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INFLUENCE OF DIFFERENT CARBON SOURCES ON THE PRODUCTION OF ALPHA AMYLASE BY *ASPERGILLUS ORYZAE* ON KINETIC BASIS

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ABSTRACT:

The present study is concerned with the selection of carbon source for the production of alpha amylase by *Aspergillus oryzae.* Different carbon sources such as the lactose, glucose, maltose, xylose and sucrose were tested for the production of enzyme. Of all the carbohydrates tested lactose at the level of 1.0 % gave the maximum production of alpha amylase (130 U/ml) after 72 h of inoculation. The kinetic analysis indicated that the volumetric production of biomass and alpha amylase was significantly higher in the presence of lactose added to the fermentation medium.

INTRODUCTION:

The ability of filamentous fungi to secrete large amounts of extra cellular protein makes them well suited for the production of industrial enzyme. An example of this is the fungus *Aspergillus oryzae* that is used for the production of industrial proteins like alpha amylase (Agger *et al.*, 2001). In developing countries *Aspergillus* sp are selected for the production of alpha amylase because of ubiquitous nature and non fastidious nutritional requirements of this organism (Ab*e et al*., 1988). The starch-degrading enzyme 'α-amylase' is widely distributed in nature. This extra cellular enzyme hydrolyses α-1, 4-glucosidic linkages randomly throughout the starch molecule in an endo-fashion producing oligosaccha-rides and monosaccharides including maltose, glucose and alpha limit dextrin (Omemum *et al*., 2005; Calik and Ozdamar, 2001; Reddy *et al*., 2003). Alpha amylases are one of most important and widely used enzymes whose spectrum of application has widen in many sectors such as clinical, medicinal and analytical chemistry. Beside their use in starch saccharification they also find applications in food brewing, detergent textile and paper industries (Ramachandran *et al*., 2004; Pandey *et al.*, 2000; Haki and Rakshit, 2003; Yovita *et al*., 2005).

The production of alpha amylase has been greatly affected by the addition of different carbon sources (Dubey *et al.,* 2000). The carbon sources affected not only the mode of amylase formation but also the velocity with which carbohydrates are metabolized (Abdullah *et al.*, 2003). Carlsen *et al.,* (2001) quantified the influence of different carbon sources such as glucose, maltose, fructose, galactose and sucrose on the production of alpha amylase by *Aspergillus oryzae.* Glucose was proved to be better carbon source for the production of alpha amylase. Lachmund *et al*., (1993) found that starch and maltose strongly increased alpha amylase productivity in

Aspergillus oryzae whereas glucose led to very low productivity in *A. oryzae*. So it is important to select suitable carbon source for the enhance production of alpha amylase.

MATERIALS AND METHODS:

Organism: *Aspergillus oryzae* GCB-32 was maintained on potato dextrose starch agar medium.

Inoculum Preparation: In the present studies conidial suspension used to inoculate the fermentation flasks was prepared in 0.005 % sterilized Monoxal O.T. Ten milliliter of sterilized Monoxal O.T. was transferred to a 72 h old slant having profuse conidial production on its surface. The test tube was shaken vigorously for breaking the clumps of conidia. The number of conidia was counted with the help of Haemacytometer, which was about $2.6x10^6$ conidia /ml.

Fermentation Technique: Twenty-five milliliter of the fermentation medium in 250 ml cotton wool plugged conical flask containing (g/l) Starch, 20; yeast extract, 8.5; NH_4Cl, 1.3g; $MgSO_4.7H_2O$, 0.12; $CaCl_2$, 0.06 (pH 5.0) was used to carry out the production of alpha amylase additional nitrogen sources such as glucose, maltose, xylose and lactose were added to fermentation media. The flasks were sterilized in an autoclave at 121°C (15 lbs/inch^2) pressure for 15 min and cooled at room temperature. One milliliter of inoculum was aseptically transferred to each flask and the flasks were placed in the orbital shaking incubator (SANYO Gallenkamp PLC, UK) for incubation at 30°C with shaking speed of 200 rpm. After fixed period of incubation, the contents of the flasks were filtered and filtrate was used for the estimation of enzyme.

