Current Science International
Volume: 13 | Issue: 03| July – Sept.| 2024

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2024.13.3.36
Journal homepage: www.curresweb.com
Pages: 419-432

Production, Immobilization and Evaluation of Xylanase's Efficacy from *Streptomyces* Sp. NRC-EAK-H26 Grown on Lignocellulosic Waste

Nouran A. Elattal[1], Fatma H. Motawe[2], Shimaa S. Hanafy[3] and Eman A. Karam[2]

[1]Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Dokki, Cairo, Egypt
[2]Microbial Chemistry Department, Biotechnology Research Institute, National Research Centre, ElBuhouth Street, P.O. 12622, Giza, Egypt.
[3]Biochemistry department, Biotechnology Research Institute, National Research Centre, ElBuohouth Street, P.O. 12622, Giza, Egypt.

Received: 14 July 2024 **Accepted:** 30 August 2024 **Published:** 20 Sept. 2024

ABSTRACT

The main objective of this research is to produce and immobilize xylanase enzyme from *Streptomyces* sp. isolated from Egyptian soil. We managed to isolate and identify *Streptomyces* sp. NRC-EAK-H26 from Egyptian soil of the Nile delta via 16S rRNA sequencing method that was further selected to produce xylanase enzyme using agriculture wastes. The highest xylanase production, 6.85 U/mL, was obtained when wheat bran was used as a sole carbon source and the medium was supplemented with 0.2% potassium nitrate. The optimal xylanase production was encountered when the media were incubated for five days at 35°C and pH 6.5. The enzyme was successfully immobilized using sodium alginate/k-carrageenan beads, achieving an immobilization efficiency of 86.9% at optimum pH shifted from pH 7 to pH 8 and optimum temperature increased from 50°C to 60°C. Interestingly, the immobilized xylanase enzyme possessed 81% of its initial hydrolytic activity after eight cycles of use, indicating excellent reusability. Thermal and pH stability of both free and immobilized xylanase were studied. The immobilized xylanase retained 55.5% of its activity at 80°C after 30 min. and was able to keep up to 33.2% of its initial activity at pH 8 after 2 h. The enzyme's stability and activity suggest its potential for producing xylo-oligosaccharides from agricultural wastes like wheat bran. The ability of the immobilized xylanase enzyme to keep hydrolytic activity over multiple uses is particularly valuable for industrial applications, where cost-effectiveness and efficiency are crucial.

Keywords: Xylanase, immobilization, thermal stability, pH stability, sodium alginate/k-carrageenan beads, *Streptomyces* sp. NRC-EAK-H26

1. Introduction

Xylan, a major component of hemicellulose found in lignocellulosic biomass, is primarily consists of multiple units of xylose bonded by *β*-1,4 glycosidic links. The hydrolysis of xylan results in the production of xylo-oligosaccharides and xylose, where xylo-oligosaccharides consist of 2–6 xylose residues (Carvalho *et al.,* 2013).

Xylanase, a hydrolytic enzyme that breaks down these *β*-1,4 glycosidic bonds, is a part of a large family of high molecular weight polysaccharides and is typically soluble in alkaline solutions (Xiao *et al.,* 2001). Interestingly, xylanase enzyme possessed several applications across various industries, including pulp and paper, bakery, food processing, detergents, textiles, animal feed, and biofuel production (Alagöz *et al.,* 2022; Althuri and Venkata Mohan, 2019; Dhivahar *et al.,* 2020; Mhiri *et al.,* 2020).

Xylanases are produced by a range of microorganisms, including fungi, bacteria, yeast, and actinomycetes (Gawande and Kamat, 1998; J *et al.,* 2020; Šuchová *et al.,* 2022; Tran *et al.,* 2024).

Corresponding Author: Eman A. Karam, Microbial Chemistry Department, Biotechnology Research Institute, National Research Centre, ElBuhouth Street, P.O. 12622, Giza, Egypt.
E-mail: eman_karam4@yahoo.com

Fungal xylanases are typically found in conjunction with cellulases, while bacterial xylanases are often cell-associated. In contrast, xylanases from actinomycetes are generally reported to be extracellular (Ding *et al.,* 2004; Kohli *et al.,* 2001). In these microorganisms, xylanase biosynthesis can be mainly regulated by low molecular mass fragments of xylan and their positional isomers.

The immobilization of xylanase enhances the enzyme's stability by protecting the active site from deactivation, facilitating repeated use, and significantly reducing operational costs through enzyme reuse and easy separation (Ariaeenejad *et al.,* 2020; Rodrigues *et al.,* 2021). Among the various immobilization methods, calcium alginate hydrogel beads are frequently used due to their cost-effectiveness, high porosity, and ease of production (Shivudu *et al.,* 2020). However, to enhance enzyme encapsulation and control the release from the gel matrix, additional polymers such as k-carrageenan or chitosan may be utilized, and coatings with agents like glutaraldehyde can be employed (Richetti *et al.,* 2012; Zhou *et al.,* 2010).

The objective of this work is to produce a high level of xylanase from *Streptomyces* sp. NRC-EAK-H26 by studying the optimum conditions for enzyme production from agriculture byproduct. Furthermore, we immobilized xylanase enzyme on sodium alginate/k-carrageenan gels and studying the effect of immobilization process on thermal and pH stability of the isolated xylanase enzyme.

2. Materials and Methods

2.1. Microorganisms

The most active *Streptomyces* strain with regard to xylanolytic activity was selected from various *Streptomyces* sp. isolated separately from different localities of the Egyptian Nile Delta soil.

2.2. Culture media

To produce xylanase enzyme, we used a modified basal salt medium (B.S.M.) of Waksman 1961 for micro-organisms fermentation. It consisted of g/L: 2.0, KNO_3; 1.0, K_2HPO_4; 0.5, $MgSO_4$; 0.5, NaC1; 3.0, $CaCO_3$; 0.01 FeSO4 and 10.0 xylan as a sole carbon source. The medium was adjusted to pH 7.0 before autoclaving. Agar slants with the same constituents were used to propagate a stock culture of *Streptomyces* sp., keeping all optimal conditions for microbial growth.

2.3. Identification of xylanase producer strain

The potential xylanase producer strain was identified through biochemical, morphological, and physiological standard methods. Thereafter, we used 16S rRNA method for genetic identification. Briefly, a set of primers were used to amplify 1421 bp fragment of the 16S rRNA region with forward primer of 5′-AGAGTTTGATCMTGGCTCAG-3′ and the reverse primer of 5′-TACGGYTACCTTGTTACGACTT-3′. Thereafter, our DNA sequence was deposited in GenBank database for a comparison with the known DNA sequences in the national Center for Biotechnology Information (https://www.ncbi.nlm. nih.gov/) using the BLAST program (Tamura *et al.,* 2011). The tree was constructed using the MEGA11 software for carrying out the neighbor joining method.

2.4. Substrates

Purified xylan was prepared from sugarcane bagasse Edfu pulp mill according to the method reported previously ensuring a suitable substrate for enzymatic assays (Sporck *et al.,* 2017).

