Production and optimization of Amylases Using *Aspergillus niger*

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Abstract -*Aspergillus niger* (NCIM 820) was grown in rice bran since it's a good carbon and starch source which has the ability to produce amylase. The optical growth of the isolate was found to be at 26 hours. Optimum amylase activity in rice bran was expressed on the fifth day of incubation as 334.51 µmol/lit min. The optimum pH was found to be 6 since the enzyme activity was higher and stable. This investigation suggests a means of production of amylase using rice bran for industrial purposes.

Index terms: amylase, *Aspergillus niger*, enzyme activity, pH, rice bran, temperature.

1 INTRODUCTION

The genus *Aspergillus* includes over 185 species and about 20 species have so far been reported as causing opportunistic infections in man [12][13]. Among these species, *A.fumigatus* is the most commonly isolated species followed by *A. flavus*[7]*A. niger. A. clavatus, A.glancus, A. oryzae, A. terreus, A. ustus* and *A. versicolor* are among the other species that are not commonly isolated and are considered as non.oppurtunistic pathogens [4][5]. *A. niger* has attracted attention for their role in fermentation of oriental food products or industrial application of hydrolytic enzymes. Also the fungus is used in the production of rice vinegars. Within the range of carbohydrolases produced by this species, the majority has been considered as α-amylase and glucoamylase1-3.

α-amylase(EC 3.2.1.1) has found its application in a range of industries including food, brewing, distilling industry, textile, paper pharmaceutical and bioconversion of solid waste etc.,[1]. Amylases have been reported to be produced by plant, animal and microbial sources, although the microbial amylase production has been reported to be most effective. Solid state fermentation which has been reported to be a bit cheaper because of the enzyme extraction procedures [2] is a ray of hope. In case of SSF, the cost of the substrate also plays a key role in deciding the cost of production. Agro industrial wastes have been reportedly used as good substrates for the effective cost cutting in the production of alpha amylases [10] and are thus attracting researchers in using agro industrial waste as a substrate for alpha amylase production. Fungal species have been studied a lot for the production of alpha amylase [1][11] because of the low cost of substrates used for the production of alpha amylases. Thus the present study was designed in search of cheaper carbon sources for the production of alpha amylase enzyme by fungal strains.

2 MATERIALS AND METHODS

2.1 Culture conditions and preparation of inocula

The pure culture (isolate) was obtained from NCIM; Pune was subcultured and maintained on Potato Dextrose agar plates. The fungus was further subcultured into test tubes of the same medium and incubated at 25°C. Seventy-two-hour-old culture of *A. niger* was used as inoculum. The culture was grown in a defined medium of the underlisted composition: $MgSO_4.7H_20$,

K_2HPO_4, KH_2PO_4, L-cysteine, biotin, thiamine and $FeSO_4.7H_20$ with added carbon and nitrogen sources (Sigma). Conical flasks containing growth medium was inoculated with 1 ml of an aqueous spore suspension containing approximately 5x104 spores per ml of the isolate. Experimental and control flasks were incubated in the rotary shaker at 120rpm at 25°C for three days.

2.2 Carbon and Nitrogen sources

In this project, certain carbon and nitrogen sources of the growth medium were used. While the tryptone was used as nitrogen source of growth, the carbon source employed was starch. However, when the carbon source of growth was maltose, the nitrogen source employed was ammonium chloride.

2.3 Rice as a source of carbon and growth medium

Rice bran was bought at the main market, Trichy, Tamilnadu, India. The rice bran was added to distilled water (1% w/v) and autoclaved at 15 Ib/in^2 at 121°C. Conical flasks (250 ml) containing 100 ml of the rice medium was inoculated with 1 ml of an aqueous spore suspension containing approximately 5x104 spores per ml of the isolate. Experimental and control flasks were incubated without shaking at 250C. On a daily basis, the contents of each flask were filtered through glass fiber filter paper (Whatman GF/A). The protein content of the filtrates was determined using the Lowry method [8].The filtrates were analyzed for amylase activity using the modified method of [15]. The filtrates were used as crude preparation.

