



ISOLATION, CHARACTERIZATION AND FERMENTATIVE PRODUCTION OF AMYLASE ENZYME FROM *ASPERGILLUS NIGER*

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Abstract - Amylase enzymes are widely used in industry, especially in the food, textile, and pharmaceutical sectors. They catalyse the hydrolysis of starch into simpler sugars. The isolation and characterization of amylase generated by *Aspergillus Niger* by fermentation are the main objectives of this investigation. Under the right fermentation circumstances, *Aspergillus niger* is known to produce a wide range of extracellular enzymes, including amylases. *Aspergillus Niger* is isolated by first choosing it from natural sources and then cultivating it in nutrient-rich culture that is most suited for the production of amylase. The amylase enzyme activity is then evaluated using standard biochemical assays, such as starch hydrolysis tests. Additionally, the characterization of the enzyme is performed to determine key properties such as optimal pH, temperature stability, and substrate specificity. The results from this study provide valuable insights into the potential of *Aspergillus Niger* species as a source of industrially relevant amylases, offering a sustainable and efficient alternative to chemical starch degradation processes.

Keywords - *Aspergillus niger*, Amylase, Enzyme production, Fermentation, Characterization.

1. Introduction

Amylases are enzymes that break down starch by hydrolyzing internal alpha 1-4 glycosidic linkages in polysaccharides while preserving the alpha anomeric structure of the final products. Regardless of kingdom, they can be found in all types of organisms. Alpha amylases are a common type of enzyme. Produced by microorganisms, plants, and animals, and they are key players in the metabolism of carbohydrates. For ages, the brewing business has used amylases derived from plants and microbes. Fungal amylases are widely used for the preparation of oriental foods. Amylases of bacteria, fungi and viruses are increasingly studied due to the relative ease of large scale production (low downstream cost as they are extracellular in nature) as compared to amylases from plants and animals and their importance in subsequent application at industry. Current research in the field of microbiology has examined the function of microbial amylases in the production of high fructose corn syrup and maltose. Amylases are also utilized in the production of maltotetrose oligosaccharide mixtures, high molecular weight branching dextrans, and the removal of starch from textiles, among other processes.

1.1 Classification Of Amylases

Amylases are a class of enzymes that catalyze the hydrolysis of starch into sugars such as glucose and maltose. Amylases are divided into three sub classes— α - β - γ -amylase according to the type of bond/link they are able to cleave (Fig. 2). α -Amylases (EC 3.2.1.1) catalyze the hydrolysis of internal α -1,4-O-glycosidic bonds in polysaccharides with the retention of α -anomeric configuration in the products. Most of the α -amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity, and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes. β -Amylases (EC 3.2.1.2) are exohydrolase enzymes that act from the nonreducing end of a polysaccharide chain by hydrolyzing α -1, 4-glucan linkages to yield successive maltose units. Since β -amylases are unable to cleave branched linkages present in branched polysaccharides, such as glycogen or amylopectin, the hydrolysis is incomplete and dextrin units remain. γ -Amylases (EC 3.2.1.3) cleave α (1-6) glycosidic linkages, in addition to cleaving the last α (1-4) glycosidic linkages at the nonreducing end of amylose and amylopectin, unlike the other forms of amylase, yielding glucose. α -Amylase is produced by several bacteria, fungi, and genetically modified species of microbes

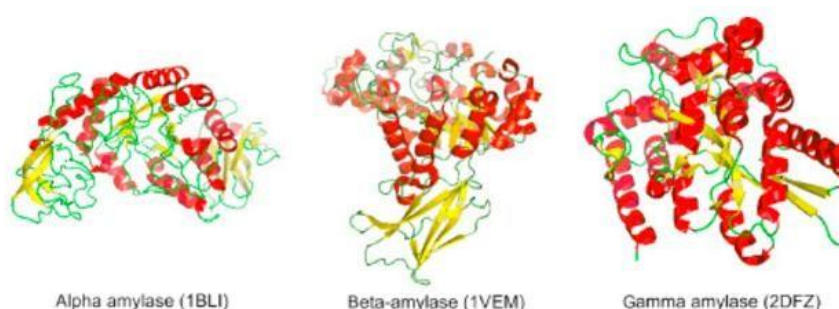


Figure 2. Structures of selected microbial amylase

1.2 Mechanism Of Action

Alpha amylases (α -1, 4-glucan-glucanohydrolase, EC 3.2.1.1) is an extracellular enzyme. This enzyme degrades α 1,4-glucosidic linkage of starch and related products in an endo fashion and produce oligosaccharides. Mode of action, properties and product of hydrolysis differ somewhat, depend on the source of enzyme. Two types of enzymes have been recognized, termed liquefying and saccharifying. The main difference between them is that the saccharifying enzyme produces a higher yield of reducing sugar than the liquefying enzyme.