2.5. Fermentation studies

To investigate the physiological and physical conditions affecting xylanolytic activity, fermentation studies were conducted in shaken cultures. A 2% (v/v) spore suspension from 7-day-old agar slants was used to inoculate Erlenmeyer flasks 250 mL containing 50 mL of the appropriate B.S.M. The cultures were incubated at 35°C with shaking at 180 rpm for 5 days. After incubation, the cultures were centrifuged to obtain supernatants for crude enzyme preparation. Two duplicates were prepared for each experiment.

2.6. Protein determination

We determined the protein content using Lowry method that uses bovine serum albumin as a protein standard (Lowry *et al.,* 1951).

2.7. Enzymes assays

Xylanase activities were determined using 1% xylan (Sigma-Aldrich, USA) in 0.5 mL of citrate phosphate buffer, (50 mM, pH 6.5) as substrates. The assay mixtures contained 0.5 mL of enzyme preparation and were incubated at 45°C for 20 min. Dinitrosalicylic acid colorimetric method (DNS method) was used to determine the reducing sugars released (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme liberating 1µmol of reducing sugars equivalent to xylose, per minute under the standard conditions.

2.8. Factors influencing xylanase production

All experiments in this study were carried out in duplicates. In order to measure the effects of incubation time on the production of enzyme and effect of different carbon sources on xylanase production, the B.S.M. was supplemented with 1% (w/v) xylan, xylose, galactose, glucose, fructose, cellulose, sucrose and maltose. Furthermore, we studied the effect of equimolar addition of different inorganic or organic nitrogen sources on xylanase production. Appropriate pH values for the optimized B.S.M. (from pH 5 to pH 10) were adjusted, to study their effects on xylanase production (Kansoh and Nagieb, 2004).

2.9. Time course of xylanase production

The isolate strain that produces the highest xylanase level was re-cultured using Erlenmeyer flasks (250 mL) containing the optimized B.S.M. (50 mL) on a rotary shaker (180 rpm) at 35°C for 1 to 8 days. Xylanase activity in the culture broth supernatant was measured at various time intervals (Sharma *et al.,* 2013).

2.10. Utilization of agriculture wastes by *Streptomyces* sp. NRC-EAK-H26 for xylanase production

In order to induce xylanase synthesis from cheap and most economic carbon sources, 5 g of seven agricultural byproducts were tested as substrates for xylanase production as wheat straw, wheat bran, corn bran, corn straw, rice straw, and sugarcane bagasse. These substrates were dried in oven at 60°C for 72 h and then ground well before being added to the fermentation medium.

2.11. Partial purification of the crude xylanase

To achieve 40% saturation, ammonium sulfate was added to the supernatant while stirring in an ice bath for 2 h, followed by centrifugation at 5,000 rpm for 10 min at 4°C, and the precipitate was collected and dissolved in phosphate buffer (pH 8). This solution was dialyzed against distilled water at 4°C overnight. The resulting supernatant was subsequently precipitated with ammonium sulfate up to 80% saturation.

2.12. Preparation of carrier (sodium alginate/k-carrageenan gel)

In order to prepare sodium alginate/k-carrageenan gel (ALG.CAR), sodium alginate/k-carragenan (1:1 w/w) was dissolved in distilled water to achieve 2% (w/v) alginate/carragenan gel final concentration. Thereafter, an overhead mechanical stirrer was used to mix thoroughly the gel solution until complete dissolution, and an Encapsulator with nozzle size 300 µm was used to drop the polymer solution into $CaCl_2$ to form uniform gel beads. The ALG.CAR gel beads were hardened using $CaCl_2$ for 3 h. To covalently immobilize xylanase enzyme, the formed ALG.CAR gel beads were dipped into a solution of polyethylenimine (4% v/v) at pH 9.5 for 3 h. The gel beads were washed with distilled water, and finally were soaked in 2.5% (v/v) glutaraldehyde solution for 3 h before become ready for immobilization (Karam *et al.,* 2018).

2.13. Immobilization of xylanase enzyme

ALG.CAR (0.25 g) was inserted into 0.5 mL xylanase enzyme at 4˚C for 24 h. Thereafter, washing with distilled water was employed to remove the unbound enzyme. The washing was stopped once no protein nor enzymatic activity were detected in the washing solution (Karam *et al.,* 2018).

2.14. Immobilization yield

Immobilized enzyme yield was calculated as U/g carrier from the equation: Immobilized enzyme (U/g carrier) = Immobilized enzyme activity (U/g carrier)/ Enzyme added (U/g carrier) - Unbound enzyme (U/g carrier) (%).

2.15. Optimization of the immobilization yield

2.15.1. Loading time

In order to achieve the best time for loading the enzyme in the carrier, xylanase free enzyme was loaded in ALG.CAR carrier at 4ºC and incubated for different time intervals, namely, 2, 6, 12, 24, 36, and 48 h. Thereafter, we washed the carrier twice with acetate buffer (0.2 M - pH 5.2) for 30 min, to get rid of any unbound enzyme. Finally, we detected the xylanase hydrolytic activity for each time interval and presented it as immobilization yield (%) (Esawy *et al.,* 2014).

2.15.2. Operational stability

To determine the stability of the formed immobilized xylanase enzyme over several runs, 0.25 g of immobilized xylanase (wet) was incubated with 0.5 mL of xylan (1% w/v) in sodium acetate buffer (0.05 M - pH 6.5) at 45°C for 20 min. Thereafter, the supernatant was assayed for xylanase hydrolytic activity and we collected the immobilized enzyme, washed with distilled water and a new run was carried out by incubation with 0.5 mL of freshly prepared substrate.

2.16. Characterization of immobilized and free enzymes

2.16.1. Optimum temperature of the free and immobilized enzymes

To detect the best temperature needed for xylanase enzyme activity, we used different temperatures (from 40ºC to 70ºC) for 20 min incubation period for both the free and the immobilized xylanase enzymes before determining the enzyme activity.

2.16.2. Thermal stability of the free and immobilized enzymes

To assess the thermal stability, we determined the residual activity for both the free and immobilized xylanases using five deferent temperatures (40ºC-80ºC) for 2 h, every 30 min time interval (Abdel Wahab *et al.,* 2018).

2.16.3. Effect of pH on the free and immobilized enzymes

Several buffer systems were used to assess the effect of pH on the activity of the free and immobilized xylanase enzymes, such as 0.1 M citrate phosphate buffer (pH 3.0-7.0), 0.1 M phosphate buffer (pH 7.0-8.0) and 0.1 M Tris-HCl buffer (pH 8.0-9.0), keeping all other experimental conditions (Karam *et al.,* 2018).

2.16.4. pH stability

In absence of the substrate, the free and the immobilized enzymes were subjected to different pH values ranging from 3 to 9 using the same buffers under the previous section for 2 h at 4ºC., keeping constant other optimized experimental conditions. The enzyme was readjusted to pH 5.2 and the residual activity was assayed for each pH at the optimized conditions (Karam *et al.,* 2018).

3. Results and Discussion

3.1. Selection and identification of xylanase producer strain

From various soil samples collected from different localities, 27 *Streptomyces* isolate strains were found to exhibit growth on the basal medium for the detection of xylanase activity. Further screening revealed that only 16 of these isolate strains showed xylanase activity (Table 1). The isolate no. 5 showed the highest xylanase activity of 4.20 (U/mL), after 5 days incubation period. Therefore, this isolate was identified and used in the following work. This isolate was recognized as *Streptomyces sp.* NRC-EAK-H26 strain based on 16S rRNA sequencing method, with similarity equal to 95.56% with an accession number of LC456059 from GenBank, as illustrated in Fig.1.

