mL)	Air inlet	Air outlet	Inlet air	Outlet air
Plate type SSF bioreactor	107.33 ± 4.2	59.5 ± 5.6	13.21 ± 0.8	19.80 ± 0.6	-	-	-	-
Conical flask (Water saturated forced aeration)	191.72 ± 8.23	34.55 ± 3.31	18.61 ± 1.39	11.25 ± 1.15	89.48 ± 1.29	88.41 ± 4.2	26.28 ± 0.4	26.41 ± 0.2
Conical flask (Forced aeration)	172.46 ± 8.12	26.24 ± 1.1	19.61 ± 0.5	10.19 ± 0.4	56.23 ±3.1	86.39 ± 1.8	26.17 ± 0.1	26.36 ± 0.2

† Humidity (%) level of the air at the inlet and outlet point.

‡ Temperature degree (°C) of the air at the inlet and outlet point.

Table (2): Impact of Ions on Xylanase Activity from Bacillus safensis MABS6 Cultivated on Tomato Pomace.

.Cation tested (0.1 M)	Relative Activity (%)
Control	100
Hg^{+2}	9.6
Cu^{+2}	21.3
$K^{+1}(SO_4)^{-2}$	43.2
$K^{+1}(Cl^{-1})$	90.6
Mn^{+2}	55.8
Ba^{+2}	74.6
Fe^{+2}	78.7
NH_4^{+1}	86.2
Ca^{+2}	86.9
Mg^{+2}	118.3

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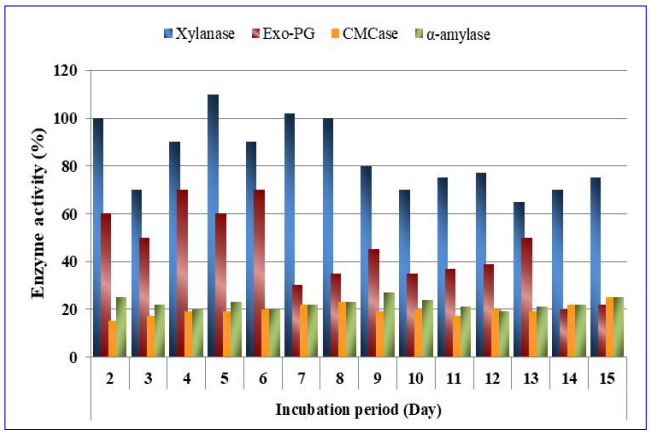

Figure (2): Comparative enzymatic analysis of xylanase, exo-polygalacturonase, cellulase, and α-amylase in Conical Flasks over 15 Days.

