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Kinetics of Glucoamylase Production by *Arachniotus sp.*

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ABSTRACT

Arachniotus sp. was cultured in waste bread fermentation medium to produce glucoamylase. The fermented biomass was harvested after 48 h of submerged fermentation (120 rpm). It was filtered and centrifuged at 10,000 rpm at –10°C. The supernatants were subjected to enzyme assay. Maximum activity of glucoamylase was 11.14 U/mL/min recorded in the optimum medium containing waste bread (2.0 g/100 mL), $(NH_4)_2SO_4$ (0.4 g/100 mL), $MgSO_4.7H_2O$ (0.1 g/100 mL), $CaCl_2.2H_2O$ (0.15 g/100 mL) and KH_2PO_4 (0.4 g/100 mL) at pH 4 and 32°C. *Arachniotus sp.* proved to be an effective glucoamylase producer in waste bread medium.

Key Words: Glucoamylase; Hydrolytic enzyme; Fermentation; *Arachniotus sp.*

INTRODUCTION

Amylases are among the industrially important hydrolytic enzymes and are of great significance in present day biotechnology. Glucoamylase finds applications in food industry including the degradation of starches into glucose for the production of syrups and whole grain hydrolysis for alcohol production (Pandey, 1995). With the advent of new frontiers in biotechnology, the spectrum of glucoamylase application has been widened in many other fields, such as clinical, medicinal and analytical chemistry. The enzyme has also got widespread applications in textile, feed, brewing and distilling industries (Pandey *et al.*, 2000). Glucoamylase can be applied for the digestion of residual beta-cyclodextrin to reduce cholesterol in hen's egg (Pramila *et al.*, 2000). Glucoamylases are produced from a wide variety of sources including plants, animals and microorganism. The microbial enzymes have advantages over plant and animal enzymes, because their production can be improved by genetic and environmental manipulations (Kwan *et al.*, 1993).

Keeping in view the extensive industrial importance of the enzyme, a study was carried out for optimization of fermentation parameters for glucoamylase production by a fungus *Arachniotus sp*. in waste bread medium.

MATERIALS AND METHODS

Arachniotus sp. was maintained (pH 4 and 30°C) on potato dextrose-agar (PDA) slants (Asghar *et al*., 2000). Conical flasks (250 mL) containing 50 mL of waste bread medium containing different concentrations of micro-nutrients were inoculated with 2.5 mL of homogenous spore suspension (1.57 x 10^7/mL) prepared from slant cultures (Asghar *et al*., 2000). The flasks were incubated at pH 4 and 30°C on a shaker (120 rpm) for optimum fermentation period. The fermented biomass was filtered and the filtrate was centrifuged. The supernatant was ultrafiltered through millipore filter and the filtrate was assayed for glucoamylase.

Optimization of culture conditions. In the first experiment, culture medium of waste bread (1.5%) was inoculated and incubated for 12, 24, 36 48 and 72 h for the optimization of fermentation period. Fermentation medium containing different levels of substrate was incubated for optimum time period in the second experiment. Varying concentrations of $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and KH_2PO_4 were then used in four subsequent experiments, respectively, to determine their optimum levels in waste bread medium for glucoamylse production by *Arachniotus sp*.

Enzyme assay. An appropriately diluted culture filtrate was used to determine glucoamylase activity in 0.1 M citrate buffer (pH 4.8) by a spectrophotometric method using starch as substrate and dintro salysylic acid (DNS) as coupling reagent (Bernfeld, 1955). One unit of enzyme activity in each case was defined as the amount of enzyme, which released one µmol of glucose per minute.

RESULTS AND DISCUSSION

Fermentation parameters were optimized for production of glucoamylase by *Arachniotus sp.* in the shake culture medium of waste bread and results have been discussed as under:

Incubation period. The glucoamylase activity increased upto 48 h (3.90 U/mL/min) of incubation and decreased thereafter (Table I). Chadha *et al*. (1997) observed maximum glucoamylase production by *Thermomyces lanuginosus* after 72 days of incubation (15 rpm) in 2% rice flour fermentation medium.