Table 1: Xylanase activity of *Streptomyces* isolates in submerged culture.

Streptomyces **isolate strain no.**	**Xylanase activity (U\mL)**	***Streptomyces*** **isolate strain no.**	**Xylanase activity (U\mL)**	***Streptomyces*** **isolate strain no.**	**Xylanase activity (U\mL)**
1	1.2	10	3.6	19	-
2	2.2	11	1.2	20	3.4
3	-	12	-	21	2.5
4	2.20	13	1.4	22	1.6
5	**4.20**	14	-	23	1.7
6	-	15	2.5	24	-
7	-	16	-	25	1.5
8	2.4	17	3.7	26	-
9	-	18	-	27	1.7

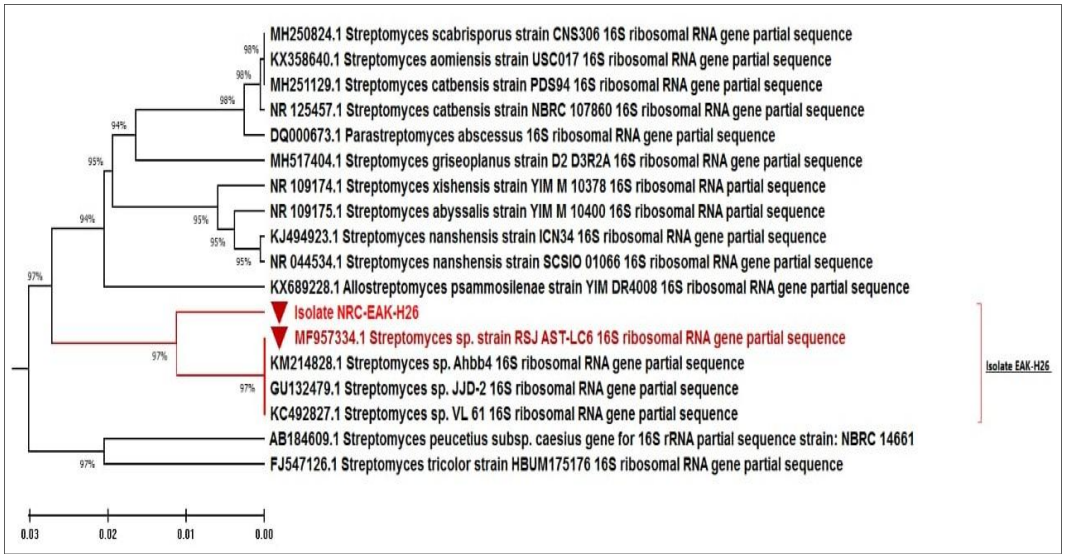

Fig. 1: Phylogenetic tree of isolate no. (-) *Streptomyces* sp. NRC-EAK-H26 based on partial 16S rDNA sequences. Neighbor joining method was carried out using the MEGA11 software to construct the tree.

3.2. Factors influencing xylanase production

Of all the tested carbon sources, xylan supported the highest level of production for *Streptomyces* sp. NRC-EAK-H26. In contrast, other carbon sources were largely ineffective, yielding low amounts of the enzyme, as shown in Fig. 2. We can suggest that xylanase is apparently inductive in nature as lowest level of xylanase production was recorded on using glucose. The production and characterization of xylanases were examined previously across four strains of *Streptomyces* sp., with strain MDS demonstrating the best production when grown in a medium containing xylan (Saratale *et al.,* 2012).

Figure 3 shows the effect of different inorganic nitrogen salts on xylanase production.The B.S.M. supplementation with 0.2% potassium nitrate was a good nitrogen source for the production of xylanase, followed by sodium nitrate. To investigate the influence of organic nitrogen sources on the production of xylanase enzyme, peptone, casein, soya bean and yeast extract were added individually to the B.S.M. and showed a better production of the enzyme comparable with different inorganic nitrogen sources. Maximum increase in enzyme production was obtain with yeast extract rather than with peptone, casein or soya bean, as illustrated in Fig. 3, which is in accordance with previously published results (Rifaat *et al.,* 2006).

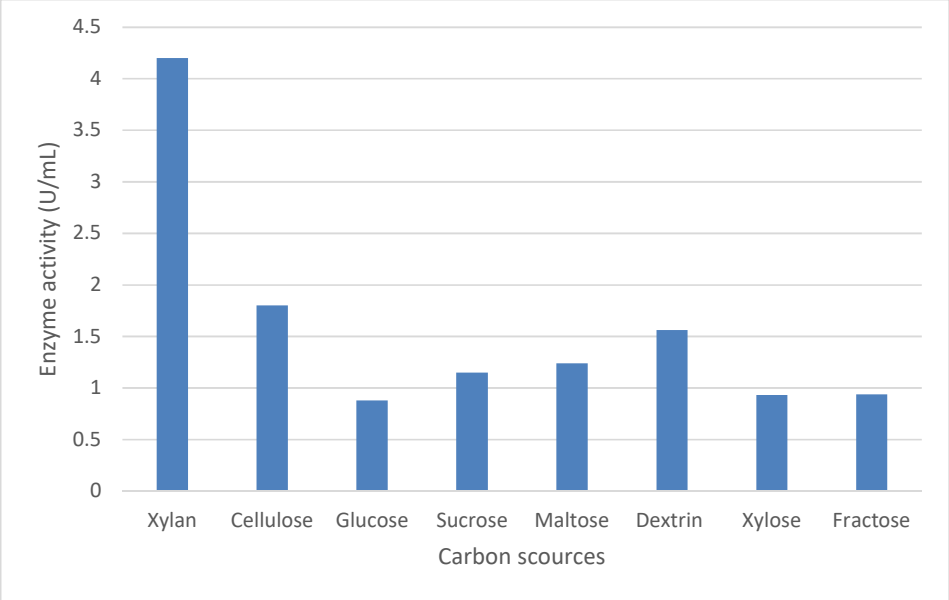

Fig. 2: Effect of different carbon sources on the production of xylanase enzyme.

