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Production of α-Amylase by *Arachniotus sp.* using Waste Bread Medium

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ABSTRACT

Optimization studies on α-amylase production in waste-bread medium by *Arachniotus sp*. under continuous shaking conditions showed that addition of $(NH_4)_2SO_4$,$MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and KH_2PO_4 to the fermentation medium enhanced α-amylase production. Maximum α-amylase (11.66 IU/mL/min) activity was recorded after 48 h of continuous shaking fermentation (120 rpm) in the culture medium of 2.5% waste bread containing $(NH_4)_2SO_4$ 0.2%, $MgSO_4.7H_2O$ 0.04%, $CaCl_2.2H_2O$ 0.05% and KH_2PO_4 0.2% at pH 4 and 32°C.

Key Words: *Arachniotus sp*.; α-amylase; Waste bread medium; Optimization; Fermentation

INTRODUCTION

Amylolytic enzymes represent a group of catalytic proteins of great importance for the food industry. These enzymes are to be produced commercially by microorganisms. In the last 15 years, many α-amylases have been isolated, purified and crystallized from a variety of sources (Poonam & Singh, 1995). The majority of these have come from microorganisms. Microorganisms can be grown under controlled conditions in large number to yield amylases that are relatively easy to isolate and purify (Mamo & Gessesse, 1999). α-amylase preparations are mainly used in food industry, brewing processes and continuous process for de-sizing of textile fabrics (Reed, 1987). Other applications include modification of starches suitable for preparation of adhesives, sizes and coatings for the paper industry, as well as manufacture of glucose and glucose syrups (Hanes & Stedt, 1988). This paper reports the optimization of fermentation parameters for α-amylase production to establish the relationship between *Arachniotus sp*. and waste bread medium.

MATERIALS AND METHODS

Microorganism and fermentation. *Arachniotus sp.* obtained from the Department of Plant Pathology, University of Agriculture, Faisalabad was maintained on potato starch-agar slants at pH 4 and 32°C (Asghar *et al*., 2000). Conical flasks with 100 mL of waste bread medium containing different concentrations of micro-nutrients were inoculated with 5 mL of homogenous spore suspension (3 x 10^6 spores/mL). The flasks were incubated at pH 4 and 32°C on a shaker (120 rpm) for optimum fermentation period. The fermented biomass in each case was filtered and the filtrates were centrifuged. The supernatant was ultra-filtered through Millipore filter and the filtrate was assayed for α-amylase.

Optimization of culture conditions. The growth medium of waste bread was fermented with *Arachniotus sp*. for different fermentation periods with varying levels of substrate, pH, temperature, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and KH_2PO_4 in shake flask. The experiments were carried out in such a way that the parameter optimized in one experiment was maintained in the subsequent investigation.

Enzyme assay. An appropriately diluted culture filtrate was used to determine α-amylase activity by a spectrophotometeric method using starch as substrate and 3, 5 dinitorsalicylic (DNS) acid as coupling reagent (Bernfeld, 1955). One unit enzyme activity was defined as the amount of enzyme, which released one μ mol maltose per minute.

RESULTS AND DISCUSSION

Different culture conditions were optimized for α-amylase production by conducting a series of experiments and the results are discussed as under:

Fermentation period. Triplicate media containing 1.5% waste bread as substrate were harvested after 24, 36, 48, 60 and 72 h of incubation at pH 4 and 32°C. The maximum activity of α-amylase (3.91 IU/mL/min) was observed in culture filtrates harvested after 48 h of continuous shaking fermentation, which declined thereafter, through 60-72 h (Table I). Results are in line with those of Chadha *et al*. (1997) who optimized shake flask culture for production of α-amylase by *Thermomyces lanuginosus* and observed maximum α-amylase activity when inoculated and cultured for 72 h in 2% rice flour medium.

