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RESEARCH ARTICLE
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BIOTECHNOLOGICAL ADVANCES IN AMYLASE PRODUCTION FROM *ASPERGILLUS NIGER* FOR POTENTIAL INDUSTRIAL APPLICATIONS

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ABSTRACT
The growing demand for efficient and sustainable enzyme production has sparked interest in microbial sources, particularly for industrial applications. Amylases are vital enzymes in modern biotechnology, with applications spanning pharmaceutical, food, and textile industries. Among the various sources of amylase, microbial production, particularly from *Aspergillus niger*, has proven to be the most efficient for industrial use. This study highlights biotechnological advances in amylase production through solid-state fermentation (SSF) using wheat bran as a substrate. A strain of *Aspergillus niger* isolated from soil was selected for its superior enzyme production, with maximum yields observed after 4 to 5 days of incubation at 40°C and pH 7.0. Key factors such as incubation time, temperature, pH, and substrate concentration were optimized to enhance the enzyme yield. Soluble starch was added which boosted amylase production. Lowry method was used to confirm the protein concentration after ammonium sulfate precipitation and dialysis were used to purify the amylase. With strong activity over a broad pH range (4.0–7.0) and temperature range (30–42 °C), the partially purified enzyme is appropriate for a number of industrial applications. These results highlight the possibility of *A. niger* amylase as an affordable and environmentally friendly option for use in textiles, starch processing, and other industries that need thermostable enzymes. The current study offers viable path for increasing the production of microbial amylase and providing economical and sustainable answers to industrial requirements.

KEYWORDS: Amylase, *Aspergillus niger*, solid-state fermentation, enzyme production, food industry, textile industry.

1.0 INTRODUCTION
Amylases are key enzymes involved in the hydrolysis of starch into simpler sugars, playing a pivotal role across a range of industrial processes, from food production to biofuel generation. Starch degrading enzymes such as amylase have been receiving a great deal of attention and interest due to

their perceived technological significance and economic benefits (Sheela *et.al.,* 2021). Although many microorganisms are able to produce this enzyme, some of the most widely used for their industrial applications are *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Aspergillus niger*, *Penicillium chrysogenum* (Saranraj & Stella, 2013). When compared to other microbial sources, the fungal amylases are preferred because of their more acceptable GRAS (Generally Recognized As Safe) status, the conditions such as hyphal mode of growth and good tolerance to low water activity (aw) and high osmotic pressure makes fungal species most efficient for bioconversion of solid substrates and thus attracting more interest as source of amylolytic enzymes (Singh *et.al.*, 2014). *Aspergillus niger*, has gained significant attention due to its prolific ability to produce amylases, making it a cornerstone of enzyme biotechnology. Among the various sources of amylases, microbial enzymes are highly sought after for their efficiency, scalability, and cost-effectiveness. *A. niger*, known for its rapid growth and high enzyme yield, has become one of the most valuable organisms in enzyme production, particularly for α-amylase and glucoamylase. Alpha amylase can be derived from several sources such as plants, animals and microbes. The microbial enzyme meets the industrial demand and a large number of them are available commercially (Pandey *et al*., 2000)

In recent years, *A. niger* has demonstrated its utility in numerous industries, including food processing, textiles, paper, and biofuel production, owing to its ability to produce industrially relevant enzymes with high activity and stability under diverse conditions. Amylase, a starch degrading enzyme have gained importance in various industrial process like pharmaceutical, food, brewing, paper, textile and chemicals. It is extensively used in pharmaceutical industries in digestive tonics, for hydrolysis of starch to produce different sugars like glucose and maltose which have several applications (Saini *et.al* 2017). The most widespread applications of α-amylases are in the starch industry, which are used for starch hydrolysis in the starch liquefaction process that converts starch into fructose and glucose syrups (Nielsen and Borchert, 2000) The use of modern biotechnological techniques, such as genetic manipulation and optimization of fermentation conditions, has enhanced the yield and functionality of amylase production from *A. niger*, opening new frontiers for its application in sustainable industrial processes.