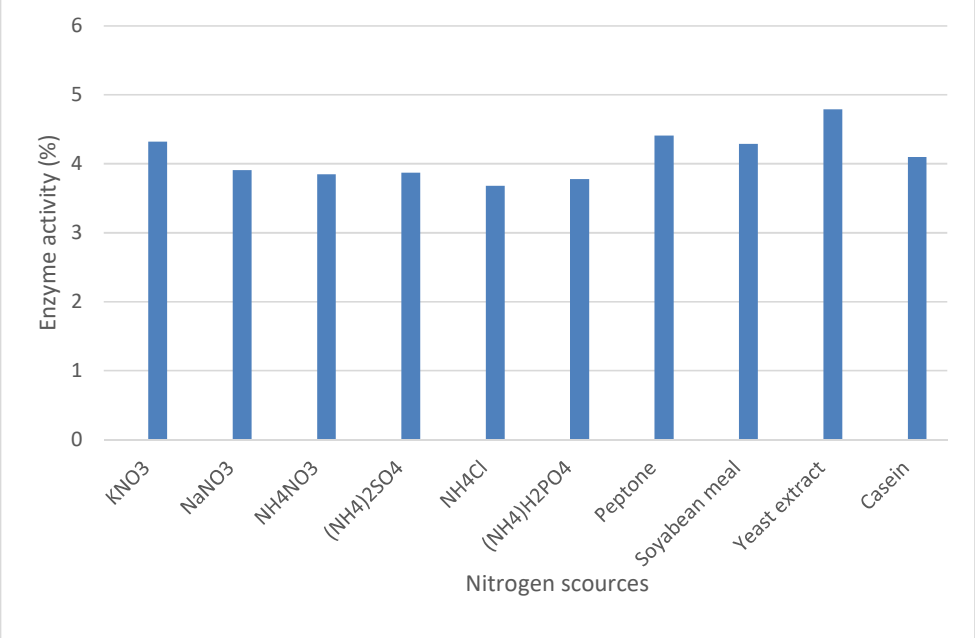

Fig. 3: Effect of different nitrogen sources on the production of xylanase enzyme.

The effect of initial pH values on the optimized media B.S.M. was detected on the 5th day of incubation period, as shown in Fig. 4. Furthermore, initial pH values of the media, ranging from pH 5.0 to pH 10.0, affected greatly xylanase production. The optimum xylanase productivity was observed at pH 6.5, whereas it was markedly reduced at higher and lower pH values. In agreement with our results, endo-β-1,4-xylanase was produced from *Streptomyces* sp. T7 at optimum pH of 6 (Li *et al.,* 2022).

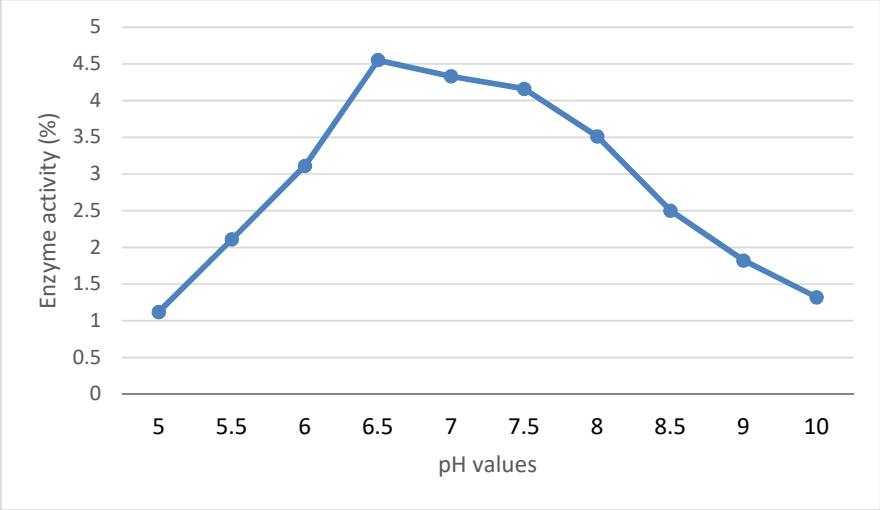

Fig. 4: Effect of pH values on the production of xylanase enzyme.

3.3. Time course of xylanase production by *Streptomyces* sp. NRC-EAK-H26

The xylanase production was determined at different times from 1 to 8 days, as seen in Fig. 5. The maximum xylanase activity (4.43 U/mL), was detected in the supernatant of culture media after 5 days of incubation at 35 ˚C, as illustrated in Fig. 5. In agreement with our results it was mentioned previously that the incubation time for optimal xylanase production by other *Streptomyces* sp. was in the range of 3–7 days (Sharma *et al.,* 2013).

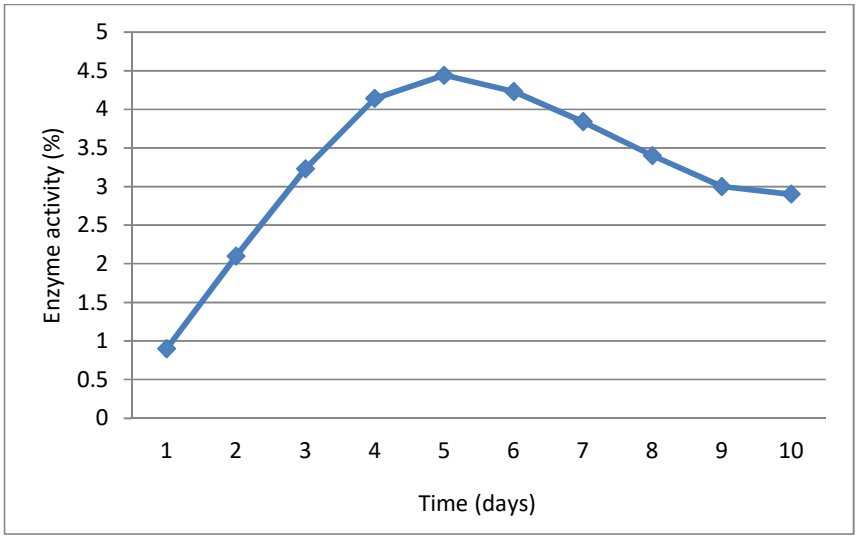

Fig. 5: Time course of xylanase production by *Streptomyces* sp. NRC-EAK-H26.

3.4. Utilization of agriculture wastes by *Streptomyces* sp. NRC-EAK-H26 for xylanase production

Different types of agricultural wastes were used as a substrate for xylanase enzyme production. Wheat bran, wheat straw, corn straw and corn cobs yielded higher enzyme activity than xylan, as shown in Fig. 6. The highest xylanase production (6.85 U\mL) was obtained when wheat bran was used as a sole carbon source. These results are in agreement with previous report indicating that the most effective carbon source for xylanase production by *Streptomyces thermocarboxydus* TKU045 was wheat bran powder (Tran and Doan, 2021). The computable effect of wheat bran for xylanase production might be due to its low lignin content and high content of hemicellulose which is more degradable (Kansoh and Nagieb, 2004).

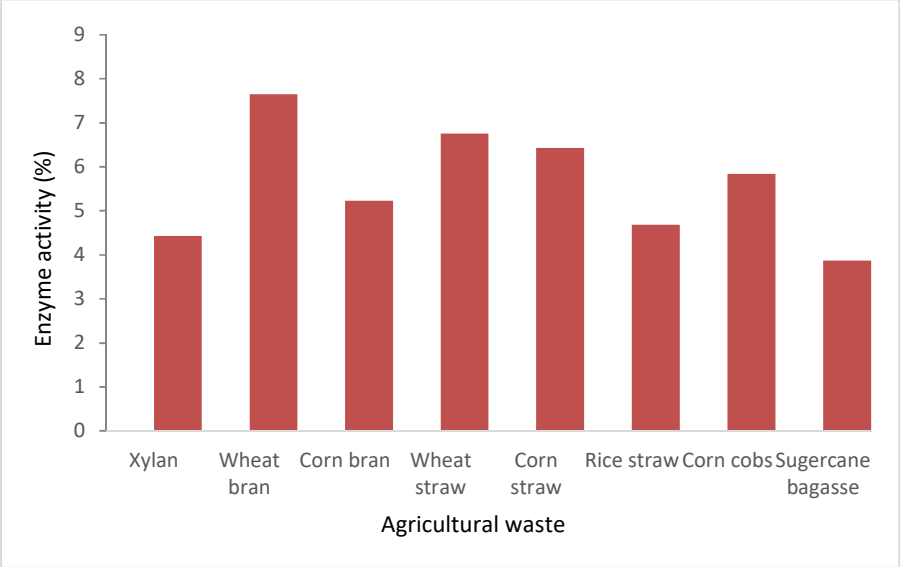

Fig. 6: Utilization of agriculture wastes by *Streptomyces* sp. NRC-EAK-H26 for xylanase production.

