

Valorization of Rice Polish Biomass through Acid and Enzymatic Hydrolysis for Fermentable Sugar Production

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Effects of acid and enzymatic hydrolysis, as well as starch content, were compared relative to the amounts of reducing sugars obtained from rice polish. The growth of yeast on various sugar profiles obtained from both hydrolysis was evaluated. The effect of pretreatments of different H₂SO₄ concentrations (1 to 5%) was examined at different incubation periods (1 to 3 h). The impacts of 1% H₂SO₄ and H₃PO₄ on rice polish were also studied, and the reducing sugar release was measured using the DNS assay. For enzymatic hydrolysis, a fungus with high starch-degrading ability was isolated from soil and tentatively identified as *Aspergillus niger*. The efficiency of amylase produced by *A. niger* via submerged fermentation was determined at various residence times (48 to 120 h), with reducing sugar release measured by a substrate-based assay and enzyme activity by a product-based assay. Finally, the yeast growth was assessed on hydrolysates from both methods. Proximate analysis revealed 79.5% starch, 35.2% sugar, and 8.5% nitrogen in rice polish. Maximum reducing sugar (19.2 mg/mL) was obtained after pretreatment with 2% H₂SO₄ after 1.0 h, and H₂SO₄ yield (1.08 g/L) outperformed H₃PO₄ (0.59 g/L). Moreover, the substrate-based assay showed optimal starch conversion at 72 h (10.6 µmol/min), and the product-based assay showed maximum enzyme activity after 72 h (409 µmol/min). The evaluation of yeast growth revealed that enzymatic hydrolysis produced more reducing sugars (8.68 mg/mL) compared to acid hydrolysis (6.61 mg/mL), highlighting its potential for ethanol production.

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INTRODUCTION

The agro-industry generates substantial amounts of waste with diverse characteristics. It is important to identify efficient valorization methods for these wastes to maximize income in this sector. Poor management of these wastes contributes to environmental degradation and the economic instability of most nations. Currently, the development of waste management plans is critical, with increasing focus on converting leftover materials into valuable products at affordable rates (Singh *et al.* 2021). The

byproduct of rice milling, *i.e.*, rice polish, is a highly cost-effective source of carbohydrates (starches) and is highly abundant in major rice-producing regions (Ambreen *et al.* 2006).

Agro-industrial waste can be used as a cost-effective, readily available substrate for amylase production and help reduce pollution (Priya *et al.* 2010). Screening microorganisms for amylase activity may lead to the discovery of new amylases suitable for commercial use (Okunwaye *et al.* 2021). Rice polish has potential for use in ethanol production. Nonetheless, in Pakistan, this by-product is not being leveraged effectively at present.

The choice of hydrolysis method significantly affects the environmental sustainability of biomass valorization. Acid hydrolysis is rapid and efficient, but it requires high temperatures and can produce corrosive by-products that increase environmental impacts. On the other hand, enzymatic hydrolysis is a 'greener' alternative because it proceeds under mild conditions and generates less toxic by-products. However, as shown in high-quality Life Cycle Assessments (LCAs), *e.g.*, Murali and Shastri 2022, the energy required to make enzymes can be a major environmental drawback. Thus, for a process to be sustainable, it needs to come up with sufficiently high sugar yield to compensate for these production expenses. This makes comparison of these two methods especially important in the context of the valorization of rice polish.

Selecting appropriate microorganisms is important in the production of specific enzymes (Jinu 2017). Frac *et al.* (2018) reported that extracellular enzymes degrade complex starch and nitrogenous compounds in the soil to produce humus, including reducing sugars, enhancing soil fertility, and releasing nutrients to be used by other organisms. These enzymes are used in a variety of industrial applications, and they are widely researched. The glycosidic bonds in starches are broken by amylases to yield end products depending on amylase type (Dhanya *et al.* 2009). While amylases occur in microorganisms, animals, and plants, industrially important ones are primarily produced by microorganisms (Pandey *et al.* 2000). Fungi and bacteria secrete the extracellular proteins such as amylases (Kazunari *et al.* 2011). The extracellular enzymes derived from fungi are used in pharmaceutical production, textiles, detergents, medical therapy, food production, and molecular biology (Wosten 2019).

Rice polish is highly complex in structure, making its sugar recovery challenging. Anwar *et al.* (2012) reported that the cellulose and lignin contents in this biomass are about 21.4% and 27%, respectively. The high lignin content is a barrier that makes the cellulose hard to access. Hence, it is important to consider whether acid hydrolysis or enzymatic hydrolysis is more effective in overcoming this recalcitrance. Although acid hydrolysis can be faster, enzymatic hydrolysis can provide a potentially less polluting pathway for breaking down sugars. To determine which method is more efficient for reducing sugar release for downstream valorization, a direct comparison must be carried out. Anwar *et al.* (2012) reported only cellulose and lignin contents, but a comprehensive analysis of rice polish usually shows 20 to 30% hemicellulose.

To obtain starch with specific functionalities, it is vital to examine the effect of acid hydrolysis on the function and structure of starch. Wang *et al.* (2014) showed that acid hydrolysis is an effective technique for understanding the structure of the starch granules. The enzymatic hydrolysis of the starch is used in biological processes to convert starch to glucose. This technique is more attractive because the process of its breakdown is environmentally friendly, as it requires no solvents or chemical reagents (Das *et al.* 2019). Rice polish can cause pollution, yet it also serves as a substrate to reduce pollution. To achieve sustainable bioethanol production, valorization of agricultural by-products

including rice polish is crucial. Recently, several green and enzymatic methods have been developed to recover the bioactive and fermentable values from biomass with maximum recovery (Tang and Huang 2024). New technologies such as ultrasound-assisted processing are being investigated to alter the functional characteristics of these substrates, to enhance the hydrolysis efficiency (Wang *et al.* 2025).

This research aimed to isolate starch-degrading microorganisms using the agro-industrial waste (rice polish) to produce alpha-amylase enzyme. Rice polish was then used to produce valuable bioproducts and to compare acid and enzymatic hydrolysis for converting starch in rice polish to reducing sugars.

EXPERIMENTAL

Collection of Soil Samples

Soil samples were obtained from Khwaja Rice Mills (Circular Road, Narowal), Bajwah Rice Mills (near Sahara Hospital, Narowal), Gorla Rice Mills (Sakna Manak, Narowal), and Zafar Rice Mills (Sakna Manak, Narowal) in Narowal district, Punjab, Pakistan. These samples were utilized for the isolation of microorganisms. Using a sterile spatula, soil samples were collected from the subsurface at a depth of 3 to 4 cm near the wastewater outlet of the rice mill. The samples were placed in sterile polythene bags, shipped aseptically to the lab, and kept at the ambient temperature until needed. The research was conducted at the Food and Biotechnology Research Centre (FBRC), Pakistan Council of Scientific and Industrial Research Centre (PCSIR), Lahore.