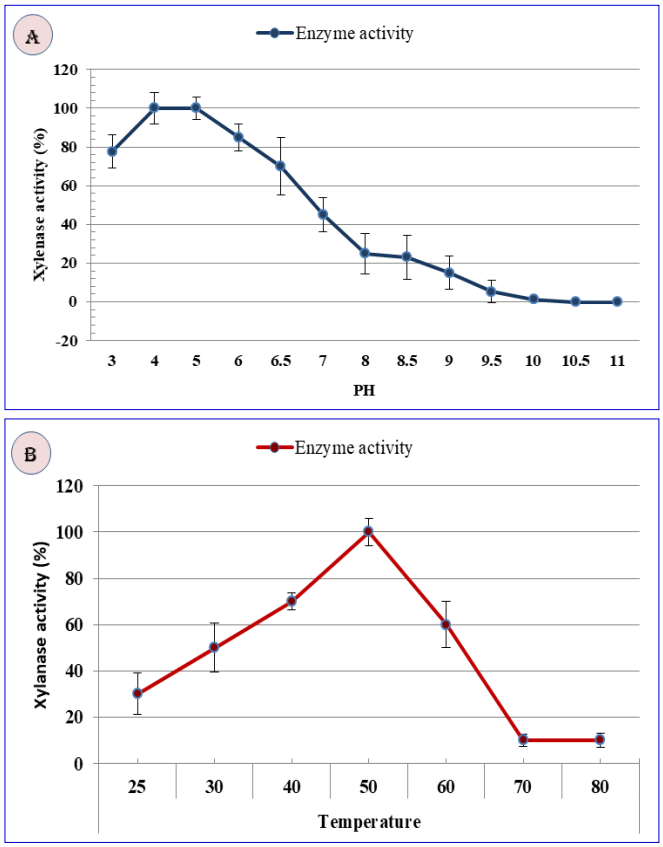

Figure (3): Optimal pH and Temperature for Xylanase activity from *Bacillus safensis* MABS6 cultivated on tomato pomace. A, enzyme activity at different pHs; B; enzyme activity at different temperatures.

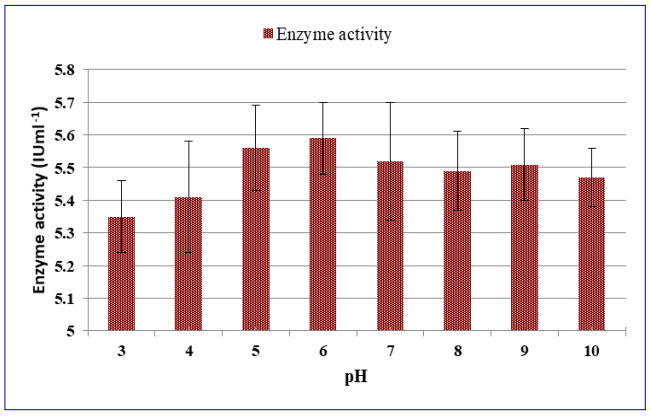

Figure (4): pH stability of xylanase activity at different pHs.

within the temperature range of 30 ºC to 45 ºC. However, a noticeable decline of approximately 50% in enzyme activity was observed at 50 ºC, followed by a more substantial decrease at 60 and 70 ºC.

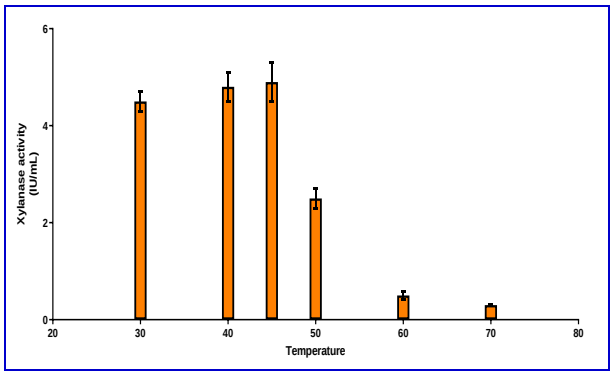

Figure (5): Thermal stability of xylanase enzyme produced by *Bacillus safensis* MABS6.

DISCUSSION

Numerous scholarly publications have documented the recurring phenomena of a dramatic surge followed by a subsequent decline in enzymatic activity during the course of the cultivation period. One of the explanations that has been claimed is the early breakdown of the strain, resulting in a reduction in enzyme activity. It is possible that xylanase or exo-PG enzymes synthesized in the first phase of fermentation undergo degradation or denaturation.

The moisture content in SSF is mostly influenced by the nature of the substrate and its ability to retain water. Consequently, this phenomenon has a substantial influence on the dynamics of microbial proliferation. The present investigation determined that the ideal substrate-to-moisture ratio was 1:3 (w/v), which aligns with the findings reported by Kumar *et al*. (2018). In previous studies conducted by Sindhu *et al*. (2006) and Kamble and Jadhav (2011), the authors reported the use of a 1:1.5 and 1:1.8 (w/v) ratio, respectively, for the synthesis of xylanase by solid-state fermentation using wheat bran as the substrate. Sanghi *et al*. (2007) used a substrate-to-moisture ratio of 1:1 (w/v) with wheat bran, whereas Virupakshi *et al*. (2005) employed a ratio of 1:2 (w/v) with rice bran. It is apparent that the particular microbial strain employed in the research influences the variance in the substrate-to-moisture ratio.

