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Prof. Dr. Banu KUTLU



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SUSTAINABLE PRODUCTION OF FUNGAL α -AMYLASE USING AGRO-INDUSTRIAL WASTE IN SOLID STATE FERMENTATION

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ABSTRACT

The commercial production of industrial enzymes using inexpensive substrates has received considerable attention in recent times. In the current research work, the process of α -amylase production by fungus was studied using solid state fermentation (SSF) and agro-industrial waste materials as substrates. *Aspergillus flavus* was used as the organism for production, and enzyme production was optimized based on several substrates and nutritional variables. Among the various agro-based residues, palm kernel cake produced the highest enzyme activity (0.210 U/mL). Nutritional optimization showed that sucrose was more effective in promoting enzyme production, producing an enzyme activity level of 0.421 U/mL. Glucose, on the other hand, showed relatively lower enzyme production. The two nitrogen sources studied, namely ammonium nitrate and glycine, showed higher enzyme production from ammonium nitrate (0.231 U/mL). It is also important to observe that the use of sucrose and ammonium nitrate in combination has resulted in an increase in α -amylase activity (0.821 U/mL). This shows the importance of synergistic action between the optimum use of carbon and nitrogen sources in controlling enzyme biosynthesis. The results obtained from this research confirm that agro-industrial wastes can be used as cheap raw materials for producing higher α -amylase through solid-state fermentation. Also, this study illustrates the significance of optimizing nutrition for increased enzyme production.

Keywords: Amylase, Agro-industrial waste, solid state fermentation, palm kernal cake.

INTRODUCTION

The valorization of agricultural by products and residues is a strategic approach for the development of sustainable economic and industrial systems, especially in developing countries. These residues are produced on a large scale and their poor management is serious challenge for the environment, economy and logistics. Therefore, the conversion of agricultural residues into valuable biotechnological products has become a high priority research area. These residues can be used as low-cost raw materials for the production of high value compounds using innovative bioprocessing and biorefinery concepts. These compounds include industrial enzyme, bioethanol and other alcohols, biohydrogen, biopesticides and organic acids. This approach not only resolves the issue of waste disposal but also helps in the development of sustainable industries and circular economy models (Melnichuk *et al.*, 2020).

Among the industrially important biocatalyst, α - amylases represent one of the most widely used enzyme families owing to their prime role in starch hydrolysis and fermented food production. Apart from their prime role in the food and starch industry, α - amylases find extensive use in various industrial processes such as paper and pulp bioprocessing, textile desizing and other biotechnological applications. These enzymes catalyse the endo hydrolysis of α - 1,4 glycosidic bonds in Starch, thus facilitating the large-scale production of starch derived value-added products such as glucose, maltose, high fructose corn syrup and cyclodextrins (Kunamneni *et al.*, 2005).

The demand for amylases is steadily increasing across the globe, especially in the paper, pulp and textile sectors, where they improve processing efficiency and quality. In fact, amylase constitute about 30 % of the total global enzyme output, thereby establishing their immense industrial develop α - amylases with improved catalytic efficiency, heat stability and operational durability in challenging industrial environments, while also focusing on cost effective production methods. α - amylases are secreted by a broad spectrum of biological materials such as microorganisms, algae, plants and animals, where they fulfil vital roles in carbohydrates metabolism. Conventionally, amylases of plant and microbial origin have been widely used as food processing agents. For instance, barely amylases have long been an essential component of brewing technology, while fungal amylase is widely used in preparation and processing of traditional fermented and oriental foods (Mishra *et al.*, 2020). The α - amylases production has been widely explored in both submerged (SmF) and solid-state fermentation (SSF). Although Smf is still currently in use on a large scale, the use of artificial media has substantially increased the production cost, thus making it less economically viable. As a result, there has been a growing interest in the use of low cost agricultural and agro-industrial byproducts as novel substrates, especially in SSF processes, where these wastes act as nutrient rich and economically feasible materials for enzyme production. Recently, SSF has received considerable attention owing to its advantages over Smf. These include easier process engineering, lower capital investment, lower production cost, higher enzyme production with improved physicochemical properties, reduced catabolite inhibition and easier down stream processing. Additionally, SSF more closely the natural environments of many filamentous fungi and some bacteria, which often leads to higher enzyme productivity and stability (Saxena and Singh, 2011). The Present work focuses on the production of α - amylases through solid state fermentation (SSF) using coconut oil cake , sunflower oil cake and palm kernel cake as agro industrial substrates, with emphasis on optimizing crucial process parameters to improve the productivity of the enzyme.

MATERIALS AND METHODS

Microorganism

A fungal strain of *Aspergillus flavus* was used for the production of α -amylase in this study. The organism was grown on Czapek-Dox agar slants and subcultured periodically to maintain viability.

Screening for Enzyme Production (Naziz *et al.*, 2024)

Preliminary qualitative screening for α -amylase production was carried out using starch agar plate assays. The presence of a clear hydrolysis zone around the fungal colony, as revealed by staining, indicated the presence of amylase in the culture, and the highly productive strain was chosen for further analysis under solid-state fermentation (SSF).

