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PRODUCTION OF AMYLASE ENZYME THROUGH SOLID STATE FERMENTATION BY *MUSA ACCUMINATA*

Praveen Kumar Dasari*, Kumar Raja Jayavarapu, Chevula Jashuva, Medidhi Thanmai, Surabi Pavani and Suravarapu Gayatri Devi

Mother Teresa Pharmacy College, Sathupalli, Khammam, Telangana.

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Corresponding Author: Praveen Kumar Dasari

Address: Mother Teresa Pharmacy College, Sathupalli, Khammam, Telangana.

ABSTRACT

Amylase, an enzyme that breaks down starch into simpler sugars, has diverse applications, including digestion, diagnosis of pancreatic problems, and industrial processes like food processing, textile desizing and detergent manufacturing. The main aim of the present research work was optimization of process parameters for the production amylase enzyme using *musa accuminata* leaves as substrate in a solid state fermentation by using microorganism *Bacillus lechniformis*. Solid-state fermentation was explained as an activity that occurs on a non-soluble material may acts both as support and a source of nutrients, with a reduced amount of water under the process of fermentation. For the production of amylase enzyme different parameters like incubation time, incubation temprature, pH, inoculum level and moisture content and were optimized the incubation time of 48 hours, the temperature of 30℃, pH 7, inoculum level of 80% v/w and moisture content of 80% v/w were marked optimum for the production of amylase. Different carbon components were screened for the enzyme production; they are glucose, fructose, maltose, sucrose and lactose used as carbon supplements. To determine nitrogen effect on enzyme production, potassium nitrate was taken and 0.2 w/w was observed optimum for that enzyme production. Final conclusion was that *musa accuminata* could be a promising substrate for industrial application since it produces a significant alpha amylase activity in solid sate fermentation.

KEYWORDS: *Musa acuminata, Bacillus lichinifirmis, amylase*, solid state fermentation.

INTRODUCTION

Amylase is a crucial enzyme that catalyzes the breakdown of starch and other polysaccharides into simpler sugars such as maltose, glucose, and dextrins. It is a type of hydrolase enzyme, meaning it functions by catalyzing the hydrolysis of glycosidic bonds in carbohydrates. Amylase plays a vital role in a variety of biological processes, including digestion, energy production, and biotechnology applications. Amylases are naturally produced by a wide range of organisms, including plants, animals, and microorganisms. There are different types of amylase enzymes, each with specific characteristics and mechanisms of action**.** Sources of Amylase Enzyme Amylases are produced by a variety of organisms: Humans and Animals: In humans, amylase is secreted primarily by the salivary glands (salivary amylase) and pancreas (pancreatic amylase), where it plays an essential role in the digestion of dietary starches. Microorganisms: Bacteria (e.g., *Bacillus*, *Streptomyces*, and *Bacillus subtilis*) are significant sources of amylases, especially for industrial production. Plants: Many plants, especially those that store starch (e.g., tubers, seeds), produce amylases during seed germination to convert starch into sugars that can be utilized for growth. Industrial Importance of Amylase, Amylases have vast industrial applications: Food Industry: Amylases are crucial in the production of glucose syrups, maltodextrins, beer, bread, and baby food. In baking, they help break down starches in flour, which improves fermentation and the rising of dough. Bioethanol Production: Amylase plays a key role in the bioethanol industry by converting starch from crops like corn into fermentable sugars, which are then used by yeast to produce ethanol. Detergent Industry: Amylases are used in laundry detergents to break down starch-based stains on clothes, improving cleaning efficiency. Textile Industry: Amylase helps in the desizing of fabrics, which involves removing starch-based sizing agents from textiles. Pharmaceuticals: Amylase is used in the production of digestive enzyme supplements to aid in the digestion of carbohydrates, particularly in individuals with enzyme deficiencies. The main aim of the present research work was optimization of process parameters for the production amylase enzyme using *musa accuminata* leaves as substrate in a solid state fermentation by using microorganism *Bacillus lechniformis*.

MATERIAL AND METHODS

Substrate: *Musa accuminata* leaves were collected from our college ground sathupalli, Telangana and they were naturally dried and powdered, packed and stored until further use.

Microorganism: *Bacillus lechniformis* was used for the optimization of process parameters for the production of amylase enzyme using *musa acuminata* as substrate. Nutrient agar medium was used for the maintenance and sub culturing of the microorganism.