3.5. Partial purification of the crude xylanase

Preliminary purification using ammonium sulfate precipitation indicated that saturation of the crude preparation of xylanase enzyme with 40% and 60% ammonium sulfate resulted in precipitates with low enzymatic activity and high protein content, as illustrated in Table 2. Therefore, these precipitates were discarded. Xylanase enzyme was precipitated with 80% ammonium sulfate saturation from the remaining supernatant, with a purification fold of 7.09 and 60.28% recovery of enzymatic activity. These data are in agreement with those reported previously that showed purification of xylanase with 8.7-fold and recovery of 62.79% with 60–80% ammonium sulphate saturation (Kansoh and Nagieb, 2004).

Table 2: Partial purification of the crude xylanase preparation from *Streptomyces* sp. NRC-EAK-H26

Purification steps	Total protein	Total Unit	Specific activity	Yield (%)	Purification fold
-Culture filtrate	44.7	465.5	10.41	100	-
-Ammonium sulfate precipitation					
0-40%	15.8	35.7	2.26	7.67	0.22
40-60%	8.5	70.8	8.33	15.21	0.83
60-80%	3.8	280.6	73.8	60.28	7.09

3.6. Immobilization yield of xylanase

Table 3 illustrated the immobilization of the partial purified xylanase enzyme with a yield of the immobilization process of 86.91%. In agreement with our results, immobilized xylanase enzyme produced by *Streptomyces olivaceoviridis* E-86 that was immobilized using Eudragit S-100 polymer, possessed immobilization efficiency of 90% (Ai *et al.,* 2005).

Table 3: Immobilization of the partial purified xylanase preparation from *Streptomyces sp.* NRC-EAK-H26

Added enzyme (U) (A)	Protein of added Enzyme (mg) (C)	Bounded enzyme (U) (I)	Unbounded enzyme (U) (B)	Protein for bounded enzyme (D)	Protein for unbounded enzyme (C-D)	Immobilization yield (%) I/(A-B)%
28.06	0.38	16.87	8.65	0.25	0.13	86.91

3.7. Effect of loading time of enzyme.

Fig. 7 depicts the highest loading capacity which was 77% at 24 h and stayed nearly constant till 48 h.

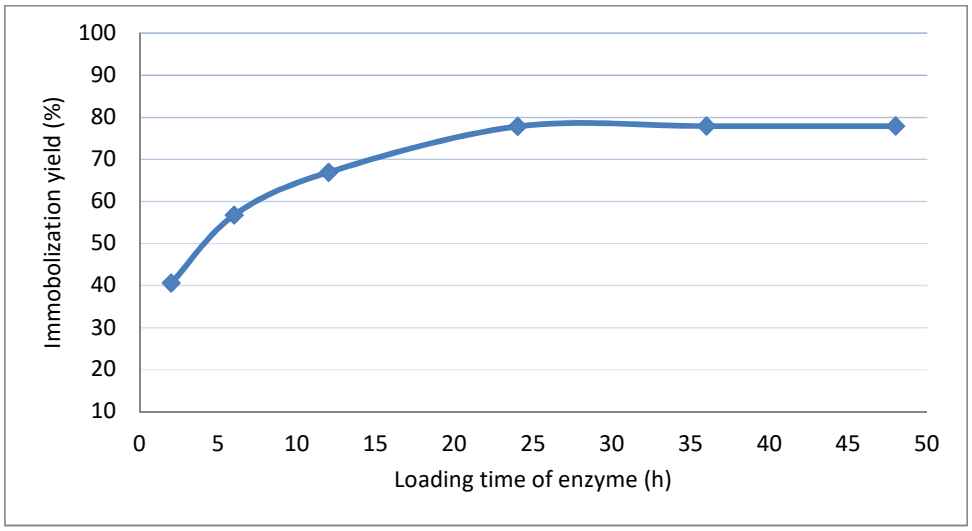

Fig. 7: Effect of loading time of xylanase enzyme.

3.8. Operational stability

Figure 8 shows the residual activity of the immobilized xylanase enzyme used for 10 runs. It was about 80% of activity after eight runs. It was reported previously that 40% of the initial catalytic activity of the immobilized xylanase on Eudragit S-100 was lost after five cycles of assays (Teng *et al.,* 2021). The immobilized xylanase kept about 56% of its initial catalytic activity after 10 successive cycles of hydrolysis as illustrated in Fig. 8.

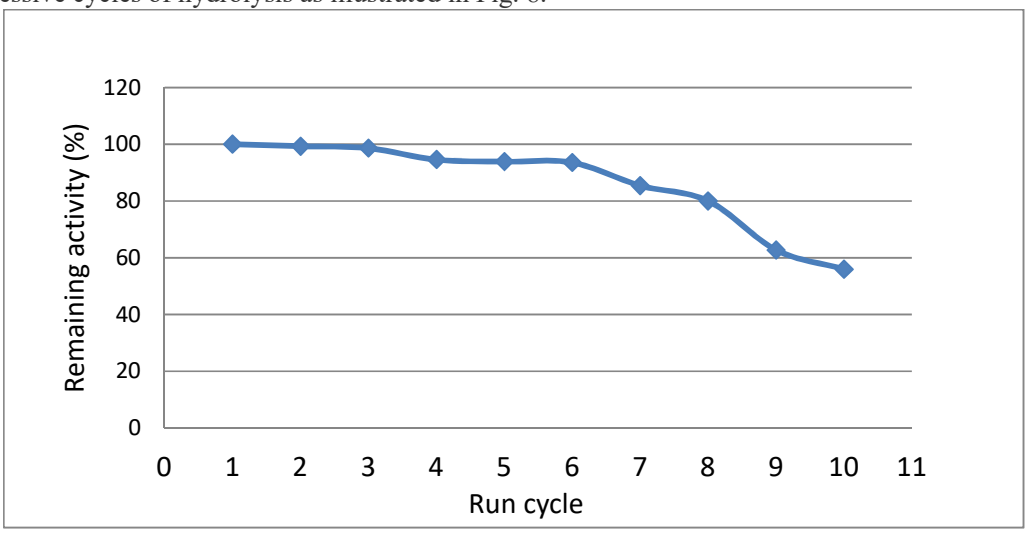

Fig. 8: Operation stability of immobilized xylanase.