Preparation of Media

According to the literature, starch agar media is suitable for the isolation of starch-degrading microbes. About 100 mL of starch agar media was prepared with the following composition (g/100 mL): Starch ($C_6H_{10}O_5$)_n 2.5 g, sodium chloride (NaCl) 0.025 g, ammonium sulphate ($(NH_4)_2SO_4$) 0.75 g, magnesium sulphate ($MgSO_4$) 0.025 g, potassium dihydrogen phosphate (KH_2PO_4) 0.05 g, agar 1.5 g, and yeast extract 0.75 g. These components were dissolved in 100 mL of distilled water. After adjusting the medium's pH to 5.6 using 0.1N NaOH/HCl, the medium was autoclaved for 15 min at 121 °C.

Isolation of Starch-Degrading Microorganisms

The serial dilution and pour plate methods were used to isolate starch-degrading microbes from soil samples (Clark *et al.* 1958). Initially, 10 g of soil was homogenized in 90 mL of sterile 0.85% saline and shaken at 30 °C and 300 rpm for 45 min. Serial dilutions of the sample were then prepared up to 10^4 to 10^5 . Approximately 0.1 mL of each dilution was placed on the corresponding Petri dishes. Starch agar media (25 mL) was poured into oven-sterilized Petri dishes (160 °C for 2 h), and 2 to 3 drops of chloramphenicol were added to each plate to inhibit bacterial growth. These plates were vigorously agitated to ensure thorough homogenization, allowed to solidify at ambient temperature, and then incubated at 37 °C for 3 to 4 days.

Screening of Amylolytic Fungal Strains

Starch agar media was prepared with the composition mentioned above. Isolated strains were streaked onto this medium and incubated for 3 to 5 days. The absence or presence of the starch-degrading microbes was determined through the analysis of clear

zones around the colonies using the iodine flooding test. After growth occurred, plates were flooded with iodine. The development of clear halo zones around colonies indicated amylase production. When iodine staining turned the plate blue, it showed starch was present, but had not been used by the microbe. On the other hand, when there is no color change after iodine flooding, it indicates that starch had been utilized by the microbe, confirming the presence of a starch-degrading microbe in the sample. The initial colonies that had formed distinct areas of starch hydrolysis were chosen and transferred to the potato dextrose agar (PDA) slants. To preserve and maintain the cultures, the slants were stored in a refrigerator at 4 °C.

Identification of Starch-Degrading/Amylase-Producing Fungal Isolates

Starch-degrading microbes were identified through morphological and microscopic studies (Azar 2024). For morphological identification, features such as the color, size, texture, margins, and nature of the colonies were observed. The size, shape, and arrangement of hyphae, conidia, and conidiophores were also examined using microscopy. These features were compared with mycological identification keys and taxonomic descriptions.

Proximate Analysis of Rice Polish Substrate

The substrate's total sugar, starch, and nitrogen contents were then ascertained *via* characterization. For this purpose, 1.0 g of rice polish was mixed with 80 mL of 0.7% KOH solution, and the mixture was heated for 30 min at 90 °C in a water bath. A final volume of 100 mL was obtained by mixing 50 mL of this combination with 50 mL of 5% acetic acid solution. Using this mixture, the phenol-sulfuric acid method was used to determine the overall starch and sugar present in the rice polish (DuBois *et al.* 1956). The iodine test was used to determine the total starch level (McCready *et al.* 1950). Additionally, the Kjeldahl method was used to calculate the total nitrogen content in the rice polish (Kirk 1950).

Effect of Pretreatments of H₃PO₄ and H₂SO₄ on Reducing Sugars

Different acids, specifically phosphoric acid (H₃PO₄) and sulphuric acid (H₂SO₄), were used to hydrolyze rice polish. In several test tubes, 1.0 g of rice polish and 9 mL of distilled water were mixed to make the slurry. After that, samples were incubated for 1, 2, and 3 h at 90 °C with varying concentrations of concentrated sulphuric acid (w/v), including 1%, 1.5%, 2%, 2.5%, 3%, 4%, and 5%. Specifically, separate sets of samples were incubated for each of the time periods: one set for 1.0 h, another set for 2 h, and a third set for 3 h. Following that, these samples were centrifuged for 20 min at 4 °C and 6000 rpm. After centrifugation, pellet and supernatant from each sample were kept in separate test tubes, and the amount of reducing sugars in each was calculated using the following formula:

$$N \text{ (Reducing sugar)} = \frac{\text{Absorbance} \times \text{Standard Factor} \times \text{Dilution Factor}}{1000}$$

Estimation of reducing sugars in the supernatant

To estimate the reducing sugar content from starch, 0.1 mL of supernatant was added to 0.9 mL of distilled water, followed by the addition of 3 mL of DNS (3,5-dinitrosalicylic acid) reagent to develop the color. The spectrophotometer (SP-300 Optima, Tokyo, Japan) was then utilized to calculate the absorbance at 540 nm after the mixture

had been boiled in hot water for over fifteen min and cooled down. A blank was used as a reference.

Estimation of reducing sugars in the pellet

Sulfuric acid and phosphoric acid tests were conducted to evaluate their effects on rice polish pellets, and the amount of resulting reducing sugars was measured using the DNS reagent.

Phosphoric acid test

To estimate starch transformed into reducing sugar, 1.0 g of rice polish pellet was homogenized in 20 mL of 1% phosphoric acid. The mixture was then heated at 90 °C for 60 min, followed by centrifugation at 6000 rpm for 20 min. Following centrifugation, 3 mL of the DNS reagent was added into 1 mL of the supernatant, and the resultant mixture was subjected to heating in boiling water for 15 min. Then, absorbance was measured at 480 nm.

Sulphuric acid test

Concentrated H₂SO₄ (0.1 mL) was added to 1.0 g of rice polish pellet. The mixture was then heated at a temperature of 90°C for 1.0 h and then centrifuged. The optical density was measured at 480 nm using the spectrophotometer.

Fungal Amylase Production by Submerged Fermentation

Using rice polish as the substrate, submerged fermentation was employed to prepare the fermentation medium (agar starch medium) for the synthesis of amylase by *Aspergillus niger*. Fermentation medium (100 mL) was prepared with the composition mentioned above. After that, the pH was adjusted to 5, and the medium was autoclaved at 121 °C for 15 min. *Aspergillus niger* culture was inoculated into the medium using a sterilized inoculum needle and incubated for 6 to 7 days at 37 °C. The biomass produced by submerged fermentation was filtered through a muslin cloth and then through filtering assemblies, one at a time, to collect the crude amylase. The enzyme filtrate was gathered following 20 min of centrifugation at 5,000 rpm. The supernatant was carefully collected to avoid spore development. The crude amylase extract was stored at 4 °C to maintain enzymatic stability until further use. The extracted crude enzyme was used in an enzyme assay (the assay procedure is described in the enzyme assay section), and the resulting filtrate was then used for the enzymatic hydrolysis described below.

Saccharification of Rice Polish by Crude Amylase

The hydrolysis process was monitored by withdrawing 1.0 mL aliquots of the hydrolysis reaction mixture (substrate-buffer-enzyme blend) at 48, 72, 96, and 120 hours. The aliquots were then processed, and the amount of reducing sugars released was determined. These aliquots were heated in the water bath for 10 min, then allowed to cool and centrifuged for 5 min at 10,000 rpm. The enzyme activity and glucose concentration were calculated using both substrate-based and product-based assays.