Isolate *Bacillus safensis* MABS6 exhibited maximum xylanase production over a 72-hour incubation period, using wheat bran as the substrate. The alignment of this ideal incubation length is consistent with the results presented in the investigations conducted by Kamble and Jadhav (2011), Khandeparkar and Bhosle (2006), and Virupakshi *et al*. (2005). The influence of the moistening agent on xylanase titer in SSF has been revealed in previous investigations conducted by Battan *et al*. (2006) and Sanghi *et al*. (2007). Modifications were made to the moistening agent in

order to enhance xylanase production under SSF conditions in the instance of *Bacillus safensis* MABS6.

The xylanase enzymes synthesized by *Bacillus safensis* MABS6 exhibited their highest efficiency in circumstances characterized by a pH of 5.0 and a temperature of 50°C. The enzymatic features of *Bacillus safensis* MABS6 are differentiated from those documented in earlier research due to its unique pH and temperature profile. In a study conducted by Khusro *et al*. (2016), it was discovered that the xylanases exhibited optimum pH at 7.0 and temperature at 35°C. In a study, Chaturvedi *et al*. (2015) emphasized the characteristics of xylanase, noting that it exhibits an optimal pH of 6.5 and a temperature optimum of 45°C. Teeravivattanakit *et al*. (2016) documented the presence of a xylanase enzyme that demonstrated an ideal pH of 7.0 and a temperature optimum of 50°C, which is consistent with the results obtained in our study. In a study conducted by Boucherba *et al*. (2017), the researchers observed the characteristics of xylanase, noting that its ideal pH was 7.0 and its optimal temperature was 55°C. In a study conducted by Adigüzel and Tunçer (2016), a xylanase enzyme was identified that exhibited a temperature optimum of 60°C, which is similar to the xylanase enzyme we have previously reported. However, it should be noted that the pH optimum of this xylanase was found to be 6.0. The observed fluctuations in pH and temperature optima serve to highlight the wide range of enzymatic characteristics shown by xylanases derived from various microbial origins.

Isolate *Bacillus safensis* MABS6 demonstrated maximum xylanase production after a 72-hour incubation period using wheat bran as the substrate. This optimal incubation duration aligns with findings from previous investigations conducted by Kamble and Jadhav (2011), Khandeparkar and Bhosle (2006), and Virupakshi *et al*. (2005). The impact of the moistening agent on xylanase titer in Solid State Fermentation (SSF) has been explored in earlier studies by Battan *et al*. (2006) and Sanghi *et al*. (2007). Modifications were made to the moistening agent to enhance xylanase production under SSF conditions for *Bacillus safensis* MABS6.

The xylanase enzymes produced by *Bacillus safensis* MABS6 exhibited their highest efficiency at a pH of 5.0 and a temperature of 50°C. These enzymatic characteristics differ from those reported in previous research. Khusro *et al*. (2016) discovered that xylanases demonstrated optimum pH at 7.0 and temperature at 35°C. Chaturvedi *et al*. (2015) emphasized that xylanase displayed an optimal pH of 6.5 and a temperature optimum of 45°C. Teeraviv-attanakit *et al*. (2016) reported a xylanase enzyme with an ideal pH of 7.0 and a temperature optimum of 50°C, which aligns with our study's results. Boucherba *et al*. (2017) observed a xylanase with an optimal pH of 7.0 and an optimal temperature of 55°C. Adigüzel and Tunçer (2016) identified a xylanase enzyme with a temperature optimum of 60°C, similar to our previously reported xylanase, but with a pH optimum of 6.0. These variations in pH and temperature optima underscore the diverse enzymatic characteristics exhibited by xylanases derived from different microbial sources.