This research article explores the biotechnological advancements in the production of amylase from *Aspergillus niger*, focusing on its industrial potential. By examining enzyme production processes, enzymatic properties, and the role of *A. niger* in various sectors, this study aims to provide insights into optimizing amylase production and its wide-ranging applications, contributing to the development of efficient and eco-friendly industrial practices.

2.0 MATERIALS AND METHODS

2.1 Microbiological media and reagents

2.1.1 PDA (Potato Dextrose Agar): Used for culturing *Aspergillus niger*. Potato starch, iodine (indicator for starch hydrolysis), wheat bran (substrate), peptone, yeast extract, agar, soil samples.

2.1.2 Salts and chemical agents

Potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), sodium dihydrogen phosphate ($Na_2H_2PO_4$), magnesium sulfate ($MgSO_4$), NaCl, ammonium sulfate, ethanol, $BaCl_2$, $MgCl_2$, $CaCl_2$, $FeSO_4$, $CuSO_4$, EDTA, methanol, acetone, chloroform, petroleum ether, polyethylene glycol, Tween 80, sodium sulfate, sodium tripolyphosphate.

2.1.3 Purification and characterization chemicals

Ammonium sulfate, 0.1 M phosphate buffer, PAGE reagents, NaCl, phosphorylase, ovalbumin, carbonic anhydrase, trypsin inhibitor, lysozyme, SDS-PAGE reagents, peptide mapping reagents, Tris buffer, iodine solution (2% KI), 0.2 N HCl, chloroform, ethanol, sodium sulfate, acetone, Tween 80.

2.1.4 Materials for industrial applications

Cotton fabrics for desizing, wheat flour, salt, sugar, water, oil, yeast (5g).

2.2 Isolation of Microorganism

Aspergillus niger strains were isolated from soil samples collected from various locations using serial dilution and baiting techniques (Abe *et al*., 1988). The isolated fungal species were cultivated on PDA medium.

2.2.1 Screening of Isolates

Screening of amylase-producing fungi was conducted using starch agar plate method. *Aspergillus niger* strains that showed significant starch hydrolysis were selected for further study.

2.2.2 Inoculum Preparation

Spore suspensions of *Aspergillus niger* were prepared by suspending seven loopfuls of spores in 10 ml sterile distilled water. This spore suspension was adjusted to an optical density of 0.8 (~7×10^5 spores/ml).

2.3 Solid-State Fermentation (SSF) for Enzyme Production

5 g wheat bran was mixed with 8 ml mineral salt medium (MSM) in 250 ml flasks. After autoclaving at 121°C for 15 minutes, the medium was inoculated with 1 ml of the spore suspension and incubated at 30°C for 4-5 days. After incubation, the fungal medium was mixed with water, filtered, and centrifuged at 12,000 rpm. The supernatant, containing the enzyme, was collected for further analysis.

2.4 Media Optimization Parameters

2.4.1 Temperature and pH Optimization

Enzyme activity was assessed at different pH (3.6–9.0) and temperature (25–70°C) levels to determine the optimal conditions for amylase production.

2.4.2 Protein Estimation

Protein content was estimated using the Lowry method (Lowry *et al*., 1951), with bovine serum albumin (BSA) as the standard. Enzyme activity was calculated in μg/ml protein.

2.4.3 Incubation Time

The effect of enzyme-substrate reaction time (5–60 minutes) on enzyme activity was studied. To ascertain the effect of incubation time on enzyme activity the enzyme substrate reaction mixture was incubated for different incubation periods of 5, 10, 20, 30, 40, 50 and 60 min and enzyme activity was studied.

2.4.4 Purification of Amylase:

Amylase was purified from the crude extract using ammonium sulfate precipitation. The precipitate was dialyzed in phosphate buffer (pH 7.0) for 4-5 days with daily buffer changes.

2.4.5 Enzyme Characterization

The purified amylase was characterized based on its thermal stability, optimal pH, and substrate affinity.

2.4.6 Spectrophotometric Assay of Amylase Activity

Amylase activity was determined using the Dinitro Salicylic acid method. After stopping the reaction with DNSA reagent, the absorbance was measured at 540 nm using a spectrophotometer for standard and test samples. The graph was plotted with a concentration of glucose against absorbance (OD value). From the graph, the concentration of amylase in the enzyme samples was determined

2.5 Applications of Amylase

The worldwide application of alpha amylase across various industries like detergents, food, paper and pulp, and textiles is contributing to market growth.

