

RESEARCH ARTICLE

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Comparative Evaluation of Culture Media and pH Conditions for Xylanase Production in *Aspergillus niger*

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Abstract

Xylanase plays a crucial role in the degradation of hemicellulose and holds significant promise across a variety of biotechnological industries. This study investigates the effects of culture medium composition and initial pH on xylanase production by *Aspergillus niger*. Five different media; Mandels', Vogel's, Marciel's, Okafor's, and a modified Czapek-Dox, were evaluated for their ability to support enzyme synthesis. Additionally, initial pH levels ranging from 4.0 to 8.0 were tested to identify optimal production conditions. Among the tested media, Mandels' medium supported the highest xylanase activity, while the optimal pH range for enzyme production was between 6.0 and 7.0. These findings provide valuable insights for optimizing large-scale xylanase production using *A. niger*, contributing to the development of more efficient and cost-effective bioprocesses. Notably, this study incorporates biomass-specific productivity metrics (IU/g biomass), allowing for a more accurate evaluation of the strain's enzymatic efficiency. This quantitative approach may help inform future efforts to develop cost-effective and scalable bioprocesses, particularly when applied to low-cost agro-industrial substrates.

Keywords: Xylanase, Hemicellulose Degradation, Culture Medium Optimization, Biomass-specific Productivity

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Citation: Pakaweerachat P, Chysirichote T. Comparative Evaluation of Culture Media and pH Conditions for Xylanase Production in *Aspergillus niger*. J Pure Appl Microbiol. 2025;19(3):2260-2270. doi: 10.22207/JPAM.19.3.53

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INTRODUCTION

Hemicellulose, a major polysaccharide component of plant biomass, represents a significant renewable resource for the production of value-added bioproducts.^{1,2} Xylan, the most abundant form of hemicellulose,³ is composed of a α -1,4-linked xylose backbone with various side groups and requires a synergistic set of enzymes for its complete hydrolysis. Among these, xylanases (endo- β -1,4-xylanases; EC 3.2.1.8) catalyze the random cleavage of the xylan backbone, making them critical for the efficient degradation of lignocellulosic biomass.⁴ Owing to their efficiency and versatility, xylanases are widely employed in several industrial sectors, including pulp and paper processing (for environmentally friendly bleaching), animal feed improvement, food and beverage clarification, textile processing, and biofuel production.^{5,6}

Given the growing interest in sustainable bioprocessing, microbial production of xylanase has received considerable attention. Filamentous fungi, particularly *Aspergillus* species, are regarded as prolific producers due to their high secretion capacity and well-established fermentation profiles.^{7,8} *Aspergillus niger* has emerged as a leading organism for industrial enzyme production⁸ due to its Generally Recognized as Safe (GRAS) status, robust growth on diverse substrates, and ease of genetic manipulation.⁹ However, the production yield and activity of fungal xylanases are highly sensitive to cultivation conditions, especially the composition of the culture medium^{10,11} and environmental pH.^{12,13}

Among these variables, the composition of the culture medium plays a decisive role in modulating enzyme synthesis, not only by supplying essential nutrients but also by influencing regulatory pathways involved in enzyme induction or repression.¹⁴ For instance, the utilization of agro-industrial residues like wheat bran and corn cob has been shown to enhance xylanase production in *A. niger* strains under solid-state fermentation conditions.^{15,16} Additionally, pH plays a crucial role in enzyme production¹⁷ by influencing nutrient solubility, membrane transport, enzyme stability, and the activity of regulatory proteins.^{18,19} Studies have demonstrated that *A. niger* can produce xylanase over a broad pH range, with

optimal activity observed in both acidic and alkaline conditions, depending on the strain and cultivation parameters.^{20,21}

Although numerous studies have investigated xylanase production, comprehensive comparative analyses of the effects of different culture media and pH conditions on *A. niger* remain limited. In this study, five culture media; Mandels', Vogel's, Marciel's, Okafor's, and a modified Czapek-Dox medium, were evaluated for their ability to support xylanase production by *A. niger*. In addition, the influence of initial pH (4.0-8.0) was examined to determine optimal conditions for enzyme synthesis. The findings provide valuable insights for the biotechnological optimization of *A. niger* xylanase production, with potential applications in diverse industrial sectors.

