



## Production of Amylase Enzyme Using *Aspergillus niger*

### KEYWORDS

Isolation of *Aspergillus niger*, fermentation, separation of product

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**ABSTRACT** Amylase is an enzyme produced by different fungi and bacteria. For the production of the amylase enzyme the fungus was isolated from soil by dilution method. The medium used for the isolation was Czapek-Dox medium. From onion roots the same fungus was isolated and characterized. The fungus characterized on the Potato Dextrose Agar medium containing 1% starch. The *Aspergillus niger* colonies showed maximum zone of clearance after pouring iodine were picked up and used. In this fermentation for the production of the amylase enzyme, *Aspergillus niger* isolated from soil as well as onion roots was used. The production was carried out using 5lit fermenter on lab scale. The media for the fermentation is optimized in the laboratory and used for the isolated *Aspergillus niger*. The product produced using fermentation for 72hrs at 30°C. The produced product was separated by filtration, centrifugation and salt precipitation method.

### INTRODUCTION

$\alpha$ -Amylase enzyme is  $\alpha$ -1,4 glucan, 4 glucanohydrolases ( E. C. 3.2.1.1) that is important enzyme in the starch processing industries for the hydrolysis of polysaccharides into simple sugar constituents (Akpan et al., 1999; Fogarty and Kally, 1980; Haq et al., 2002; Nigam and Singh, 1995). The quality and quantity of organic materials present in the soil have a direct effect on the fungal population of the soil. The development of microfungi is especially favoured by soils having an acidic reaction and where the aerobic condition is likely to be present near the surface. (Kavita Sharma, 2010).

*Aspergillus niger* can be isolated using Czapek-Dox medium and isolated fungi is used for the production of the amylase enzyme.  $\alpha$ -Amylase can be produced using special medium containing starch in it. The enzyme production is carried out using lab scale fermenter using submerged fermentation process. The produced enzyme separated using filtration, centrifugation and salt precipitation.

The produced enzyme has various applications in different fields. For example, liquefaction of starch, manufacturing of maltose, manufacture of high fructose containing syrups, manufacture of oligosaccharides mixture, manufacture of maltotetraose syrup etc (Prasanna V. Aiyer, 2005)

### MATERIALS AND METHODS

#### Isolation of fungi.

*Aspergillus niger* was isolated from two different sources. From soil the fungi isolated using serial dilution method on Czapek- Dox medium following culturing on the Potato dextrose agar containing 1% starch in the medium for characterization. From onion roots the black fungi was picked up and cultured on Czapek-Dox medium for isolation, and characterized using Potato dextrose agar containing 1% starch.

#### Screening of isolated fungi

*Aspergillus niger* produces  $\alpha$ - amylase enzyme which breaks starch. Starch when reacted with iodine produces violet bluish color. So the screening is done using iodine test.

#### Media optimization

For the production the media is prepared in the lab. Ingredients used are  $\text{KH}_2\text{PO}_4$  1.4gm,  $\text{NH}_4\text{NO}_3$  10gm, KCL 0.5gm,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.01gm,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.01gm, Soluble starch 20gm, Tween 80 1ml. pH maintained at 6.5.

### Fermentation

For fermentation the media prepared 4lit and autoclaved at 121°C for 20min. The isolated culture is mixed in the medium using nicrome wire loop. The temperature is set at 30°C. The fermentation process is carried out for 72hrs.

### Separation of enzyme

The culture obtained after incubation was crushed and mixed with phosphate buffer (pH 7.2) and filtered using Whatman filter paper No 1. Ammonium sulfate is mixed and kept overnight at 4°C. The mixture was centrifuged at 2000 x g for 30min at 4°C. The supernatant obtained after filtration was used as crude enzyme source.

### RESULT AND DISCUSSION

#### Results

During the incubation fungal colonies were observed. The results of present investigation reveal with various work done by researchers. *Aspergillus niger* is isolated from soil of forest and studied soil. Microbial analysis of different soil samples of selected site in Latur found *Aspergillus niger* as a dominant fungi, The fungi is isolated from mesophilic temperature ranges of 28 °C to 35 °C.

To differentiate between cell-bound and extracellular amylases, the cells were removed from broth by centrifugation, and ammonium sulfate added to the broth and kept overnight at 4°C. The white yellowish precipitate was isolated from broth.



Figure 1: Fermentation of Amylase enzyme using *Aspergillus niger* (Lab scale fermenter, BioEra 5L, College of Computer Science and Technology, Latur)

**Discussion.**

Extracellular amylase production was greatly stimulated by the addition of Tween 80 to the medium. The surfactant also affected an increase in extracellular amylase production from *Aspergillus niger*. Reese and Maguire postulated that Tween 80 alters cell permeability, resulting in increased enzyme yields. Total amylase production occurred during the logarithmic phase of growth.

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