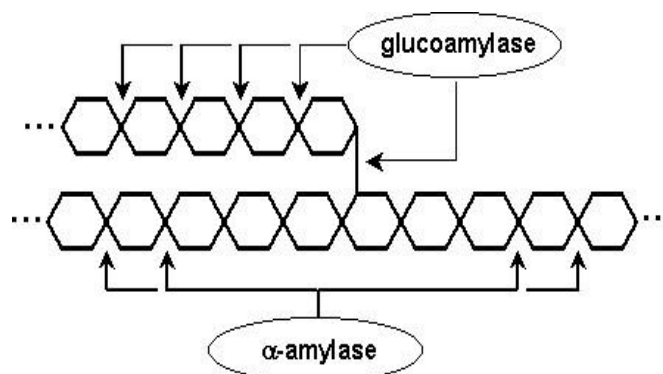


Figure - 1 Mode of action of α -amylase and glucoamylase on α -D-(1-4) and α -D-(1-6) - glucosidal bonds of starch.

2. Objectives Of The Present Study

1. Optimization of submerged fermentative process parameters like pH, temperature, inoculum size, incubation period etc.
2. Identification and evaluation of different agro wastes for economic production of amylase enzyme from isolated *Aspergillus species*.

3. Methodology

To identify the starch-degrading amylase-producing strain based on the zone of hydrolysis on starch-containing media plates, the primary goal of this experiment was to isolate an amylase-producing *Aspergillus niger* from various soil samples collected from various locations and the dump yards of starch processing industries in and around Bilaspur. This was done by growing the samples in synthetic media like SDA, PDA, Czapeck Dox agar, etc. enriched with starch.

3.1 Materials And Methods

This section describes the materials and methods used in the present study.

3.1.1 Materials

Different agro-industrial waste materials (green gram, rice bran, and wheat bran) were collected from the local market and processed using.

3.2 Collection

For isolation of amylase enzyme from *Aspergillus niger* species, samples were collected from various places located around bilaspur.

3.3 Screening For Amylolytic Activity

Placing the isolated strains on a specified medium that contained (g/l) Sucrose 30.0, Starch 10.0, Magnesium Sulphate 0.5, Potassium Chloride 0.5, Potassium Phosphate 1.0, Ferrous Sulphate 0.01 and Peptone 5.0 allowed for the screening of their amylolytic activity. These plates were incubated at 30 °C for 24 h. After adding iodine solution to PDA starch agar plates, fungi exhibiting distinct zones of hydrolysis were found to create amylase. A small number of colonies were chosen based on the hydrolysis zone and kept at 4 °C on the PDA medium slants.

3.4 Characterization Of Amylase Producing Isolate

According to Bergey's Manual of Determinative Bacteriology, the cultural, morphological, and physiological traits of the strain *Aspergillus niger* were studied by using a variety of media and biochemical reactions.

3.5 Medium For Amylase Production

In case of submerged fermentations, the defined medium and growth conditions mentioned elsewhere in materials and methods was used for amylase production studies. as substrate unless otherwise mentioned.

4 Results And Discussion

The results obtained during the course of present study are presented and discussed in this Paper. This part of the thesis elucidates isolation of microbes from different habitats, screening for amylase activity, identification of the isolate by Bergey's manual of Bacteriology and ribotyping, media optimization for amylase production in submerged and solid state fermentation. In addition, purification and characterization of the amylase and its application potentials are discussed.

4.3 Isolation And Screening For Amylase Producing Organisms

Soil was suspended in sterile water, mixed, and incubated on sterile Potato dextrose starch agar plates. Plates were incubated with soil solution, checked for microbial colonies, and over 30 strains were selected and grown on starch-containing agar slants.