MATERIALS AND METHODS

Strain and inoculum preparation

A. niger ATCC 16888 was used as the enzyme-producing strain. It was cultivated on potato dextrose agar (PDA) at 30 °C for 7 days. Spores were harvested using sterile water, and the spore suspension was adjusted to 10⁸ spores/mL.

Culture media and pH conditions

Five liquid media were tested: Mandels' medium, Vogel's medium, Marciel's medium,²² Okafor's medium,²³ and modified Czapek-Dox medium (CDM)²⁴ as presented in Table 1. Birchwood xylan (1.0% w/v) was used as the carbon source. Initial pH was set to 5.6. To assess pH influence, Mandels' medium was adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0 using acetate buffer (pH 4.0-5.0) and phosphate buffer (pH 6.0-8.0).

Cultivation technique

Fifty milliliters of sterilized culture medium (e.g., Mandels' medium) were dispensed into 250 mL Erlenmeyer flasks, adjusted to the desired initial pH (ranging from 4.0 to 8.0), and autoclaved at 121 °C for 15 minutes. After cooling, the media were inoculated with *A. niger* spore suspension at a concentration of 10⁷ spores/mL. The cultures were incubated at 30 °C for 5 days with shaking at 120 rpm to ensure adequate aeration. Samples were collected at regular intervals to assess biomass accumulation and

Table 1. Composition of the media for *A. niger* cultivation

Composition (g/L)	Mandels'	Vogel's	Marciel's	Okafar's	Modified CDM
Urea	0.30	-	-	-	-
Peptone	0.75	-	-	-	-
Yeast extract	0.25	-	-	-	5.00
(NH ₄) ₂ SO ₄	1.40	-	-	-	-
NH ₄ NO ₃	-	2.00	-	-	-
KH ₂ PO ₄	2.00	5.00	-	1.00	1.00
K ₂ HPO ₄	-	-	0.23	-	1.00
MgSO ₄ ·7H ₂ O	0.30	0.20	0.05	-	0.50
CaCl ₂ ·2H ₂ O	0.40	0.10	0.01	-	-
NaNO ₃	-	-	0.05	3.00	3.00
KCl	-	-	-	0.50	0.50
Na ₃ -citrate	-	2.50	-	-	-
Trace elements (mg/L)					
FeSO ₄ ·7H ₂ O	5.00	0.75	9.00	10.00	10.00
ZnSO ₄	1.40	3.00	2.00	1.00	-
MnSO ₄	1.60	0.50	12.00	12.5	-
CoCl ₂ ·6H ₂ O	20.00	-	-	-	-
CuSO ₄ ·5H ₂ O	-	0.30	-	0.50	-
H ₃ BO ₃	-	0.05	-	-	-

xylanase activity. All experimental conditions were tested in triplicate to ensure accuracy and reproducibility.

Enzyme extraction and assay

Samples were centrifuged at 6,000 rpm for 10 minutes, after which the supernatant was collected for enzyme assays, and the biomass dry weight was measured. Xylanase activity was determined according to the method of Bailey et al.²⁵ using birchwood xylan (1% w/v) as the substrate. The reaction mixture contained 0.5 mL of appropriately diluted enzyme solution and 0.5 mL of substrate prepared in 50 mM sodium citrate buffer (pH 6.0). The mixture was incubated at 50 °C for 10 minutes.

The amount of reducing sugars released was quantified using the 3,5-dinitrosalicylic acid (DNS) method,²⁶ with absorbance measured at 540 nm. One unit (IU) of xylanase activity was defined as the amount of enzyme required to release 1.0 μmol of xylose per minute under the assay conditions.

Determination of biomass

Fungal biomass obtained from the cultivation was harvested by filtration using pre-weighed Whatman No. 1 filter paper, then washed

thoroughly with distilled water to remove residual medium. The biomass was dried at 80 °C for 24 hours or until a constant weight was achieved and subsequently weighed to determine dry biomass. All measurements were performed in triplicate to ensure accuracy and reproducibility.