2.5.1 Food industry

In food industry amylase is largely used to enhance the quality of dough and improve its volume and texture of baked food items. It breaks down complex starch molecules into simpler sugars, so that elasticity of dough improved and diminishes the need for chemical added substances.

2.5.2 Making of bread

500g of wheat flour was added in two different containers. Sugar, salt and yeast in appropriate amount were added in both the containers. In one of the plate, selected enzyme was added, and the dough in both the plates was prepared with water. After kneading, little oil was applied on the dough and allowed to rest 10-15 minutes. The baking process was started.

2.6 In Textile industry

2.6.1 Desizing of cloth

For desizing process, two pre-weighted equal size clothes were taken. The solution in both the plates should be in equal amount. In first plate phosphate buffer and in second plate, enzyme: phosphate buffer (1:1) was added. The plates were incubated at room temperature for few hours to absorb the

enzyme. The cloths were removed from plate and kept overnight to dry. Then the next day iodine was added to the cloth.

3.0 Results & Discussion:

Production of amylase was done by solid state fermentation using wheat bran as the substrate. *Aspergillus niger* is a filamentous fungus that mostly grow on Potato Dextrose Agar (PDA). Fungal species produce black spores and back colonies on the PDA plates as in Figure 1. Starch hydrolysis process is a direct indication by *Aspergillus niger*.

3.1 Selection of strain and screening of isolates:

Different fungal cultures AN1, AN2, AN3, AN4, and AN5 were isolated from soil sample and screened for amylase production. When starch agar medium was inoculated with the organism and frequently iodine solution is added, production of amylase is indicated by the zone of clearance around the microbial growth. On the basis of the area of clearance on the plate, *Aspergillus niger* (AN2) was selected for further studies on amylase production (figure 2).

Figure 1: Growth on PDA plate after 2 days before adding iodine

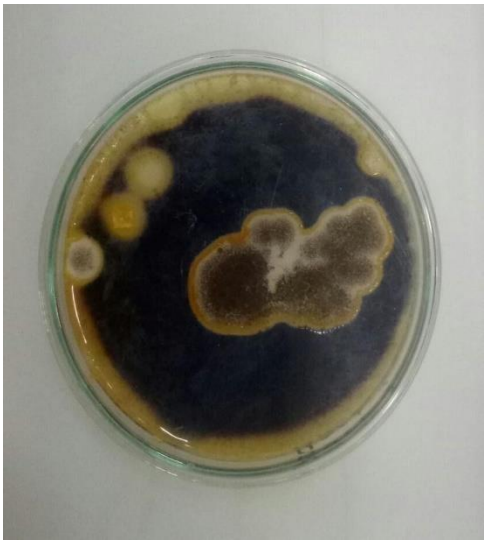

Figure 2: Growth on PDA plate after adding the iodine on plate, which confirms the amylase positive test

3.2 Solid State Fermentation

Wheat bran substrate was used as solid substrates for SSF (figure 3). It was inoculated with *A. niger* spores and incubated for four days at room temperature. Adjusting the pH 7, the enzyme was extracted by using phosphate buffer. The enzyme was filtered and its protein content and enzyme activity was

determined. Wheat Bran supplemented shows the higher resulted for amylo-glucoamidase production by SSF using fungi [Pandey, 1990]. According to Tunga and Tunga, 2003, a lower yield of extracellular amylase production under SSF by *A. oryzae* using sugarcane bagasse has been reported. Biomass production has high amylolytic activity of *Aspergillus niger*. Ikenebomeh and Chikundu, 1997 found that *Aspergillus niger* was superior to other species of *Aspergillus* and strains of fungi in biomass yield from agricultural waste.

Figure 3: *Aspergillus niger* growth in SSF and Submerged fermentation

3.2.1 Enzyme production

By comparison of solid state and submerged fermentations of the five fungi subjected to *Aspergillus niger* AN2 was found to be the best amylase producer as in Table 1. So, for further optimization of culture conditions this potential strain was selected and produced.

