

Screening of Polysaccharide-Lytic Enzymes by *Aspergillus Oryzae* on Corncob (*Zea mays*) and Banana Peel (*Musa acuminata* Colla)

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ABSTRACT

Polysaccharide-lytic enzymes (PLEs)—xylanase, pectinase, amylase, β -glucosidase and endoglucanase—play key roles in the food, feed and bioenergy sectors by converting lignocellulosic waste into fermentable sugars. This study evaluated the ability of *Aspergillus oryzae* to produce these five PLEs on two abundant yet under-utilised agro-industrial residues: corn cobs (*Zea mays*) and banana peels (*Musa acuminata* balbisiana Colla). Submerged fermentation was performed using 1 % (w/v) substrate at 30 °C and 120 rpm. Enzyme activities were determined colorimetrically, and differences between substrates were analyzed using Mann–Whitney U test whereas the starch- and pectin-rich banana peels favour pectinase, amylase, glucosidase and endoglucanase, Spearman–Kendall correlation, and linear regression. The highest xylanase titre (12.8 ± 0.2 IU mL⁻¹) was achieved with corn cobs—about 1.5-fold higher than with banana peels ($p < 0.05$). Conversely, banana peels significantly boosted pectinase (2.5 IU mL⁻¹), amylase (14.0 IU mL⁻¹), β -glucosidase (18.1 IU mL⁻¹) and endoglucanase (15.2 IU mL⁻¹) compared with corn cobs ($p < 0.05$). Strong negative correlations ($\rho \approx -0.85$ to -0.95) were observed between xylanase and the other enzymes, while the latter showed very strong positive intercorrelations ($\rho \approx 0.81$ – 0.98). Linear regression confirmed that substrate type significantly affected enzyme yields ($R^2 > 0.90$; $p < 0.001$). These findings indicate that corn cobs are the most effective substrate for xylanase production, whereas banana peels better support pectinase, amylase, β -glucosidase, and endoglucanase synthesis. The results highlight the importance of selecting appropriate agro-waste substrates to maximise enzyme production and sustainably valorise agricultural residues.

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1. INTRODUCTION

Polysaccharide-degrading enzymes, or Polysaccharide-Lytic Enzymes (PLEs), play a pivotal role across several industrial sectors—including food, animal feed, and bioenergy. PLEs comprise a variety of enzymes, among them xylanase, pectinase, amylase, β -glucosidase, and endoglucanase, which collectively break down complex polysaccharide compounds into oligosaccharides or monosaccharides [1], [2]. The chief advantage of PLEs lies in their ability to harness natural feedstocks—particularly lignocellulosic waste—to generate simple sugars that are valuable for subsequent fermentation processes [3]–[5].

Aspergillus oryzae is widely regarded as outstanding for hydrolytic-enzyme production thanks to its ability to biosynthesize a diverse and stable array of enzymes [6], [7]. Its capacity to utilise a wide variety of carbon substrates results in high enzyme activities that are vital for biomass conversion and waste treatment. *Aspergillus oryzae* also produces amylase, which breaks complex carbohydrates into simple sugars and thus supports fermentation [8]. Its application in the bioremediation of starchy wastes further promotes sustainability [6], [7], [9].

Agricultural residues such as banana peels (*Musa acuminata* Colla) and corn cobs (*Zea mays*) are abundant biomass resources, yet their utilisation remains limited. Most of this waste ends up in landfills or is burned, causing environmental problems such as greenhouse-gas emissions and pollution [10], [11]. Both banana peels and corn cobs are rich in complex carbohydrates—including cellulose, hemicellulose, pectin and starch—that can be hydrolysed into simple sugars by polysaccharide-degrading enzymes. Harnessing *Aspergillus oryzae* together with these agricultural wastes is expected to reduce production costs while meeting sustainability demands in industrial processes.

Aspergillus oryzae has shown considerable potential for producing polysaccharide-degrading enzymes when cultivated on various agricultural waste substrates. Studies reveal that incubating *A. oryzae* with lignocellulosic materials such as sugarcane bagasse or rice bran results in a marked increase in enzymes like pectinase and xylanase [12]. This indicates that the specific substrate composition can boost the synthesis of particular enzymes, which is essential for efficient biomass degradation.

