

**PROCESS ECONOMIZATION FOR α -AMYLASE PRODUCTION BY BACILLUS
MEGATERIUM KLMI 4 THROUGH SUBMERGED FERMENTATION USING BASAL
MEDIUM**

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ABSTRACT

After the optimization of basic parameters (pH, Temperature, Inoculum size and Starch concentration with activity 93 IU) the economic production of α -amylase through submerged fermentation by *Bacillus megaterium* KLMI4 was studied with the addition of different sugars as carbon source, organic nitrogen source, inorganic nitrogen source, and different metal salts. Arabinose, raffinose, meso-inositol, sucrose & galactose do not support amylase production since they are non-metabolizable sugars. Maximum activity was observed with fructose (252 IU), and maltose (240 IU). Addition of peptone with different concentrations, 1.5% proved to be beneficial for enzyme production with the highest yield of α -amylase (161 IU). Amongst all the organic nitrogen sources supplemented, peptone was observed to be most beneficial for α -amylase production with an yield of 175.0 IU. Urea suppressed the enzyme yield. The order of beneficiality of the sources is in the following manner: peptone>beef extract>tryptone>yeast extract>corn steep liquor>soluble casein>hydrolysed casein. Ammonium bisulphate was observed to be most beneficial, α -amylase activity of 125 IU after 48 h of incubation period was recorded. Amongst other salts, only sodium nitrate and ammonium sulfate were beneficial, that to a small extent. Other salts were either not beneficial or were slightly suppressive. Amongst the metal salts supplemented, zinc sulphate, manganese sulphate and ferrous sulphate were observed to exhibit suppressive action over α -amylase production by the bacterium. Other salts enhanced α -amylase production, maximum yield (181 IU) being observed with calcium sulphate. The beneficial influence of these salts was observed to be in the following order: calcium sulphate>calcium chloride>copper sulphate>magnesium sulphate.

KEYWORDS: Alpha-amylase, carbon, nitrogen (organic and inorganic), and influence of metal salts.

INTRODUCTION

Carbon sources play an important role in the process of α -amylase production by an organism. Hence, it was felt worthwhile to evaluate the influence of various sugars as C sources and various organic and inorganic N sources on α -amylase production by the organism under submerged fermentation studies.

Nitrogen sources (both organic and inorganic) play an important role in the maintenance and activities of the bacteria and living organisms in general. Peptone, various other complex organic compounds like tryptone, yeast extract, beef extract, corn steep liquor, casein (both hydrolyzed and soluble) and urea are known to influence bacterial activity.

Various inorganic salts are known to stimulate growth and activity of microorganisms. They also enhance soil fertility. Hence, studies were carried out to evaluate the

influence of especially some nitrogen based and other inorganic salts (sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride, ammonium sulphate and ammonium bisulphate) on the α -amylase production by the bacterium under consideration.

Some metal ions act as co-factors for microbial enzymes. It is also known that some microorganisms are dependent on calcium for their activities. Many enzymes are known to require metal ions to increase their activity, hence they are known as metalloenzymes. They bind strongly with the metal ions or require metal ions to maintain stability and native state. Some enzymes bind weakly to the metal ions only during the catalytic state. Under both the states the enzymes act as electrophilic catalysts so therefore α -amylases are referred to as metallo-enzymes.

MATERIALS AND METHODS

A. Influence Carbon sources on Enzyme Production

The influence of different carbon sources was studied maintaining all the optimized physico-chemical parameters of the fermenting medium constant, i.e. pH at 8.0, temperature at 37⁰ C, inoculum size of 3x10⁶ cfu/ml. The sugars supplemented were separately to the medium at 1% concentration (w/v) each of fructose, meso-inositol, glucose, galactose, sucrose, arabinose, raffinose, lactose, maltose, dextrin, xylose, glycerol and trehalose. After conducting the SmF studies under optimized conditions, samples were withdrawn at intervals of 24 h and α -amylase activity was estimated by DNS method earlier.

B. Influence Nitrogen sources on Enzyme Production

Studies were conducted to evaluate the influence of these organic compounds on α -amylase activity of the bacterial strain under consideration through submerged fermentation using basal medium as the substrate.

Influence of Peptone on α -Amylase Production

Peptone is the most common organic nitrogen source in many media. Hence its influence was studied maintaining all the optimized physico-chemical parameters of the fermenting medium constant, i.e. pH at 8.0, temperature at 37⁰ C, inoculum size of 3x10⁶ cfu/ml and substrate (starch) concentration of 1.0%. Different concentrations of peptone, i.e., 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% (w/v), were added separately to the fermenting medium. Appropriate controls were maintained without peptone addition.