Inoculum Preparation

Spores from actively growing Czapek-Dox agar plates were collected using sterile distilled water to obtain a uniform spore suspension. One milliliter of this standardized spore suspension was used as inoculum for each fermentation flask.

Substrate

Three different agro-industrial substrates, namely coconut oil cake, sunflower oil cake, and palm kernel cake, were purchased from the local market and used as substrates for SSF. The substrates were dried and powdered to get a uniform particle size suitable for fungal growth.

The production of enzymes was tested separately for each substrate to identify the best one for the highest production of α -amylase.

Solid-State Fermentation (SSF) Technique

SSF was performed in 250 ml Erlenmeyer flasks with 2.5 g of dried substrate. A sterilized salt solution was used to maintain the desired level of moisture. The contents were autoclaved at 121°C for 15 minutes and then cooled to room temperature. Each flask was inoculated with 1 ml of spore suspension, mixed well, and incubated under static conditions for 72 hours.

Enzyme Extraction

The enzyme was extracted from the fermented substrate by adding distilled water to it and shaking it in a rotary shaker for 1 hour. The contents were then centrifuged at 7000 rpm for 10 minutes, and the supernatant was collected as the crude enzyme extract.

Enzyme Assay

α -Amylase activity was measured by DNS (3,5-Dinitrosalicylic acid) method. The reaction mixture contained 1.25 ml of 1% soluble starch solution, 0.25 ml of acetate buffer, 0.25 ml of distilled water, and 0.25 ml of crude enzyme extract. The reaction mixture was incubated for 10 minutes. The reaction was stopped by adding 3 ml of DNS reagent and 1 ml of sodium potassium tartrate. The tubes were boiled in a boiling water bath for 5 minutes. The tubes were cooled, and the absorbance was measured at 575 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μ mol of reducing sugar per minute. All experiments were carried out in triplicate, and mean values were calculated.

Optimization Studies for Enzyme Production

Optimization of enzyme production was done step by step by assessing the effect of various physicochemical factors under SSF conditions (Balakrishnan *et al.*, 2021).

Effect of Substrate

Coconut oil cake, sunflower oil cake, and palm kernel cake were tested individually to find out the most effective substrate for α -amylase production.

Effect of Carbon Sources

The effect of the presence of additional carbon sources, such as glucose and sucrose, on the production of the enzyme was also studied.

Effect of Nitrogen Sources

The role of the addition of nitrogen sources, such as ammonium nitrate and glycine, was also explored to increase the production of the enzyme.

Effect of Combination of Carbon and Nitrogen Sources

The effect of various combinations, such as sucrose and ammonium nitrate, and glucose and glycine, was also studied to explore their combined effect on the production of α -amylase.

RESULT AND DISCUSSION

Effect of Different Substrates:

Table 1 shows the effect of different substrates on enzyme activity. Among the substrates tested, palm kernel cake recorded the highest enzyme activity (0.219 U/ml), followed by sunflower oil cake (0.138 U/ml) and coconut oil cake (0.102 U/ml). The control values for coconut oil cake, sunflower oil cake, and palm kernel cake were 0.021, 0.018, and 0.023 respectively, while the test values increased to 0.123, 0.156, and 0.242. The highest net increase was observed with palm kernel cake, indicating its superior suitability for enzyme production.

The variation in enzyme activity suggests that substrate composition significantly influences microbial growth and enzyme synthesis. Palm kernel cake may provide better nutrient availability, including essential carbon and nitrogen sources, enhancing enzyme secretion. In contrast, coconut oil cake showed comparatively lower activity, possibly due to lower nutrient accessibility or inhibitory factors. Thus, substrate selection plays a crucial role in optimizing enzyme production. The increased enzyme activity measured in palm kernel cake is in line with the findings of Ng et al. (2013), which showed efficient hydrolysis of palm kernel cake protein because of its optimal nutrient composition. This validates the importance of substrate selection in improving microbial growth and efficiency of enzyme production.

Table : 1. Effect of Different Substrates:

Substrate	Control	Test	Enzyme activity (U/ ml)
Coconut oil cake	0.021	0.123	0.102
Sunflower oil cake	0.018	0.156	0.138
Palm Kernel cake	0.023	0.242	0.219

Effect of Carbon Sources

The Table 2 shows the effect of different carbon sources on enzyme activity. Among the tested sources, sucrose produced higher enzyme activity (0.421 U/ml) compared to glucose (0.215 U/ml). The test value for glucose was 0.261 U/ml, with a control of 0.046 U/ml, resulting in a net activity of 0.215 U/ml. In contrast, sucrose showed a test value of 0.476 U/ml and a control of 0.055 U/ml, giving the highest net enzyme activity of 0.421 U/ml. The results indicate that carbon source significantly influences enzyme production. Sucrose appears to enhance enzyme synthesis more effectively than glucose, possibly due to reduced catabolite repression and better metabolic regulation. Glucose, being a simple sugar, may partially suppress enzyme formation. Therefore, sucrose was found to be the most suitable carbon source for maximizing enzyme activity under the given experimental conditions. The results are in accordance with Begum and Munjam (2021), who found that carbon sources have a significant effect on pectinase enzyme production by *Aspergillus niger* in submerged fermentation. Sucrose was found to be a better carbon source for enzyme production compared to glucose. This is because glucose, being a readily metabolizable sugar, can repress the production of inducible enzymes.