Numerous studies have shown that *Aspergillus oryzae* can produce individual hydrolytic enzymes on single substrates—xylanase on corn cobs, for instance,[13] or amylase on banana-peel waste[14]. Yet no report has systematically compared the full multi-enzyme polysaccharide-degrading profile (xylanase, pectinase, amylase, β -glucosidase, and endoglucanase) of *A. oryzae* grown on two different substrates—banana peels and corn cobs—under identical fermentation conditions. Although co-production studies exist, they involve non-*oryzae* species such as *A. fumigatus* MS16 on banana peels [15]. Banana peels are rich in pectin and starch, whereas corn cobs contain higher levels of cellulose and hemicellulose, presenting distinct opportunities and challenges for enzyme production [16]. Our study addresses this gap by evaluating both agro-industrial wastes as targeted enzyme sources. Tailoring the substrate in this way can boost enzyme yields and thus improve the overall efficiency of converting agricultural residues into valuable

products[17]. Specifically, the research assesses the potential of *A. oryzae* to produce polysaccharide-degrading enzymes on banana peels and corn cobs so that the relative effectiveness of each substrate in promoting enzyme synthesis can be determined.

The findings are expected to guide the choice of the best substrate for industrial-scale polysaccharidase production while encouraging sustainable use of agricultural waste and reducing environmental impact.

2. RESEARCH METHODS

2.1 Materials

a. Biomass feedstock

Banana-peel biomass was collected from household waste, while corn cobs (*Zea mays*) were sourced from maize farmers in Jogorogo, Ngawi. Before use, the banana peels were cut into 5–8 cm pieces with a stainless-steel knife and soaked in 1 % (w/v) sodium thiosulfate for 5 h at 30 °C to inactivate polyphenol oxidase and prevent melanin formation [18]. The pieces were then rinsed with de-ionised water until a BaCl₂ test showed residual sulphite < 5 ppm, and dried at 60 °C. A control batch without sodium thiosulfate was prepared in parallel to assess the effect of soaking. The cellulose, hemicellulose, pectin and starch contents before and after soaking did not differ significantly ($p > 0.05$) (data not shown). Ground corn cobs were immersed in 0.5 % (w/v) sodium hypochlorite for ~ 6 h at 30 °C for delignification, followed by hot-air-oven drying [19], [20]. Dried banana-peel and corn-cob fragments were then sieved through a < 1 mm mesh and stored for subsequent use.

b. Culture medium

The liquid growth medium contained (all % w/v): 1 % carbon source (banana peels or corn cobs), 0.25 % KH₂PO₄, 0.10 % yeast extract, 0.035 % MgSO₄·7H₂O, 0.035 % urea, 0.10 % Tween 80, 0.005 % FeSO₄·H₂O, 0.001 % MnSO₄·H₂O, and 0.10 % NaCl. The initial pH was adjusted to 5.6. A 5 mL inoculum containing 3 % calcium carbonate was transferred into a 100 mL Erlenmeyer flask holding 50 mL of medium. Cultivation was carried out at 30 °C with rotary shaking at 120 rpm.

c. Saccharification of *Aspergillus oryzae* biomass substrates

Reducing sugars were quantified with the Somogyi–Nelson method (Somogyi, 1952). Protein concentration was determined by the Lowry assay using bovine serum albumin as a standard (Lowry et al., 1951) or by measuring optical density at 280 nm.

Activities of xylanase, pectinase, amylase, β -glucosidase, and endoglucanase in cultures grown on banana-peel or corn-cob biomass were assayed as follows: a 100 μ L crude-enzyme aliquot was incubated with the relevant 1 % (w/v) substrate—beechwood xylan for xylanase, citrus pectin for pectinase, starch for amylase, salicin for β -glucosidase, and carboxymethyl cellulose (CMC) for endoglucanase—in 50 mM acetate buffer (pH 5.0) to a final reaction volume of 1.0 mL. The resulting reducing sugars were measured with the Somogyi–Nelson method against xylose, galacturonate and glucose standards. One

international unit (U) of enzyme activity was defined as the amount of enzyme that releases 1 μmol of reducing sugar per minute under the assay conditions. The enzymes produced are presumed to participate in degrading the polysaccharide constituents of banana-peel or corn-cob biomass.

