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ORIGINAL ARTICLE

Production of Alpha Amylase from Banana Peel Using *Aspergillus niger*

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ABSTRACT

Background: Various chemicals and dyes used in paper production are xenobiotic in nature and pose significant environmental concerns.

Objective: In this study, a fungal strain was isolated from contaminated paper mill effluent and identified as *Aspergillus niger* through standard microbiological and morphological analyses.

Methodology: The strain was screened for α-amylase production using banana peel—a low-cost agro-residue—as the sole carbon source. Optimization of physicochemical parameters, including incubation temperature (30 °C), initial pH (6.0), and incubation time (72 h), yielded maximal enzyme activity. Crude α-amylase activity was quantified spectrophotometrically by measuring reducing sugars released from soluble starch. Partial purification was achieved via ammonium sulfate precipitation, with 50% saturation providing the highest specific activity and yield.

Application: The applicability of the partially purified α-amylase was demonstrated by de-inking waste office paper; treatment with the enzyme significantly improved paper brightness and reduced ink load, as confirmed by standard gravimetric and spectrophotometric assays.

Conclusion: These findings indicate that banana peel is a feasible and cost-efficient substrate for α-amylase production by *A. niger*. The α-amylase has promising potential for sustainable paper waste management and bioremediation in the pulp and paper industry.

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INTRODUCTION

Different types of chemicals and dyes are used for paper production. Some of which are water insoluble, accumulate, and cause serious environmental issues. Traditionally, papers were manufactured using tree pulp and recycled papers as raw material. This trend has increased in the previous 10 to 12 years worldwide. Industry analyses report that deinked pulp represented about 55% of the reclaimed-pulp market in 2023[1]. Therefore, there is a need to find new cost-effective techniques to avoid such issues. For these reasons, enzymatic methods like amylase are introduced, which are effective, safe, and economical[2].

Amylases are a class of enzymes that are found in plants, animals, and microbes. Amylase are involved in the conversion of starch into mono-, di-, and trisaccharide. Such processes are of high significance in microbiology and biotechnological industries. These amylases have approximately 25% of commercial-level enzyme products in the market[3,4]. The amylase enzyme has been divided into three main divisions, i.e., alpha, beta, and gamma amylase. α-amylase is grouped as family 13 of the

glycosidic hydrolases. They have similar structures, catalytic sites, and catalytic mechanisms. The α-amylase has a 3D structure resembling a barrel shape with three characteristic domains—A, B, and C[5,6]. Amylase is obtained from a variety of microorganisms, such as bacterial and fungal sources[7]. The most stable origination of amylase was discovered in fungal as compared to the bacterial sources[8]. Microbial amylase was obtained with much ease by fermentation. These days scientists are more interested in manipulating microorganisms with methods such as genetic engineering to synthesize enhanced amounts of the desired product[9,10]. In comparison of microorganisms to both plants and animals, the cellular replication of microbial cells was much faster than both plants and animals. In a few minutes, it divides much more rapidly, and multiple copies of the enzyme are made[11]. Amylase alone has applications in the industrial fields like paper, food items, brewing, and the pharmaceutical industries[12].

Aspergillus niger belongs to the genus *Aspergillus*. It is normally found in the form of saprophytes growing on stored grain, dead leaves, and other decaying vegetative materials. Its appearance is in the form of dark brown patches or carbon black. It is extensively produced and utilized in the food industry for producing numerous enzymes such as α-amylases, amyl glycosidase, cellulase, lactase, and acid proteases[13,14]. In the present scenario, solid-state fermentation is used for fungal growth on a moist substrate, and it has been utilized by increasing the yield of amylase[15]. Starch is the best source for fungal growth and fermentation[16].

Starch is composed of polymers for the regulation and storage of carbohydrates in plants. Starch is used by the plants to store energy for themselves. Starch in plants are amyloplasts and are present in leaves, roots, seeds, embryos, and fruits[17-20]. It contains amylose and amylopectin in structural components. Amylose molecules have a helical, unbranched, and linear shape with five hundred twenty thousand D-glucose monomer molecules. With enlarged shapes, they have 7-22 nm of hydrodynamic radii, dependent on the source, with oxygen numbers of 2 to 6 atoms present on the outside of the glucose ring[21]. Amylopectin molecules have high branches of glucose polymer around 2,000 to 200,000 glucose units; the inner branched chain has nearly 30 glucose units. Glucose molecules combine with each other as alpha glucose sugar units and serve an important function, as they are water soluble[22].

An instant method for amylase production by microbes is Remazol brilliant blue starch (RBBS) as a substrate in solid-state media. RBBS anthraquinone dye is included in nutrient agar, and amylase production is confirmed by the dissipation of the color throughout the colonies. This process is apocalyptic, which allows for direct isolation and visualization of amylase-producing fungus and bacteria from the favorable condition without pre-replication of colonies[23].

This study has been conducted to produce alpha amylase by *Aspergillus niger*. Subsequently, different physicochemical parameters, including temperature, pH, and incubation time, were optimized. The enzyme was purified. The purified enzyme was used in the deinking process to recycle the waste papers.

