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Optimization of Cellulase Enzyme Production from Corn Cob Waste by Cellulolytic Fungus Aspergillus niger

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Abstract

Corncob is an agricultural waste rich in cellulose, making it a promising alternative substrate for cellulase enzyme production, which is widely used in various industrial applications such as bioenergy, food, and textile industries. Aspergillus niger is a well-known cellulolytic fungus capable of efficiently producing cellulase enzymes. This study aimed to optimize cellulase production by A. niger using corncob powder (Zea mays) as a substrate through submerged fermentation (SmF), with variations in temperature (29.5°C and 30.5°C) and pH (4.5 and 5.5). The cellulolytic activity of A. niger was tested on CMC agar medium. Cellulase activity was analyzed based on the concentration of reducing sugars using the DNS method. The highest enzymatic activity was recorded at 1.125 IU/mL with a biomass yield of 0.754 mg/mL at 30.5°C and pH 4.5. These findings suggest that optimizing environmental conditions and utilizing corncob waste as a carbon source can enhance cellulase production by A. niger, offering a sustainable and cost-effective approach for enzyme-based industries.

Keywords: Aspergillus Niger; Cellulase Enzyme; Cellulolytic Fungus; Cellulose; Corn Cob

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INTRODUCTION

Corn (*Zea mays*) is a major agricultural commodity in Indonesia, with 2024 production reaching 15.14 million tons—a 2.47% increase from the previous year (Badan Pusat Statistik, 2025). Despite this, about 90% of corn cob waste is still discarded or burned, contributing to air pollution (Halbi, 2021). Yet, corn cobs contain valuable lignocellulosic compounds—cellulose (40–60%), hemicellulose (20–30%), and lignin (15–30%)—with potential for industrial uses, such as cellulase enzyme production (Susanti & Rahmi, 2020).

Cellulase, which includes endoglucanase, exoglucanase, and β-glucosidase, works synergistically to break down cellulose into glucose. It is widely used in industries such as bioenergy, textiles, animal feed, and waste treatment. The global demand continues to increase and is projected to reach USD 2.99 billion by 2028 (Research and Markets, 2024). However, the high production cost, mainly due to expensive raw materials and fermentation processes, remains a challenge. Therefore, utilizing lignocellulosic waste as a low-cost substrate and optimizing fermentation conditions are essential for more efficient and economical enzyme production (Maftukhah & Abdullah, 2018).

One of the promising cellulase-producing microorganisms is *Aspergillus niger*, a cellulolytic fungus known for its ability to degrade cellulose into glucose. Several studies have reported that *A. niger* exhibits higher cellulase activity compared to other fungal species such as *Trichoderma harzianum*, *Penicillium chrysogenum*, *Aspergillus flavus*, and *Aspergillus terreus* (Bukar *et al*., 2016; Ezeagu *et al*., 2023).

Given the high potential of corn cobs as a low-cost and abundantly available alternative substrate, their utilization in cellulase production by *Aspergillus niger* presents a research opportunity to address cost-efficiency challenges in the enzyme industry. One of the critical aspects of enzyme production is the influence of fermentation conditions, particularly temperature and pH, which play a vital role in determining the enzymatic activity. Therefore, optimizing temperature and pH parameters is expected to enhance cellulase activity, thereby supporting more efficient and sustainable production. This study aims to contribute scientifically to the valorization of agricultural waste and the development of microorganism-based bioproducts.

RESEARCH METHOD

Research location

The research was conducted from December 2024 to April 2025 at the Biotechnology Research Laboratory, Biology Study Program, Faculty of Mathematics and Natural Sciences (FPMIPA), Universitas Pendidikan Indonesia.

Materials

The fungal sample used in this study was obtained from the pure culture isolation of the cellulolytic fungus *Aspergillus niger*, provided by PT Agritama Sinergi dan Inovasi (AGAVI). Sweet corn (*Zea mays* var. *saccharata*) cobs were collected from a corn plantation located in Saguling Subdistrict, West Bandung Regency, West Java, Indonesia.