2.2 Experimental Design and Research Methodology

Table 1. Experimental Design

Replicate	Treatment P0 (Corn cobs)	Treatment P1 (Banana peels)
1	BJ0	KP1
2	BJ0	KP1
3	BJ0	KP1

This study employed a Completely Randomized Design (CRD) with a single main factor—the type of substrate—at two treatment levels: P0 (corn cobs) and P1 (banana peels). Each treatment was performed in triplicate to minimise data variability and enhance the validity of the results. The design was chosen to give a comprehensive view of how substrate type influences the enzyme activities produced.

The response variables were the activities of xylanase, pectinase, amylase, β -glucosidase, and endoglucanase secreted by *Aspergillus oryzae* in each experimental unit. The study aimed to evaluate the effect of corn-cob and banana-peel substrates on the production of these enzymes. Statistical analyses included tests for normality, the Mann–Whitney test, correlation analysis, clustering, predictive regression and data visualisation to clarify patterns of enzyme production as a function of substrate.

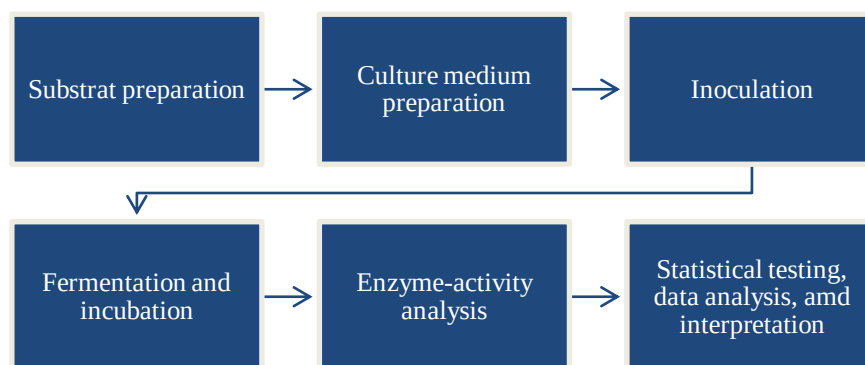


Figure 1. Research Flowchart

3. RESULTS AND DISCUSSION

3.1 Substrate-Dependent Enzyme Production

Because the normality test indicated non-normal data ($p < 0.05$), enzyme activities on corn cobs and banana peels were compared with the Mann–Whitney U test. The results show that xylanase production is significantly higher on corn cobs, whereas pectinase, amylase, β -glucosidase, and endoglucanase are significantly higher on banana peels ($p < 0.05$ for all enzymes).

Table 2. Mann–Whitney U test

Enzyme	U-statistic	<i>p</i> -value	Conclusion
Xylanase	16.0	0.029	Significant
Pectinase	0.0	0.026	Significant
Amylase	0.0	0.029	Significant
β -Glucosidase	0.0	0.029	Significant
Endoglucanase	0.0	0.028	Significant

Box-plot visualisation corroborated these findings: corn cobs (code 0) showed the highest median xylanase activity (12.8 ± 0.2 IU mL⁻¹), roughly 1.5-fold greater than that on banana peels (8.4 ± 0.3 IU mL⁻¹). Conversely, banana peels (code 1) markedly boosted pectinase (2.5 vs 2.0 IU mL⁻¹), amylase (14.0 vs 2.7 IU mL⁻¹), β -glucosidase (18.1 vs 11.3 IU mL⁻¹) and endoglucanase (15.2 vs 7.4 IU mL⁻¹). This pattern aligns with the higher pectin and starch content of banana peels and the greater xylan content of corn cobs.

Figure 2 A–E compares the five enzyme activities on the two substrates, while **Figure 3** (mean $\pm$ SD, $n = 3$) highlights the same trend: corn cobs (code 0) excel in xylanase production, whereas banana peels (code 1) dominate in pectinase, amylase, β -glucosidase and endoglucanase. Asterisks denote significant inter-substrate differences (t-test/Mann–Whitney, $p < 0.05$).

These findings indicate that corn cobs are optimal for xylanase production, whereas banana peels are better suited for the other four enzymes—information that is valuable for selecting substrates in enzymatic fermentation processes.