In a recent study by Bansal *et al*. (2022), it was found that the efficiency of enzymatic hydrolysis does not significantly impact the generation of fermentable sugars compared to acidic hydrolysis. Enzymatic hydrolysis is commonly utilized as an initial step in certain processes to minimize chemical usage and energy consumption. This approach helps reduce costs and address environmental concerns associated with chemical hydrolysis, as highlighted by Sreeraj *et al*. (2022). Previous research has also demonstrated the use of xylanase in the production of fermentable sugars from various lignocellulosic materials.

In conclusion, the collected results indicate that tomato pomace holds potential as a suitable substrate for the production of hydrolytic enzymes through solid-state fermentation. Specifically, xylanase and exo-PG have shown promising results in this context. Utilizing this residue or combinations of it with other materials for the production of xylanase or exo-PG could offer an innovative and cost-effective alternative, especially considering the substantial amounts of tomato residue generated by tomato processing industries annually. Consequently, the expenses associated with fermentation media during microbial enzyme production can be significantly reduced.

CONCLUSION

The study results highlight the potential for cost-effective production of xylanase through Solid-State Fermentation (SSF) using readily available agro-industrial residues and a highly efficient bacterial isolate, *Bacillus safensis* MABS6. This investigation demonstrated that the cultivation of this bacterium on tomato pomace, under carefully controlled conditions, resulted in the synthesis of a substantial concentration of high-quality xylanase and other enzymes. These results suggest that *Bacillus safensis* MABS6 holds promise for scaling up xylanase production through solid-state fermentation. The remarkable stability of the synthesized enzyme makes it suitable for diverse industrial applications. Moreover, the investigated xylanase showed superior effectiveness in generating fermentable sugars from both untreated and pre-treated agricultural wastes, surpassing previous findings. This makes it highly favorable for industrial applications with similar requirements.

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الإنتاج المستدام وصديق للبيئة لانزيم xylanase وإنزيمات الأخرى من معجون الطماطم عن طريق التخمر الصلب باستخدام *Bacillus safensis* MABS6

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الملخص العربي

اهتمت هذه الدراسة باستخدام بقايا الطماطم، كمصدر رئيسي ووفير في صناعات الطعام الزراعية، لتخليق إنزيم exo- xylanas و polygalacturonase (exo-PG), cellulase (CMCase), α-amylase. وتضمنت التجارب العمل بتقنية التخمير في الحالة الصلبة باستخدام بكتريا ***Bacillus safensis* MABS6**. واثبتت الدراسة ان أعلى أنشطة للانزيمات موضع الدراسة كانت في الأيام الأولى للزراعة، حيث بلغت القيم تقريبًا 110 و 70 وحدة دولية/مل على التوالي. على الجانب الآخر، استمرت إنتاجيات cellulase (CMCase) و α-amylase وبشكل ثابت نسبيًا خلال عملية التخمير، بقيم متوسطة تبلغ 19.8 و 22.4 وحدة دولية/مل على التوالي. في التجارب التالية، التي أُجريت في مفاعل بيولوجي من نوع الأطباق على نطاق مختبر، كشفت النتائج عن ترابط إيجابي بارز بين التهوية وأنشطة الانزيمات المختبرة وهم انزيم exo-polygalacturonase (exo-PG) و xylanas . كما اثبتت الدراسة ان إنزيم xylanas، الذي يُعتبر إنزيمًا هامًا، اظهر مستويات تخليق مرتفعة، مما دفعنا إلى دراسة الظروف المثلى لقياس نشاطه بدقة. أظهرت النتائج أيضًا أن الرقم الهيدروجيني الأمثل للإنزيم هو 5، وأن أفضل درجة حرارة هي 50 درجة مئوية. كما لوحظ تسهيل نشاط الإنزيم بوجود أيونات المغنيسيوم، بينما لوحظ تثبيط كبير بوجود أيونات الزئبق والنحاس. وعلاوة على ذلك، أظهر النشاط الإنزيمي ارتفاعًا مستدامًا أثناء التخزين ضمن نطاق الرقم الهيدروجيني من 3 إلى 12 وتحت شروط درجة حرارة تتراوح بين 30 درجة مئوية و60 درجة مئوية.