Data analysis

All results are expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons at a 95% confidence level (p < 0.05). All statistical analyses were performed using Minitab 19 software (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Effect of culture media composition on the biomass and xylanase production

The composition of the culture medium had a significant impact on xylanase activity and fungal biomass production by *A. niger*, as shown in Figures 1 and 2. Among the tested media, Mandels' medium produced the highest biomass production and xylanase activity at 72 and 96 hours, respectively. This suggests that its

balanced nutrient composition effectively supports both fungal growth and enzyme biosynthesis.²⁷ Mandels' medium includes multiple nitrogen sources, including urea, peptone, yeast extract, and ammonium sulfate, that together provide a rich mixture of amino acids, peptides, and vitamins, all of which promote extracellular enzyme production.^{28,29}

Modified Czapek-Dox (CMD) medium also supported relatively high xylanase activity. However, its dependence on sodium nitrate as the sole nitrogen source may have reduced nitrogen uptake efficiency.³⁰ In contrast, Vogel's, Marciel's, and Okafor's media yielded significantly lower enzyme activity. This can be attributed to their limited nitrogen diversity and the lower

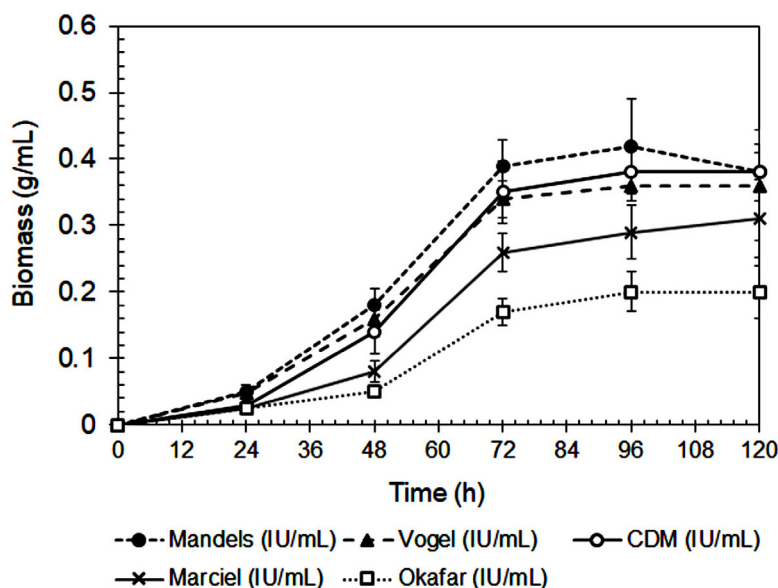


Figure 1. Biomass production of *A. niger* during cultivating in different media

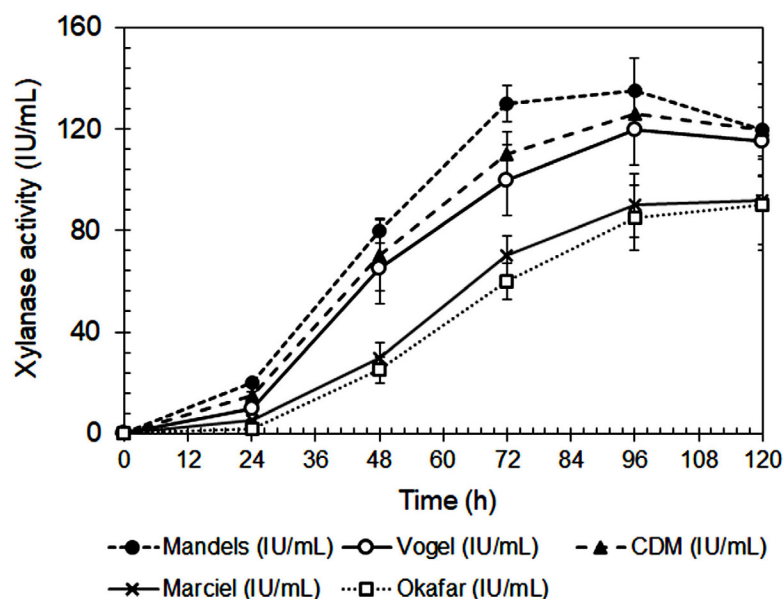


Figure 2. Xylanase activity from *A. niger* during cultivating in different media

bioavailability of their nitrogen sources. Vogel's medium primarily uses ammonium nitrate, while Marciel's and Okafor's formulations rely heavily on sodium nitrate.