MATERIAL AND METHODS

Materials

Starch, malt extract, ammonium chloride, yeast extract, glucose, ammonium nitrate, dipotassium phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), citric acid, acetate buffer, dinitro salicylic acid (DNS) reagents, Bradford chemical, sodium hydroxide, and hydrochloric acid were utilized in this study.

Fungal Strain

In this study, a fungal strain was isolated from contaminated paper mill effluent. This strain was identified as *Aspergillus niger* through standard microbiological and morphological analyses.

Enzyme Assay

1.67 g of K_2HPO_4 and 0.05 g of KH_2PO_4 were added to 100 mL of water. DNS was primed by adding 10 g of NaOH, 182 g of potassium sodium tartrate, 0.5 g of sodium sulfate, 10 g of DNS reagent, and 2 mL of phenol into 500 mL of autoclaved distilled water. Phenol was heated at 60˚C for about 40 min to dissolve it in water. The DNS was kept on a magnetic stirrer at room temperature for about 6-10 h and stored in the refrigerator.

Preparation of Fermentation Medium

Production medium was prepared by adding 1.5 g of malt extract, 1 g of starch, and 1.5 g of banana peel as a carbon source into 100 mL of distilled water, and the medium was autoclaved. *Aspergillus niger* slant was taken, and about 5 mL of 0.45 % saline solution was added into it and scratched with the help of an autoclaved wire loop. About 5% v/v of *A. niger* was added into the fermentation medium. The flask was kept in a shaking incubator at 30°C. Samples were taken after every 24 h to check the fungal growth.

Alpha Amylase Assay

Enzyme assay was performed with some modification. Then optical density (OD) was measured at 540 nm and calculated[24].

Purification of Alpha Amylase

Purification of alpha amylase was done through method described by Arunachallam[24]. Different concentrations (20, 30, 40, 50, 60 and 70%) of ammonium sulfate were added to the supernatant and subjected to centrifugation. The pellet obtained was suspended in the dialyzing buffer overnight and kept at 4°C. Then deinking was performed by two methods, including the non-heating chemical method/accept & reject method. Second was the heating/chemical and enzymatic method.

RESULTS

Alpha Amylase Production

Alpha amylase by *Aspergillus niger* was produced from banana peel as a carbon source with pre-optimized conditions. Multiple values were obtained over different time periods, mostly over a 24 h interval. Maximum production of enzyme was obtained at 72 h.

Enzyme Assay

After time intervals of 24 h, readings were obtained at 540 nm (Figure. **1**). The highest reading was measured at 96 h.

Enzyme Purification

The best value was obtained at 50% dissolution of ammonium sulfate in enzymes. Figure. **2** shows the enzyme activity obtained at different percentages of ammonium sulfate. Results obtained by deinking are as follows.

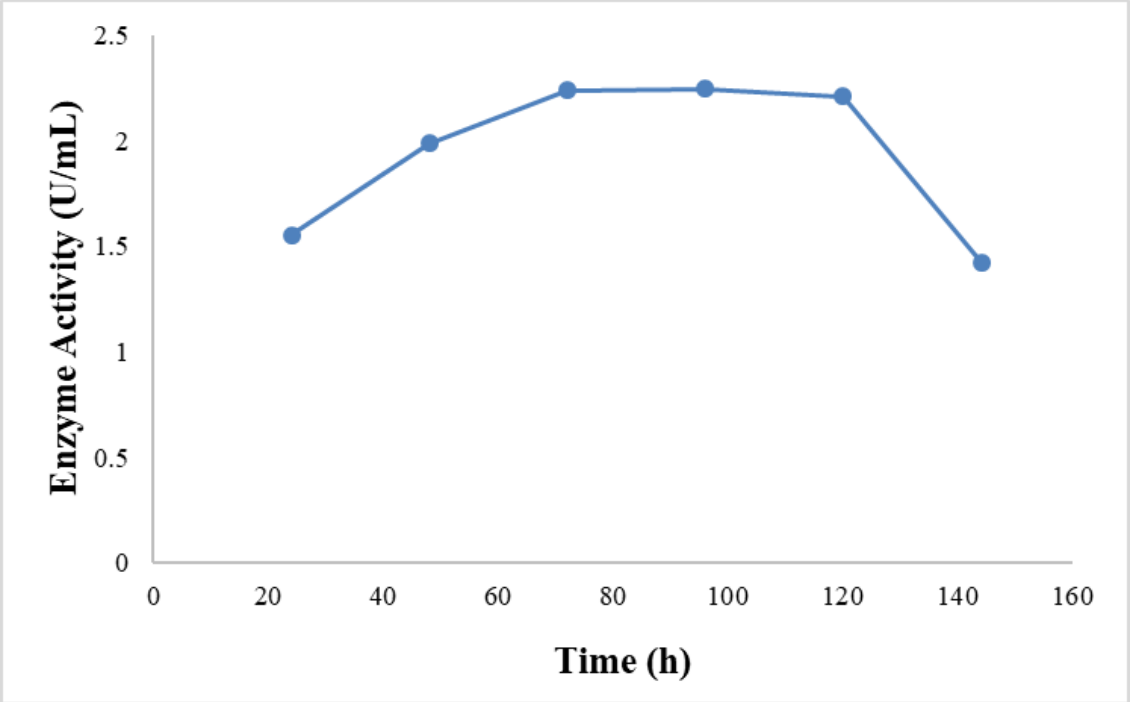

Figure. 1. Effect of time on amylase activity produced by *Aspergillus niger*.