Preparation of inoculum: streaking was done on pure nutrient agar slants from the old cultures of *Bacillus lechniformis* and incubated them at 30℃ for 2 days.

Development of inoculum: 10 ml of sterile distilled water were added to the 2 days old cultured slants, from the 1 ml of suspension was used as the inoculum and placed it into each flask containing approximately 10^7 spores / ml.

Fermentation condition: Solid state fermentation was carried out in 250 ml conical flask containing 10 grams of substrate with 10ml of production medium containing potassium heptahydrogen phosphate 0.4 g/l, NaCl 0.5g/l , magnesium sulphate heptahydrate 0.02g/l, calcium chloride 0.02g/l. Inoculum was placed to the production medium and incubated with continues shaking. Shaker fermentation was accomplished at 30℃ with controlled agitation. At the end of the fermentation period. the supernatant was collected and used for their experiments.

Determination of Enzyme Activity

Enzyme extraction:- At the end of the fermentation the enzyme produce in the culture was extracted with 100 ml of phosphate buffer by stirring for 20 minutes using magnetic stirrer. The harvester culture was filtered through whatmann no.1 filter paper and centrifuged at 8000 rpm for 15min. for 1ml of enzyme extraction 3 ml of dinitro salicylic acid was added to stop the enzymatic reaction. The absorbance was measured at 540nm and related to the amylase activity.

RESULTS AND DISCUSSION

Amylases have diverse applications in several industries: Amylases are crucial in the production of sweeteners (like glucose syrup), brewing, baking (improving dough fermentation), and in the preparation of baby foods, sauces, and soups. In laundry detergents, amylase is used to break down starch-based stains on fabrics, making it an important ingredient in household cleaning products.

To determine the effect of incubation time on amylase enzyme production, the medium incubate at different time intervals and the maximum amylase activity was optimised at 48

hours. After 48 hrs, due to depletion of nutrient materials enzyme production was decreased. Amylase production at various time intervals was show in the fig 1.

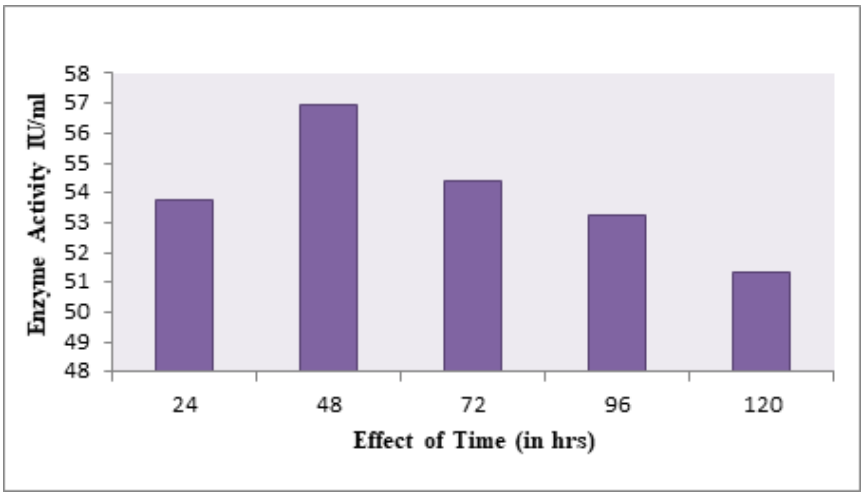

Fig. No. 1: Effect of Time.

The temperature was an important and very critical for the production of enzyme in solid state fermentation as it ultimately influence the growth of the micro organism. The maximum yield of amylase was observed at 30°C temperature fig 2.