Substrate level. The experiment was carried out to study the effect of varying substrate levels. Maximum glucoamylase (4.02 U/mL/min) production by *Arachniotus spp*. was observed with 2% substrate (W/V) in the medium (Table I). Goto *et al.* (1998) used 3% (w/v) oat bran as optimum substrate for maximum production of glucoamylase by *Aspergillus fumigatus*. The results clearly

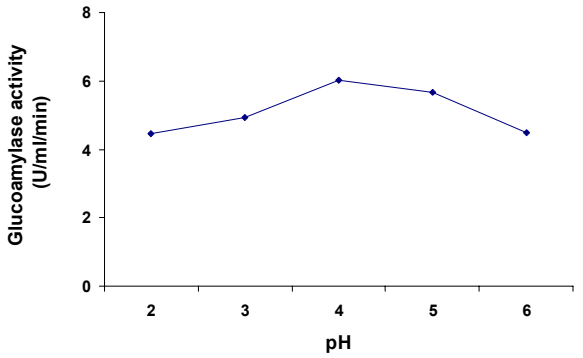
Table I. Activity of glucoamylase at varying incubation periods and substrate levels

Fermentation Period (hours)	Glucoamylase activity (U/mL/min)	Substrate level (%)	Glucoamylase activity (U/mL/min)
24	3.26	0.5	2.70
36	3.39	1.0	3.81
48	3.90	1.5	3.91
60	3.49	2.0	4.02
72	2.80	2.5	3.85

indicated that when substrate level increased beyond a certain limit the enzyme activity started decreasing. This decline may be attributed to partial adsorption of enzyme to the substrate or stearic hindrance.

pH. There was a gradual increase in enzyme production by *Arachniotus sp.* upto pH 4, and the medium adjusted at initial pH 4 yielded maximum glucoamylase activity. Further increase in pH of the medium resulted in less enzyme production (Fig. 1). Results of our study are in line with those of Sayed *et al.* (2000) who produced glucoamylase using *Monascus purpurens* ATCC *16437* as organism of choice and 4.2 as the optimum pH.

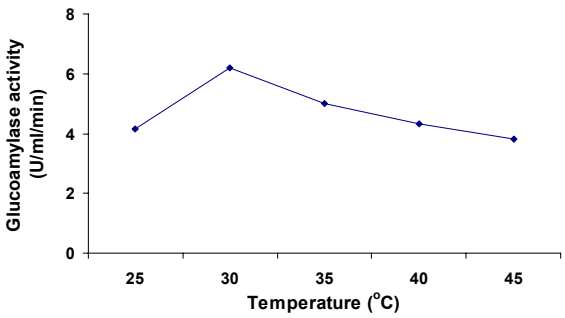
Fig. 1. Effect of pH of the medium on glucoamylase production



Temperature. Duplicate flasks of the growth media containing 2.0% substrate were fermented with *Arachnoitus spp*. at pH 4 for 48 h at different conditions of temperature. Maximum production of glucoamylase (6.19 U/mL/min) was observed in the medium incubated at 32°C found to decrease by a further increase incubation temperature (Fig. 2). Our results are in agreement with the work of Feroza *et al.* (1998) who produced glucoamylase form *Aspergillus niger* in culture medium of potato starch and observed maximum enzyme production at 35°C and pH 6. Moreira *et al*. (1999) reported 42°C as the optimum temperature for amylase production by *Aspergillus awamori* in shake culture of 1% (w/v) starch.

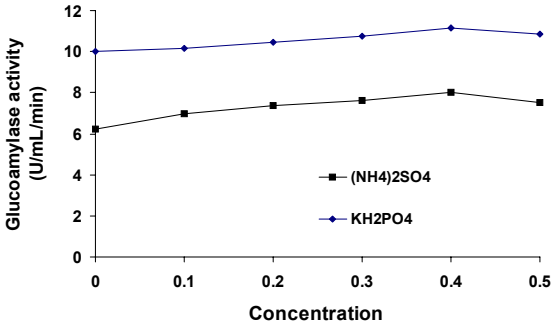
$(NH_4)_2SO_4$. Secretion of glucoamylase by *Arachniotus* sp.