Inorganic nitrogen sources like nitrate (NO_3^-) require enzymatic reduction to nitrite (NO_2^-) and then to ammonium (NH_4^+) before the fungus can assimilate them.³⁰ This multi-

step reduction, mediated by nitrate and nitrite reductases, consumes energy and may delay nitrogen assimilation during early fungal growth and enzyme synthesis.³¹ As a result, media that rely mainly on nitrate may not support optimal xylanase production.

In contrast, organic nitrogen sources, such as those found in Mandels' medium are more

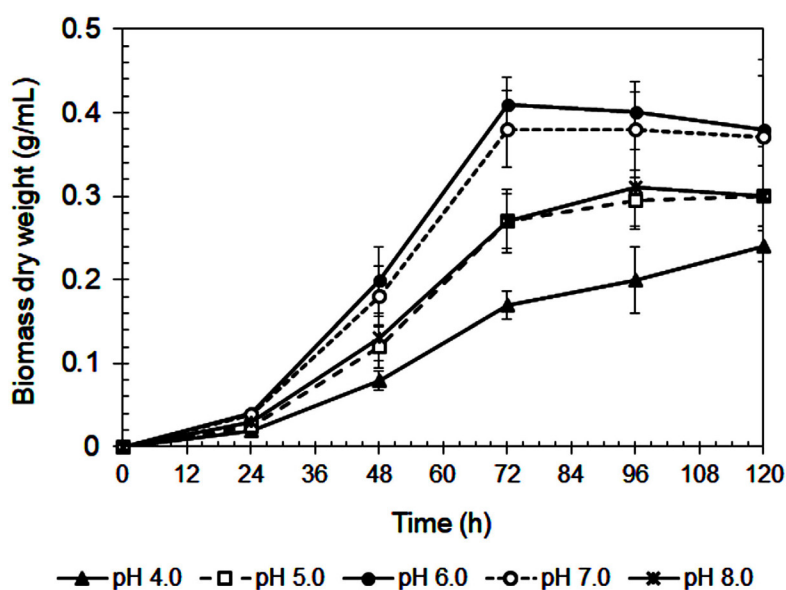


Figure 3. Xylanase activity of *A. niger* during cultivating in the Mandels' medium at various initial pHs

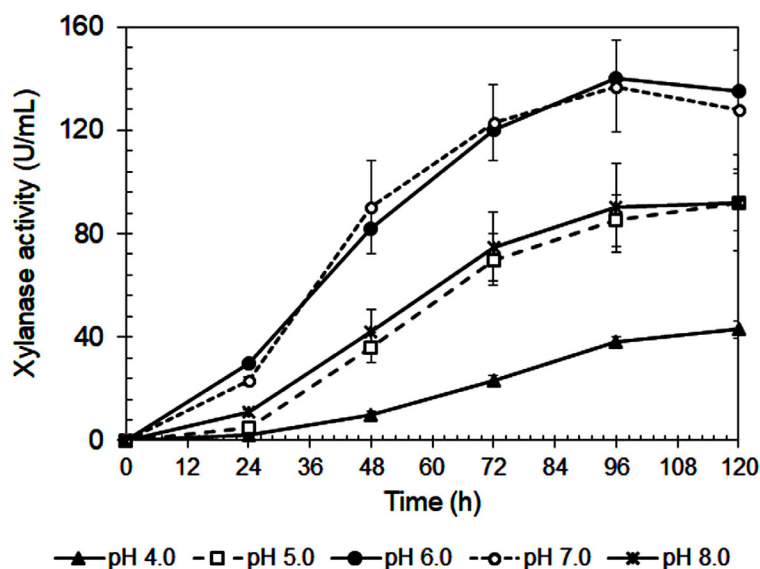


Figure 4. Biomass production of *A. niger* during cultivating in the Mandels' medium at various initial pHs

easily metabolized and require less energy for assimilation. They supply readily available building blocks for protein and enzyme biosynthesis, which enhances both fungal growth and secondary metabolite production.³² The lowest biomass yield and xylanase activity were observed in Okafar's medium, further underscoring the importance of nitrogen source composition and bioavailability in supporting efficient enzyme production.