3.9. Effect of pH on the free and immobilized xylanase

Fig. 9 presents the impact of pH on the activity of the free and immobilized xylanase enzymes. The optimum pH of the immobilized xylanase shifted from pH 7 to pH 8. This might be due to the anionic nature of the immobilization matrix (Meryandini *et al.,* 2009). Abou-Dobara *et al.,* 2013 showed that optimum pH of immobilized xylanases became more acidic when compared to the free xylanase changing from pH 6.5 to pH 6.0. This might be due to the used cationic matrix that

possessed positive charges (Abou-Dobara *et al.,* 2013). Furthermore, our data were expressed as relative activities to controls which were set as 100% at pH 7 and pH 8 for the free and the immobilized xylanase enzymes, respectively. In agreement with our results, xylanase highest activity was measured at pH 7 for xylanase crude enzyme produced from *S. thermocarboxydus* TKU045′s (Tran and Doan, 2021).

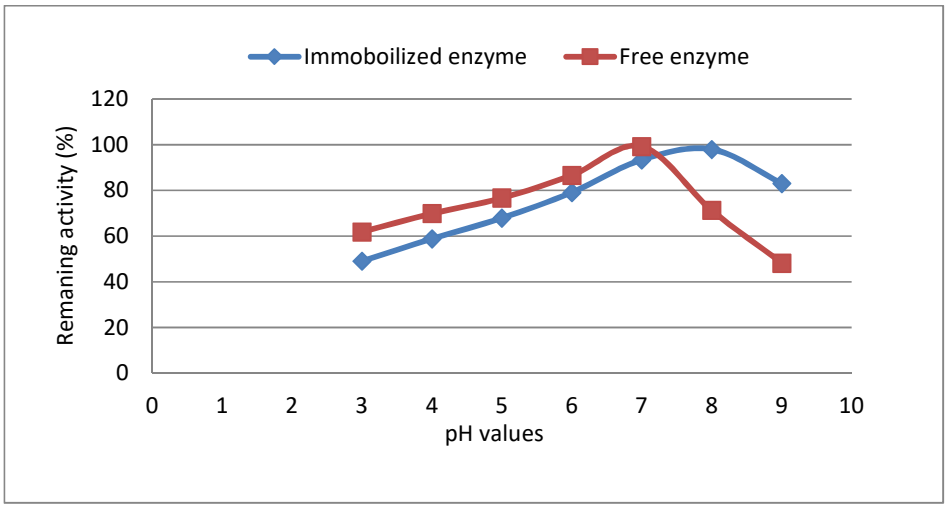

Fig. 9: Effect of pH on the activity of free and immobilized xylanases on sodium alginate/k-carrageenan beads.

3.10. Effect of different temperatures on the activity of the free and immobilized xylanases

Immobilized xylanase enzyme optimal reaction temperature was found to be changed from 50°C to 60°C, as indicated in Fig. 10, which is similar to previously reported results (Abou-Dobara *et al.,* 2013; Sharad *et al.,* 2016). Ai *et al.,* 2005 showed an optimum temperature shift for xylanase activity from 60°C to 65°C during immobilization of *S. olivaceoviridis* E-86 xylanase on Eudragit S-100 (Ai *et al.,* 2005).

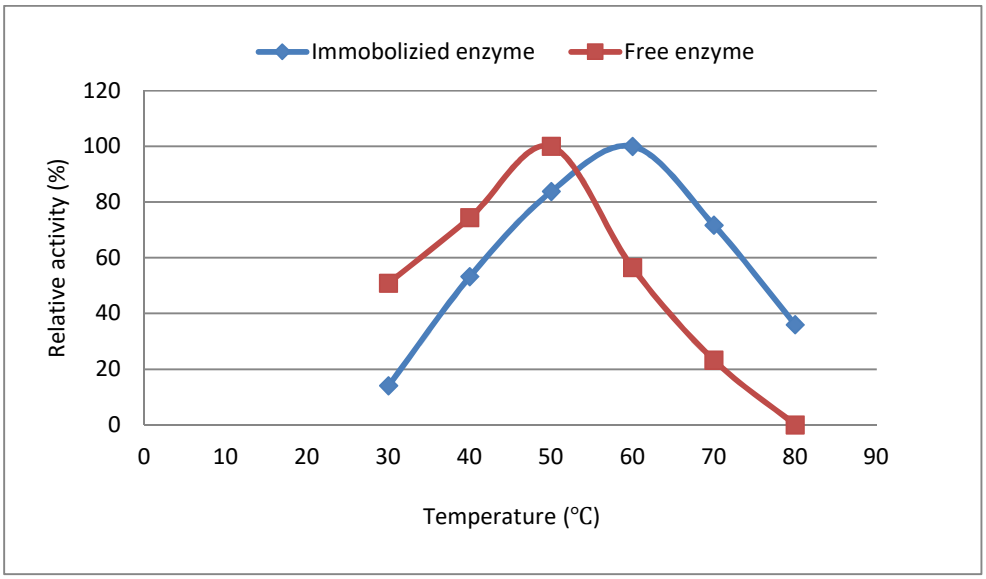

Fig. 10: Effect of temperature on the activity of free and immobilize xylanases on sodium alginate/k-carrageenan beads.

3.11. Thermal stability of free and immobilized xylanase enzymes

Effect of thermal stability on xylanase activity is shown in Figs. 11a & 11b. The immobilized xylanase retained 55.5% of its activity at 80°C after 30 min whereas the free enzyme kept 19.4% of its activity at the same temperature that suggests thermal stability of the immobilized enzyme. Similar results were reported previously that showed that the immobilized xylanase with silica gel and modified with glutaraldehyde from *Trichoderma longibrachiatum* possessed higher thermal stability than the free xylanase enzyme (Alagöz *et al.,* 2022). Furthermore, calcium alginate-immobilized xylanase enzyme isolated from *Bacillus licheniformis* S3 exhibited thermal stability when compared with agar–agar-immobilized enzyme and kept 57.1% of its initial activity up to 150 min at 80°C (Irfan *et al.,* 2020).