The equipment used in this study included an autoclave, beaker glass, petri dishes, centrifuge, incubator, laminar air flow cabinet, refrigerator, oven, Erlenmeyer flasks, measuring cylinders, Bunsen burner, shaker, magnetic stirrer with hot plate, spectrophotometer, test tube rack, Durham tubes, analytical balance, blender, microscope, vortex mixer, micropipette, 100-mesh sieve, test tubes, inoculation loop, and mortar and pestle. The materials used in this study included a cellulolytic fungal isolate of *Aspergillus niger* from a pure culture, Carboxymethyl Cellulose (CMC) medium, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), various agar media (starch, gelatin, lipid, and phosphate), carbohydrate fermentation media, Dinitrosalicylic Acid (DNS) reagent, NaOH 6% solution, NaCl solution, Congo red 0.1% solution, and corn cob waste.

Fermentation step

Pre-treatment of corn cobs and delignification

Pre-treatment and delignification of corn cobs began with three days of sun-drying, followed by oven drying at 100°C for another three days. The dried cobs were crushed, sieved (100 mesh), and treated with 6% NaOH (1:10 ratio), then autoclaved at 121°C for 15 minutes. The mixture was neutralized with H_2SO_4 and oven-dried at 105°C for 8 hours. The resulting delignified powder was used for enzymatic hydrolysis to produce hydrolysate sugars (Sari *et al*., 2018).

Isolation and identification of cellulolytic fungi

The cellulolytic fungus was isolated from a pure culture and cultivated on solid

Potato Dextrose Agar (PDA) medium using the spread plate method. After incubation at 37°C for 72 hours, fungal colonies were transferred to slant tubes containing PDA to obtain pure cultures, followed by incubation at the same temperature for another 72 hours.

Cellulolytic activity assay on CMC Medium

Fungal isolates purified on PDA were tested for cellulolytic activity on CMC agar medium using the cellulolytic index as an indicator. After inoculation, fungi were incubated at 37°C for 7 days, then flooded with 0.1% Congo red for 30 minutes and rinsed with 1% NaCl. Cellulolytic activity was shown by clear zones around colonies. The cellulolytic index (CI) was calculated using a specific formula (Talantan *et al*., 2018):

$$\text{Cellulolytic Index (CI)} = \frac{\text{clear zone diameter (mm) - colony diameter (mm)}}{\text{colony diameter (mm)}}$$

Cellulase enzyme production using Submerged Fermentation (SmF) method

Cellulase production used the Submerged Fermentation (SmF) method with three replications. Three loops of *Aspergillus niger* colonies were inoculated into Potato Dextrose Broth and incubated on a shaker at 135 rpm. The fermentation medium contained $(NH_4)_2SO_4$, KH_2PO_4, urea, $CaCl_2$, $MgSO_4$, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $CoCl_2$, peptone, and 7.5 g corn cob substrate in 1000 mL of 0.2 M phosphate buffer. After autoclaving at 121°C for 15 minutes, 2% inoculum was added, and fermentation occurred at 135 rpm for 96 hours under two temperature (29.5°C and 30.5°C) and pH (4.5 and 5.5) conditions. Crude cellulase was obtained by centrifuging at 4000 rpm for 5 minutes to separate fungal cells (Oktariani, 2017).

Measurement of Fungal Biomass Quantity

Fungal biomass during fermentation was measured using the Optical Density (OD) method with a spectrophotometer at a wavelength of 610 nm. A total of 1 mL of fermentation medium from each sample was transferred into a cuvette, while a cuvette containing distilled water was used as a blank for comparison (Lizayana & Iswandi, 2016).