2.4 Determination of pH

The substrate along with the inoculate was subjected to various levels of pH ranging from 4- 8 to determine the optimum pH.

2.5 Solid state fermentation

Conical flask containing rice bran and the isolate were incubated at various temperatures ranging from 30-45°C for 3-7 days.

2.6 Enzyme Assay-Amylase

Enzyme activity was defined in units. One unit of enzyme activity was defined as the amount of enzyme which produced 0.1% reduction in the blue colour of the starch-iodine complex.

3 RESULTS

pH	4	5	6	7	8
Enzyme Activity(µmol/litmin)	334.66	351.23	366.51	343.59	329.11

Table 1: Optimization of pH for enzyme activity

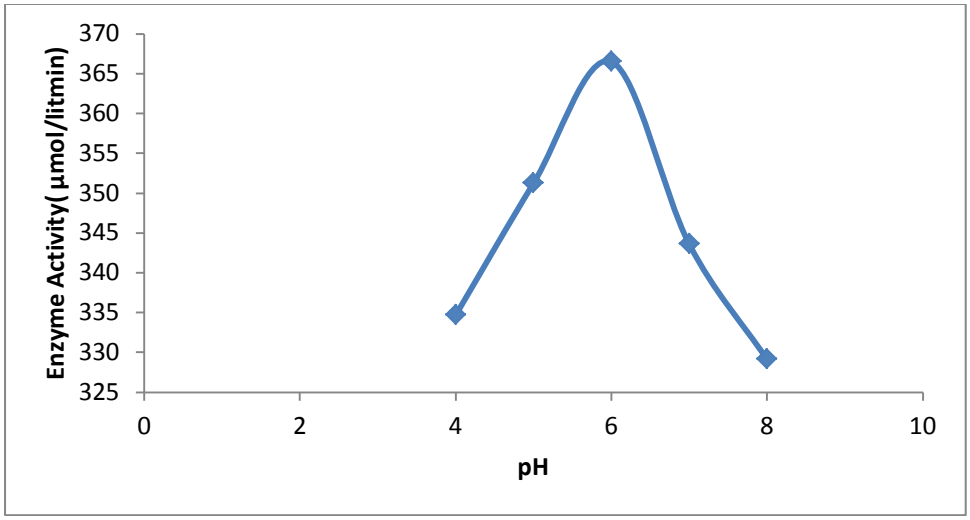

Figure 1: optimization of pH for enzyme activity

Temperature(°C)	30	35	40	45
Enzyme Activity(µmol/litmin)	323.24	356.48	310.18	301.18

