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INFLUENCE OF CULTURE CONDITIONS ON I3-AMYLASE PRODUCTION BY *Aspergillus niger* IN WASTE BREAD MEDIUM

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Addition of urea. MgSO$_4$.7H$_2$O. KCI and KHl04 enhanced the production of r~-amylase by *Aspergitlu» Ilig!'r* from waste bread. whereas lnS0$_4$.7H$_2$0 depressed the enzyme production under optimum conditions. rI-amylase activity (22.651 U/ml/min) was maximum after 48 hr incubation in 1.5% waste bread medium. containing n.";"/" urea. ().O~C% MgS0$_4$.7Hp. 0.1% KCI and 0.2% KHl04. at pH 4 and 37°C.

Key words: *Aspergillus niger.* ~-amylase. continuous shaking fermentation. waste bread

INTRODUCTION

Amylases have extensive applications in food and beverage industries to convert starch into maltose and glucose (Chatterjee et al.. 1988). The amylases (a. & In along with cellulase and hemicellulase are used in cereal processing industry as natural means of improving feed utilization and controlling pollution by recycling the agricultural residues and wastes. This is reflected by rapid increase in the use of enzymes by poultry feed industry (Ahmad et al.. 1993). li-amylases that produce maltose from linear dextrins are entering the market and can be used in combination with debranching enzyme to make pure maltose syrups (Brown et al.. 1987). Quality and freshness of bread are priority considerations for most consumers and anti-staling properties of amylases modify the flour fraction in baking (Callcjo and Maria. 1997).

Traditionally, starch and starchy waste materials were/arc still converted into low molecular weight dextrins and glucose by acid hydrolysis but enzymes have several advantages. The specificity of enzymes allows the production of sugar syrups with well defined physical and chemical properties. Furthermore. milder enzymatic hydrolysis results less in side reactions and causes less browning (Aktinson and Mavituna. 1991). The present paper reports the effect of culture conditions on li-amylase production by *Aspergillus niger* in waste bread medium.

MATERIALS AND METHODS

Suhstratc: The waste bread was obtained from a students mess in the University of Agriculture. Faisalabad. It was dried and ground to powder form (40mm mesh) and used as a substrate for amylase production.

Fcrmentativc Organism: Pure culture of fungus *Aspergillus niger* procured from the Department of Veterinary Microbiology was raised on potato starch-agar slants. sporulation medium (Irshad, 1999) and incubated aerobically (pH 4: 37°C) for 72 hours. For the preparation of inoculum. the spores were directly transferred into the conical flask containing 100ml prcstcnhzcd glucose solution (inoculum medium) by disposable syringe from sporulaltion slants. Conical flask containing IOOml of waste bread medium (1.5%) and varying concentrations of urea and micro nutrients were inoculated with Sml of homogeneous spore suspension (1.52 x 10' spores/nil] The flasks were incubated at pH 4 and :noe under continuous shaking conditions (120 rpm) for optimum fermentation period. The fermented biomass samples were filtered and the filtrates centrifuged at 10.000 rpm at _100e. The supernatants thus collected were subjected to enzyme assay.

Optimization of Conditions: In the first experiment. the growth medium of waste bread (I %) was -fermented for 12. 24. 36. 48 and 72 hr for optimization of fermentation period with *Aspergillus niger.* Fermentation medium containing different levels of waste bread \\as incubated at pH 4 and 37°C for optimum time period (~X hr) in the second experiment. In the third expcri mcnt.. growt h media (1.5% waste bread) adjusted at different pH values were fermented for ~8 hr. Varying concentrations of urea and ionic salts (MgS01'7Hp. KCL KHlO, and ZnS(\.7H~O) were then used in five independent expcri mcnts. in such a way that concentration of a nutrient oprunizcd in one experiment was maintained in subsequent imcstigations.

Enzyme Assays: Culture filtrates were assayed for ri-amylase activity by spectrophotomct ne method of Bernfeld (1955). The rate at which maltose was liberated from starch by enzyme solution was measured by its ability to reduce 3. 5-dinitrosalicylic acid (DNS) reagent. The absorbance of coloured complexes was read at 540nm against reagent blank. One unit enzyme activity was defined as the amount of enzyme which released I umole of maltose in one nunuic.

RESULTS AND DISCUSSION

Fermentation Period: It was observed that initially the enzym~ production increased by increasing fermentation period up to 48 hr but decreased thereafter (Table I). The growth medium harvested after 48 hr showed maximum [1-amylase (5.971 U/min/ml) production. These results arc in line with Lealem and Gashe (1994) who obtained maximum enzyme activity (961U/ml) after 72 hr. when *Bacillus* sp. A-OOI was grown in starch salt medium. Chiou and Jeang (1995) noted maximum li-amylase (220 IU/ml) activity after 24 hr incubation of *Cytophaga* sp. in raw corn starch medium.