Cellulase enzyme activity analysis

Cellulase activity assay on corn cob powder substrate

The cellulase activity assay mixed 0.01 g corn cob powder, 1 mL phosphate buffer (pH 4.5 and 5.5), and 1 mL crude enzyme extract, then incubated at 29.5°C and 30.5°C for 30 minutes. The reaction was stopped by heating at 100°C for 15 minutes, followed by centrifugation at 5000 rpm for 10 minutes. Then, 1 mL DNS reagent was added to 1 mL supernatant and incubated at 100°C for 10 minutes. Enzyme activity was measured at 540 nm with a UV-Vis spectrophotometer and calculated using a specific formula (Murtiyaningsih & Hazmi, 2017):

$$\text{Enzyme activity (IU/mL)} = \frac{C \; x \; Df \; x \; 10}{t \; x \; BM \; glucose}$$

Description: C = concentration of reducing sugar; t = incubation time (30 minutes); Df = dilution factor; BM = BM glucose (180 dalton)

Data Analysis Step

Statistical data analysis was performed using SPSS 20 for Windows to evaluate the effect of pH and temperature on cellulase enzyme activity. The analysis included tests for normality and homogeneity. If the data were normally distributed and homogeneous, a Two-Way ANOVA was conducted. However, if the data were not normally distributed, the non-parametric Friedman test was applied, followed by the Wilcoxon test for pairwise comparisons

RESULT AND DISCUSSION

Cellulolytic Activity of fungi on CMC medium

The cellulolytic activity assay showed all *Aspergillus niger* isolates had high activity, indicated by clear zones around colonies (Figure 1). Isolate AN8 had the highest cellulolytic index (4.00), while AN3 had a lower but still high value (2.66) (Table 1). These results confirm *A. niger's* enzymatic potential, consistent with Sutari (2020), who stated that isolates with an index above 3.00 have high cellulase production potential.

However, the variation in indices among isolates indicates physiological differences such as spore density and growth rate (Sari *et al*., 2017). Murtiyaningsih & Hazmi (2017) stated that the higher the cellulolytic index, the greater the cellulolytic activity. This result is further supported by Belal et al. (2021), who reported that *A. niger* produced the highest cellulolytic index compared to *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp., *Fusarium* sp., *Rhizopus* sp., *Aspergillus flavus*, *Mucor* sp., and *Alternaria* sp. Therefore, the

biotechnological potential of *A. niger* for cellulase production is highly competitive compared to other fungi.

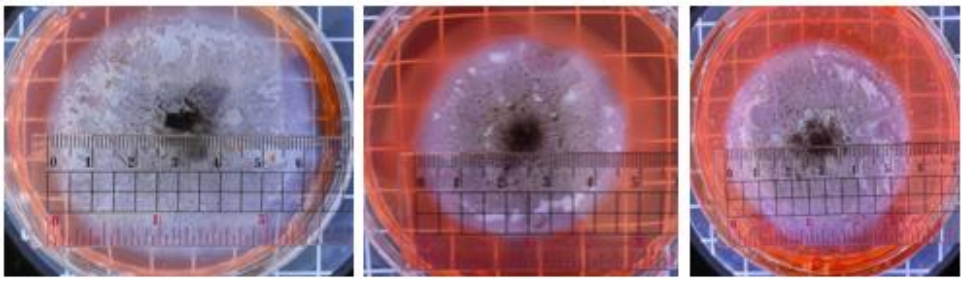

Figure 1. Results of the Cellulolytic Activity Test of *Aspergillus niger* Isolates AN8, AN6, and AN3

Table 1. Cellulolytic Activity Test Results of *Aspergillus niger* Isolates

Isolate Code	Clear Zone Diameter (mm)	Colony Diameter (mm)	Cellulolytic Index	Category
AN8	65	13	4	High
AN6	47	10	3,7	High
AN3	55	15	2,66	High

Effect of temperature and pH on cellulase enzyme activity

The results showed that at a temperature of 29.5°C, cellulase enzyme activity progressively increased up to 96 hours at both pH 4.5 and 5.5, in line with biomass accumulation (Figure 2). The highest activity was observed at pH 4.5, reaching 1.074 IU/mL. This finding is consistent with the study by Sari et al. (2022), which stated that a greater number of active cells resulting from high biomass accumulation leads to higher enzyme production. Kusuma *et al*. (2019) also reported that a sharp increase in cellulase enzyme activity indicates the full adaptation of fungal cells to environmental conditions and the optimization of metabolic pathways for cellulase production.