Fig. 2. Effect of incubation temperature on glucoamylase production



increased with the addition of $(NH_4)_2SO_4$ in to the waste bread medium. Maximum glucoamylase activity (8.0 U/mL/min) was noted in the medium supplemented with 0.4% $(NH_4)_2SO_4$ (Fig. 3). The results are comparable to Feroza *et al.* (1998) who produced maximum glucoamylase from *Aspergillus niger* by using 0.3% $(NH_4)_2SO_4$ as optimum additional nitrogen source in shake culture (150 rpm) of potato starch.

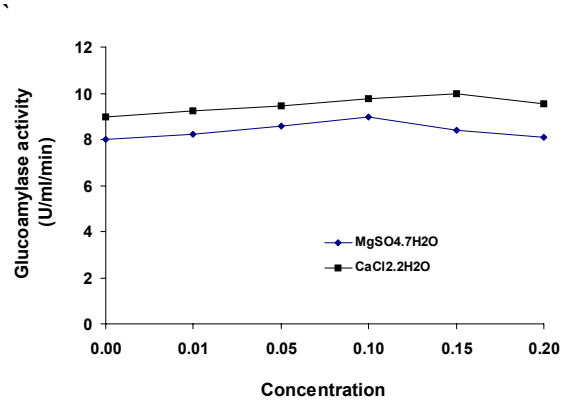
Fig. 3. Effect of varying concentrations of $(NH_4)_2SO_4$ and KH_2PO_4 on glucoamylase production



$MgSO_4.7H_2O$. Waste bread medium containing five different concentrations of $MgSO_4.7H_2O$ along with pre-optized nutrients were fermented with *Arachniotus sp*. at pH 4 and 32°C for 48 h. Addition of $MgSO_4.7H_2O$ enhanced the enzyme production and maximum glucoamylase activity (9.99 U/mL/min) was recovered from the culture filtrate of the medium containing 0.1% $MgSO_4.7H_2O$ (Fig. 4). The results are well in line with the work of Jensen *et al.* (1987) who produced maximum extracellular amylase with 0.05% $MgSO_4.7H_2O$ in growth medium fermented with *Thermomyces laniuginosus*.

$CaCl_2.2H_2O$. Glucoamylase production by *Arachniotus sp.* was studied with varying concentrations of $CaCl_2.2H_2O$ in the preoptimized waste bread medium. An enhancement in enzyme production by the fungus was observed with the addition of $CaCl_2.2H_2O$ into the medium and maximum activity of glucoamylase (9.99 U/mL/min) was observed with 0.15% level of the salt (Fig. 4). Further increase in the

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



level of $CaCl_2.2H_2O$ in the medium caused a gradual decrease in enzyme production. The results are in agreement with Fujio and Morita (1996) who reported that glucoamylase production and its activity in liquid culture supplemented with calcium was higher than recorded for extracts from calcium free culture of *Rhizopus sp*.

KH_2PO_4. The growth media containing different concentration of KH_2PO_4 were fermented for 48 h under pre-optimized culture conditions. Maximum glucoamylase activity (11.14 U/mL/min) was recorded in the medium containing 0.4% KH_2PO_4. The enzyme production by the fungus increased steadily with the addition of KH_2PO_4 upto 0.4% level and decreased, thereafter (Fig. 3). Our results are comparable to the results of Feroza *et al.* (1998) who produced maximum glucoamylase from *Aspergillus niger* and used 0.3% KH_2PO_4 in the growth medium of potato starch powder. Xiangli *et al.* (1984) produced maximum extra cellular glucoamylase in growth medium of low molecular weight dextrin containing 0.05% KH_2PO_4.

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