These results are consistent with earlier studies showing that complex nutrient sources enhance xylanase secretion, especially when

agro-industrial residues are used as carbon sources. For example, Izidoro and Knob³³ reported that brewer's spent grain significantly increased xylanase production by *A. niger*, particularly in nutrient-rich media. Similarly, Fasiku et al.³⁴ found that *A. niger* GIO achieved the highest xylanase activity during solid-state fermentation (SSF) using alkaline-pretreated maize straw. In that study, optimal enzyme activity occurred at 40 °C and pH 5.0, demonstrating that agro-waste pretreatment can improve nutrient availability and enzyme yield.

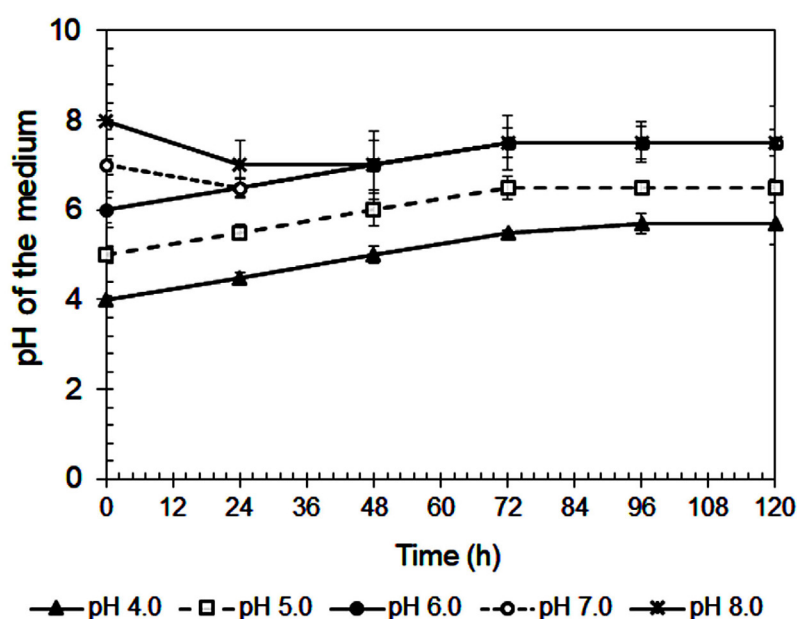


Figure 5. pH change of the medium during cultivating in the Mandels' medium at various initial pHs

Table 2. Summary of maximum biomass production and xylanase activity, and xylanase production capacity of *A. niger* cultivated in different media

Media	Maximum biomass (g/mL), Time (h)	Maximum xylanase activity (IU/mL), Time (h)	Xylanase production capacity of <i>A. niger</i> (IU/g biomass)	
			72 h	96 h
Mandels	0.39 ± 0.04 ^a , 72 h	135.8 ± 7.1 ^a , 96 h	333.3 ^a	378.2 ^b
Vogel	0.34 ± 0.05 ^b , 72 h	120.1 ± 4.0 ^c , 96 h	294.1 ^d	333.3 ^c
CDM	0.35 ± 0.03 ^b , 72 h	126.0 ± 4.6 ^b , 96 h	314.3 ^c	331.6 ^c
Marciel	0.26 ± 0.03 ^c , 72 h	90.2 ± 12.6 ^d , 96 h	269.2 ^d	310.3 ^d
Okafar	0.17 ± 0.02 ^d , 72 h	85.4 ± 2.8 ^e , 96 h	352.9 ^a	425.0 ^a

Note: Data are presented as mean ± standard deviation. Different superscript letters in the same row indicate significant differences ($p < 0.05$).

The carbon source's structure and concentration also play a critical role. Tan et al.³⁵ showed that corn cob xylan at 3.5 g/L resulted in higher xylanase activity than empty fruit bunches during *A. niger* fermentation. This highlights the importance of substrate accessibility and composition in inducing enzyme production.