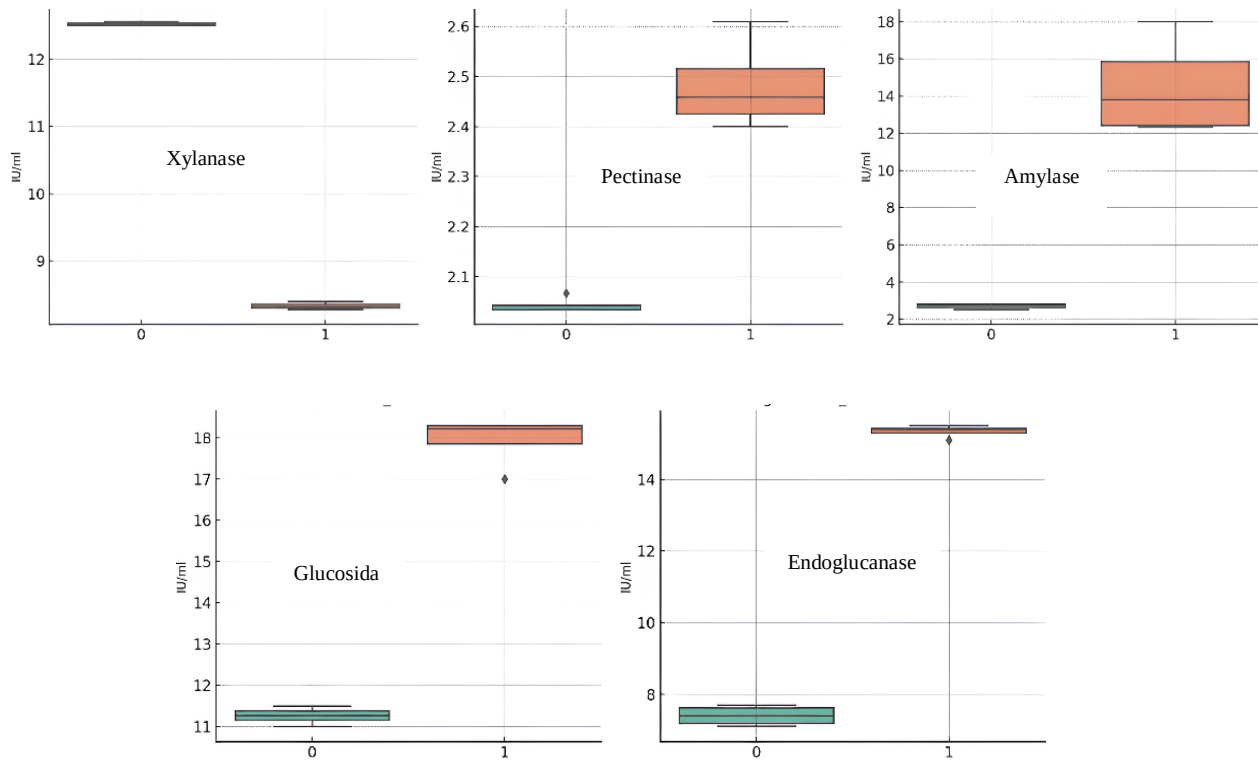


Figure 2. Box-plot visualisation of enzyme-production differences by substrate

The higher xylanase yield on corn cobs can be explained by the substrate's rich lignocellulosic matrix, which favours the growth of enzyme-producing microorganisms. Earlier studies have shown that lignocellulosic materials are excellent substrates for xylanase production because they supply the structural components required for the enzyme to act [21], [22]. Banana peels, by contrast, are better suited to the production of pectinase and related enzymes. Their high pectin content has been proven to stimulate pectinase formation, as microorganisms such as *Aspergillus niger* thrive in pectin-rich environments [23], [24].

These findings align with previous work on the enzymatic potential of agro-industrial residues. Sethi et al. (2016) reported that using banana peels as a substrate boosts pectinase output and highlighted the advantages of the fermentation system employed. This is increasingly relevant because banana peels are a cost-effective substrate, thanks to both their availability and nutrient profile [22], [25]. Moreover, exploiting banana peels not only aids enzyme production but also helps manage waste from the fruit-processing industry, which typically discards large quantities of peels [26].

