World Journal of Chemistry 4 (1): 89-91, 2009
ISSN 1817-3128

Amylase Production on Submerged Fermentation by *Bacillus* spp

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Abstract: The production of extracellular amylase by *Bacillus spp* was optimized in a submerged fermentation. The production of the enzyme was maximum at 10 h after inoculation. The effect of incubation period, pH of the medium and incubation temperature was optimized. The maximum production of enzyme were obtained at 35°C and pH 7.

Key words: Amylase · Submerged fermentation

INTRODUCTION

Amylases are enzymes that break down starch or glycogen. The amylases can be derived from several sources such as plants, animals and microbes. The major advantage of using microorganisms for production of amylases is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics [1]. The microbial amylases meet industrial demands; a large number of them are available commercially; and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry [2] Although many microorganisms produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis, Bacillus amyloliquifaciens* and *Aspergillus niger.* Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries. They are mainly employed for starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup and maltotetraose syrup. In detergents production, they are applied to improve cleaning effect and are also used for starch de-sizing in textile industry [3, 4].

The use of the submerged culture is advantageous because of the ease of sterilization and process control is easier to engineer in these systems. Depending on the strain and the culture conditions, the enzyme can be constitutive or inducible, showing different production patterns.

The purpose of this work was to study the production of amylase by *Bacillus sp*., in submerged cultures and optimized the cultural conditions for the production of amylase.

MATERIALS AND METHODS

Microorganism: *Bacillus* spp was isolated from environment and maintained on nutrient agar slants and sub cultured for every 10 days.

Inoculum and Fermentation Medium: The inoculum was prepared by the addition of sterile distilled water in to the freshly grown nutrient agar slants, from this 0.5 ml of cell suspension was inoculated in to 100 ml of sterilized fermentation medium and incubated at 35°C for 10 hrs. The composition of the fermentation medium was[g/l] 6.0 g Bacteriological peptone; 0.5 g $MgSO_4.7H_2O$; 0.5 g KCl; 1.0 g Starch-, pH 7.

Extraction of Amylase from the Fermentation Medium: After incubation the fermentation medium was harvested by centrifugation at 5000 rpm for 20 minutes at 4°C. The supernatant was collected and subjected to estimate the amylase activity.

Effect of Temperature: To study the effect of temperature on amylase production the submerged fermentation was carried out at different temperatures (25°C, 30°C, 35°C and 40° C)

Effect of pH: The fermentation medium was prepared by varying the pH values (5.0, 6.0, 7.0 and 8.0) for the production of amylase.

Assay of Amylase: The amylase activity was determined following the method of Bernfeld [5]. An assay mixture containing, enzyme extract, starch as substrate and DNS as coupling reagent was used. One unit of α- amylase activity was defined as the number of µ moles of maltose liberated by 1 mL of enzyme solution per minute.

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RESULTS AND DISCUSSION

Amylase Production in Submerged Fermentation: In submerged fermentation the production of amylase was reached maximum of 4 U/ml at 10 h of incubation period (Fig. 1). Further increase in incubation period did not show any significant increase in enzyme production rather it was decreased. Thus optimum time of enzyme synthesis was to be 10 h after inoculation. Ramesh and Lonsane[6] reported the enzyme production was initiated at about 6 h in the media containing 0.2 or 1.0% soluble starch.

Effect of Temperature: Results from Fig. 2 shows the effect of different incubation temperature on the production of amylase by *Bacillus spp.,* The maximum production of amylase was obtained at 35°C. The optimum temperature was observed for the production of α-amylase from Banana stalk using *B. subtilis* was also 35°C as reported by Krishna and Chandrasekaran [7]. Increase in incubation temperature, decreased the production of enzyme. The production of the enzyme was greatly inhibited at 40°C. It might be due to that at high temperature, the growth of the bacteria was greatly inhibited and hence, enzyme formation was also prohibited [8, 9].

Table 1: Effect of varying pH of the medium on amylase production

pH	Amylase activity U/ml
5.5	3.2
6	4.1
6.5	4.9
7	11.0
7.5	9.0
8	7.0

Effect of pH: In our study the amylase production by *Bacillus spp.,* was found maximum at 7.0 (11 U/ml) (Tab.1). Further increase in the pH resulted decrease in the activity of amylase. However, the pH of the fermentation medium was found to be optimum at 7.0. When pH is altered below or above the optimum the activity is decreased or becomes denatured. Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth [10]. Terui [11] who reported 6.8 as optimum pH for the production of α-amylase by *B. subtilis.*

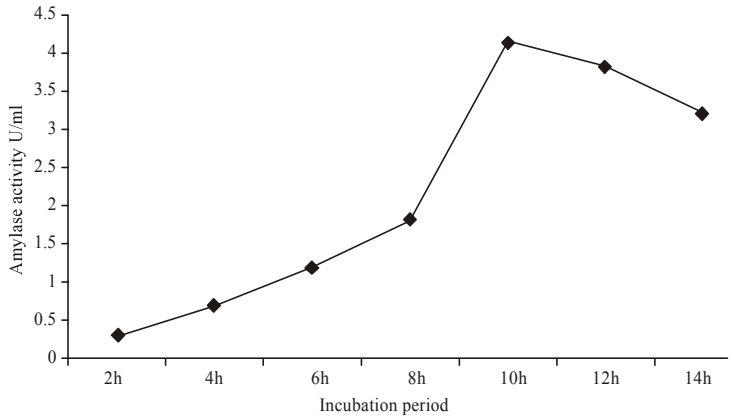

Fig. 1: Amylase production in various incubation periods

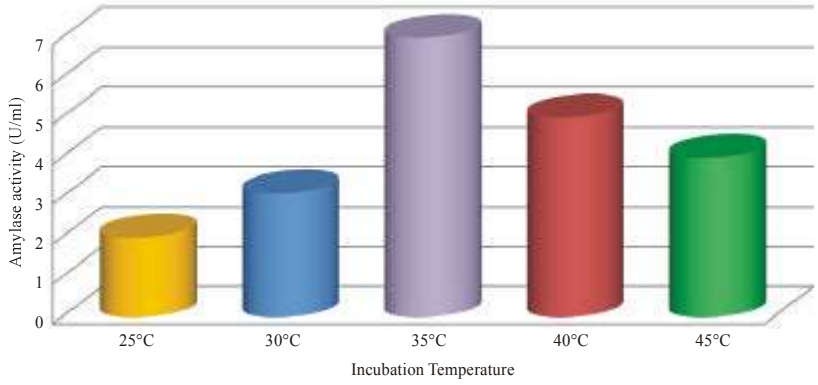

Fig. 2: Effect of varying incubation temperature on amylase production

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