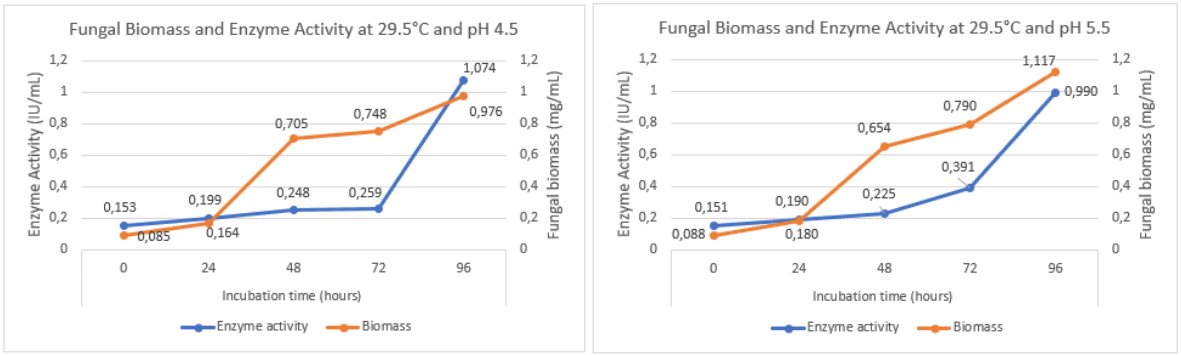

Figure 2. Comparison of Biomass of *Aspergillus niger* and Cellulase Enzyme Activity at 29.5°C with pH Variations of 4.5 and 5.5

In contrast, at a temperature of 30.5°C, cellulase enzyme activity reached its peak earlier, at 48 hours, and subsequently declined, despite continued biomass accumulation (Figure 3). The highest cellulase activity was recorded at pH 4.5, reaching 1.125 IU/mL. This discrepancy suggests that biomass growth does not always correlate directly with enzyme production.

Compared to the study conducted by Purkan et al. (2015), which used rice husk substrate and reported maximum cellulase activity of 0.709 IU/mL at pH 4 and 50°C before declining, the results of this study demonstrate higher enzyme activity, reaching 1.125 IU/mL at 30.5°C and pH 4.5 at 48 hours. These findings indicate that pH conditions of 4.5 and 5.5 at 30.5°C in this study provided more optimal effectiveness for cellulase enzyme production than previously reported conditions.

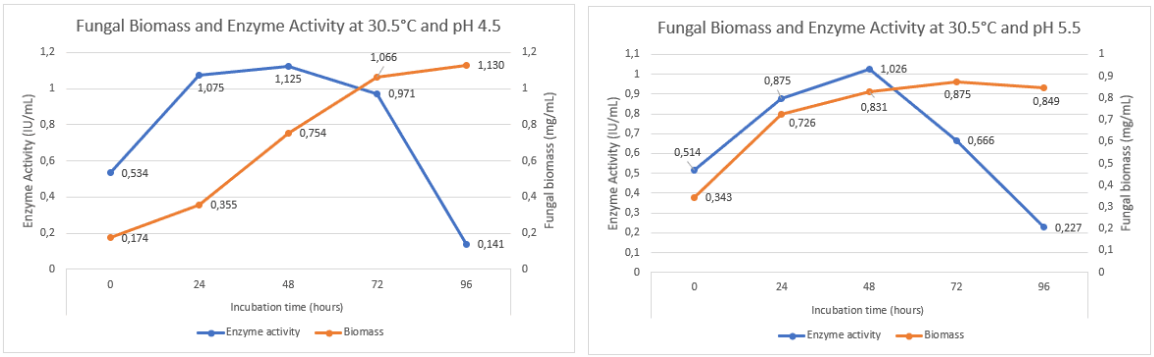

Figure 3. Comparison of Biomass of *Aspergillus niger* and Cellulase Enzyme Activity at 30.5°C with pH Variations of 4.5 and 5.5