Product-based assay

About 0.5 mL of the crude enzyme was mixed with 0.5 mL of 1% starch stock solution and allowed to sit at room temperature for 15 min. After adding 3 mL of the DNS reagent, the reaction mixture was heated to the boiling point for 15 min. Following color

development, absorbance was measured at 540 nm compared to the blank, and the enzyme activity was calculated using the following Eq. 1:

$$E_a = \text{Conc.} \times \text{DF} \times 1000 / \text{Mol. Wt. of the standard} \times \text{incubation time} \quad (1)$$

where E_a denotes enzyme activity, and DF is the dilution factor.

Substrate-based assay

A reaction mixture containing 0.4 mL of 1% starch stock solution and 0.4 mL of the crude enzyme was incubated at room temperature for 15 min. The mixture was then combined with 0.2 mL of 1 M HCl and 1.0 mL iodine reagent. After the color was developed, the absorbance at 580 nm was measured against the blank. The amount of reducing sugars was measured through the following Eq. 2,

$$\text{Substrate (Reducing sugar)} = (A_1 - A_2) \times \text{standard factor} / \text{incubation time (t)} \quad (2)$$

where A_1 denotes the absorbance of the blank containing starch with no enzyme, and A_2 is the absorbance of the sample after enzymatic reaction, and the standard factor is derived from a glucose calibration curve.

Comparison of Yeast Growth on Different Sugar Profiles

The supernatants from both acid and enzymatic hydrolysis were employed to quantify initial concentrations of reducing sugars available for fermentation. About 3 mL of the DNS reagent was admixed with 1.0 mL of each supernatant, which was then heated in boiling water for 15 min. Using a spectrophotometer, the optical density was determined at 540 nm to calculate glucose levels in both supernatants.

The hydrolysis supernatants (100 mL) were then used to prepare the modified YP-hydrolysate medium. The medium's pH was adjusted to 4.5 by adding 0.1 N NaOH/HCl and boiled for 20 min to create a uniform solution. After covering the flask with aluminium foil and plugging it with cotton, it was autoclaved for 15 min at 121 °C. Following sterilization, the media were inoculated with yeast and incubated for 3 to 5 days. Serial dilutions were carried out up to 10^9 to 10^{10} dilutions, and the ninth and tenth dilutions of both cultures were used. Dilutions (0.1 mL) were transferred to the petri plates, and 25 mL of media was poured onto them using the pour plate method. To ensure a uniform dispersion of YP-hydrolysate medium, petri plates were rotated both clockwise and counterclockwise, allowed to solidify at room temperature, and incubated for 3 to 4 days at 37 °C. Using the Miller DNS method at a controlled pH of 3.5 with sodium acetate buffer, a precise map was generated of the amount of sugar consumed and biomass produced by the yeast using pour-plate colony counts. This enabled a quantitative comparison of the efficiency of enzymatic vs. acid derived streams of sugars. After growth was completed, the culture was filtered. 0.1 mL of this filtrate sample was added to 0.9 mL of the sodium acetate buffer (pH = 3.5), and then 3 mL of DNS reagent was added to stop the reaction. The mixture was then boiled for 15 min. The OD value was determined at 540 nm against the blank. The concentration of reducing sugar (mg/mL) and the enzyme activity were calculated using Eqs. 3 and 4, respectively.

$$\text{Reducing sugar (mg/mL)} = \text{OD value} \times \text{Standard factor} \times \text{Dilution factor}/1000 \quad (3)$$

$$E_a = \text{Product (}\mu\text{g) released} \times 1000 / \text{Mol. wt of the standard} \times \text{Incubation time} \quad (4)$$

Analytical Procedures

Estimation of carbohydrate fractions

A glucose standard curve was prepared to estimate the reducing sugar content of rice polish. About 1.0% glucose stock solution (1.0 g/100 mL of distilled water) was made and utilized to create dilutions of different concentrations. Each standard solution (1.0 mL) with concentrations ranging from 100 to 1000 $\mu\text{g/mL}$ (100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 $\mu\text{g/mL}$) was taken, and 3 mL of the DNS reagent was added to it. All test tubes, including a blank, were mixed and then placed in the boiling water for 15 min. The solutions were allowed to cool at room temperature. The optical density was measured at 540 nm relative to the blank after the color developed. The standard curve was then plotted using glucose concentrations and the corresponding absorbance values to construct baseline reducing sugars, as shown in Fig. 1A.

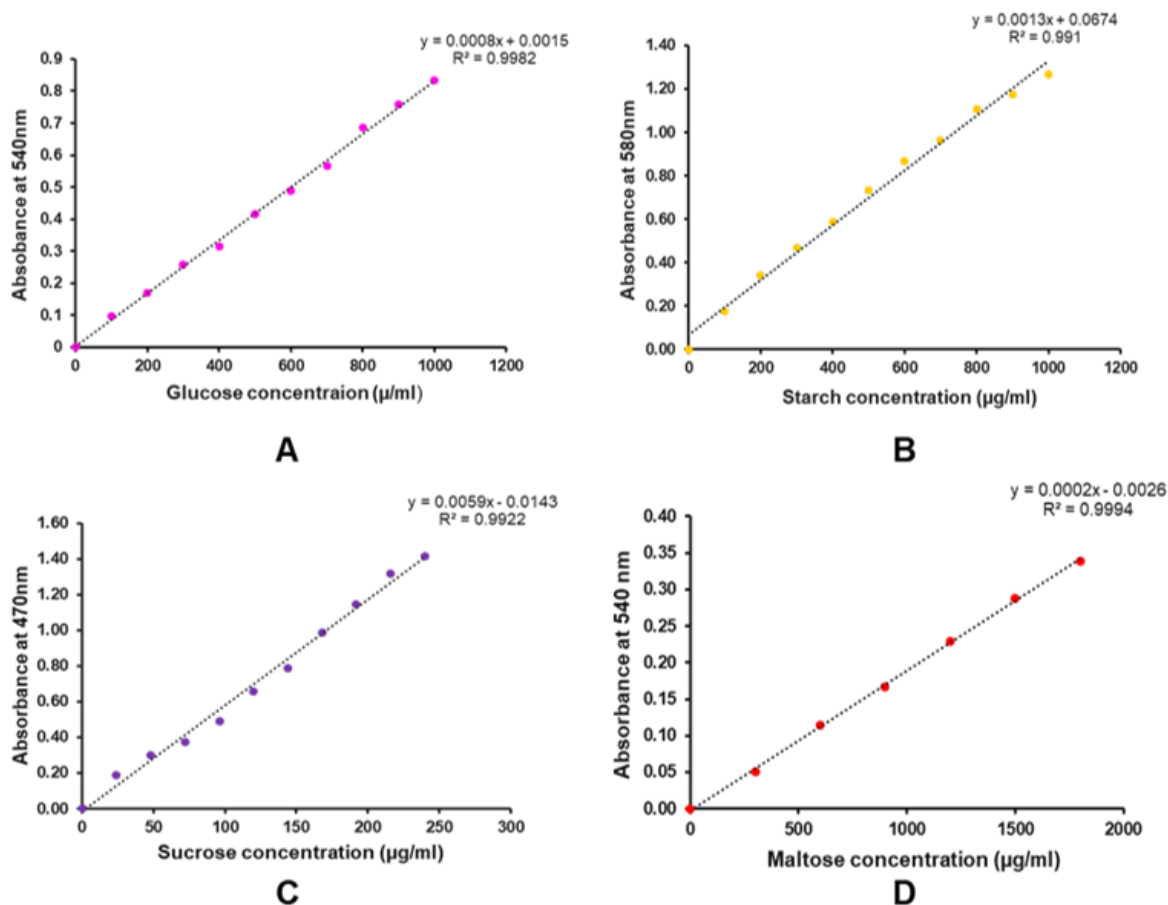


Fig. 1. Standard calibration curves for quantitative biomass measures. (A) Glucose standard curve used for measuring the amount of reducing sugars; **(B)** Starch standard curve for checking validity of the proximate analysis; **(C)** Sucrose standard curve for baseline total soluble sugar quantification; **(D)** Maltose standard curve for monitoring the activity of fungal amylase and for enzymatic saccharification. The linear regression equation ($y = mx + c$) as well as the coefficient of determination (R^2) are shown for all curves.