Influence of Other Organic Nutrients on α -Amylase Production

Peptone at 1.5% effected optimum α -amylase production. Hence the influence of other organic nutrients (tryptone, yeast extract, beef extract, corn steep liquor, both hydrolyzed, soluble casein and urea including peptone) on α -amylase production by the bacterium was evaluated by adding them at 1.5% concentration (w/v) individually and separately to the fermentation medium and autoclave sterilizing before inoculating the organism. Samples from the individual flasks were withdrawn at intervals of 24 h and α -amylase production was estimated.

Influence of Inorganic Nutrients on α -Amylase Production

The influence of different inorganic nitrogen sources was studied maintaining all the optimized physico-chemical parameters of the fermenting medium constant, i.e. pH 8.0; temperature 37⁰ C; inoculum size 3x10⁶ cfu/ml and substrate (starch) concentration, 1.0% (w/v). Different inorganic salts (sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride, ammonium sulphate and ammonium bisulphate) were supplemented separately at a concentration of 1.0% to the fermenting medium. Appropriate controls were maintained without

nitrogen salt supplementation. Samples were withdrawn aseptically at 24 h intervals and α -amylase production was estimated.

C. Influence of Metal Salts on α -Amylase Production

Studies were carried out to evaluate the influence of some metal ions and calcium salts on the potency of the bacterium to produce α -amylase through SmF. The metal ions selected for the study were magnesium sulphate (MgSO₄.7H₂O), manganese sulphate (MnSO₄.7H₂O), copper sulphate (CuSO₄), zinc sulphate (ZnSO₄.7H₂O), ferrous sulphate (FeSO₄.7H₂O) and chloride as well as sulphate forms of calcium (CaCl₂ and CaSO₄). Each of these was dispensed into 100 ml fermenting medium separately at the rate of 1% (w/v) and autoclave sterilized before inoculating the fermenting organism. Then the flasks were incubated at 37⁰C. At intervals of 24 h, samples were withdrawn from the flasks for estimation of the enzyme.

RESULT

A. Influence of Carbon Sources

The results of the enzyme activity after 48 h of incubation are presented in Fig.1. Maximum activity of α -amylase was observed with fructose (252 IU/ml), closely followed by that with supplementation of maltose (240 IU/ml). Several sugars caused lower enzyme activity but higher than that observed in the controls with starch alone: dextrin (196 IU/ml), glucose (135 IU/ml), glycerol (131 IU/ml), trehalose (123 IU/ml) and xylose (102 IU/ml). The non-metabolizable sugars like arabinose, raffinose, meso-inositol, sucrose and galactose do not support α -amylase production as indicated by enzyme activities much lower than that in the control, while with lactose lower enzyme activity is observed as the organism is basically a non-lactose fermenter.

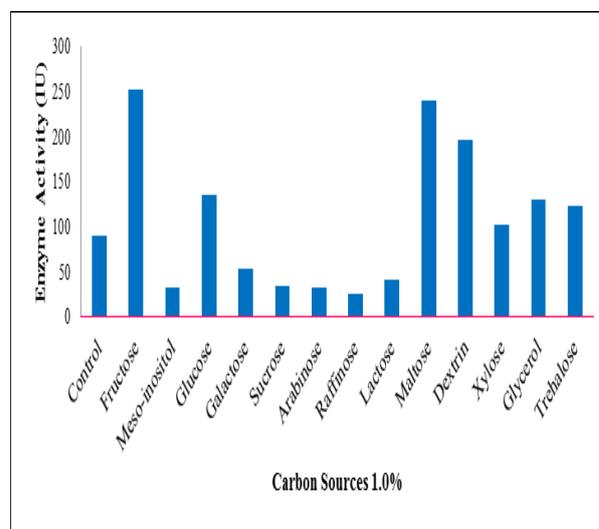


Fig. 1. Influence of Carbon Sources.

B. Influence of Organic Nitrogen Sources

Influence of Peptone

The results of the enzyme production at each concentration of peptone was estimated after 48 h of

incubation and presented in Fig.2. Peptone proved to be beneficial for enzyme production and enzyme yield increased gradually as peptone concentration increased upto 1.5%. Thereafter it decreased as peptone concentration increased, the enzyme yield being suppressed at 3.5 and 4.0% peptone concentrations. The highest yield of α -amylase (161 IU/ml) was recorded at 1.5% peptone.