In addition to macronutrients, trace elements such as iron, zinc, and manganese are essential for xylanase biosynthesis.³⁶⁻³⁸ These act as cofactors that maintain enzyme stability and support catalytic function.³⁹ Mandels' and CMD media, which produced higher enzyme activity, included a more balanced profile of these trace elements. In contrast, Vogel's medium contained fewer essential minerals, which may explain its lower enzyme yields.

Recent advances in strain improvement have also demonstrated the potential for enhancing xylanase production beyond medium optimization. Sharma et al.⁴⁰ reported that *A. niger* mutants developed through UV irradiation and 5-bromouracil treatment exhibited up to a fourfold increase in xylanase activity compared to wild-type strains. These improved mutants were selected based on colony morphology, stability, amino acid requirements, and enzyme output. Combining genetically enhanced strains with nutrient-rich media, such as Mandels', offers a synergistic approach to improving industrial xylanase yields.

Marciel's medium, which lacks nitrogen source diversity, showed the lowest enzyme activity among the tested media. Although Okafor's medium contains a relatively high concentration of sodium nitrate, it was less effective than Mandels' or CMD in supporting xylanase production. This is likely due to the slower uptake of nitrate compared to ammonium and organic nitrogen sources like peptone and yeast extract, as nitrate must be enzymatically reduced before it can be assimilated.⁴¹

Mandels' and CMD media also provided a better balance of trace elements, likely contributing to enhanced xylanase stability and catalytic efficiency. In contrast, Vogel's medium, with fewer essential minerals, resulted in lower enzyme production. These findings support the use of *A. niger* as a preferred microorganism for enzyme production. Its GRAS status, rapid growth, and ability to metabolize lignocellulosic

substrates make it highly suitable for industrial-scale fermentation.⁴⁰ Enzymes from *A. niger* are widely applied in the paper and pulp industry, food processing, and biofuel production, emphasizing the importance of process optimization strategies such as those presented here.

The data summarized in Table 2 demonstrates the xylanase production capacity, expressed as enzyme activity per gram of biomass (IU/g), provides a more accurate assessment of the fungus's efficiency in enzyme secretion independent of biomass quantity. Based on Table 2, the highest specific xylanase productivity was observed in Okafor's medium (425.0 IU/g biomass at 96 h), despite its low overall xylanase activity and biomass yield. This indicates that under nitrogen-limited conditions, *A. niger* may redirect metabolic energy toward secondary metabolite synthesis rather than growth, a phenomenon often reported in filamentous fungi during nutrient stress.⁴⁰ Mandels' medium, which supported the highest overall xylanase activity and biomass production, showed a moderate xylanase production capacity. While the total enzyme output is greater, the enzyme yield per unit biomass is slightly lower than that in Okafor's medium, likely due to the nutrient-rich conditions favoring both primary and secondary metabolism. CMD medium also exhibited relatively balanced performance with a xylanase activity of 331.6 IU/g biomass, suggesting efficient enzyme production under semi-defined nutrient conditions. Vogel's and Marciel's media had lower capacities, consistent with their limited nitrogen availability and reduced metabolic flexibility.

These findings underscore the inverse relationship that can sometimes occur between biomass yield and enzyme productivity per biomass unit. Therefore, optimizing culture conditions should not focus solely on maximizing biomass or enzyme titer alone but rather on enhancing specific productivity, particularly in industrial settings where enzyme yield per input biomass is economically critical.

Effect of culture pH on the biomass and xylanase productions of *A. niger*

The influence of initial pH on xylanase production by *A. niger* is shown in Figure 3. The highest enzyme activity (141 IU/mL) occurred at

pH 6.0, consistent with previous studies.⁴² Enzyme activity declined sharply at more acidic pH values (≤ 5.0), likely due to poor fungal growth (Figure 4) and reduced enzyme stability.^{43,44} A similar reduction at pH 8.0 suggests that alkaline conditions are also unfavorable for enzyme synthesis.⁴⁵

Xylanase showed optimal stability and productivity in a slightly acidic to neutral pH range, which is ideal for food industry applications, such as baking and beverages, where controlled pH is important.⁴⁶ The steep decrease in activity at pH 4.0-5.0 may be due to enzyme denaturation, conformational changes, and inhibited fungal metabolism.^{29,31} On the other hand, reduced enzyme production at pH ≥ 7.0 could result from suppressed fungal growth or disruption of metabolic pathways.⁴⁶