The decline in enzyme activity may be triggered by glucose depletion, which leads to the utilization of cellulose as a carbon source and inhibits enzyme function through feedback inhibition (Sembiring, 2019). Catabolite repression may also suppress cellulase production when glucose levels in the medium remain high, hindering cellulose degradation as the fungus preferentially utilizes glucose as its primary carbon source (Yogyaswari *et al.*, 2016). A study by Brown et al. (2024) emphasized that the accumulation of cellobiose in the medium can inhibit cellulase enzyme activity, particularly endoglucanase and β-glucosidase.

The results of this study demonstrate that temperature and pH play crucial roles in cellulase enzyme activity. Bendaoud et al. (2024) reported similar findings, indicating that *Aspergillus niger* produced the highest cellulase activity under optimal conditions of 30°C and pH 4, outperforming *Trichoderma longibrachiatum*. Bakare et al. (2022) also

observed maximum cellulase activity at 30°C, yielding 0.72 IU/mL, although at a different pH level of 6.

The findings of Lubis et al. (2024) further support this study by stating that enzyme protein structural stability is highly influenced by pH. Acidic environments facilitate enzyme release, while high pH levels can hinder it, requiring careful pH adjustment to maintain optimal enzyme activity. Kurniawati et al. (2021) also noted that temperature affects the rate of enzymatic reactions up to an optimal point, beyond which enzyme denaturation may occur.

Effectiveness of Corncob as a Substrate

The selection of corncob as a substrate has proven effective in enhancing cellulase enzyme activity, supported by its high cellulose content and microporous structure, which facilitates enzyme accessibility (Isa et al., 2020). These findings are consistent with those of Pandey et al. (2015), who reported that corncob substrate yielded the highest cellulase activity of 1.21 IU/mL compared to wheat bran, sucrose, and maltose when using *Trichoderma harzianum*. Similar results were reported by Effiong et al. (2024), showing that corncob substrate generated higher enzyme activity than rice bran and sorghum bran. Therefore, corncob holds strong potential as an alternative substrate in enzymatic bioprocesses.

Data analysis results

The statistical analysis results indicated that the data were not normally distributed ($p < 0.05$); therefore, non-parametric Friedman and Wilcoxon tests were applied (Table 2). The Friedman test revealed significant differences among the temperature and pH treatments ($p = 0.000 < 0.05$), indicating a substantial effect on enzyme activity. The Wilcoxon test also confirmed that the differences between treatment pairs were statistically significant ($p = 0.000 < 0.05$), validating that variations in environmental parameters had a meaningful impact. These statistical significance values demonstrate that temperature and pH variations influence cellulase enzyme activity, emphasizing the importance of optimizing these two factors to enhance enzyme production and activity.

Table 2. Results of Statistical Tests

Statistical Test		α	Significance
Normality Test (Shapiro-Wilk)	pH	0.05	0.000
	Temperature		0.000
	Enzyme activity		0.003

Friedman Test		0.05	0.000
Wilcoxon Test	Temperature – enzyme activity	0.05	0.000
	pH – enzyme activity	0.05	0.000

CONCLUSION

This study shows that corn cob waste can be used as a substrate for cellulase production by *Aspergillus niger*, with the highest enzyme activity of 1.125 IU/mL at 30.5°C and pH 4.5. Optimizing fermentation and using low-cost substrates can improve cellulase production efficiently and sustainably. These findings support the use of lignocellulosic waste as an alternative raw material, promoting eco-friendly bioconversion and reducing industrial production costs.

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