Table: 2. Effect of Carbon Sources

Carbon source	Test (U/ ml)	Control	Enzyme activity (U/ ml)
Glucose	0.261	0.046	0.215
Sucrose	0.476	0.055	0.421

Effect of Nitrogen Sources

Effect of nitrogen source illustrates (Table 3) the effect of different nitrogen sources on enzyme activity. Among the nitrogen sources tested, ammonium nitrate showed higher enzyme activity (0.231 U/ml) compared to glycine (0.212 U/ml). The test value for ammonium nitrate was 0.272 U/ml, while the control was 0.041 U/ml, resulting in a net enzyme activity of 0.231 U/ml. In the case of glycine, the test value was 0.249 U/ml and the control was 0.037 U/ml, giving a net activity of 0.212 U/ml. The results indicate that nitrogen source plays an important role in enzyme production.

Table: 3. Effect of Nitrogen Sources

Nitrogen source	Test (U/ ml)	Control	Enzyme Activity (U/ ml)
Ammonium nitrate	0.272	0.041	0.231
Glycine	0.249	0.037	0.212

Ammonium nitrate, being an inorganic nitrogen source, may be readily assimilated by microorganisms, thereby enhancing enzyme synthesis. Glycine, an organic nitrogen source, also supported enzyme production but to a slightly lesser extent. Thus, ammonium nitrate was found to be more effective for maximizing enzyme activity under the given experimental conditions. The findings on the significance of nitrogen source in enzyme production are in agreement with Jiang et al. (2026) in *Microorganisms*, who found that varying forms of nitrogen have a significant impact on nutrient utilization and the biological microenvironment. The study found that inorganic nitrogen sources, such as ammonium-based sources, are easily assimilated and can increase metabolic activity. Likewise, ammonium nitrate can potentially increase efficient enzyme production because of easy microbial uptake. While organic nitrogen sources, such as glycine, can also increase metabolic activity, their assimilation can be relatively slower, leading to slightly lower enzyme production.

Effect of Combination of Carbon and Nitrogen Sources

Table 4 presents the effect of combined carbon and nitrogen sources on enzyme activity. The combination of sucrose and ammonium nitrate showed the highest enzyme activity (0.821 U/ml), whereas the combination of glycine and glucose recorded lower activity (0.455 U/ml). The test value for sucrose and ammonium nitrate was 0.870 U/ml with a control of 0.049 U/ml, resulting in a net enzyme activity of 0.821 U/ml. In comparison, glycine and glucose showed a test value of 0.490 U/ml and a control of 0.035 U/ml, giving a net activity of 0.455 U/ml. The results clearly indicate that the combined effect of suitable carbon and nitrogen sources significantly enhances enzyme production. Sucrose and ammonium nitrate may provide an optimal balance of energy and nitrogen for microbial growth and enzyme synthesis. In contrast, glycine and glucose were comparatively less effective. Thus, proper nutrient combination is essential for maximizing enzyme yield.

Table: 4. Effect of Combination of Carbon and Nitrogen Sources

Carbon and nitrogen	Test (U/ml)	Control	Enzyme Activity (U/ ml)
Sucrose and Ammonium nitrate	0.870	0.049	0.821
Glycine and Glucose	0.490	0.035	0.455

The significant increase in enzyme production with the combination of sucrose and ammonium nitrate is consistent with the results of Wang et al. (2025). Their results indicated that microbial metabolism has distinct preferences for carbon and nitrogen sources, which directly affect the efficiency of metabolism and enzyme production. A combination of easily accessible carbon and inorganic nitrogen sources enhances metabolic flux and protein synthesis. This suggests that sucrose and ammonium nitrate may provide conducive regulatory environments for enzyme production, whereas glucose and glycine may not be as effective in supporting metabolic pathways, leading to relatively lower enzyme production.

SUMMARY AND CONCLUSION

The substrate carbon and nitrogen nutrient supplementation have been found to be potentially influence enzyme production. Compare to all other substrate palm kernel cake was found to the best substrate, while sucrose (carbon) and ammonium nitrate (nitrogen) were best combination for enzyme production. The combine supplementation of sucrose and ammonium nitrate was found to be significantly increase the enzyme activity, compared with other treatments. Hence, optimization of pH, temperature, substrate concentration and nutrient supplementation is important for increasing the enzyme production.

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