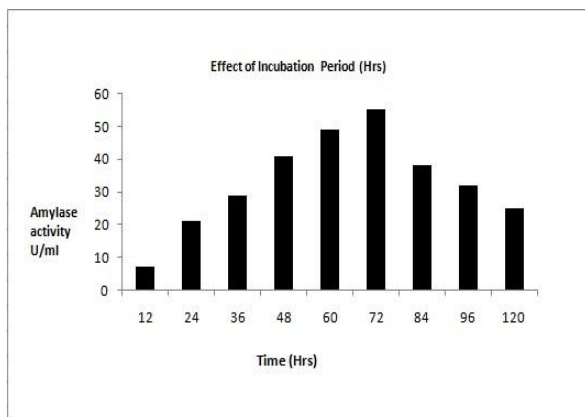
Each isolate was confirmed for production pattern using Potato dextrose starch agar plate.

4.4 Characterization Of The Isolate

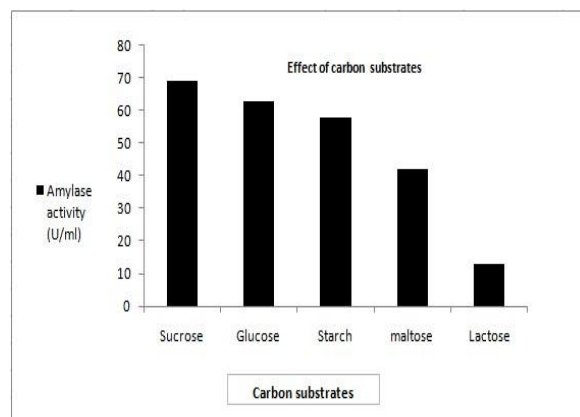
The media was inoculated with a loop-full of spore suspension of *Aspergillus niger* isolated and then incubated in 30°C at 200 RPM for 96 h. The media was centrifuged at 5000 RPM for 15 min and the crude enzyme isolate was used for amylase analysis. Further studies were carried out with pH 5.0

and 2 % inoculum, which was grown overnight by incubating at 200 rpm at 30°C. The parameters were optimized in such a fashion. The parameters were optimized in such a fashion that one parameter optimized was used for subsequent experiments. The following Parameters were optimized:

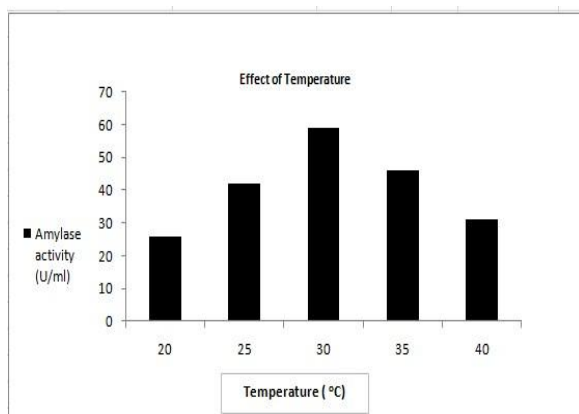
- Effect of Incubation time
- Effect of substrates
- Effect of pH
- Effect of inoculums level
- Effect of Temperature
- Screening of Carbon and Nitrogen sources