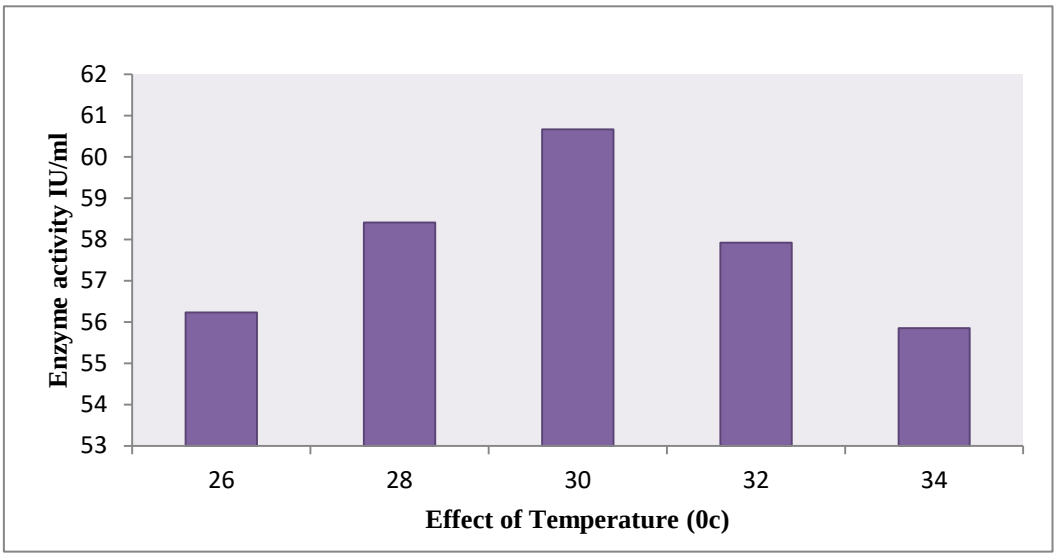

Fig. No. 2: Effect of temperature.

Every enzyme has an optimum pH, an increasing or decreasing pH reduces enzyme activity by changing the ionization. To optimize the effect of pH on enzyme production, the nutrient medium was prepared with different pH ranges 5, 6, 7, 8 and 9. The maximum production of amylase enzyme was noted at 7 pH fig 03.

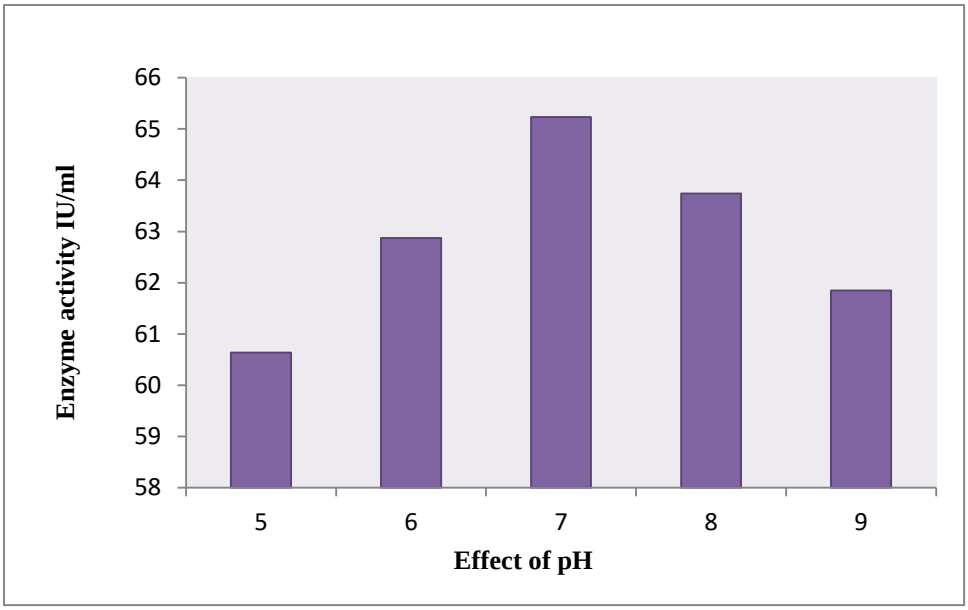

Fig. No. 3: Effect of pH.

When the inoculum size was increasing there was increase in enzyme production but there after the enzyme activity was decreased because depletion of nutrients by the increase biomass, which resulted diminishing in metabolic activity different inoculum levels were prepared for the production of amylase enzyme 60%, 70%, 80%, 90% and 100% v/w. The maximum enzyme production was observed at 80% v/w of inoculum fig 4.

