

STUDIES ON THE PRODUCTION OF α - AMYLASE BY *Bacillus subtilis*

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ABSTRACT

Cell growth of *Bacillus subtilis* and the production of α -amylase in the medium were examined. Based on the amylase productivity level in shake flask cultures after 72 hours of growth, the growth medium containing starch and peptone was selected as the best medium.

Our results show that the amylase production is higher in the presence of optimum Carbon and Nitrogen ratio. The production of the enzyme was maximum (370U/mg) at 72 h after inoculation. The effect of incubation period, pH of the medium and incubation temperature was optimized. The maximum production of enzyme was obtained at 30°C and pH 7.

KEYWORDS:- α - amylase, *Bacillus subtilis*, Batch cultures, Enzyme production, Growth.

INTRODUCTION

Enzymes are protein molecules, which are necessary for life. Amylases are enzymes that break down complex carbohydrates. There are different sources to produce amylases. Plants, animals and microbes can produce amylases [1]. These enzymes account for about 30% of the world's enzyme production and have a great significance with extensive biotechnological applications in bread and baking, food, textile, and paper industries [2, 3]. Today, amylases are available commercially in the large number and they have almost completely replaced chemical hydrolysis of starch processing and reduce the production of chemicals used in carbohydrate hydrolysis. Microorganisms produce different kinds of industrial enzymes. Because of their biochemical diversity and the ease with environmental and genetic manipulation, they have replaced enzymes, which traditionally have been isolated from complex eukaryotes [4]. *Bacillus subtilis* is one of the most widely used bacteria for the production of specific chemicals and industrial enzymes and also a major source of amylase and protease enzymes. [5, 6] 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]. There are some factors, which influence the nature of their metabolic process and the enzyme production. The composition and concentration of media and physical parameters greatly affect the growth and production of extracellular amylase in bacteria [7]. Optimization of cultural conditions is important for maximum production of microbial strains [8]. *Bacillus* species and other forms of microorganisms grow at different rates with specificity to different substrates in the culture medium. The growth conditions also influence their enzymatic activities [9]. The present work describes the batch culture production of α -amylase by *Bacillus subtilis* under controlled conditions.

MATERIAL AND METHODOLOGY

Bacillus subtilis Cultures were obtained from Microbial Type Culture Collection. In this study, the cultures were grown in water soluble medium containing Beef extract, Yeast extract, Peptone, and NaCl. Production media containing equal volumes of carbon and nitrogen source was taken in flask. The inoculum was taken 1% of the production media size. The cells were inoculated into nutrient broth and incubated at 30°C for 48 h. These cells were transferred to micro centrifuge tubes and centrifuged at 4000 rpm for 15 min. Supernatant was separated from cell debris and used as the crude enzyme. Bernfeld procedure was used to determine amylase activity. Samples were taken at 3, 6, 12, 24, 32, 48, 60, 72, 84, and 96 h. The reaction mixture containing 200 μ l of 1% substrate (w/v) in 0.1 M phosphate buffer and 150 μ l of enzyme solution was incubated for 30 min at 30°C. The reaction was stopped by adding 400 μ l of 3, 5-dinitrosalicylic acid solution. Then this mixture was heated in a boiling water bath and cooled at room temperature to develop brown colour. 8 ml of deionized water was added to this solution. Then the absorbance was measured at 540 nm with a spectrophotometer.

RESULTS AND DISCUSSION

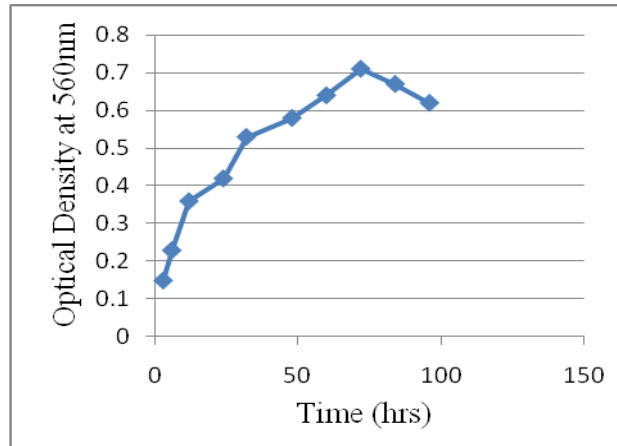


Figure 1: Growth of *Bacillus subtilis* with respect to Time (hrs)