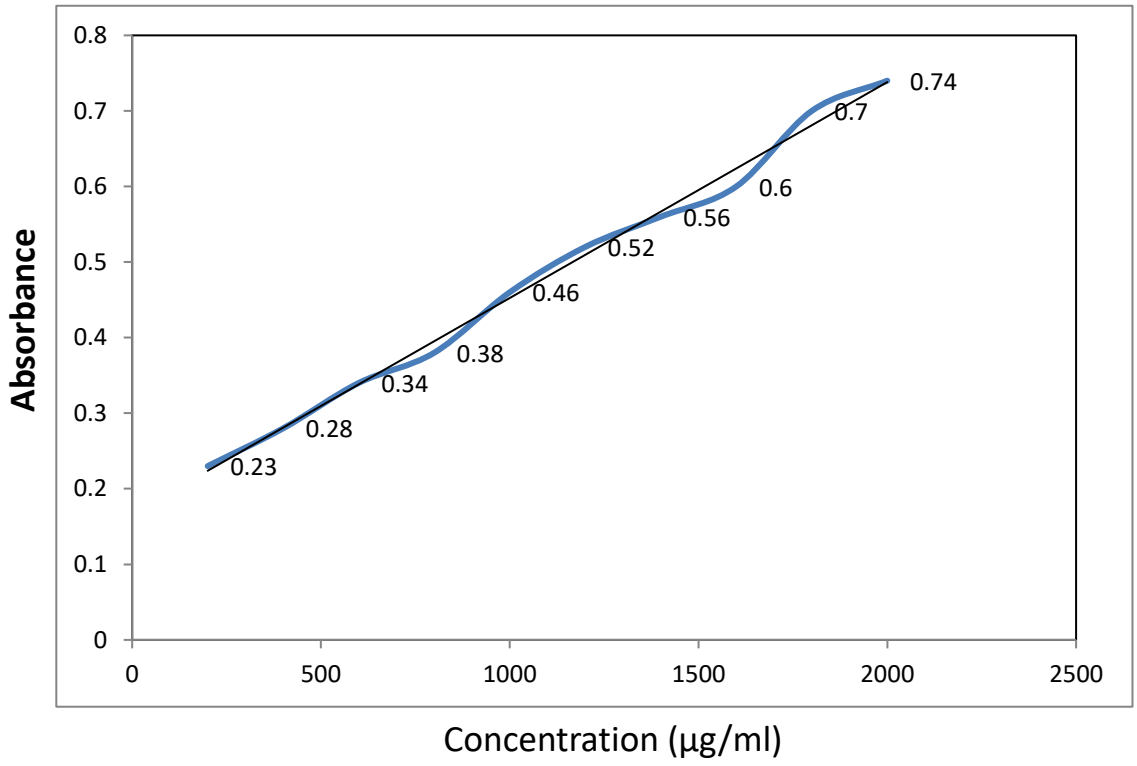

Figure 4: Graph showing Concentration of glucose in microgram/ml and Absorbance

Table 1: Concentration of glucose in test samples

Samples	Concentration(µg/ml)
AN1	800
AN2	1710
AN3	1110
AN4	1320

3.2.2 Effect of pH

The selected fungal strain, *Aspergillus niger* was inoculated into substrates and incubated at room temperature for four days. The enzyme was filtered and extracted to check the specific activities of the amylase produced at different pH (Figure 5). The maximum yield of amylase production was observed at pH 7. There was marked increase in the yield till pH 9 (Figure 6), and then there was very minimum activity at pH 1. Bacterial amylases having alkaline pH optima were reported. It is seen that amylase production is increasing at pH 5 (Gupta *et al*., 2008). In our study the effect of pH on the enzyme activity indicates that the amylase is active in the pH range 5 – 9, both acidic to alkaline. Observing these results, it can be predicted that, the enzyme would be useful if it requires wide range of pH from acidic to slightly alkaline range and vice versa.