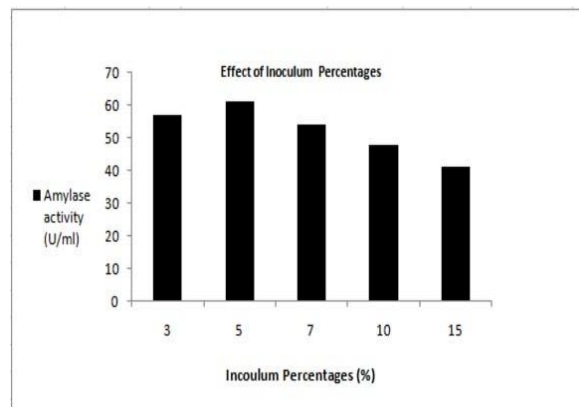
Graph-1



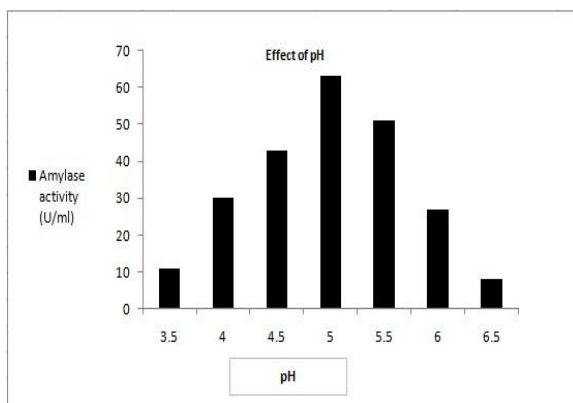
Graph-2



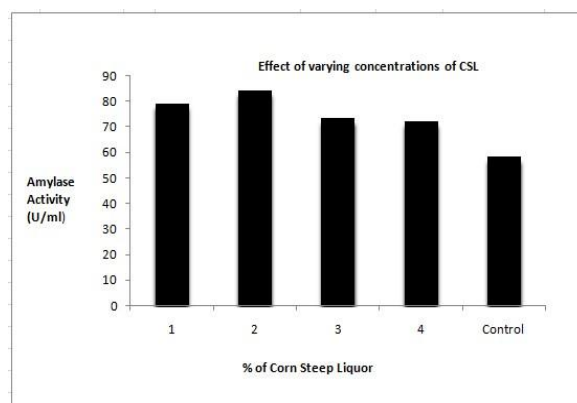
Graph-3



Graph-4



Graph-5



Graph-6

Table - 1

Effect of Incubation Period on Amylase Activity	
Incubation period (Hrs)	Amylase activity (U/ml)
12	7
24	21
36	29
48	41
60	49
72	55
84	38
96	32
120	25

The maximum amylase activity by the isolated *Aspergillus niger* strain was observed at 72 h and the maximum amylase activity obtained was 55 U/ml. Samples were collected at regular intervals of 12 h periods. The growth pattern revealed an increase in enzyme production from 12 h to 72 h and on further incubation the amylase production declined indicating 72 h as optimal incubation period for the isolated strain.

Table-2

Amylase Activity at Different Temperatures	
Temperature (°C)	Amylase activity (U/ml)
20	26
25	42
30	59
35	46
40	31

The role of incubation temperature on growth and amylase production by *Aspergillus niger* was studied by incubating the culture broth at different temperatures ranging from 20 to 40°C with an increment of 5°C. The fermentations and assays were carried out in triplicate and average values are reported as described earlier. The results indicated that the organism had potential to grow in all the tested incubation temperatures and produced amylase enzyme. However, effective enzyme production was noticed in the temperature range of 30 to 35°C. The maximum growth was observed at 30°C with amylase production of (59 U/ml) in 24 h as shown in figure and table 2. Hence fermentation experiments for optimization were performed at this incubation temperature.

Table-3

Amylase Activity At Different pH	
pH	Amylase activity (U/ml)
3.5	11
4	30
4.5	43
5	63
5.5	51
6	27
6.5	8

The effect of initial medium pH on biomass growth and amylase yield was studied at 30°C. Different initial medium pH values (3.5-6.5) were used for the study. The fermentations and assays were carried out in triplicate as per the general procedures. Growth and amylase production were observed in all studied pH environments. However, the influence of pH on growth and enzyme production could be seen. Maximum biomass and enzyme production (63 U/ml) were observed at pH 5.0 and minimum production (<10 U/ml) was noticed at pH 6.5. These results are shown in figure 3.

Table-4

Varying Percentages of Inoculum levels	
Inoculum Levels (%)	Amylase activity (U/ml)
3	57
5	61
7	54
10	48
15	41

Maximum enzyme production (61 U/ml) was noticed with inoculum (5%) and further increase in inoculum level did not increase any enzyme production. Approximately 30 % variation in enzyme production was observed with increase of inoculum concentration from 3 to 5 %.

Table-5

Effect of Different Carbon Substrates	
Carbon substrate	Amylase activity (U/ml)
Sucrose	69
Glucose	63
Starch	58

Maltose	42
Lactose	13

Results showed different impact on enzyme production with different substrates. The maximum enzyme production obtained was 69 U/ml with 3% w/v Sucrose. Glucose and starch when supplemented as additional carbon substrate to the medium has resulted in enhanced enzyme production. Among the tested substrate sucrose and glucose resulted in enhanced enzyme production. Varying carbon substrates effect on enzyme production by the isolated *Aspergillus niger* are reported in figure and table 5.

Conclusions

The *Aspergillus niger* isolated from soil exhibited significant amylase production under optimized conditions. The enzyme showed desirable characteristics, including stability at moderate pH and temperature, making it suitable for industrial applications. An efficient amylase producing microbial species belonging to *Aspergillus niger* was isolated from soil sample collected from dump yards of a local starch processing industry near bilaspur. The *Aspergillus niger* was characterized for amylase production using conventional and statistical software programmed. The enzyme revealed stability for more than 24 hrs in different pH environment and active in the pH range of 4.5 to 6.5.

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