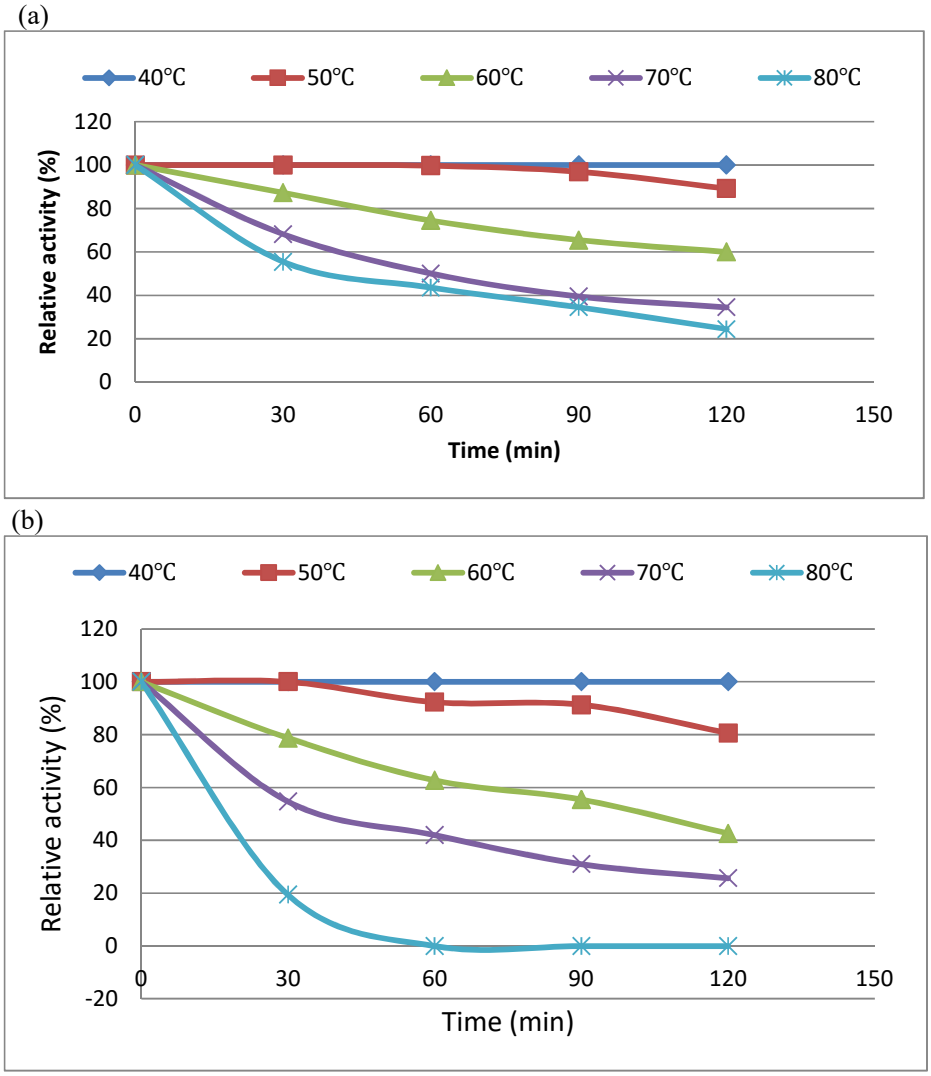

Fig. 11: Thermal stability of free **(a)** and immobilized xylanases on sodium alginate/k-carrageenan beads **(b)**.

3.12. Effect of pH stability on the free and immobilized xylanase enzymes

In alkaline pH (pH 8), the immobilized xylanase enzyme was able to keep up to 33.2% of its initial activity when compared with free enzyme after incubation at room temperature for 2 h, as illustrated in Fig. 12. Enhanced stability by immobilization suggests greater industrial applications and multiple uses for xylanase enzyme (Gupta *et al.,* 2022; Sharad *et al.,* 2016). Similarly, it was reported that the immobilized xylanase enzyme exhibited higher pH stability than the free xylanase enzyme by 2- and 6-fold at pH 5 and pH 9, respectively (de Albuquerque *et al.,* 2016).

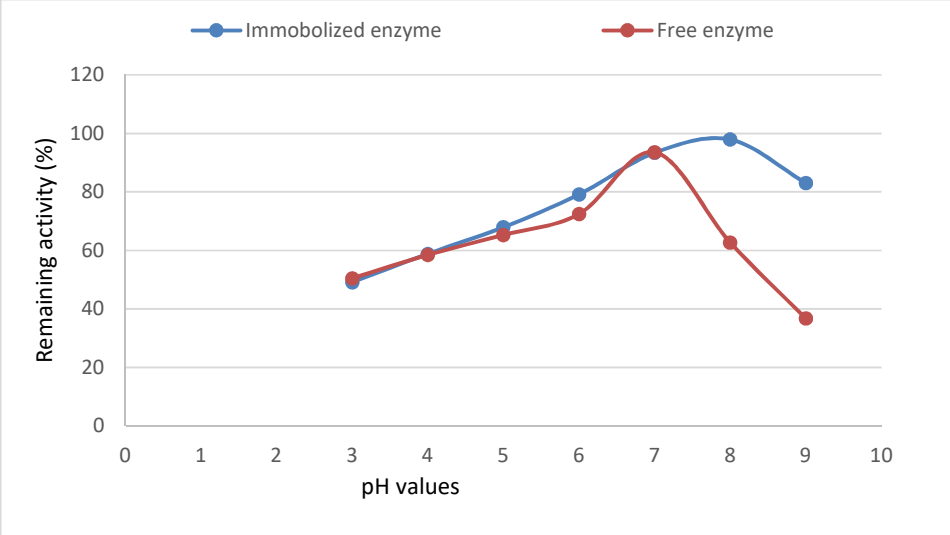

Fig. 12: pH stability of free and immobilized xylanases on sodium alginate/k-carrageenan beads.

4. Conclusion

Xylanase enzyme isolated from *Streptomyces* sp. NRC-EAK-H26 showed enhanced thermal and pH stabilities especially when immobilized with sodium alginate/k-carrageenan beads. Furthermore, we used an eco-friendly method for fermentation and culture of the isolated *Streptomyces* sp. NRC-EAK-H26 by using wheat bran as a carbon source. Overall, this work provides valuable insights into the production and application of xylanase from a native microbial source, contributing to the field of biotechnology and sustainable agriculture. Additionally, evaluating the enzyme's efficacy on various lignocellulosic substrates could broaden its application scope.

Acknowledgments

Thanks go to the National Research Centre, Giza, Egypt for providing all instruments used during this study.

Author Contributions

All authors participated equally in all issues related to the article.

Funding

Not applicable **yet.**

Availability of data and materials

This article includes all data related to this research.

Data Availability Statement

Supporting data for this research article are available upon reasonable request from the corresponding author, but with restriction use and so are not publicly available.

Declarations

Ethics approval

Not applicable.

Consent to publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

References

Abdel Wahab, W.A., E.A. Karam, M.E. Hassan, *et al.,* 2018. Optimization of pectinase immobilization on grafted alginate-agar gel beads by 24 full factorial CCD and thermodynamic profiling for evaluating of operational covalent immobilization. International Journal of Biological Macromolecules, 113: 159-170.

Abou-Dobara, M., A. El-Sayed, A. El-fallal, and N. Omar, 2013. Immobilization and characterization of Streptomyces griseorubens xylanase. Egyptian Journal of Botany, 485-493.

Ai, Z., Z. Jiang, L. Li, *et al.,* 2005. Immobilization of Streptomyces olivaceoviridis E-86 xylanase on Eudragit S-100 for xylo-oligosaccharide production. Process Biochemistry, 40(8): 2707-2714.

Alagöz, D., N.E. Varan, A. Toprak, *et al.,* 2022. Immobilization of xylanase on differently functionalized silica gel supports for orange juice clarification. Process Biochemistry, 113: 270-280.

Althuri, A. and S. Venkata Mohan, 2019. Single pot bioprocessing for ethanol production from biogenic municipal solid waste. Bioresource Technology, 283: 159-167.

Ariaeenejad, S., A. Sheykh Abdollahzadeh Mamaghani, M. Maleki, *et al.,* 2020. A novel high performance in-silico screened metagenome-derived alkali-thermostable endo-β-1,4-glucanase for lignocellulosic biomass hydrolysis in the harsh conditions. BMC Biotechnology, 20(1): 56.