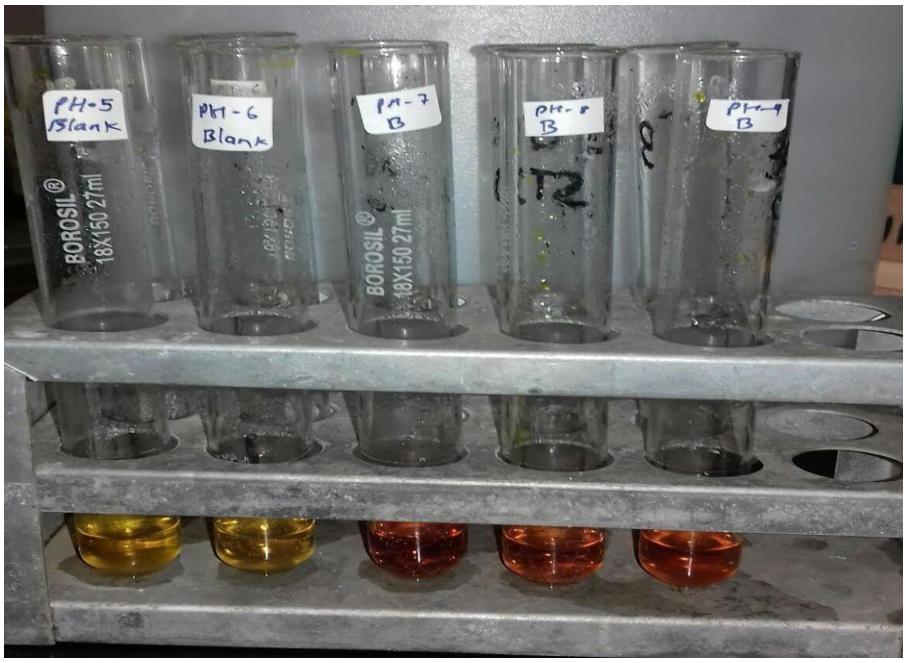

Figure 5: Different tubes for pH optimization

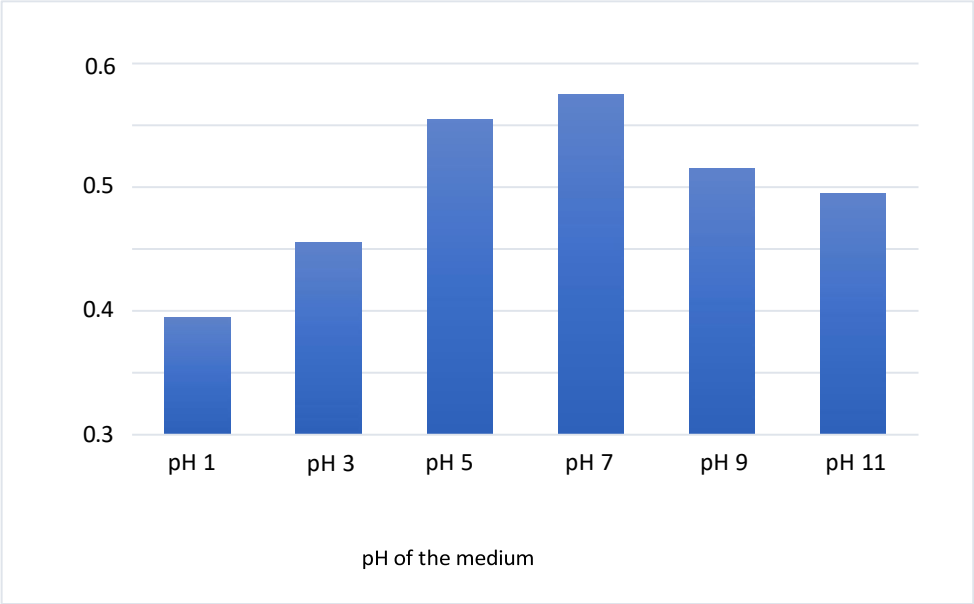

Figure 6: The effect of pH on amylase enzyme by *Aspergillus niger*

3.2.3 Effect of temperature

Aspergillus niger inoculated at different temperature of 0°C, 30°C, 37°C, 40°C, 50°C, 60°C, 70°C and 80°C. Temperature (40°C) was found to be the best for enzyme production in solid state fermentations (Figure 7). Then a gradual decrease in yield was observed. It was observed that the best enzyme production in *Aspergillus niger* was at room temperature for both in SMF and SSF as also reported by Varalakshmi *et al.*, 2009. And Kathiresan and Manivannan, 2006 reported 30°C to be the best for enzyme production by *Penicillium fellutanum*. The highest yield of amylase was reported between 30°C - 37°C (Ueno *et al.*, 1987; Kundu *et al*., 1973).