The yield of fungal amylase activity and subsequent enzymatic hydrolysis metrics were expressed as maltose equivalents (0 to 2000 µg/mL) using the corresponding calibration curve made with the same DNS heat-development method (Fig. 1D). Proximate analysis metrics were calculated using baseline calibrations, with total soluble sugars (reported as total sucrose equivalents) quantified *via* the phenol-sulfuric acid method at 470 nm (DuBois *et al.* 1956) and total starch quantified at 580 nm *via* iodine-potassium iodide (I₂-KI) assay (McCready *et al.* 1950) using starch (0 to 1000 µg/mL) and sucrose (0 to 250 µg/mL) standard curves (Figs. 1B and 1C). From the calibration curves, the amount of carbohydrates in each sample was determined by using the following formula:

$$\text{Carbohydrate Conc. (}\mu\text{g/mL)} = \text{Absorbance} \times \text{Average standard factor} \quad (5)$$

Enzyme Assay

To measure the amylase activity, the techniques outlined by Ghose (2005) were used. A 250 mL flask containing 2 g of rice polish and a sodium acetate buffer (pH = 5.6) was used for the enzymatic hydrolysis. Aliquots of 1.0 mL were heated for 10 min in the water bath, then allowed to cool before being centrifuged at 6000 rpm for 20 min. Aliquots of 1.0 mL were taken from the buffered substrate-enzyme mixture at 48 h, 72 h, 96 h, and 120 h in order to monitor the progress of the enzymatic hydrolysis. After the hydrolysates were collected, glucose concentration was measured. The enzyme activity was calculated using Eq. 4 as mentioned above.

Statistical Analysis

To determine whether there were statistically significant differences among the samples, one-way ANOVA was performed, subsequent to Duncan's multiple range tests ($p = 0.05$). Each experiment was performed in triplicate, and the data were presented as mean values with standard deviation (SD).

RESULTS

Isolation of Starch-Degrading Fungus from the Soil Samples

Starch-degrading fungi were isolated from soil samples using serial dilution and pour plate techniques. The fungal isolates obtained from the soil samples comprised seven distinct strains, as illustrated by Fig. 2.

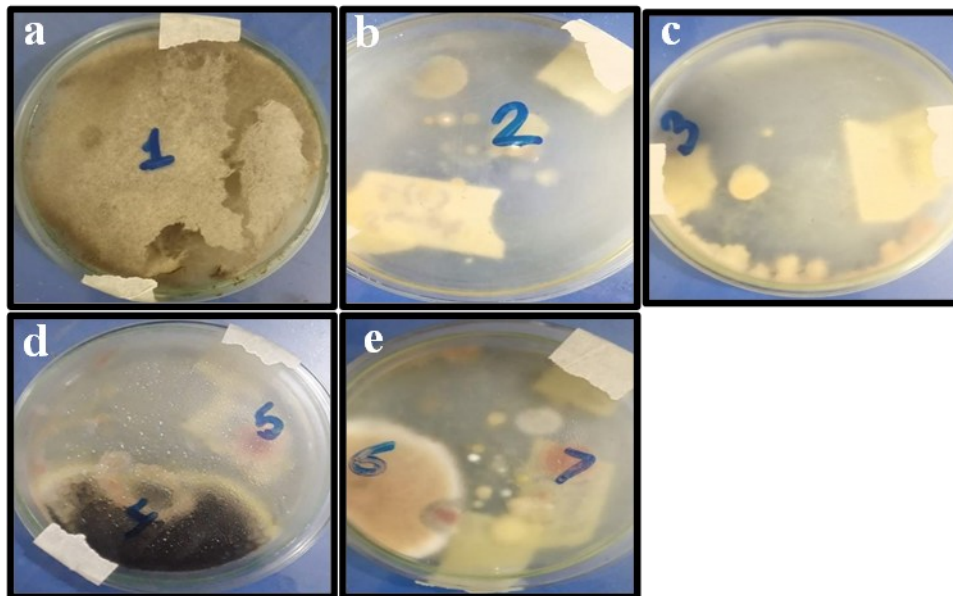


Fig. 2. Growth of the fungal strains isolated from soil samples of different rice mills. (a) *Aspergillus flavus*; (b) *Mucor racemosus*; (c) *Aspergillus terreus*; (d) *Aspergillus niger*; *Aspergillus fumigatus*; and (e) *Trichothecium roseum*; *Mucor hiemalis*. Microbial isolates 1 to 7 are characterized morphologically. The images (a-e) represent the growth of the colonies on the starch agar media after 3 to 4 days. Note: White rectangular objects visible at the edge of the plates are adhesive tape used in the laboratory for sealing and labelling purposes.

Screening for Amylolytic Fungal Strains

Amylolytic fungal strains were screened *via* iodine flooding to assess their starch-degrading capabilities. Among seven isolated fungal strains, *A. niger* showed a clear zone for starch hydrolysis and was chosen for further experiments.

Morphological Identification

Morphological analysis was used to identify the starch-degrading isolates. Characteristics of the isolated strains included colony morphology aspects including size, color, shape, colony nature, and pigmentation. Figure 2(d) depicts the dark brown and black color of the *A. niger* colony, which had a powdery and velvety texture. It produced brown and black pigment. Conidia were round, rough-walled, and either black or dark brown. *Aspergillus fumigatus* colonies were initially fluffy and white, but they eventually turned green or yellowish-green. With chains of conidia at the tips, conidiophores were long, unbranched, and protruded upward from the colony. Conidia have a pale to bluish green color. *Aspergillus flavus* colonies ranged in color from yellow-green to olive-green. Velvety to granular or powdery texture was present. Conidiophores had rough walls, were lengthy, and had no branches. Conidia were round, yellowish green, and had rough walls. *Aspergillus terreus* colonies possessed smooth-walled conidiophores, a suede-like feel, and a cinnamon-brown or sand-brown color. The conidia of *A. terreus* were pale yellow, globose, and tiny. *Trichothecium roseum* colonies were powdery, granular, and flat. After being white at first, they turned pale pink to peach. Conidiophores appeared alone or in loose clusters, were upright, and lacked branches. Initially white, *M. hiemalis* colonies eventually turned greyish-brown, fluffy, and cottony. Sporangiophores exhibited variable shapes, whereas sporangia were typically round or pear-shaped.

