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INVESTIGATION OF ALPHA-AMYLASE PRODUCTION FROM BACILLUS SUBTILIS IN DIFFERENT MEDIA

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ABSTRACT

Microbial enzymes are widely used in the world. Alpha-amylase is one of these enzymes and it is used in textile industry. Alpha-amylase production and its activity was compared in two different media.

Our aim was getting good yield of alpha-amylase with high activity. Therefore we inves tigated the effect of chemicals found in the media to enzyme production and activity. When $CaSO_4$ was used inplace of $CaCl_2$, enzyme activity increased 7 %. It was found that 15 % glycerol was optimal concentration for bacterial growth. C/N is important for enzyme production. Maximum production was obtained in 45/15 C/N.

According to our findings we developed a medium for high yield of alpha-amylase production from *B. subtilis* with high activity. In this new medium (ESXCÇ)**. 60 % increase alpha-amylase activity was established.

INTRODUCTION

Some of the microbial enzymes produced in large quantities for industrial purposes (Bailey and Ollis, 1977). Bacterial alpha-amylases are used in de-sizing process in textile industry (Tarakçıoğlu, 1979). In order to get firm and smoot texture, sizing process is applied. Starch is used in this process. Excess starch is removed from the texture by de-sizing process.

Bacterial amylases are resistant to high temperatures, they can active in wide pH ranges and also they can manage de-sizing in a short time.

Because of the above properties bacterial amylases are widely used in de-sizing process. *Bacillus subtilis* is usually used for amylase production (Radley, 1976).

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In this study different growth conditions were investigated in order to produce alpha-amylase with high yield from *B. Subtilis*. Also effect of different chemicals, pH and temperature to enzyme production and activity were studied.

MATERIALS AND METHODS

B. subtilis used in this study obtained from Molecular Biology Dept. of Hacettepe University. Bacteria preserved on nutrient agar stock cultures.

Two different media Pfueller and Elliott (1969) and Andersson et. al. (1985) were used for enzyme production.

Pfueller and Elliott (1969) growth medium (medium I) in a liter: 5.0 gr kazein hydrolysate, 0.5 gr yeast extract, 3.0 gr K_2HPO_4. 1.0 gr KH_2PO_4, 1.0 gr soluble starch and a 10 ml of mixed salt solution, is used. Mixed salt solution contains in a liter: 3.0 gr $FeCl_3$, 0.5 gr $MgCl_2.6H_20$, 0.85 $CaCl_2$. 6 H_20, 100 gr NH_4 Cl, 100 gr NaCl.

Andersson et. al. (1985) growth medium (medium II) in a liter: 10 gr D-glucose, 5.0 gr peptone, 2.0 gr yeast extract, 1.5 gr NaCl, 0.5 gr KH_2PO_4, 0.5 gr $MgS0_4$. $7H_20$, 0.1 gr $CaCl_2$ and 15 % v/v sterile glycerole.

For enzyme production, soluble starch was used in place of D-glucose in growth medium. pH of the Pfueller and Elliott's medium (medium I) adjusted to pH: 7. with 0.1 N HCl solution and medium of Andersson et. al. (II. medium) with 3 M Na0H or 3 M HCl. The pH of the growth media adjusted to pH: 7 before sterilization Growth in the medium I is held at 37° C incubator with shaker at 150 r.p.m and medium II is held at 37° C incubator with shaker at 175 r.p.m.

According to the experimental results medium II was used as a basal growth medium. Bacterial growth was measured as absorbance at 600 nm with Coleman Junior II Model.

Alpha-amylase activity was established by dextrogenic method (Pfueller and Elliott, 1969) in growth medium after removing the cells by centrifugation. Enzyme activity was calculated as International Unit (IU) (Pfueller and Elliott, 1969).

In this study we progressed a new medium called (ESXCÇ medium) to get alpha-amylase productively with maxiamal activity. ESXCÇ

medium containing per liter: 30 gr soluble starch, 10 gr peptone, 5.0 gr yeast extract, 1.5 gr NaCl, 0.5 gr KH_2P0_4, 05 gr $MgS0_4$. $7H_20$, 0.2 gr $CaS0_4$.

RESULTS

In medium I maximal bacterial growth was obtained at 18th hour. Secretion of alpha-amylase started in logarithmic phase and reached to the maximal activity at 16th hour (Fig. 1). However in medium II it was found that maximal bacterial growth was at 16th hour and enzyme activity reached maximum at 18th hour (Fig. 2).