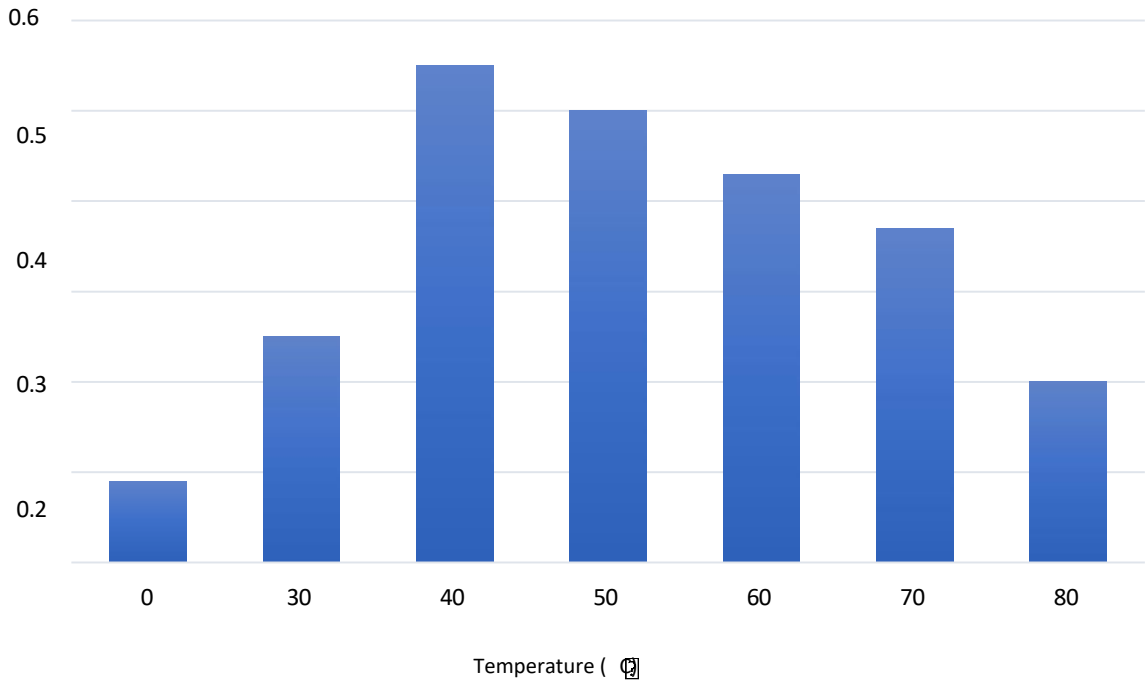

Figure 7: The effect of temperature on amylase enzyme by *Aspergillus niger*

3.2.4 Enzyme stability

The enzyme showed highest stability at 40°C and the least stability at 80°C retaining only 60% of its original activity at 30°C. Comparable results were detailed from our prior studies (Alva *et al*., 2007) on *Aspergillus* sp. JGI 12.

3.2.5 Effect of Time of Incubation

Aspergillus niger was inoculated into medium such as wheat bran substrate for SSF. The cultures were incubated at room temperature for 4- 5 days. The enzyme was extracted and the specific activity of the amylase produced at different days of incubation were seen (Figure 8). Production of enzyme started after 24h of inoculation and increased with incubation time. The maximum production was observed after 96 hours of incubation and after that it started to decline. Ely *et.al*., 2002 observed that the mycelial growth on starch reached high level after five days and maximum amylase activity was produced after two days of cultivation.