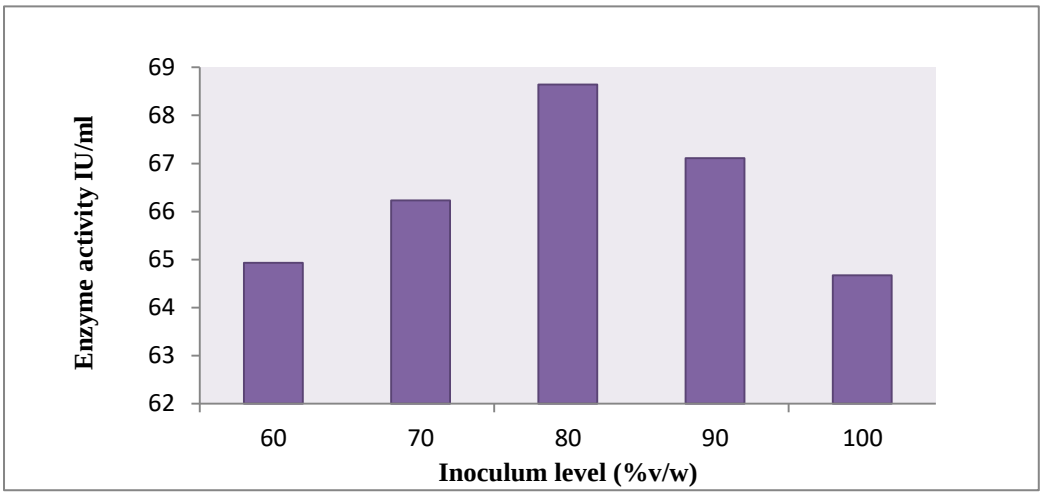

Fig. No. 4: Effect of Inoculum Level.

Moisture content plays an important role in solid state fermentation for the production of enzyme. High moisture content observes decreased substrate porosity, which prevents oxygen penetration. Different moisture contents were prepared with 60-100% v/w. The maximum enzyme activity was optimized at 80% v/w of the moisture content fig. 5

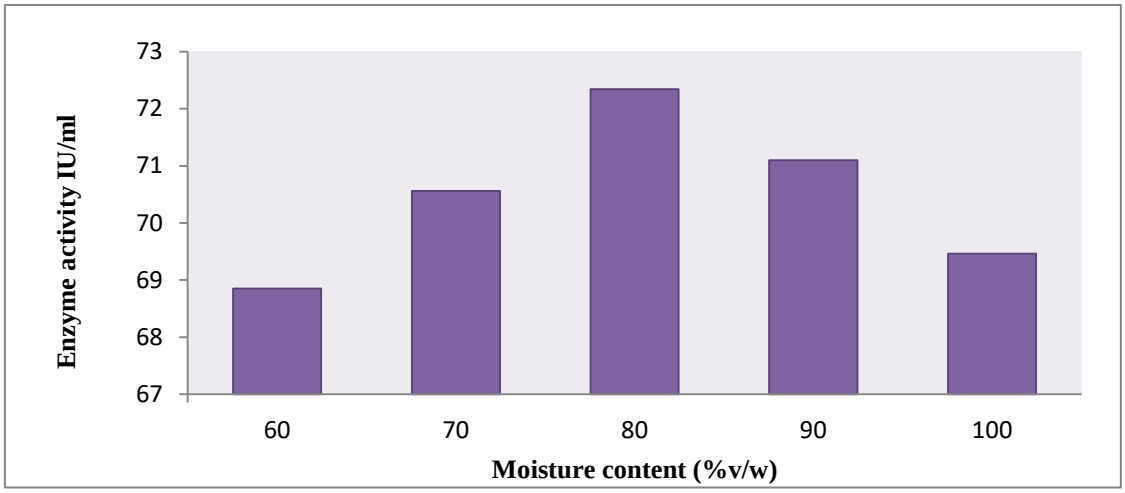

Fig. No. 5: Effect of Moisture content.

Different carbon components were screened for the enzyme production; they are glucose, fructose, maltose, sucrose and lactose utilized as carbon supplements. Among the carbon source, lactose give best production when compared with other carbon supplements. The result shows that maximum enzyme production was observed for lactose concentration fig. 6