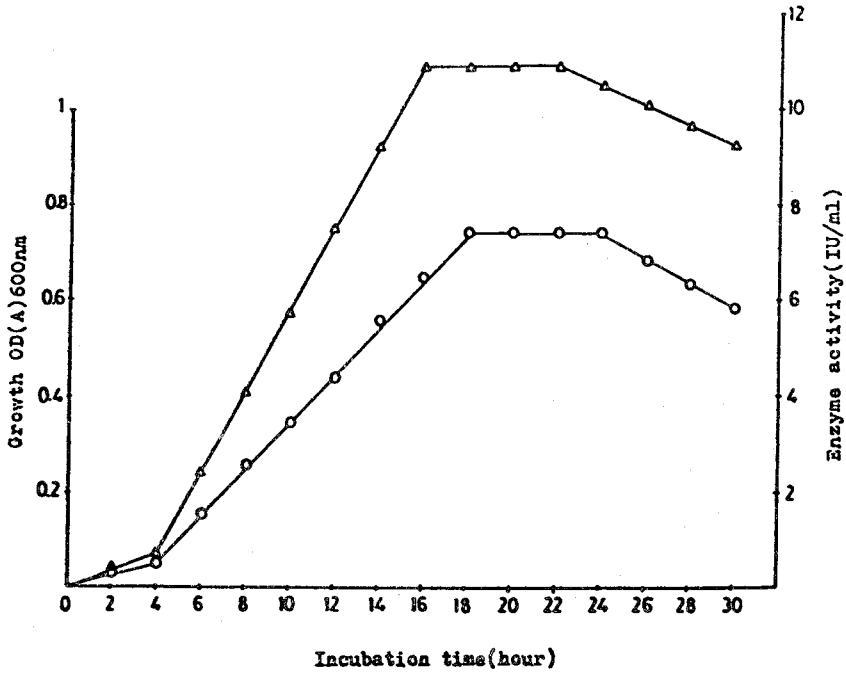

Figure 1- In medium I maximal bacterial growth and enzyme activity.
0 — 0: Bacterial growth
△ — △: Enzyme activity

Bacterial growth and alpha-amylase activity in two media were compared. B. subtilis cells grew 16 % better in medium II than medium I. Also in medium II alpha-amylase activity was 28 % higer than the medium I.

In a medium which C/N ratio was 45/15, alpha-amylase activity was 35.7 % more than the control medium (C/N 10/7).

Final concentration of 15 % glycerol was found optimal both for growht and enzyme activity. 7 % increase was obtained in alpha--amylase activity when $CaSO_4$ was added in a medium in place of $CaCl_2$.

Maximal enzyme activity was obtained at pH: 6 (Fig. 3) and at 65° C (Fig. 4).

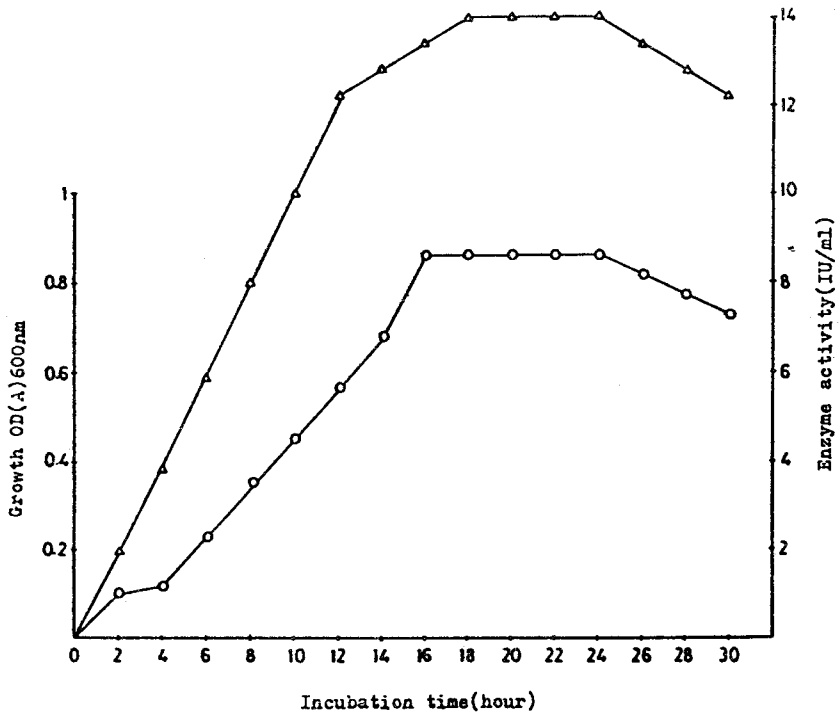

Figure 2– In medium II maximal bacterial growth and enzyme activity.
o — o: Bacterial growth
△ — △ :Enzyme activity

Bacterial growth and alpha-amylase activity reached maximum level at 18th hour in ESXCÇ medium. Also in this medium 60 % increase in alpha-amylase activity was obtained comparison to the medium II.