Substrate level. Fermentation media containing 1.0, 1.5, 2.0, 2.5 and 3.0% waste bread were incubated for 48 h at pH and 32°C. It was observed that 2.5% waste bread in the fermentation medium yielded maximum (5.98 IU/mL/min) α-amylase activity (Table I) after 48 h. A further increase in substrate level caused a gradual decrease in enzyme yield. Our results are in agreement with those of Bajpai *et al*. (1992) who reported 2% corn starch and 3% corn gluten as

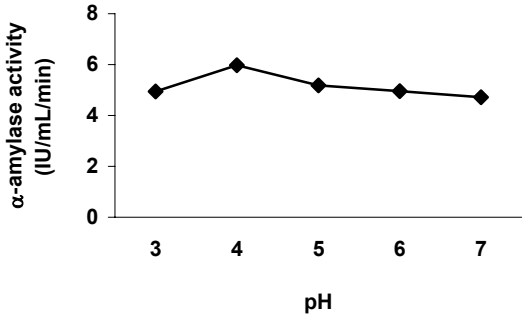
Table I. Effect of varying fermentation period and substrate level on α-amylase production by *Arachniotus sp*.

Fermentation Period (hours)	α-amylase activity (IU/mL/min)	Substrate level (%)	α-amylase activity (IU/mL/min)
24	2.87	1.0	2.86
36	3.52	1.5	3.94
48	3.91	2.0	5.15
60	3.57	2.5	5.98
72	2.82	3.0	5.37

optimum substrate level for production of α-amylase by *Bacillus sp*.

Effect of pH. The results showed maximum activity of α-amylase (5.97 IU/mL/min) in the fermentation medium adjusted at pH 4. At pH 3, the enzyme activity was low due to acidic environment not suitable for optimum growth of *Arachniotus sp*. At pH 5 and 6, a decrease in enzyme production was observed because increase in pH of the medium beyond pH 4 did not favor the secretion of enzyme by the fungus (Fig. 1). Moreira *et al*. (1999) optimized the fermentation medium of starch by *Aspergillus tamarii* and optimal α-amylase activity was observed in the starch medium at pH 4.5.

Fig. 1. Effect of pH on α-amylase production



Temperature. The results showed maximum activity of α-amylase (5.98 IU/mL/min) in the waste bread medium (2.5%) incubated at 32°C for 48 h at pH 4. A further increase in temperature caused a decrease in enzyme production by *Arachniotus sp*. (Fig. 2). Our results are in line with those of Upton and Fogarty (1977) who produced maximum α-amylase using *Aspergillus oryzae* in 8% starch medium, under optimized conditions at 30°C after 4 days.

$(NH_4)_2SO_4$. Five different concentrations of ammonium sulphate *viz.* 0.1, 0.2, 0.3, 0.4 and 0.5% were used as an additional nitrogen supplement in the fermentation medium of waster bread (2.5%). Maximum activity of α-amylase (6.97 IU/mL/min) was recorded in the medium supplemented with 0.2% $(NH_4)_2SO_4$. All the other media showed lower activities of the enzyme (Fig. 3). Results are comparable to those of Krishna and Chandrasekaran (1996) who produced α-amylase by *Bacillus substilus* using banana fruit stalk as a substrate and observed maximum activity of

Fig. 2. Effect of temperature on α-amylase production

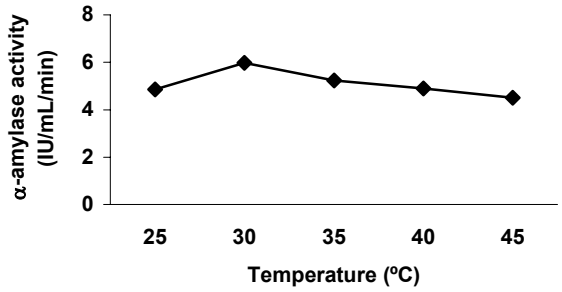
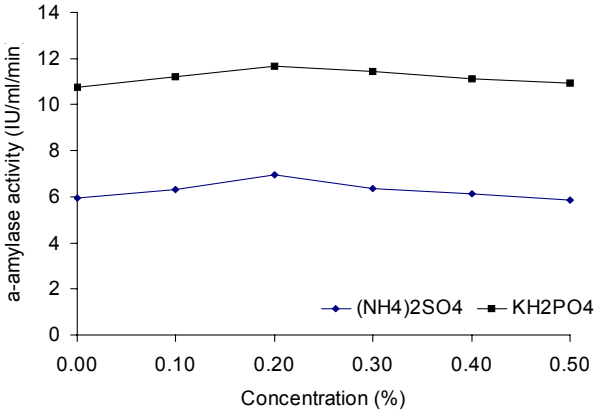