Substrate Level: Growth media containing varying substrate levels (0.5, 1.0. 1.5. 2.0 and 2.5%) were fermented with *Aspergillus niger* for 48 hr. The results showed that li-amylase activity increased steadily up to 7.73 IV/ml/min with 1.5% waste bread and decreased thereafter to 5.251 U/min/ml with 2.5% substrate (Table I) due to partial adsorption of the enzyme to the substrate. The results are in accordance with Sani et al. (1992) who used 2% (w/v) cassava peel for the maximum production of li-amylase by *Aspergillus niger*. Chiou and Jeang (1995) used 0.2% raw corn starch for maximum production of [i-amylase by *Cytophaga sp*.

Growth Medium 1)8: Growth media of waste bread (1.5%) with different pH values viz. 2. 3. 4, 5 and 6 were inoculated. incubated (pH 4, 37°C) and harvested after 48 hr. Maximum [i-amylase production was (6.931 U/ml/min) recorded in the medium adjuested at pH 4 (Fig. 1). An initial increase in enzyme production was observed by initial increase in pH from 2 to 4. A further increase in pH (from 4-6) caused a decrease in enzyme yield. These results are in agreement with those of Aslam (1997) who produced maximum li-amylase (7.73 IU/ml/min) by *Arachniotus* sp. at pH 4. Lin et al. (1998) obtained maximum [i-amylase activity at pH 8.5 and 55°C by *Bacillus* sp. TS23. The variation may be due to difference in substrates and microorganisms used by different researchers.

Urea: Four different levels of urea i.e. 0.1. 0.2, 0.3 and 0.4(X, were used as additional nitrogen source in optimum (1.5%) waste bread medium at pH 4. Results showed that li-amylase production was enhanced by the addition of urea and tLl% level gave optimum enzyme production (13.35IU/ml/min) by *Aspergillus niger*. Further increase in urea concentration up to 0.4% led to a steady decrease in yield of ri-amylase (Fig.2). Pazlorova and Votruba (1996) used ammonium-sorbing zeolite as nitrogen source for maximum production of amyalse with *Bacillus al1lylolique.faciens*. Kclly et al. (1997) used 2% *(w/v)* yeast extract as additional nitrogen source for maximum production 01' c\:traccllular ~-amylase by *Bacillus flavothernrus*,

MJ?;SO4.782°: Different concentrations of MgSO,7H-<l (0.03,0.04.0.05 and 0.06%) were added to the fcnucutauou medium along with pre-optimized substratc (I 'i"", and urea (0.3%). Maximum ~-amylase activity (15.~() IU/m11 min) was obained with 0.04% $MgSO_4.7H_2O$ (Table 21 A further increase in. its concentration resulted in decreased ri-amylase activity. Jensen et a1. (1987) pro-duced maximum extracellular amylascs WIth O.05'}';, $MgSO_4.7H_2O$ in growth medium of dextran fermented with *Thermomvces lanuginosus*, Aslam (I ')1')7) reported maximum enzyme activity (8.0 lll/ml/minl \\ ith 0.1105% $MgSO_4.7H_2O$ in corn stover medium fermented by *Arachniotus* sp.

KCI: Addition of KC! to the growth medium enhanced the production ofl1-amylase and 0.1 % KCI facilitated highcr activity (18.13 IU/ml/min) than all other COIKcnlrations tested (Table 2). These results are in line with those of Sen and Chakarbarty (1987) who optimivcd o.5'}';, KCI for r1-amylase by *l.actobacillus cellobiosus* D-,\I)' ill I(III'h soluble starch medium. Abouzeid (1997) opiiuuvcd I·~/nKCI for amylase production from 10% banana fruit starch bv *Aspergillus flavus*.

KH_2PO_4: It was observed that production of 1~-am~lasc by *Aspergillus niger* increased with the addition ofKHYO into the optimum growth medium. Maximum I~-alllylase activity (22.65 IU/ml/min) was observed with O.2'~Ic, KHlO,. The activity increased gradually with increase in its concentration up to 0.2% and decreased thereafter (Table 3). Results of present investigation arc in agrccmcnt with Xiangli etal, (1984) who produced extracellular amylase by immobilized *Aspergillus niger* in a medium containing 0.05% KHlO, Jcnsen et al. (1987) optimizcd 01 n~, KH,PO, in the growth medium fermented b~ *i ıl (TII/O/IIV'('(S . lanuginosus*.