The box-plot analysis confirming the enzyme-production gap between the two substrates further underlines the distinct enzymatic profiles. The visualised data show that xylanase reaches its highest titres on corn cobs, whereas the other enzymes are produced predominantly on banana peels. This observation is critical for optimising fermentation processes, because it demonstrates that each substrate can be selected strategically according to the target enzyme [27].

From a practical standpoint, being able to generate specific enzymes from these two substrates has major implications for food processing, biofuel production, and waste management. High xylanase output from corn cobs, for instance, can be exploited for hemicellulose degradation in biomass, facilitating the conversion of lignocellulosic matter into fermentable sugars for bio-ethanol production [22], [27]. Meanwhile, the enzyme set derived from banana peels can be applied in various food-processing operations, such as juice clarification or the development of functional foods enriched with bioactive compounds [28].

3.2 Enzyme Production by Substrate

To identify which substrate is most efficient for each enzyme, mean enzyme activities were compared between the two feedstocks. The analysis shows that corn cobs are superior for xylanase production, whereas banana peels are more effective for pectinase, amylase, β -glucosidase and endoglucanase. This pattern is supported by the work of Elegbede and Lateef, who demonstrated that corn-based substrates—corn cobs among them—significantly enhance xylanase production [29].

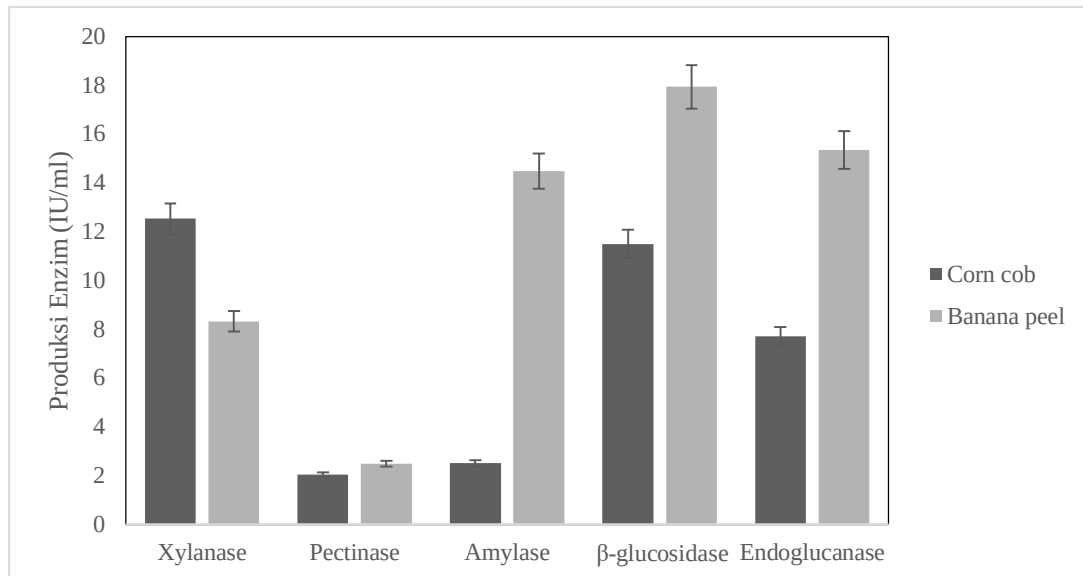


Figure 3. Enzyme Production by Substrate

3.3 Relationships among Enzymes

Xylanase shows a strong negative correlation with the other four enzymes ($\rho = -0.95$ to -0.85 ; $p \leq 0.032$). In contrast, pectinase production is very strongly and positively correlated with amylase, β -glucosidase, and endoglucanase ($\rho = 0.81$ – 0.90 ; $p \leq 0.050$). In this context, the growth conditions of *Aspergillus oryzae* and substrate availability play decisive roles in enzyme expression.