Interestingly, although maximum activity was recorded at pH 6.0, xylanase production remained relatively stable between pH 6.0 and 7.0.⁴⁷ This suggests that small pH fluctuations within this range may not significantly affect enzyme output, a beneficial trait for industrial processes where exact pH control is difficult.⁴⁵

The tendency for fermentation pH to stabilize between 6.2 and 6.4 during cultivation, regardless of the starting pH (Figure 5), further supports process robustness. This buffering effect is likely caused by fungal metabolism and the release of organic acids or bases.⁴⁶ In addition, changes in colony color (black under acidic and white under alkaline conditions) suggest that pH affects fungal physiology, possibly influencing sporulation or metabolic behavior.⁴³

As summarized in Table 3, pH also influenced the efficiency of enzyme production in relation to fungal biomass. Although pH 6.0 led to the highest xylanase titer and biomass yield, pH 7.0 produced the highest xylanase productivity per gram of biomass (325.3 IU/g), suggesting more efficient enzyme synthesis at this pH. In contrast, extreme acidic (pH 4.0) and alkaline (pH 8.0) conditions resulted in much lower specific productivity (195.9 and 277.0 IU/g, respectively). These findings confirm that maintaining culture pH between 6.5 and 7.0 is ideal for maximizing enzyme production efficiency in submerged fermentation systems.

Table 3. Summary of maximum biomass production and xylanase activity, and xylanase production capacity of *A. niger* cultivated in Mandels' medium at different pH values

pH	Maximum biomass (g/mL)	Maximum activity (IU/mL)	Fermentation time (h)	Xylanase production capacity of <i>A. niger</i> (IU/g biomass)
4.0	0.22 \pm 0.02 ^c	43.1 \pm 3.4 ^c	120	195.9 ^c
5.0	0.27 \pm 0.04 ^b	70.3 \pm 9.8 ^b	72	292.9 ^a
6.0	0.41 \pm 0.03 ^a	120.0 \pm 9.6 ^a	72	292.7 ^a
7.0	0.38 \pm 0.05 ^a	123.6 \pm 14.7 ^a	72	325.3 ^a
8.0	0.27 \pm 0.03 ^b	74.8 \pm 13.5 ^b	72	277.0 ^b

Note: Data are presented as mean \pm standard deviation. Different superscript letters in the same row indicate significant differences ($p < 0.05$).

CONCLUSION

This study underscores the critical influence of culture medium composition and initial pH on xylanase production by *Aspergillus niger*. Among the evaluated media, Mandels' formulation proved most effective, primarily due to its balanced combination of organic and inorganic nitrogen sources and essential trace

elements. Optimal enzyme yields were achieved within a pH range of 6.0-7.0, conditions that also supported robust fungal growth and enhanced enzyme productivity. These findings provide a solid framework for scaling up fermentation processes, with potential to increase process efficiency, lower production costs, and ensure consistent xylanase output.

Given the broad industrial relevance of xylanase, the implications of this work are far-reaching. In the pulp and paper industry, *A. niger* derived xylanase enables eco-friendly bio-bleaching and improves fiber quality. In food and feed sectors, it enhances product texture, nutrient release, and digestibility. Furthermore, in lignocellulosic biorefineries, xylanase is vital for the hydrolysis of hemicellulose into fermentable sugars, facilitating the sustainable production of biofuels and bio-based chemicals.

The demonstrated compatibility of *A. niger* with low-cost agro-industrial substrates, its resilience under mildly acidic to neutral pH, and its GRAS (Generally Recognized as Safe) status reinforce its value as a workhorse for industrial enzyme production. Overall, this study contributes to the optimization of key bioprocess parameters and supports the development of scalable, sustainable, and economically viable strategies for industrial xylanase manufacturing.

ACKNOWLEDGMENTS

The author gratefully recognizes the Department of Food Engineering, School of Engineering, King Mongkut's Institute of Technology Ladkrabang for providing equipment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

TC conceptualized the study and applied methodology. PP and TC performed formal analysis. TC performed data curation, visualization, validation and supervision. TC wrote the manuscript. TC and PP reviewed and edited the the manuscript. Both authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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