Proximate Analysis of Rice Polishing

The phenol-sulfuric acid method was utilized to evaluate total soluble sugar content as sucrose in rice polishing. Both the water bath and hot plate methods were used to treat rice polish for optimal outcomes. Findings indicated that the average polysaccharide-derived sucrose content in rice polish was $35.2 \pm 0.03\%$. The rice polish had an average starch content of $79.5 \pm 0.04\%$, determined by the iodine-potassium iodide (I_2 -KI) assay. The Kjeldahl method was employed to measure the total nitrogen content, revealing that the rice polish had an 8.5% nitrogen/protein concentration.

Acid Hydrolysis (1:9)

The optimal H_2SO_4 concentration for maximizing the yield of total fermentable sugars from rice polish was found by testing various concentrations of H_2SO_4 (1%, 1.5%, 2%, 2.5%, 3%, 4%, and 5%). These tests were conducted at 90 °C with different incubation times (1, 2, and 3 h). A mixture of 1.0 g of rice polish in 9 mL of distilled water was used. Concentrations of H_2SO_4 were selected based on existing literature.

Table 1 shows how different H_2SO_4 concentrations affect the hydrolysis of rice polish (ratio 1:9). After incubating for 1 h at 90 °C, the reducing sugar levels were: 9.14 ± 0.07 , 16.2 ± 0.03 , 19.2 ± 0.6 , 18.6 ± 0.08 , 11.1 ± 0.02 , 19.0 ± 0.09 , and 19.2 ± 0.07 mg/mL for 1%, 1.5%, 2%, 2.5%, 3%, 4%, and 5% H_2SO_4 , respectively. After a 2 h incubation period at 90 °C, the reducing sugar amounts were 10.5 ± 0.15 , 19.0 ± 0.06 , 18.2 ± 0.08 , and 19.0 ± 0.01 mg/mL for 1%, 2%, 3%, 4%, and 5% H_2SO_4 . After a 3 h incubation period at 90 °C, the reducing sugar amounts were 10.0 ± 0.02 , 18.3 ± 0.03 , 19.0 ± 0.12 , and 19.1 ± 0.45 mg/mL for 1%, 2%, 3%, 4%, and 5% concentrated H_2SO_4 . The results showed that the reducing sugar was highest in the sample with 2% concentrated H_2SO_4 collected after a 1 h incubation period, compared to other pretreatments, and lowest in a sample with 3% concentrated H_2SO_4 after a 1 h incubation period.

There was a significant statistical difference found between different concentrations of H_2SO_4 in reducing sugar yield obtained after 2 h and 3 h of residence time ($p < 0.05$). Whereas, the yield after a 1 h incubation period showed no significant difference among 2 %, 2.5 %, and 5% concentrations. This indicates that initial hydrolysis was limited due to biomass penetration time rather than acid strength. Conversely, extending the residence time to 2 h and 3 h provided enough time for acids to penetrate the rice polish matrix fully. This gave enough time to break the tough structural carbohydrates, resulting in statistical significant difference in reducing sugar yields as time passes.

Table 1. Effect of Varying Concentrations of H₂SO₄ on the Hydrolysis of Rice Polish (1:9) at 90 °C with the Residence Time of 1, 2, and 3 h

H ₂ SO ₄ Conc.	1 h	2 h	3 h
	Reducing sugar (mg/mL ± SD)*	Reducing sugar (mg/mL ± SD)*	Reducing sugar (mg/mL ± SD)*
Blank	4.74 ^a ± 0.04	4.86 ^a ± 0.05	5.39 ^a ± 0.03
1%	9.14 ^b ± 0.07	10.5 ^b ± 0.15	10.0 ^b ± 0.02
1.5%	16.2 ^d ± 0.03		
2%	19.2 ^e ± 0.6	19.0 ^e ± 0.06	18.3 ^c ± 0.03
2.5%	18.6 ^e ± 0.08		
3%	11.1 ^c ± 0.02	18.2 ^c ± 0.08	19.0 ^e ± 0.12
4%	19.0 ^f ± 0.09		
5%	19.2 ^e ± 0.07	19.0 ^d ± 0.01	19.0 ^d ± 0.45

*Values are the mean of three independent replicates (n = 3) ± standard deviation (SD). Means within column followed by different lowercase superscript letters (a, b, c, d, e, f) indicate statistically significant differences according to Duncan's Multiple Test (p < 0.05).

Phosphoric and Sulfuric Acid Tests

After subsequent acid hydrolysis, the remaining pellet was tested for reducing sugars using 1% H₂SO₄ and 1% H₃PO₄, as shown in Table 2. Results showed that 5.38 ± 0.09 g/L of total fermentable sugars were released during the first acid hydrolysis with 1% concentrated H₂SO₄. Subsequently, the 1% phosphoric acid test yielded 0.59 ± 0.02 g/L, and the 1% sulphuric acid test yielded 1.08 ± 0.06 g/L of reducing sugar.

Table 2. Concentration of Reducing Sugars (g/L) from Rice Polish following Phosphoric and Sulfuric Acid Hydrolysis

Acid Hydrolysis		1% H ₃ PO ₄ Test	1% H ₂ SO ₄ Test
Sample	Reducing Sugar (g/L)	Reducing Sugar (g/L)	Reducing Sugar (g/L)
Rice Polish	5.38 ± 0.09	0.59 ± 0.02	1.08 ± 0.06
*Values are the mean of three independent replicates (n = 3) ± standard deviation			

Fermentation at Flask Level and Enzymatic Hydrolysis with *A. Niger*

After 48, 72, 96, and 120 h, the enzyme activity (E_a) and the quantity of the reducing sugars in the substrate were calculated. A product-based assay (DNS assay for analyzing enzyme activity) and a substrate-based assay (iodine test for assessing the quantity of reducing sugars in the substrate) were conducted at various intervals. Results from product-based assay demonstrated that the peak enzyme activity occurred at 72 h (409 ± 0.07 $\mu\text{mol/min}$), followed by 48 h (403 ± 0.07 $\mu\text{mol/min}$), 96 h (245 ± 0.03 $\mu\text{mol/min}$), and 120 h (123 ± 0.03 $\mu\text{mol/min}$), as illustrated in Table 3. The substrate-based assay revealed that the highest conversion rate of the starch to reducing sugars happened after 72 h, with a rate of 10.6 ± 0.02 $\mu\text{mol/min}$. Subsequent measurements were taken at 48 h, yielding a rate of 7.26 ± 0.04 $\mu\text{mol/min}$, at 96 h with a rate of 6.27 ± 0.03 $\mu\text{mol/min}$, and at 120 h with a rate of 4.66 ± 0.03 $\mu\text{mol/min}$, as depicted by Table 4.

Statistical analysis showed a significant difference in amylase activity among different incubation periods ($p < 0.05$). The significant peak in the reducing sugar yield at 72 h showed the substrate's starch optimum saccharification threshold by *Aspergillus* amylase. The subsequent significant decline at 96 h and 120 h may be due to progressive thermal inactivation and loss of enzyme stability during prolonged incubation. Additionally, the high amount of reducing sugars produced during 72 h likely induced product inhibition, slowing further hydrolysis, or resulted in chemical reversion reactions, where free reducing sugars condense to form complex oligosaccharides, leading to a decreased detectable reducing sugar yield.