Enzyme Assay: Alpha amylase activity was estimated according to the method of Rick and Stegbauer (1974). One unit activity is defined as the amount of enzyme, which liberates reducing group from 1% soluble starch corresponding to 1mg maltose hydrate in 10 min.' All the analytical grade reagents and mean values were repeated in the results.

Kinetic Study: Kinetic parameters for batch fermentation were determined according to the method of Pirt (1975) and Lawford and Rouseau (1993). Different kinetic parameters were studied such as **a**) The value of μ (h^{-1}) was calculated from plot of In (x) vs time of fermentation **b**) Product yield coefficient namely Yp/x was determined by the equation: Yp/x= dP /dx **c**) Qp (IU/ml/ min) was determined from the maximum slope of enzyme produced vs time of fermentation **d**) Qx (g cell mass /l/h) was determined from the maximum slope of cell mass formation vs time of fermentation and **e**) Specific rate of product formation was determined by the equation $q_p=\mu$ x Y p/x.

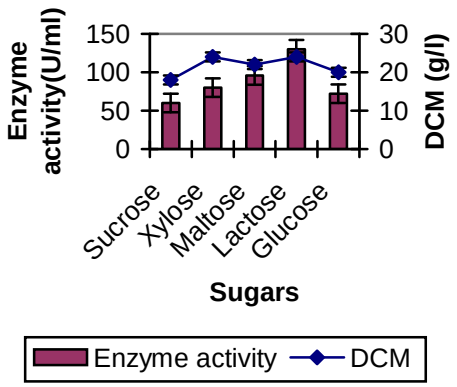
RESULTS AND DISCUSSION:

The nature and amount of carbon source in culture media is very important for the growth and production of extracellular amylase. The fermentation of alpha amylase is greatly affected by the addition of different carbon sources (Dubey *et al*., 2000; Carlsen and Nielsen, 2001). In the present study the effect of addition of different carbon sources such as glucose, lactose, maltose, xylose and sucrose were tested for the production of alpha amylase (Fig 1). These sugars

were added to fermentation media at the level of 1.0 %. Of all the sugars examined lactose gave maximum production of alpha amylase (130 U/ml). The production of the enzyme by the addition of lactose to the fermentation medium was found to be highly significant and varied signi-ficantly ($p<0.05$) with other sugars. Eratt *et al.* (1984) reported that alpha amylase production was induced by carbo-hydrates containing α 1-4 glucosidic bond e.g. maltose, maltodextrin or starch and effectively repressed by glucose. Hashem *et al.*, (1993) stated that addition of lactose to the culture medium stimulate the production of alpha amylase.

In the present study lactose along with starch is proved to be good carbon source. Complex carbo-hydrates such as starch and lactose are slowly metabolized by micro-organisms, which increase the accumulation of inducible alpha amylase in fermentation media (Nguyen *et al.*, 2000; Calik and Ozdamar, 2001).

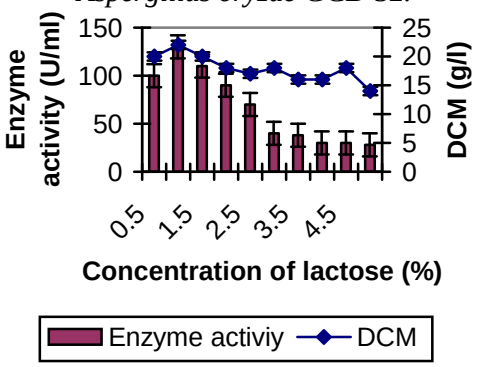
Fig 1:Effect of different carbon sources on the production of alpha amylase by *Aspergillus oryzae* GCB-32.



Each value is an average of three parallel replicate. Y error bars indicated the standard error from mean value. The values in vary significantly at $p< 0.05$.