Carvalho, A.F.A., P.D.O. Neto, D.F. da Silva, and G.M. Pastore, 2013. Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. Food Research International, 51(1): 75-85.

de Albuquerque, T.L., S. Peirce, N. Rueda, *et al.,* 2016. Ion exchange of β-galactosidase: The effect of the immobilization pH on enzyme stability. Process Biochemistry, 51(7): 875-880.

Dhivahar, J., A. Khusro, B. Ahamad Paray, M.U. Rehman, and P. Agastian, 2020. Production and partial purification of extracellular xylanase from Pseudomonas nitroreducens using frugivorous bat (Pteropus giganteus) faeces as ideal substrate and its role in poultry feed digestion. Journal of King Saud University - Science, 32(4): 2474-2479.

Ding, C.H., Z.Q. Jiang, X.T. Li, L.T. Li, and I. Kusakabe, 2004. High activity xylanase production by Streptomyces olivaceoviridis E-86. World Journal of Microbiology and Biotechnology, 20(1): 7-10.

Esawy, M., A. Kansoh, Z. Kheiralla, *et al.,* 2014. Production and Immobilization of Halophilic Invertase Produced from Honey Isolate Aspergillus niger EM77 (KF774181). International Journal of Biotechnology for Wellness Industries, 3: 36-45.

Gawande, P.V. and M.Y. Kamat, 1998. Preparation, characterization and application of Aspergillus sp. xylanase immobilized on Eudragit S-100. J. Biotechnol, 66(2-3): 165-75.

Gupta, G.K., M. Dixit, R.K. Kapoor, and P. Shukla, 2022. Xylanolytic Enzymes in Pulp and Paper Industry: New Technologies and Perspectives, 64(2): 130-143.

Irfan, M., J. Kiran, S. Ayubi, *et al.,* 2020. Immobilization of β-1,4-xylanase isolated from Bacillus licheniformis S3., 60(7): 600-612.

Khusro, J.D., A.L. Elancheran, *et al.,* 2020. Photo-mediated biosynthesis and characterization of silver nanoparticles using bacterial xylanases as reductant: Role of synthesized product (Xyl-AgNPs) in fruits juice clarification. Surfaces and Interfaces, 21: 100747.

Kansoh, A.L. and Z.A. Nagieb, 2004. Xylanase and Mannanase enzymes from Streptomyces galbus NR and their use in biobleaching of softwood kraft pulp. Antonie van Leeuwenhoek, 85(2): 103-114.

Karam, E., M. Hassan, M. Moharam, and A. Kansoh, 2018. Immobilization of Inulinase Produced by Rhizopus oligosporus NRRL 2549 for continuous fructose production. Journal of Materials and Environmental Science, 9: 2315-2321.

Kohli, U., P. Nigam, D. Singh, and K. Chaudhary, 2001. Thermostable, alkalophilic and cellulase free xylanase production by Thermoactinomyces thalophilus subgroup C. Enzyme and Microbial Technology, 28(7): 606-610.

Li, Y., X. Zhang, C. Lu, *et al.,* 2022. Identification and Characterization of a Novel Endo-β-1,4-Xylanase from Streptomyces sp. T7 and Its Application in Xylo-Oligosaccharide Production. Molecules, 27(8).

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J Biol Chem, 193(1): 265-75.

Meryandini, A., T. Sunarti, F. Mutia, N. Gusmawati, and Y. Lestari, 2009. Immobilized Xylanase from Streptomyces sp. 45I-3 in Corncob Xylan Hydrolysis. Journal of applied and industrial biotechnology in tropical region, 2.

Mhiri, S., A. Bouanane-Darenfed, S. Jemli, *et al.,* 2020. A thermophilic and thermostable xylanase from Caldicoprobacter algeriensis: Recombinant expression, characterization and application in paper biobleaching. Int J Biol Macromol, 164: 808-817.

Miller, G.L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 31(3): 426-428.

Richetti, A., C.B. Munaretto, L.A. Lerin, *et al.,* 2012. Immobilization of inulinase from Kluyveromyces marxianus NRRL Y-7571 using modified sodium alginate beads. Bioprocess and Biosystems Engineering, 35(3): 383-388.

Rifaat, H., Z. Nagieb, and Y. Ahmed, 2006. Production of xylanases by Streptomyces species and their bleaching effect on rice straw pulp. 4.

Rodrigues, P., I. Dorresteijn, J. Guilherme, *et al.,* 2021. Predicting the impacts of human population growth on forest mammals in the highlands of southwestern Ethiopia. Biological Conservation, 256: 109046.

Saratale, G.D., R.G. Saratale, and S.E. Oh, 2012. Production and characterization of multiple cellulolytic enzymes by isolated Streptomyces sp. MDS. Biomass and Bioenergy, 47: 302-315.

Sharad, K., I. Haq, A. Yadav, J. Prakash, and A. Raj, 2016. Immobilization and Biochemical Properties of Purifi ed Xylanase from Bacillus amyloliquefaciens SK-3 and Its Application in Kraft Pulp Biobleaching. Journol of Clinical Microbiology and Biochemical Technology, 26-34.

Sharma, P., D.V. Kumar, B. Naik, and G. Bisht, 2013. Screening of xylanase activity of Streptomyces albidoflavus PSM-3n isolated from Uttarakhand. EurAsian Journal of BioSciences, 7: 30-40.

Shivudu, G., K. Chandraraj, and P. Selvam, 2020. Production of xylooligosaccharides from xylan catalyzed by endo-1,4-β-D-xylanase-immobilized nanoscale carbon, silica and zirconia matrices. Molecular Catalysis, 484: 110745.

Sporck, D., F.A.M. Reinoso, J. Rencoret, *et al.,* 2017. Xylan extraction from pretreated sugarcane bagasse using alkaline and enzymatic approaches. Biotechnology for Biofuels, 10(1): 296.

Šuchová, K., A. Chyba, Z. Hegyi, M. Rebroš, and V. Puchart, 2022. Yeast GH30 Xylanase from Sugiyamaella lignohabitans Is a Glucuronoxylanase with Auxiliary Xylobiohydrolase Activity. Molecules, 27(3): 751.

Tamura, K., D. Peterson, N. Peterson, *et al.,* 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol., 28(10): 2731-9.

Teng, C., H. Tang, X. Li, *et al.,* 2021. Production of xylo-oligosaccharides using a Streptomyces rochei xylanase immobilized on Eudragit S-100. Biocatalysis and Biotransformation, 39(6): 408-417.

Tran, T.N. and C.T. Doan, 2021. Conversion of Wheat Bran to Xylanases and Dye Adsorbent by Streptomyces thermocarboxydus. 13(2).

Tran, T.N., C.T. Doan, T.K.L. Dinh, *et al.,* 2024. Optimization Production of an Endo-β-1,4-Xylanase from Streptomyces thermocarboxydus Using Wheat Bran as Sole Carbon Source. Recycling, 9(3): 50.

Xiao, B., X.F. Sun, and R. Sun, 2001. Chemical, structural, and thermal characterizations of alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw. Polymer Degradation and Stability, 74(2): 307-319.

Zhou, Z.-d., G.-y. Li, and Y.-j. Li, 2010. Immobilization of Saccharomyces cerevisiae alcohol dehydrogenase on hybrid alginate–chitosan beads. International Journal of Biological Macromolecules, 47(1): 21-26.