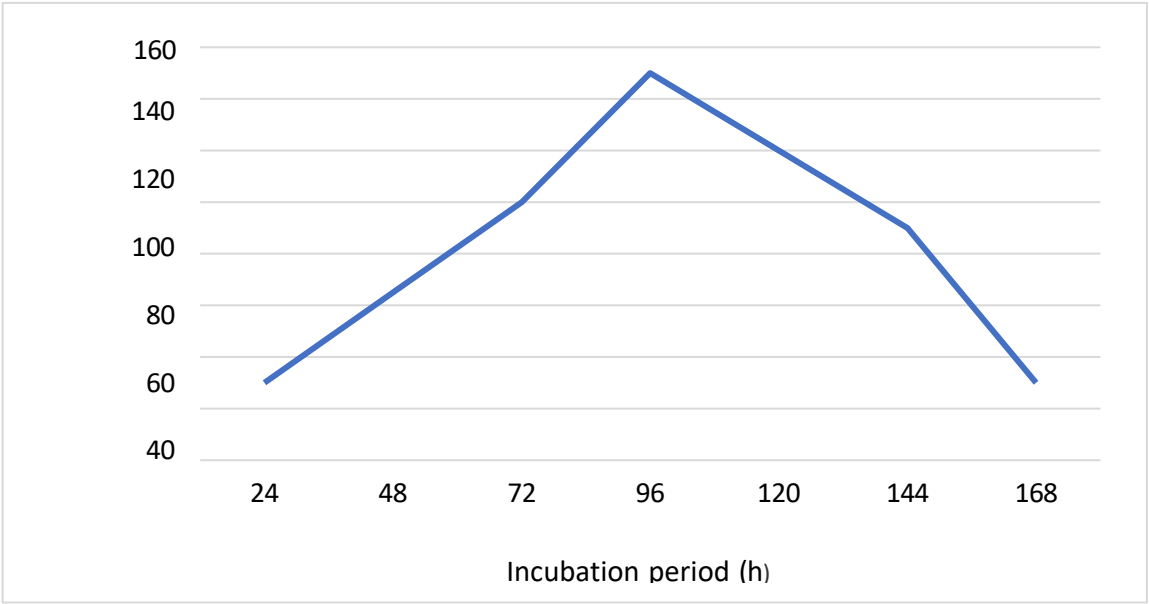

Figure 8: Effect of incubation period on amylase enzyme

3.2.6 Effect of carbon source

The amylase activity was checked on different carbon source such as glucose, sucrose, maltose, lactose, and soluble starch. Leaving starch other supplements did not show remarkable change in the yield (Figure 9). Amylase activity was best seen when starch was added as substrate. Glucose and sucrose supplementation resulted in decreased production of enzyme. Similar results of catabolite suppression were obtained by Varalakshmi *et al.,* 2009 and Nandakumar et al. (1999). in solid state fermentation and in submerged fermentation.

Figure 9: The effect of Carbon source on enzyme amylase by *Aspergillus niger*

3.3 Applications of amylase

3.3.1 In bread

The ingredients of both the batter are same except that one dough was having amylase enzyme and other without enzyme. After baking, it was observed that, the dough volume increase which was prepared with enzyme and improved texture of bread and dough (Figure 10 & 11). Amylase creates extra sugar within the batter, which moves forward to improve the taste, colour and toasting qualities of the bread. Amylases have an anti-stalling impact in bread baking and they improve the softness retention of baked products and expand the shelf life of these products. When amylase was added to the dough it resulted in upgrading the rate of fermentation and diminishment of the consistency of mixture(dough).

3.3.2 In textile

After adding iodine solution on the cloth it was observed that cloth treated with amylase enzyme has a more spreading effect than the cloth without enzyme (Figure 12). This means the starch on the cloth is removed, and this enzyme can be used as desizing agent (figure 13). Textile industries broadly uses amylase to hydrolyse and solubilize the starch, which then are washed out of the cloth for increasing the stiffness of the finished products. Amylase is utilized as desizing agent for evacuating starch from the cloth.