Table 3. Time-Course Monitoring of Absorbance and Amylase Activity ($\mu\text{mol/min}$) during Product-based Submerged Fermentation

Sr. no	Time (h)	Absorbance	Enzyme Activity ($\mu\text{mol/min} \pm \text{SD}$)
1	48	1.638	$403^c \pm 0.07$
2	72	1.659	$409^d \pm 0.07$
3	96	1.178	$245^b \pm 0.03$
4	120	0.809	$123^a \pm 0.03$
*Values are the mean of three independent replicates (n = 3) ± standard deviation. Means within column followed by different lowercase superscript letters (a, b, c, d) indicate statistically significant differences according to Duncan's Multiple Test ($p < 0.05$).			

Table 4. Absorbance and Amylase Activity ($\mu\text{mol}/\text{min}$) Measurements during Substrate-based Submerged Fermentation

Sr. no	Time (h)	Absorbance	A_1-A_2 ($\mu\text{mol}/\text{min} \pm \text{SD}$)
1	48	0.748	$7.26^c \pm 0.04$
2	72	0.369	$10.6^d \pm 0.02$
3	96	0.868	$6.27^b \pm 0.03$
4	120	1.057	$4.66^a \pm 0.03$

*Values are the mean of three independent replicates ($n = 3$) $\pm$ standard deviation. Means within column followed by different lowercase superscript letters (a, b, c, d) indicate statistically significant differences according to Duncan's Multiple Test ($p < 0.05$).

Assessment of Yeast Fermentation and Enzyme Activity

Yeast growth was examined using different sugar profiles derived from both the acid and enzymatic hydrolysis, as shown in Fig. 3. Supernatants from both hydrolysis methods were analyzed for reducing sugar content. Acid hydrolysis was found to yield more broken-down sugars along with inhibitors, whereas enzymatic hydrolysis results in more specific sugar release with fewer inhibitors. Alongside enzyme activity measurements, the results revealed that the reducing sugar concentration in the supernatant from enzymatic hydrolysis was 8.68 mg/mL. In contrast, the supernatant from acid hydrolysis contained a reducing sugar amount of 6.61 mg/mL. Enzyme activity in acid hydrolysis was 304 $\mu\text{mol}/\text{min}$, and in the enzymatic hydrolysis, it was 566 $\mu\text{mol}/\text{min}$. Notably, enzymatic hydrolysis resulted in higher release of the reducing sugars.

Colony Counts for Yeast Growth

Yeast proliferation in the acid hydrolysis from the 9th dilution supernatant was 1.59×10^{11} , and in the 10th dilution, it was 1.21×10^{12} . Conversely, the yeast growth in the 9th dilution of the supernatant from enzymatic hydrolysis was 0.48×10^{11} , and in the 10th dilution, it was 0.22×10^{12} .

DISCUSSION

Waste bioconversion offers a natural route of recoupling resources and makes an ecological recycling approach possible. Since rice is a main food crop in the world, its production leads to a large amount of biomass remnants, and these can be used in the production of valuable products (Brites *et al.* 2025). Considering the value of the agricultural industrial waste, this study has highlighted the potential of the rice polish, a starch-rich agro-industrial waste, as a valuable resource to achieve the production of alpha-amylase enzyme using microbial fermentation. The main objective was to isolate starch-degrading microbes from soil samples and use them to produce alpha-amylase. Moreover, this research aimed to compare the valorization efficiency of enzymatic and acid hydrolysis of the substrate's starch content to reducing sugars. Lastly, the potential of sugars made by both hydrolysis methods was assessed to support the yeast growth, providing information on the viability of using the rice polish as a yeast substrate in making value-added products.

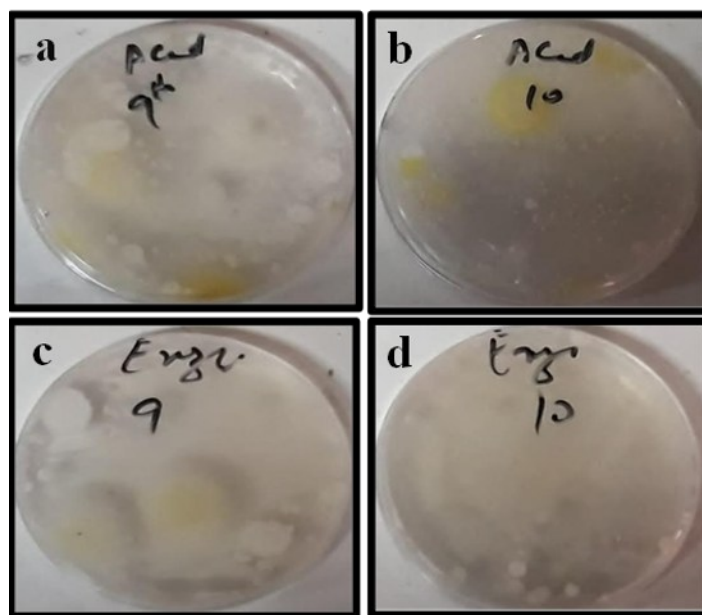


Fig. 3. Comparative growth estimation of yeast in hydrolysis supernatants. The figure shows the yeast colony formation at 10^9 and 10^{10} dilutions which is the last stage of the bioproduct. Note that the enzymatic hydrolysis samples (c and d) had more vigorous colony growth than the acid hydrolysis samples (a and b) suggesting a more suitable substrate for yeast growth. **(a)** Yeast growth in 10^9 dilution of acid hydrolysis supernatant; **(b)** yeast growth in 10^{10} dilution of acid hydrolysis supernatant; **(c)** yeast growth in 10^9 dilution of enzymatic hydrolysis supernatant; and **(d)** yeast growth in 10^{10} dilution of enzymatic hydrolysis supernatant.

The seven overall fungal strains were isolated from soil samples using the serial dilution and pour plate techniques. Among seven isolated fungal strains, one isolate exhibited maximum starch degradation on iodine flooding. It was identified as *Aspergillus niger* based on morphological and microscopic examination. The use of *Aspergillus* as a starch-degrading fungus is well known. Rekha *et al.* (2013) reported that amylases are produced by filamentous fungi. According to Ogbonna *et al.* (2014), these fungi contribute to the co-mineralization of nitrogenous substances through the breakdown of starch, which is beneficial to rhizosphere plants. The study analyzed the composition of rice polish, including nitrogen, sugar, and starch contents. The results showed that the sugar content was 35.2%, the starch content was 79.5%, and the nitrogen content was 8.5%, respectively. In a study carried out by Rosin *et al.* (2002), nitrogen content was 8.5%, starch content was 24.4% to 50.0%, and sugar content was 6.3% to 35.2%. Measurements of fresh weight of glucose, fructose, and total starch were 3.07 to 38.4 mg/100 g, 3.77 to 55.9 mg/100g, and 9.28 mg/100g, respectively. The difference in the content of carbohydrates in rice polish varies depending on the origin of the country. The total soluble sugar (sucrose) content was about 0.5% of fresh weight, which was not high, which is in accordance with the results of Li *et al.* (2002).