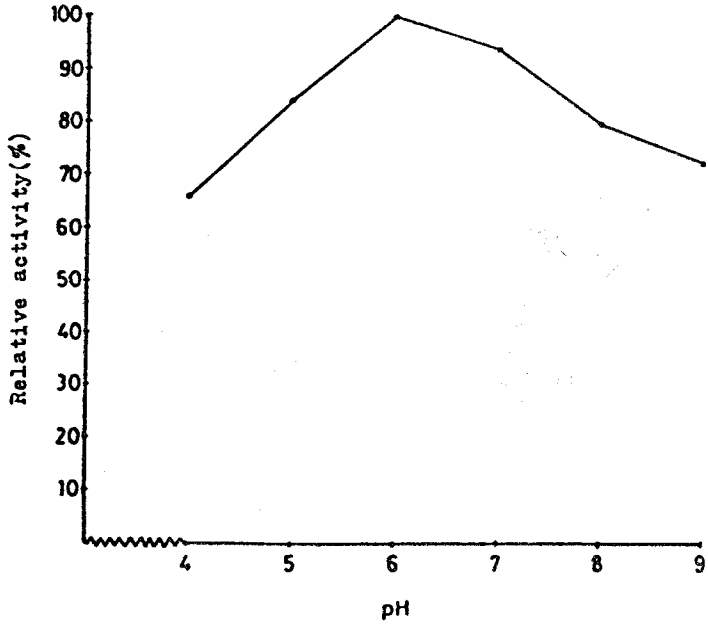

Figure 3- Effect of pH to enzyme activity.

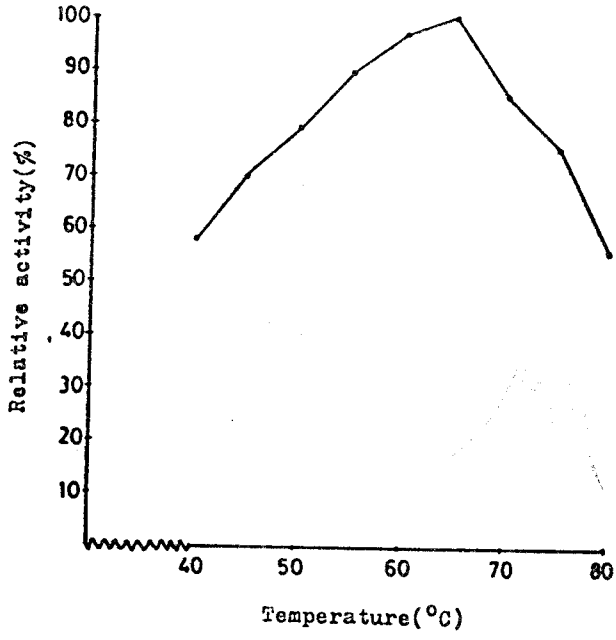

Figure 4- Effect of temperature to enzyme activity.

DISCUSSION

Alpha-amylase which hydrolyses starch is widely used in industry for a long time (Allen and Spradlin, 1974; Barfoed, 1976).

Medium II was found more productive for enzyme activity than medium I. Because in medium II bacteria grew in D-glucose containing medium overnight. This leads more healthy bacterial generation. It was obtained by various investigators that glucose increase the bacterial growth but has no effect on enzyme activity (Welker and Campbell, 1963; Sekiguchi and Okada, 1972).

In addition to starch, glycerol was used as a C source in medium II. Glycerol provides energy by feeding glycolysis (Lehninger, 1982). Final concentration of 15 % glycerol was found optimal for bacterial growth and enzyme activity. This finding is same as Andersson et. al. (1985).

Alpha-amylase is induced by maltose, fructose and galactose, but starch is the best inducer (Coleman and Grant, 1966). So, starch was used as a C source in this study. It was reported that peptone was the better N source than kazein hydrolysate (Upton and Fogarty, 1977). It was also reported that C/N affected the alpha-amylase activity (Ingle and Ericson, 1978; Kindle, 1983). We investigated various C/N ratios on enzyme production. 45/15 C/N was established the most effective one.

Alpha-amylase is an metalloenzyme. It has absolute requirement for Ca^{++} (Manning and Campbell, 1961). $CaCl_2$ was used as a Ca^{++} source in both media. But it was reported that SO_4^{-2} ions have inducing effect on enzyme activity comparison to Cl ions (Krishan and Chandra, 1983). So we used $CaSO_4$ as a Ca^{++} source in our media. We obtained 7 % increase in enzyme activity when $CaSO_1$ was used in place of $CaCl_1$.

ESXCÇ medium progressed in this study has optimal C/N ratio and glycerol concentration. It contains Ca^{++} twice as much as the medium II. Also $CaSO_4$ was used as Ca^{++} source in place of $CaCl_2$. Therefore alpha-amylase activity showed 60 % increase in this medium in comparison to medium II.

Also maximum enzyme activity was obtained at 65° C and pH: 6. This finding is in aggreement with the results of other investigators (Moseley and Keay, 1970).

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