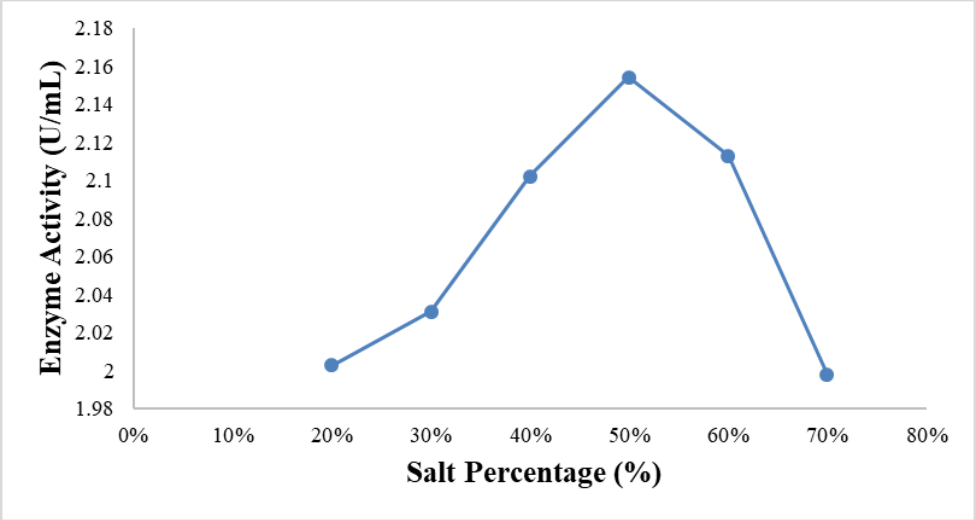

Figure. 2. Effect of ammonium salt concentration on amylase activity produced by *Aspergillus niger*.

Table 1. The Change/Difference in Weight of Paper After Analysis.

Foaming Agents	Accept	Reject	Feed
SDS	0.743	1.641	2.5
Tween	0.619	1.790	2.5

Table 2. Overview of SDS, Tween, and Feed in Terms of Dots Per Square Centimeter (cm²).

Agents	Accept	Reject	Feed
SDS	2	14	15
Tween	1	15	14

Table 3. Weight Difference of Paper After Treatment.

Agents	Weight (g)
Enzymes	2.260
H_2O + SDS	2.198
Enzymes + SDS	2.051
H_2SO_4 + SDS	2.247

Method I

Method I was based upon the accept/reject method with the help of foaming agents, including the weight of the hand sheet of 104 cm² (Table **1-2**). The total weight of the paper before the experiment was 4.5 g.

Method II

Method II was based upon an enzymatic and heating method with the help of foaming agents, including the weight of the hand sheet of 104 cm² (Table **3**). The total weight of the paper before the experiment was 4.5 g.

DISCUSSION

In this study, banana peel was used as a carbon source for amylase production from *Aspergillus niger.* We obtained the enzyme activity of 2.154 to 2.247 U/mL/min in the presence of banana peel as a carbon source. Previously, in another study, better production of amylase was obtained with wheat bran as a carbon source[25]. *A. niger* is reported to produce 1.7 U/mL cellulase using sawdust as raw material. Whereas in another study, *A. niger* gave 0.292 U/mL cellulase using coir waste and sawdust as a raw material. Miranda *et al*. found that *Aspergillus niger*

produced 2.7 U/mL cellulase using sugarcane waste as a raw material. Pineapple and orange peels were also used as a raw material for the cellulase production by *A. niger*[26-29].

In this study, *A.* niger gave maximum amylase production at pH ranging from 4.5 to 5.5. Previously, in other studies, *A. niger* gave maximum cellulase production at pH 5.0, 6.0, 4.5, 3-4, and 5.3-5.5[30-33].

The effect of temperature on amylase production by *A. niger* was also studied. *A.* niger gave maximum amylase production at 50°C. Previously, in other studies, maximum cellulase production by *A. niger* was reported at 22-25, 28, 30, 35, 40, and 45-50°C[34,35]. The effect of time on amylase production by *A. niger* was evaluated. The optimum incubation period measured for amylase production was 4 days. Previously, in other studies, the highest cellulase production by *A. niger* was found after 1, 3, 4, 5, 7, and 10 days of incubation period[36-39]. This study showed deinking of paper using amylase with better efficiency as compared to the chemical deinking method. Results of this study elucidated that amylase showed no ink spots (0.001 cm²) on paper as compared to the controlled or feed sample (14-16/cm²).

CONCLUSION

From this study, it is concluded that banana peel is a good carbon source to produce amylase by *A. niger.* The purified amylase enzyme has better deinking capabilities, thus making it a good candidate for use in the paper industry.

CONFLICT OF INTEREST

None.

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