It was found that synthesis of alpha amylase was greatly suppressed when *Aspergillus oryzae* grown either on xylose or glucose. It may be due to simple carbon sources may support respiratory metabolism with signi-ficant reduction of alpha amylase in fermentation medium. (Nandakumar *et al.*, 1999), thus the yield of enzyme was greatly inhibited. Thus lactose was selected additional carbon sources for the production of alpha amylase and its various concen-trations (0.5-5 %) were tested (Fig 2). Lactose at the level of 1.0 % was found to be best for the production of alpha amylase. Further increase or decrease in the level of lactose result decrease in the production of enzyme. It might be due to that lower level of carbon is in adequate for the growth as well as the production of alpha amylase and excess carbon is equally detrimental and cause catabolic repression.

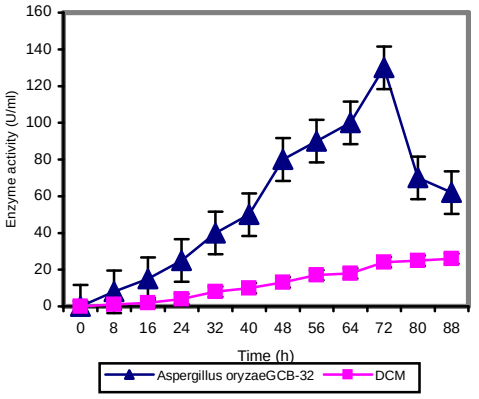
Fig 2:Effect of different concentration of lactose on the production of alpha amylase & cell mass by *Aspergillus oryzae* GCB-32.



The production of alpha amylase was increased with increase in incubation

period and found maximum after 72 h after inoculation (Fig 3). Further increase in the incubation period resulted decrease in the production of alpha amylase. It might be due to the depletion of the nutrients and production of other byproducts such as protease in the fermentation medium.

Fig 3: Effect of rate on the production of alpha amylase by *Aspergillus oryzae* in shake flask fermentation



Kinetic study indicated that the volumetric productivity of alpha amylase and cell mass formation was best in the presence of lactose at 1.0 % level (Table 1, 2). The yield of the enzyme was found to be best by the addition of lactose 1.0 % and varied significantly than other concentrations of lactose. Thus, the addition of Lactose at the level of 1.0 % was selected as carbon source for the maximum accumulation of alpha amylase in the fermentation medium.

Table 1:Kinetic parametric study following growth of organism for the effect of additional carbon sources on the production of α-amylase by *Aspergillus oryzae* GCB-32.

Carbon Sources	Kinetic parameters			
	Y_{px}	Q_p	q_p	Q_x
Glucose	3.60	1.00	0.05	0.27
Maltose	4.30	0.97	0.06	0.23
Xylose	4.00	1.11	0.05	0.27
Lactose	5.40	1.80	0.07	0.33
Sucrose	2.10	0.83	0.04	0.25

Yp/x= enzyme produced/g cell mass formation. Qp=enzyme produced/ml/h. Qx= g cell mass formation/l/h.

Table 2:Kinetic parametric study following growth of organism for effect of different concentration of Lactose on the production of alpha amylase by *Aspergillus oryzae* GCB32

Lactose concs.	Kinetic parameters			
	Y_{px}	Q_p	q_p	Q_x
0.5	5.0	1.30	0.060	0.277
1.0	5.9	1.80	0.080	0.305
1.5	5.5	1.52	0.070	0.277
2.0	5.0	1.25	0.069	0.250
2.5	4.1	0.97	0.057	0.236
3.0	2.2	0.55	0.030	0.250
3.5	2.3	0.52	0.032	0.222
4.0	2.0	0.44	0.027	0.222
4.5	1.6	0.41	0.023	0.250
5.0	2.0	0.38	0.027	0.194

Yp/x= enzyme produced/g cell mass formation. Qp=enzyme produced/ml/h. Qx= g cell mass formation/l/h.

CONCLUSION: From the present study it was concluded that supplementation of lactose as carbon sources to the fermentation medium resulted the maximum accumulation of alpha amylase in the fermentation medium.

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