ZnSO 4.782°: To study the effect 01 In ions on ri-amylase production. four different couccnt rat lOllS 01 $ZnSO_4.7H_2O$ viz. 0.0t. 0.05. 0.10 and (). 15'~Crespectively were added to the preoptimized fermentation medium The results showed that with the addition 0f $ZnSO_4.7H_2O$. the activity of ~-amylase decreased steadily but constantly (Tabie 3) from 22.27 .Ill/ml/mill to 12.12 IU/ml/min. The results are supported b~ Lin et al. (1998) who produced B-amylase by *BI/(. 111/1.\ Sl).* TS-23 and reported that enzyme production decreased b~ the addition of $ZnSO_4.7H_2O$. Abouzeid (llJlJ7) reported that Zn^{2+} acts as a strong inhibitor for r)-amylase production in growth medium fermented by *Aspcrsitllu> /1I\V/ls*

Table 1.Activity of ~-amylase at different fermentation periods and substrate levels

Fermentation period (hr)	~-amylase activity (ill/ml/min)	Substrate level (%)	~-amylase activity (Il.l/ml/min)
12	3.27	0.5	4.37
24	3.73	1.0	5.79
36	4.69	1.5	7.73
48	5.97	2.0	6.73
60	4.67	2.5	5.25

Table 2.Activity of ~-amylase with varying concentrations of $MgSO_4 \cdot 7H_2$ and KCI under optimum conditions*

$MgSO_4 \cdot 7HP$ (%)	~-amylase activity (ill/ml/min)	KO (%)	~-amylase activity (lU/ml/min)
'0.03	14.95	0.01	16.83
0.04	15.86	0.05	17.23
0.05	14.09	0.10	18.13
0.06	13.77	0.15	16.67

* Waste bread. 1.5%: urea. 0.03%; pH 4.0 and 37°C.

Table 3.Activity of ~-amylase with varying concentrations of KH_2P0_4 and $ZnS0_4.7H_20$ under optimum conditions*

K~PO" (%)	f1-amylase activity (ill/ml/min)	$ZnS0_4 \cdot 7Hp$ (%)	~-amylase activity (lUlml/min)
0.1	20.21	0.01	19.67
0.2	22.65	0.05	17.45
0.3	21.97	0.10	14.38
0.4	16.77	0.15	12.92

* Waste bread. 1.5%; urea. 0.03%; $MgS0_4.7Hp$. 0,4%; pH 4.0 and noe.

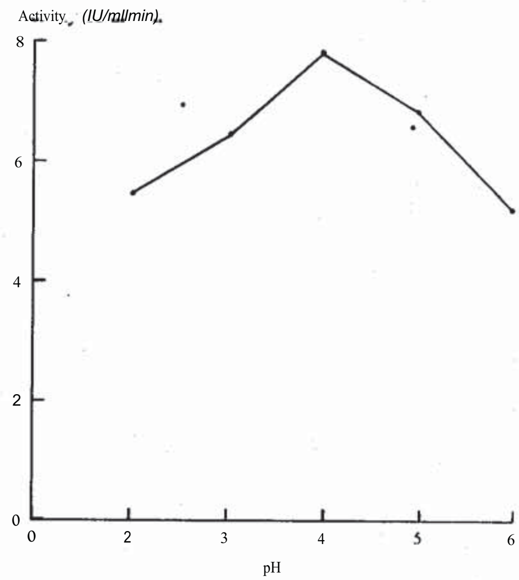
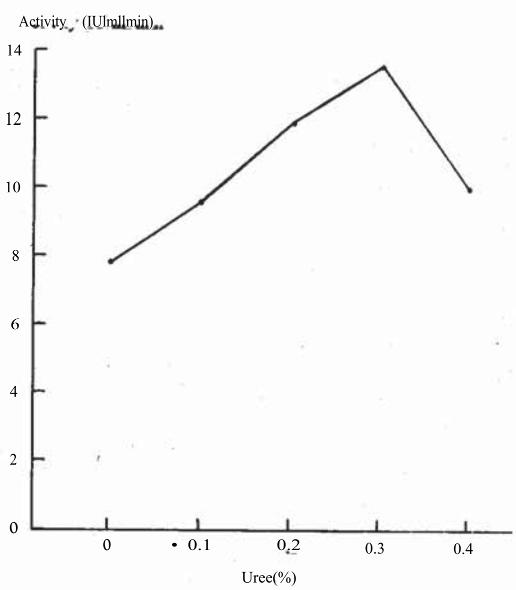


Fig. i, Effect or varyiag pH values on f3-am.ylase productioa



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