Corn cobs, for example, tend to enhance xylanase output because of their high hemicellulose (primarily xylan) content, thereby triggering metabolic pathways that prioritise this enzyme. However, this substrate is less suitable for stimulating pectinase, amylase, glucosidase and endoglucanase, presumably because its nutrient composition and

physicochemical properties do not support the metabolic synergy required for those enzymes. Banana peels, by contrast, provide a substrate matrix richer in starch, pectin and other organics that simultaneously boosts pectinase, amylase, glucosidase and endoglucanase. The high positive correlations among these four enzymes suggest a mutually supportive regulatory network, whereby higher production of one enzyme facilitates or amplifies the synthesis of others acting on similar or complementary substrates.

The simultaneous induction of pectinase–amylase– β -glucosidase–endoglucanase on banana peel can be explained by the hierarchy of Zn(II)₂Cys₆ trans-activators typical of *A. oryzae*: XlnR (responding to xylose/xylan), AmyR (amylose/glucose) and the pectin regulator GaaR. As reviewed in *Functional Genomics of A. oryzae* [31], XlnR activation not only up-regulates the xylanase gene cluster but also represses clusters controlled by AmyR and GaaR; conversely, abundant galacturonate and starch stimulate GaaR–AmyR while de-activating XlnR, hence the concerted rise of the four non-xylanase enzymes. The chemical profile of banana peel (pectin + starch, low xylan) versus corn cob (high xylose/xylan) therefore triggers two antagonistic regulatory “programmes” and yields the observed negative xylanase correlation ($\rho \approx -0.87$) with the other enzymes.

To test this carbon-flux hypothesis, we plan an *in silico* simulation using the genome-scale metabolic model of *A. oryzae*. The model already includes pentose-phosphate, starch and pectin pathways and should predict flux redistribution when the substrate is switched from corn cob to banana peel, before further fermentation trials [32].

These findings underline that choosing an appropriate substrate is crucial for maximising enzyme production by *A. oryzae*. If the primary goal is high xylanase titres, corn cobs are the substrate of choice. Conversely, when simultaneous production of pectinase, amylase, glucosidase and endoglucanase is required, banana peel is more suitable because it drives the expression of those enzymes. Overall, understanding these inter-enzyme correlations and substrate effects is instrumental in designing efficient fermentation processes in which *A. oryzae* can be exploited to yield specific enzymes for industrial or research needs.

Table 3. Correlations among Enzymes

Enzyme pair	Spearman ρ	p -value	Kendall τ	p -value
Xylanase $\leftrightarrow$ Pectinase	−0.95	0.003	−0.87	0.011
Xylanase $\leftrightarrow$ Amylase	−0.85	0.032	−0.67	0.071
Xylanase $\leftrightarrow$ β -Glucosidase	−0.89	0.019	−0.73	0.048
Xylanase $\leftrightarrow$ Endoglucanase	−0.89	0.019	−0.73	0.048
Pectinase $\leftrightarrow$ Amylase	0.81	0.050	0.67	0.071
Pectinase $\leftrightarrow$ β -Glucosidase	0.84	0.035	0.73	0.048
Pectinase $\leftrightarrow$ Endoglucanase	0.84	0.035	0.73	0.048
Amylase $\leftrightarrow$ β -Glucosidase	0.79	0.063	0.60	0.125
Amylase $\leftrightarrow$ Endoglucanase	0.98	0.0004	0.87	0.011
β -Glucosidase $\leftrightarrow$ Endoglucanase	0.74	0.091	0.60	0.125

Growth conditions and substrate availability are key determinants of enzyme expression. Corn cobs, rich in hemicellulose, boost xylanase production yet do not favour the other enzymes owing to their nutrient composition [17]. Banana peels, by contrast, supply a substrate matrix better suited to the simultaneous enhancement of pectinase, amylase, glucosidase and endoglucanase activities, thanks to their abundant starch and pectin [33]. Selecting the appropriate substrate is therefore essential for maximising the desired enzyme output from *Aspergillus oryzae*.

3.4 Enzyme-Production Prediction Based on Substrate

Table 4 summarises a linear-regression analysis with substrate as the predictor variable. The negative regression coefficient for xylanase (-4.20 ; $p < 0.001$) indicates that using banana peels is inversely related to xylanase output, confirming that corn cobs are more effective in triggering its expression. Conversely, the four other enzymes show significant positive coefficients—pectinase (0.44 ; $p < 0.001$), amylase (11.78 ; $p < 0.001$), glucosidase (6.68 ; $p < 0.001$) and endoglucanase (7.94 ; $p < 0.001$)—meaning banana peels contribute positively to their production. Very high R-squared values (all above 90 %) indicate excellent predictive power, allowing enzyme yields to be forecast with high accuracy simply from the substrate selected.