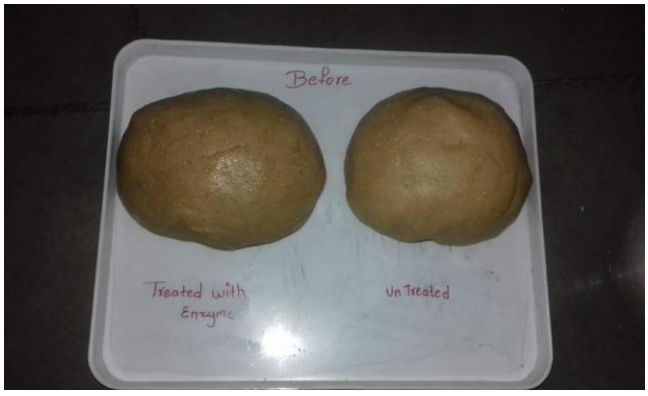

Figure 10: Before treating with an enzyme amylase

Figure 11: After treating with an enzyme amylase

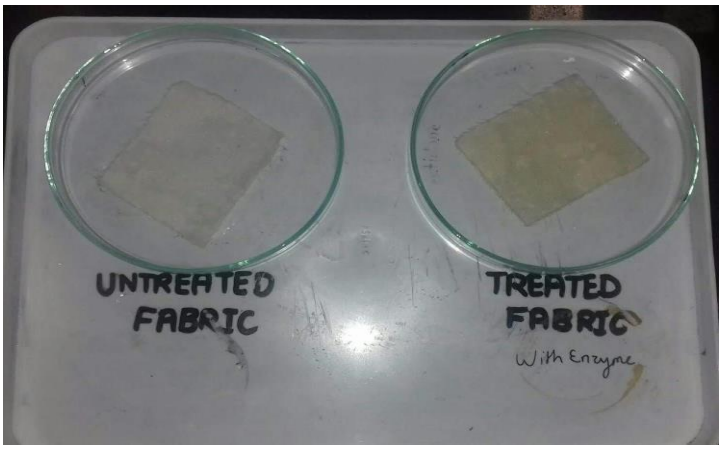

Figure 12: Before treating with an enzyme

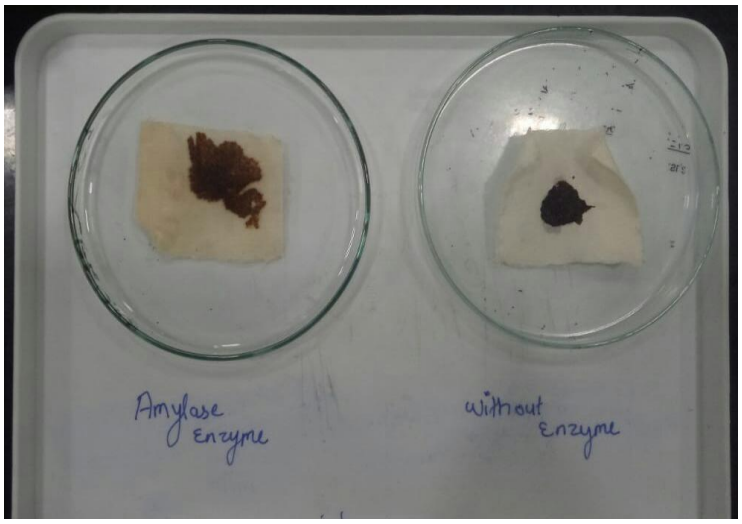

Figure 13: After treating with an enzyme and addition of iodine solution on cloth

4.0 CONCLUSION

This study demonstrates the potential of *Aspergillus niger* as a highly effective and sustainable microbial source for industrial amylase production. *Aspergillus niger* strain was isolated from fungi on starch agar plates which was capable of amylase production. The strain showed a large zone of clearance which show that it was able to produce significant amount of amylase. The strains was compared for amylase activity in solid state fermentations and submerged fermentation. The enzyme activity of *A. niger* was also found to be good in solid state fermentation. The research highlights optimized conditions for amylase yield, such as pH, temperature, and substrate concentration, and validates the role of solid-state fermentation with wheat bran as a promising approach for large-scale enzyme production. The partially purified amylase extracted from *A. niger* showed substantial activity across a broad pH and temperature range, making it suitable for various applications, including the food and textile industries, where stability and efficacy are crucial. This work provides insights into environmental friendly enzyme production methods that reduce dependency on chemical additives while enhancing the efficiency of industrial processes. Overall, the findings underscore the viability of *Aspergillus niger*-derived amylase as a cost-effective and versatile enzyme, paving the way for future biotechnological applications in sustainable industrial practices.

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