Table 2: Optimization of Temperature for enzymatic activity

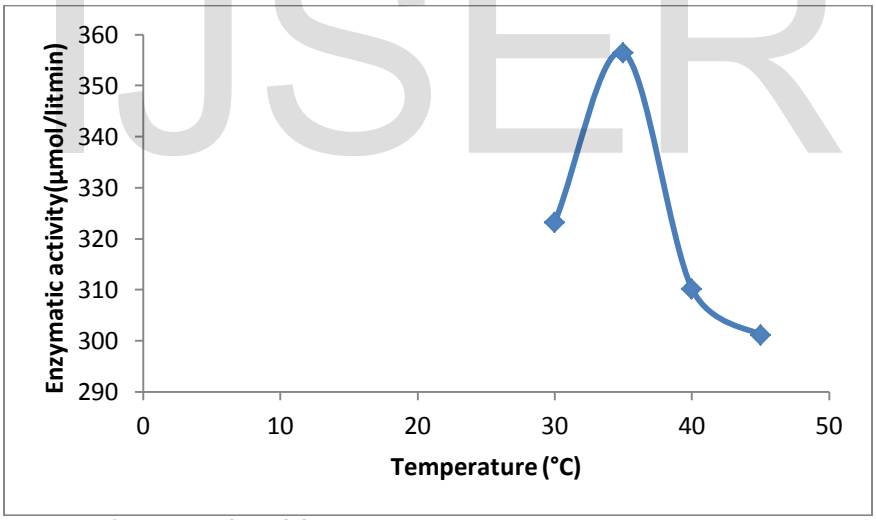

Figure 2: Optimization of Temperature for enzymatic activity

Days	3	4	5	6	7
Enzyme Activity(µmol/litmin)	292.32	315.25	334.51	321.18	301.09

Table 3: SSF performed from days 3-7 for enzymatic activity

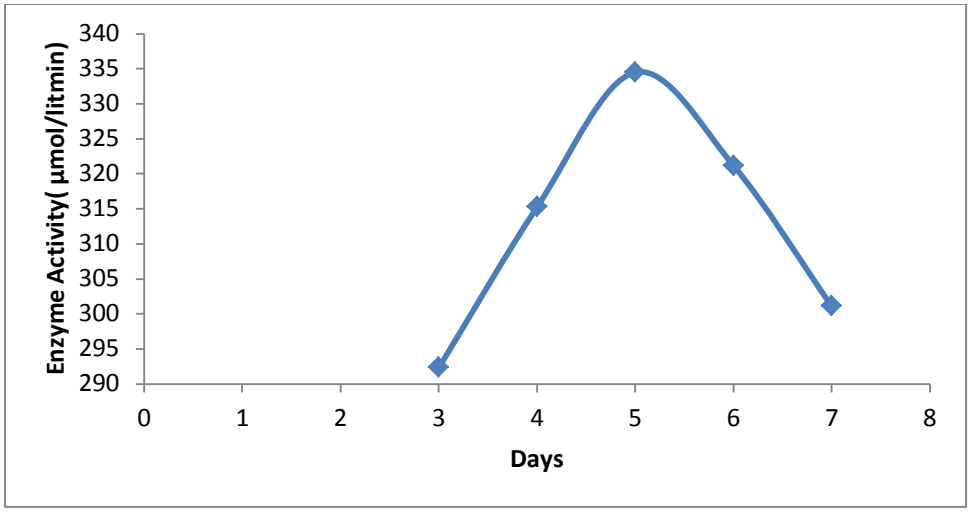

Figure 3: SSF performed from days 3- 7 for enzymatic activity

4 DISCUSSION

Amylase was produced by *A. niger* in growth medium containing rice as carbon and growth source at 35°C. Production of amylase activity was optimum on the fifth day. Conditions of growth of the organism seemed viable for amylase production. Enzymes occur in every living cell, hence in all microorganisms. Each single strain of organism produces a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature [12]. Bacterial α-amylases are produced at a much wider range of temperature. *Bacillus amyloliquefaciens*, *B. subtilis*, *B. licheniformis* and *B. stearothermophilus* are among the most commonly used *Bacillus* sp. reported to produce α- amylase at temperatures 37-60°C [9]. Temperature plays an important role in terms of amylase production and organism growth. Hence, the optimum temperature depends on whether the culture is mesophilic, thermophilic or psycrophilic. Among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25–37°C [6]. The carbon sources (starch and maltose) employed in this investigation, incorporated into the growth medium, induced amylase production by *A. niger*. Bread, starch and maltose, sucrose, lactose, glucose and galactose as carbon sources with potassium nitrate as nitrogen source supported growth and α-amylase production by *Lasiodiplodia theobromae* [14].An extremely important use for fungal amylases is in conversion of partially acid hydrolyzed starch to sweet syrups. Acid hydrolysis is a random action whereas enzymic hydrolysis is a patterned one. To meet the demand of industries, low-cost medium is required for the production of α-amylase. Both solid state fermentation (SSF) and submerged fermentation (SmF) could be used for the production of α-amylases, although traditionally these have been obtained from submerged cultures because of ease of handling and greater control of environmental factors such as temperature and pH. SSF has been used for long to convert moist agricultural polymeric substrates such as wheat, rice, soy, cassava, etc. into fermented food products including industrial enzymes [10]. Conclusively, amylase can be produced for industrial purpose from *A. niger* grown in rice bran and the defined synthetic growth medium with starch as carbon source and tryptone as nitrogen source.

5 REFERENCES

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