The impact of varying concentrations of the acid (H_2SO_4) pre-treatment (1%, 1.5, 2, 2.5, 3, 4, and 5%) was studied on the amount of reducing sugars in rice polish over incubation time (1, 2, and 3 h). After hydrolysis, the amount of free sugars in the supernatant was determined. The amount of recovered sugar was directly proportional to the incubation period, with highest results achieved after using 2% of H_2SO_4 in 1 h, with a total free sugars yield of 19.2 mg/mL. These findings were in line with Suryawanshi *et*

al. (2018), who also showed elevated reducing sugar release in an acid treatment of 2.5% which is in close alignment with this study. To determine the effects of sulfuric and phosphoric acid on rice polish, comparative tests were performed using 1% sulfuric and phosphoric acids. The DNS assay was used to determine reducing sugars. The conversion of glucose to reducing sugars was maximum with sulfuric acid (1.08 g/L) compared to phosphoric acid (0.59 g/L). These findings are in line with Yuliani *et al.* (2018) results, who observed that sulfuric acid yielded more reducing sugars compared to other acids.

In enzymatic hydrolysis, the activity of amylase was assessed at different residence times (48, 72, 96, and 120 h). The amount of reducing sugars was measured using a substrate-based assay (I₂-KI assay), and enzyme activity was observed *via* a product-based assay (DNS assay). The rate of liberation of reducing sugars by amylase was 10.6 µmol/min after 72 h of incubation. The corresponding enzyme activity was 409 µmol/min. Tanaka *et al.* (2006) reported similar results using rice bran, a starch-rich agricultural byproduct. Soni *et al.* (2003) also examined the high enzyme activity with 322 µmol/min in the product-based assay and 16.0 µmol/min in the substrate-based assay. Their results were quite similar to our results, with maximum enzyme activity in the product-based assay compared to the substrate-based assay.

The effect of acid and enzymatic hydrolysis was tested on yeast growth by examining the sugar profiles obtained from both methods. Sugar levels and the byproduct produced were directly correlated. The DNS assay was used to determine enzyme activities with a mean of sugars released as maltose and sucrose in enzyme and acid hydrolysis of 304 and 566 mol/min, respectively, using rice polish. There was a significant difference found in enzyme activity that affected the yield of yeast-based products. Enzymatic hydrolysis was found to yield 8.68 mg/mL of reducing sugars compared to the acid hydrolysis, which was 6.61 mg/mL. Research conducted by Yuliani *et al.* (2018) reported high yields of yeast-based product (ethanol) with acid hydrolysis using rice bran as a substrate, which is contrary to the present findings. This difference can be attributed to the specificity of the enzyme, *i.e.*, the enzyme isolated in this work may be more efficient in degrading biomass to fermentable sugars, resulting in high yields of enzymatic hydrolysis, or the rice polish composition may be conducive to enzymatic hydrolysis.

The use of H₃PO₄ and H₂SO₄, followed by enzymatic hydrolysis with fungal amylase, gave a significant amount of fermentable sugars in the present study. But according to literature, physical disruption of the biomass matrix can further improve starch accessibility, such as the acoustic cavitation and bubble dynamics in sonochemical reactors (Hu *et al.* 2025). The natural degrading ability of the soil-isolated fungus in the present work could be combined with ultrasound-assisted extraction, which could further boost hydrolysate glucose content, compared to conventional boiling or stirring methods (Deng and Huang 2025).

CONCLUSIONS

This research aimed to investigate the effect of acid versus enzymatic hydrolysis for converting rice polish starch to reducing sugars. The main goal was to find out the most efficient process for starch degradation.

1. Acid hydrolysis with 2% H₂SO₄ at 1 h was the optimal condition to extract the maximum reducing sugars. Acid types (1% H₂SO₄ versus H₃PO₄) were tested to ascertain their effects on sugar yield, with H₂SO₄ giving the best results.
2. Enzymatic hydrolysis using amylase enzyme, derived from an isolated starch-degrading fungus (*A. niger*) via submerged fermentation, resulted in a maximum reducing sugar yield of 10.6 µmol/min after 72 h of incubation.
3. A comparison of the yeast growth on the sugar profiles of hydrolysates obtained from both methods showed that enzymatic hydrolysis yielded more fermentable sugars (8.68 mg/mL), making it more suitable for producing biofuels, including ethanol.
4. The results indicated that enzymatic hydrolysis was more effective than acid hydrolysis in converting the rice polish starch to reducing sugars, with significant implications in manufacturing high-quality products, including ethanol.

Competing Interests

The authors declare that there are no conflicts of interest.

Availability of Data and Material

All the data generated in this research work have been included in this manuscript.

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REFERENCES

- Ambreen, N., Hanif, N. Q., and Khatoon, S. (2006). "Chemical composition of rice polish from different source," *Pakistan Veterinary Journal* 26(4),190-192.
- Anwar, Z., Gulfraz, M., Asad, M. J., Imran, M., Akram, Z., Mehmood, S., and Sadiq, A. (2012). "Bioethanol productions from rice polish by optimization of dilute acid pretreatment and enzymatic hydrolysis," *African Journal of Biotechnology* 11(4), 992-998. <https://doi.org/10.5897/ajb11.1145>
- Azar, M. M. (2024). "Larone's medically important fungi: A guide to identification," *Emerging Infectious Diseases* 30(3), 625-626. <https://doi.org/10.3201/eid3003.2316ww23>
- Brites, P., Luís, J., Kapusniak, K., Wojcik, M., Nunes, C., Coimbra, M. A., and Gonçalves, I. (2025). "Starch-rich rice by-products as a renewable resource for sustainable production of flexible, water tolerant, antioxidant, and UV-protective bioplastics," *Advanced Sustainable Systems* 2025, article e00278. <https://doi.org/10.1002/adsu.202500278>
- Clark, H. E., Geldrich, E. F., Kabler, P. W., and Huff, C. B. (1958). *Applied Microbiology*, International Book Company, New York 53. <https://doi.org/10.1128/am.6.5.347-348.1958>