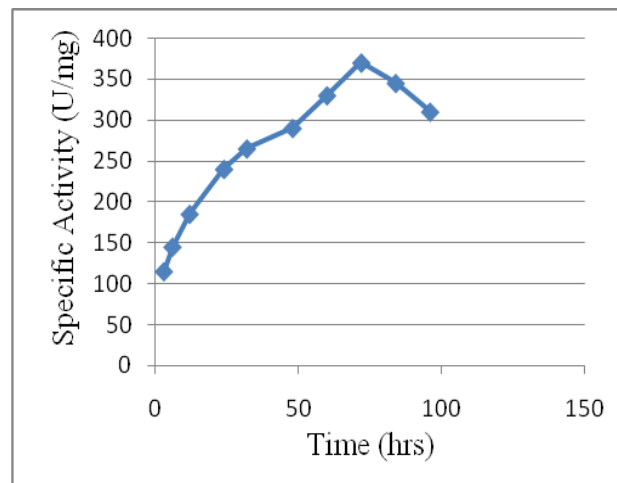


Figure 2 Production of α -amylase by *Bacillus subtilis* with respect to Time (hrs)

Optimization of culture conditions is very important for maximum microbial growth and enzyme production by microbial strains. So optimization of growth condition is a prime step in fermentation technology [10]. In the present study we observed 30°C and 7 as the optimum growth temperature and pH respectively. This could be due to the mesophilic nature of the *Bacillus subtilis*. But high temperatures and pH may inactivate the expression of gene responsible for the starch degrading enzyme [11]. Among the physiological parameters, optimum temperature, substrate concentration and pH range are the most important for enzyme production by microbes [12, 13]. Most of the starch degrading bacterial strain revealed a pH range between 6.0 and 7.0 for normal growth and enzyme activity[13]. Presently, this *B. subtilis* strain also showed maximum growth in this optimum range. The results of the time-course studies on cell growth of *B. subtilis* grown in media are shown in Fig 1. Maximum growth is shown at 72 hrs (Fig 1). The nature and amount of carbon and nitrogen sources in culture media are also important for the growth and production of extracellular amylase in bacteria along with the physical parameters [14, 15]. The levels of amylase in the crude culture supernatants varied greatly in response to the carbon and nitrogen source used for the growth of the *B. subtilis* strain. The maximal growth rate and production of extracellular amylase was obtained when the strain was grown in media. Peptone in media promoted α - amylase productivity. The main advantage of growing *B. subtilis* on this medium for amylase production is the very fast production time[16, 17, 18]. Amylase production is greatly reduced when glutamate or citrate is used and the amylase production is maximum when starch is used as a carbon source [19, 20, 21]. The growth and production of extracellular amylase production in bacteria can be affected by the composition and concentration of media [22]. Enzyme production by *B. subtilis* is directly correlated to the time period of incubation [23]. The present study observed enhanced enzyme activity with the increase in incubation time. The production of amylase was reached maximum of 370 U/mg at 72 h of incubation period (Fig. 2). 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 72 hrs after inoculation. The enzyme production was initiated at about 6 h in the media

containing 1.0% soluble starch [24]. Our results indicated that the optimum incubation period for amylase production was 72 h (Fig. 2). Enzyme production gradually declined after 72 hrs. In this experiment, the maximum α -amylase production occurred when optical cell population reached its peak. Amylase production by *B. subtilis* was found to be growth-associated as the maximum enzyme production was observed during the beginning of stationary phase, when the Optical Density reached a plateau. The production of enzyme was directly related to the growth of the *B. subtilis*.

CONCLUSION

The optimal physical conditions, concentration and composition of Nitrogen and Carbon sources are very important for studying growth of *B. subtilis* and the α -amylase production. The maximum α -amylase 370U/mg was produced during batch fermentation in shaking flask in 72 hrs of growth. The production of the enzyme was directly related to the growth of the strain. Maximum enzyme activity was obtained at the beginning of the stationary growth phase.

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