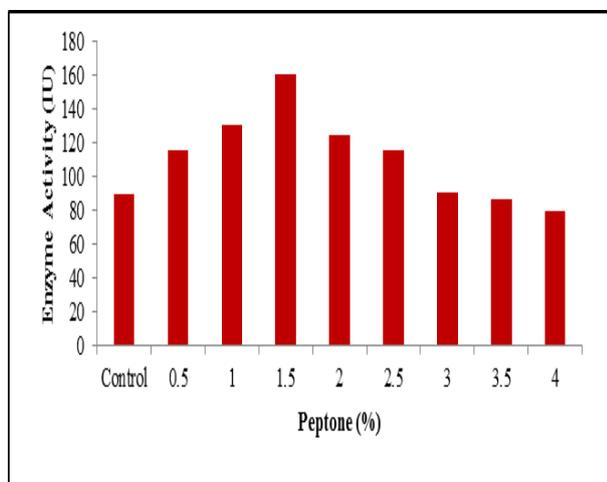


Fig. 2. Influence of Peptone.

Influence of other Organic Nitrogen Sources.

The enzyme production with different nitrogen sources was estimated after 48 h of incubation and the results are presented in Fig.3. Amongst all the organic nitrogen sources supplemented, peptone was observed to be most beneficial for α -amylase production with an yield of 175.0 IU/ml. Urea suppressed the enzyme yield. The order of beneficiality of the sources is in the following manner: peptone>beef extract>tryptone>yeast extract>corn steep liquor>soluble casein>hydrolysed casein.

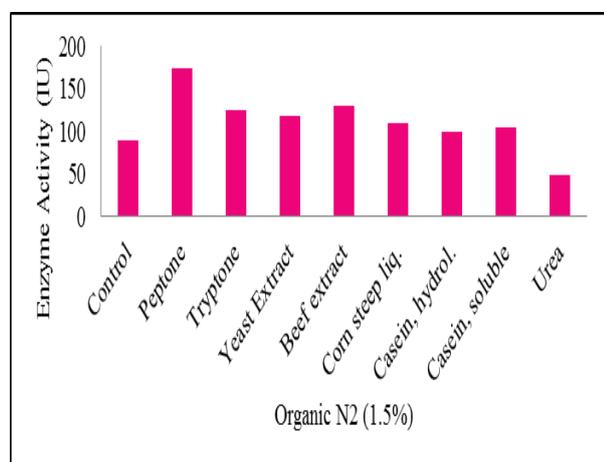


Fig. 3. Influence of Organic Nitrogen Sources.

Influence of Inorganic Nitrogen Sources

The influence of different inorganic nitrogen sources was studied maintaining all the optimized physico-chemical parameters of the fermenting medium constant, i.e. pH 8.0; temperature 37^o C; inoculum size 3x10⁶ cfu/ml and

substrate (starch) concentration, 1.0% (w/v). Different inorganic salts (sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride, ammonium sulphate and ammonium bisulphate) were supplemented separately at a concentration of 1.0% to the fermenting medium. Appropriate controls were maintained without nitrogen salt supplementation. The enzyme production as influenced by different inorganic nitrogen sources was estimated after 48 h of incubation and the results are presented in Fig.4.

Ammonium bisulphate was observed to be most beneficial amongst the salts, with α -amylase activity of 125 IU/ml at 48 h of incubation period. Amongst other salts, only sodium nitrate and ammonium sulphate were beneficial, that too a small extent. Other salts were either not beneficial or were slightly suppressive.

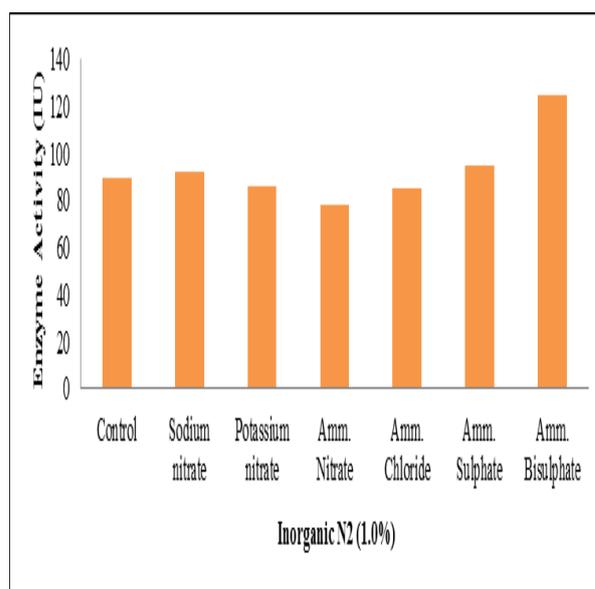


Fig. 4. Influence of Inorganic Nitrogen Sources

C. Influence of some Metal Salts on Enzyme Production

The enzyme production as influenced by different metal salts was estimated after 48 h of incubation and the results are presented in Fig.5. Amongst the metal salts supplemented, zinc sulphate, manganese sulphate and ferrous sulphate were observed to exhibit suppressive action over α -amylase production by the bacterium. Other salts enhanced α -amylase production, maximum yield of (181 IU/ml) being observed with calcium sulphate. The beneficial influence of these salts was observed to be in the following order : calcium sulphate>calcium chloride>copper sulphate>magnesium sulphate.