- Das, R., and Kayastha, A. M. (2019). "Enzymatic hydrolysis of native granular starches by a new β -amylase from peanut (*Arachis hypogaea*)," *Journal of Agricultural and Food Chemistry* 276, 583-590. <https://doi.org/10.1016/j.foodchem.2018.10.058>
- Deng, L., and Huang, G. (2025). "Ultrasound-assisted extraction, optimization, characteristics and antioxidant activity of *Piper nigrum* L. polysaccharides," *Ultrasonics Sonochemistry* 116, article e107309. <https://doi.org/10.1016/j.ultsonch.2025.107309>
- Dhanya, G., Nampoothiri, K. M., Swetha, S., and Ashok, P. (2009). "Immobilized bacterial α amylase for effective hydrolysis of raw and soluble starch," *Food Research International* 42(4), 436-442. <https://doi.org/10.1016/j.foodres.2009.02.008>
- DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry* 28(3), 350-356. <https://doi.org/10.1021/ac60111a017>
- Folorunso, A. A., Omoniyi, S. A., Adeleye, A. E. 1, and Okeke, C. E. (2016). "Proximate composition and sensory qualities of milk produced from five varieties of rice (*Oryza sativa*)," *American Journal of Food Science and Nutrition Research* 3(5), 109-112.
- Frac, M., Hannula, S. E., Belka, M., and Jedryczka, M. (2018). "Fungal biodiversity and their role in soil health," *Frontiers in Microbiology* 9, article 707. <https://doi.org/10.3389/fmicb.2018.00707>
- Ghose, T. K. (2005). "Cellulase biosynthesis and hydrolysis of cellulosic substances," in: *Advances in Biochemical Engineering*, Vol. 6, Springer, Berlin, Heidelberg, pp. 39-76. <https://doi.org/10.1016/j.biortech.2005.02.025>
- Hu, Q., Huang, G., and Huang, H. (2025). "Extraction, structure, activity and application of konjac glucomannan," *Ultrasonics Sonochemistry* 116, article 107315. <https://doi.org/10.1016/j.ultsonch.2025.107315>
- Jinu, J. (2017). "Amylases – Bioprocess and potential applications: A review," *International Journal of Bioinformatics and Biological Sciences* 5(2), 41-50. <https://doi.org/10.5958/2321-7111.2017.00006.3>
- Jung, J., Raghavendra, G. M., Kim, D., and Seo, J. (2018). "One-step synthesis of starch-silver nanoparticle solution and its application to antibacterial paper coating," *International Journal of Biological Macromolecules* 107, 2285-2290. DOI: 10.1016/j.ijbiomac.2017.10.108
- Kazunari, I., Kawase, T., Sammoto, H., Gomi, K., Kariyama, M., and Miyake, T. (2011). "Uniform culture in solid state fermentation with fungi and its efficient enzyme production," *Journal of Bioscience and Bioengineering* 3, 300-305. <https://doi.org/10.1016/j.jbiosc.2010.11.008>
- Kirk, P. L. (1950). "Kjeldahl method for total nitrogen," *Analytical Chemistry* 22(2), 354-358. <https://doi.org/10.1021/ac60038a038>
- Li, B. W., Andrews, K. W., and Pehrsson, P. R. (2002). "Individual sugars, soluble, and insoluble dietary fiber contents of 70 high consumption foods," *Journal of Food Composition and Analysis* 15, 715-723. <https://doi.org/10.1006/jfca.2002.1096>
- McCready, R. M., Guggolz, J., Silviera, V., and Owens, H. S. (1950). "Determination of starch and amylose in vegetables," *Analytical Chemistry* 22(9), 1156-1158. <https://doi.org/10.1021/ac60045a016>
- Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry* 31, 426-428. <https://doi.org/10.1021/ac60147a030>

- Murali, G., and Shastri, Y. (2022). "Life-cycle assessment-based comparison of different lignocellulosic ethanol production routes," *Biofuels* 13(2), 237-247. <https://doi.org/10.1080/17597269.2019.1670465>
- Ogbonna, C. N., Okpokwu, N. M., Okafor, C. U., and Onyia, C. E. (2014). "Isolation and screening of amylase producing fungi obtained from garri processing site," *International Journal of Biotechnology and Food Science* 2(5), 88-93.
- Okunwaye, T., Uadia, P. O., Okogbenin, B. O., Okogbenin, E. A., Onyia, D. C., and Obibuzor, J. U. (2021). "Amylase-producing fungi and bacteria associated with some food processing wastes," *Nigerian Journal of Biotechnology* 38(1), 74-82. <https://doi.org/10.4314/njb.v38i1.9>
- Okunwaye, T., Uadia, P. O., Okogbenin, O. B., Obibuzor, J. U., Okogbenin, E. A., Onyia, D. C., Idabie, E., and Asiriwa, N. U. (2021). "Isolation of microorganisms with amylolytic activity from agro-industrial wastes," in: *Proceedings of the 54th Annual Conference of Agricultural Society of Nigeria*, Alex Ekwueme Federal University Ndufu Alike, Ebonyi State, Nigeria 31st January – 4th Feb., pp, 659-665.
- Pandey, A., Nigam, P., Soccol, C. R. V. T., Soccol, V., Singh, D., and Mohan, R. (2000). "Advances in microbial amylases," *Biotechnology and Applied Biochemistry* 31, 135-152.
- Priya, S., Kaur, N., and Gupta, A. K. (2010). "Purification, characterization and inhibition studies of α -amylase of *Rhizopertha dominica*," *Pesticide Biochemistry and Physiology* 98(2), 231-237.
- Rekha, V., Saifuddin, M. D., Ahammad, S. R., and Dhal, P. K. (2013). "Isolation of amylase producing fungi from paddy-field soil," *Ecology Environmental Conservation* 19, 1041-1044.
- Rosin, P. M., Lajolo, F. M., and Menezes, E. W. (2002). "Measurement and characterization of dietary starches," *Journal of Food Composition and Analysis* 15, 367-377. <https://doi.org/10.1006/jfca.2002.1084>
- Singh, R., Das, R., Sangwan, S., Rohatgi, B., Khanam, R., Peera, S. P. G., and Misra, S. (2021). "Utilisation of agro-industrial waste for sustainable green production: A review," *Environmental Sustainability* 4(4), 619-636. <https://doi.org/10.1007/s42398-021-00200-x>
- Soni, S. K., Kaur, A., and Gupta, J. K. (2003). "A solid state fermentation based bacterial α -amylase and fungal glucoamylase system and its suitability for the hydrolysis of wheat starch," *Process Biochemistry* 39(2), 185-192. [https://doi.org/10.1016/s0032-9592\(03\)00058-x](https://doi.org/10.1016/s0032-9592(03)00058-x)
- Suryawanshi, O. P., Khokhar, D., and Patel, S. (2018). "Effect of different pre-treatment methods on reducing sugar of rice substrate to enhance the ethanol yield," *International Journal of Current Microbiology and Applied Sciences* 7, 2715-2733. <https://doi.org/10.20546/ijcmas.2018.703.314>
- Tanaka, T., Hoshina, M., Tanabe, S., Sakai, K., Ohtsubo, S., and Taniguchi, M. (2006). "Production of D-lactic acid from defatted rice bran by simultaneous saccharification and fermentation," *Bioresource Technology* 97(2), 211-217.
- Tang, Z., and Huang, G. (2024). "Antioxidant activity of polysaccharide from *Garcinia mangostana* rind and their derivatives," *BMC Complementary Medicine and Therapies* 24(1), 283. <https://doi.org/10.1186/s12906-024-04594-z>
- Wang, H., Deng, L., and Huang, G. (2025). "Ultrasound-assisted extraction and value of active substances in Muxu," *Ultrasonics Sonochemistry* 113, article 107220. <https://doi.org/10.1016/j.ultsonch.2024.107220>

- Wang, K., Hasjim, J., Wu, A. C., Henry, R. J., and Gilbert, R. G. (2014). "Variation in amylose fine structure of starches from different botanical sources," *Journal of Agricultural and Food Chemistry* 62(19), 4443-4453.
<https://doi.org/10.1021/jf5011676>
- Wosten, H. A. (2019). "Filamentous fungi for the production of enzymes, chemicals and materials," *Current Opinion in Biotechnology* 59, 65-70.
<https://doi.org/10.1016/j.copbio.2019.02.010>
- Yuliani, D., Julianto, K. A., and Jannah, A. (2018). "Acid and enzymatic hydrolyses of rice bran for bioethanol production," *Journal of Chemistry* 6(1), 24-28.
<https://doi.org/10.18860/al.v6i1.6767>

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