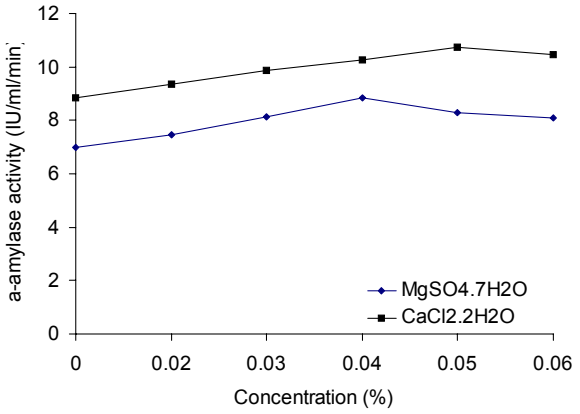
Fig. 3. Effect of varying concentrations of $(NH_4)SO_4$ and KH_2PO_4 on α-amylase production



α-amylase with 1% $(NH_4)_2SO_4$ as nitrogen source.

$MgSO_4.7H_2O$. Addition of $MgSO_4.7H_2O$ into the growth medium of waste bread (2.5%) enhanced the production of α-amylase by *Arachintous sp*. and 0.04% $MgSO_4.7H_2O$ facilitated higher α-amylase production (8.84 IU/mL/min) than all other levels of this nutrient (Fig. 4). The results are in agreement with those of Teuri (1973) who produced a-amylase using 5% starch by *Bacillius subtilis* and observed maximum activity of enzyme (30 IU/mL) with 0.05%, $MgSO_4.7H_2O$. Upton and Fogarty (1977) produced α-amylase from *Aspergillus oryzae* using 8% starch with 0.1% $MgSO_4.7H_2O$.

Fig. 4. Effect of varying concentrations of $MgSO_4.7H_20$ and $CaCl_2.2H_2O$ on α-amylase production



$CaCl_2.2H_2O$. $CaCl_2.2H_2O$ was used to enhance the fermentation rate and α-amylase production by *Arachniotus sp*. Different concentrations of $CaCl_2.2H_2O$ were added into the pre-optimized fermentation medium of waste bread. The maximum activity of α-amylase was observed in the medium supplemented with 0.05%, $CaCl_2.2H_2O$. Further increase in $CaCl_2.2H_2O$ level in waste bread medium caused a decrease in production of α-amylase by *Arachniotus sp*. (Fig. 4). Results are comparable to those of Bajpai *et al*. (1992) who produced α-amylase by *Bacillus sp*. using 2% corn starch as substrate and the maximum enzyme was produced with 0.02% $CaCl_2.2H_2O$ in optimum medium.

KH_2PO_4. Effect of varying levels of KH_2PO_4 was studied on production of α-amylase in growth medium containing optimum concentrations of waste bread (2.5%), $(NH_4)_2SO_4$ (0.2%), $MgSO_4.7H_2O$ (0.04%) and $CaCl_2.2H_2O$ (0.05%) at pH 4 and 32°C. Results revealed optimum α-amylase yield (11.66 IU/mL/min) with 0.2% KH_2PO_4 (Fig. 3) which decreased by further addition of this salt into the medium. Results are in line with those of Xiangli *et al*. (1984) who obtained maximum α-amylase by immobilized *Aspegillus niger* when the optimum medium contained 0.05%. KH_2PO_4.

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