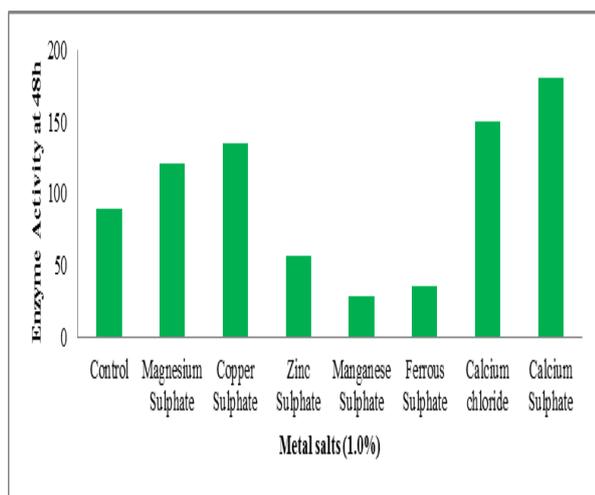


Fig. 5. Influence of Metal Salts.

DISCUSSION

The studies on the influence of carbon sources on α -amylase production indicated that fructose, maltose, dextrin, glucose, glycerol, trehalose and xylose (in the decreasing order) are beneficial to the process of enzyme production by *B. megaterium* KLMI4. The other sugars studied (arabinose, raffinose, mesoinositol, sucrose and galactose) decreased the enzyme production by the strain. Aiyer (2004) also reported that these above named five sugars suppressed enzyme production by *B. licheniformis* SPT27 since these are non-metabolizable sugars. The same appears to be good even in the present strain too. Apart from these five sugars, lactose also has been found to be inhibitory to enzyme yield by *B. megaterium* KLMI4 since this strain is basically a non-lactose fermenter.

That starch is beneficial for α -amylase production by most of the microorganisms (bacteria as well as fungi) has been well recorded by most of the researchers. The beneficial influence of different sugars on α -amylase yield by different bacterial and fungal species/strains has also been reported by many workers: fructose (Aiyer, 2004; Aqueel and Umar, 2010; Hashemi et al., 2011; Raju and Diwakar, 2012; maltose (Mamo and Gessesse, 1999; Aiyer, 2004; Anto et al., 2006; Quader, 2006; Erdal and Tskin, 2010; Gurudeeban et al., 2011), dextrin (Saito and Yamamoto, 1975; Aiyer, 2004; Narang and Satyanarayana, 2011;), glycerol (Welker and Campbell, 1963; Aiyer, 2004), trehalose (Aiyer, 2004) and xylose (Aiyer, 2004; Hashemi et al., 2011; Suribabu et al., 2014). The present observation is in concurrence with that of these researchers.

Glucose supplementation at 1% (w/v) was beneficial to *B. megaterium* KLMI4 to good extent when compared to the controls. Glucose has been observed to be beneficial to different organisms by different workers (Anto et al., 2006; Erdal and Taskin, 2010; Dey and Banerjee, 2011; Hashemi et al., 2011; Irfan et al., 2012). However, suppression of enzyme yield due to a repressive action has been reported by many workers (Bajpai and Bajpai,

1989; Ogbonnaya and Odiase, 2012; Nusrat and Rahman, 2007; Santos and Martins, 2003; Mulimani and Ramlingam, 2000; Ozdemir et al., 2009).

1.5% peptone (w/v) is optimum for α -amylase production by *B. megaterium* KLMI4 is supported by similar works by Aqueel and Umar (2007) on a strain of *B. megaterium*. However different optimum levels of peptone have been reported for different bacterial species/strains: 2% (Bozic et al., 2011), 0.2% (Unakal et al., 2012), 0.3% (Tiwari et al., 2013) and 0.5% (Riaz et al., 2013).

In the present study, amm. bisulphate was the more beneficial than amm. sulphate and sodium nitrate. Potassium nitrate, amm. nitrate and amm. sulphate were either less beneficial or slightly suppressive for the enzyme yield. Amm. bisulphate promoted the enzyme yield to 125 IU/ml after 48 h. Aiyer (2004) and Irfan et al. (2012) too reported amm. bisulphate to be more beneficial than other inorganic N_2 compounds. Oshoma et al. (2010) observed much lesser enzyme yield with sodium nitrate than with amm. sulphate.

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