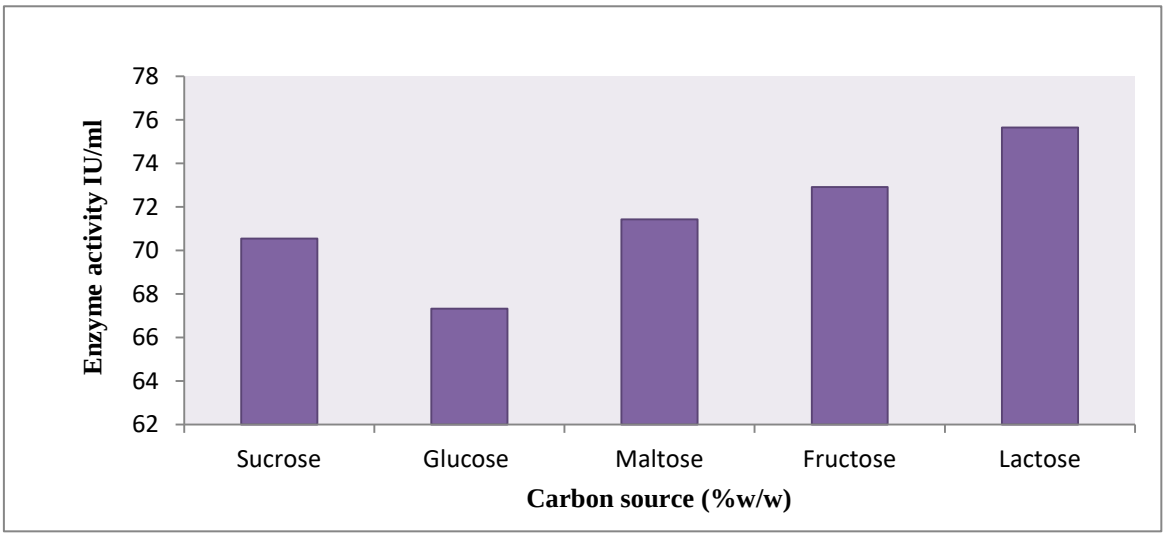

Fig. No. 6: Effect of Carbon Source.

To determine the effect of nitrogen on amylase enzyme production, the production medium was made with different concentrations of potassium nitrate like 0.1%, 0.2%, 0.3%, 0.4% and 0.5%w/w were optimized. The results indicate that maximum production of enzyme was recorded at 0.2%w/w concentration fig. 7

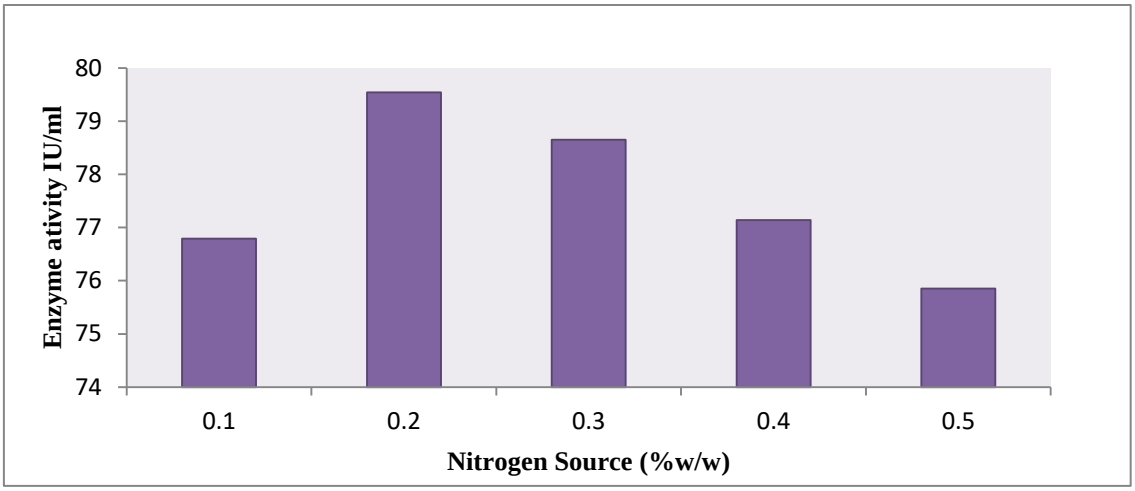

Fig. No. 7: Effect of Nitrogen Source.

CONCLUSION

Finally we concluded that *musa accuminata* leaves were promising agent, that produce significant amylase enzyme by *Bacillus lechniformis* under solid state fermentation. Amylase enzymes were distinguishing a wide diversity of source such as plants, microorganisms. They are mainly produced by numerous bacterial species. As *musa accuminata* leaves were easily available raw material and showing suitability for solid state cultivation of microorganisms,the lab scale studied on amylase production from *musa accuminata* leaves as major substrate might give the basic information of further development of large scale amylase production.

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