These findings reinforce earlier interpretations of how nutrient composition and physicochemical properties shape metabolic pathways: the hemicellulose-rich corn cobs (especially xylan) facilitate pathways prioritising xylanase synthesis, whereas the starch- and pectin-rich banana peels favour pectinase, amylase, glucosidase, and endoglucanase. Practically, a greater proportion of banana-peel biomass in the fermentation medium raises the likelihood of producing those four enzymes, while a dominant hemicellulose source is required if xylanase is the primary target. The very low p -values (< 0.001) confirm that these substrate effects are statistically significant, so even minor changes in substrate composition can result in marked differences in enzyme titres.

In short, corn cobs—rich in hemicellulose—are optimal for xylanase synthesis, whereas banana peels, with their high starch and pectin content, best support the production of pectinase, amylase, glucosidase, and endoglucanase [34], [35].

Table 4. Predictive Regression Results

Enzyme	Coefficient (Banana-peel substrate)	p -value	R-squared
Xylanase	-4.20	< 0.001	99.97 %
Pectinase	0.44	< 0.001	93.64 %
Amylase	11.78	< 0.001	92.76 %
Glucosidase	6.68	< 0.001	98.54 %
Endoglucanase	7.94	< 0.001	99.72 %

For industries that need xylanase as the primary enzyme—such as paper processing or enhancing livestock-feed digestibility—corn cobs are the more advantageous substrate. Conversely, when the objective is to produce other enzymes such as pectinase, amylase, glucosidase and endoglucanase, banana peels constitute a more effective raw material. Accordingly, the linear-regression analysis confirms that choosing the right substrate is a key factor for maximising enzyme production efficiently and selectively, while also giving a quantitative foundation for developing fermentation processes at both industrial and laboratory scales.

4. CONCLUSIONS

The present study demonstrates that corn cobs are the most effective substrate for stimulating xylanase production by *Aspergillus oryzae*, whereas banana peels more strongly enhance the activities of pectinase, amylase, β -glucosidase and endoglucanase. The strong negative correlation between xylanase and the other four enzymes indicates that, when corn cobs are used, metabolic pathways prioritise xylanase synthesis. In contrast, the high positive correlations among pectinase, amylase, β -glucosidase, and endoglucanase highlight their shared pathways or nutritional requirements, which are well supported by the composition of banana peels. Linear-regression analysis reinforces these findings, with highly significant p -values (< 0.001) and very high R-squared values ($> 90\%$), confirming that substrate choice exerts a major influence on enzyme production.

As polysaccharide-degrading biocatalysts, all five enzymes examined here play key roles in lignocellulose biodegradation. Xylanase hydrolyses hemicellulose to yield xylo-oligosaccharides and xylose, benefitting the pulp-and-paper industry and improving feed digestibility. Pectinase converts pectin to galacturonate, useful for fruit-juice clarification and agro-waste bioconversion. Amylase degrades starch to simple sugars, crucial in food processing and bio-ethanol manufacture. β -Glucosidase cleaves glucosides to release free glucose, supporting fermentation and the conversion of complex carbohydrates. Endoglucanase, a component of the cellulase system, assists in cellulose breakdown.

Future research should focus on fermentation optimisation, co-cultivation with other microorganisms (e.g. lactic-acid bacteria) and exploring these enzymes in bio-ethanol and bioplastic production to advance sustainable biotechnology in biomass-based industries. A main limitation of this study is that physical/chemical pre-treatment methods were not compared; forthcoming work will evaluate alkaline, acid and other pre-treatments to assess their impact on the enzyme profile of *A. oryzae*. Another limitation is the relatively small number of replicates ($n = 3$). Although post-hoc power analysis indicated $> 80\%$ power for large effects, future studies should employ at least five replicates and/or factorial designs (e.g. substrate $\times$ fermentation time) to improve statistical resolution.

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