Journal of Food Technology 1 (4): 187-190, 2003

Optimization and Kinetic Analysis of Carbon Sources on the Production of Alpha Amylase by *Saccharomyces cerevisiae*

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Abstract: The present study is concerned with the selection of carbon sources for the production of alpha amylase by *Saccharomyces cerevisiae* GCB-20. The lactose, glucose, maltose, xylose and sucrose as carbon sources were tested for the production of enzyme. Of all the sources tested, sucrose at the level of 0.5%was found to be best for the production of alpha amylase (530U/ml/min). The kinetic analysis indicated that the volumetric production of biomass and alpha amylase was significantly higher in the presence of sucrose (0.5%) added to the fermentation medium.

Key words: Kinetic analysis, alpha amylase, fermentation and *Saccharomyces cerevisiae*

Introduction

Alpha amylase an extracellular enzyme, degraded α 1-4 linkage of starch. This enzyme is extensively used in paper, food, pharmaceutical and detergent industries. Different microorganisms have been extensively used for the biosynthesis of alpha amylase (Fogarty and Kelly, 1980; Nigam and Sing 1995 and Haq *et al.*, 2002). The amylase of fungal origin was found to be most stable than the bacterial enzyme (Duochuan *et al.*, 1997).The production of alpha amylase by mould has been greatly affected by the addition of different carbon. (Dubey *et al.*, 2000). The carbon sources affected not only the mode of amylase formation but also the velocity with which the carbohydrates are metabolized (Prescott and Dunn's, 1987 and Carlsen *et al.*, 2001) quantified the influence of the different carbon sources such as maltose, glucose, fructose, galactose and sucrose on the production of alpha amylase by *Aspergillus oryzae*. Glucose is proved to be better carbon source for the production of alpha amylase. Most agricultural biomass containing starch can be used as a potential substrate for the production of fuels, feed protein and chemicals by microbial processes. The substrate includes corn, wheat, sorghum and other starch grains. On dry basis, corn, wheat, sorghum and other grains containing around 60-70% of starch hydrolysable to hexsoses with a significant weight increase and these offer a good resources in many fermentation processes. (Iwanno *et al.*, 1986; Mortia *et al.*, 1999 and Haq *et al.*, 2002). The objective of this study was the selection of suitable carbon sources for the production of alpha amylase by *Saccharomyces cerevisiae*.

Materials and Methods

Organism: *Sacchromyces cerevisiae* GCB-20 was obtained from Biotechnology Research Laboratory, Department of Botany, Government College University Lahore. The strain was maintained on potato dextrose starch agar medium.

Inoculum Preparation: The cell suspension was used as inoculum in case of yeast .The suspension was prepared in the sterilized monoxal O.T. (Di-Octyl ester of sulpho succinic acid). . Each ml of cells suspension contained 2.6 x 10^6 cells.

Fermentation Technique: Fifty ml of the fermentation medium containing g/l Peptone 20.0, Yeast extract 3.0, Starch 10, $CaCl_2$ 2, $MgSO_4$ 0.005 in 1000ml of phosphate buffer was transferred to each of 250 ml of cotton plugged conical flask. The flasks were sterilized in the autoclave at 15 lb $inch^{-2}$ pressure (121°C) for 15 min and cooled at room temperature. One ml of inoculum was aseptically transferred to each flask. The flasks were then placed in the rotary shaking incubator (200 rpm) at 30 °C for 72 h. After 72h the fermented broth was centrifuged at 7000 rpm for 30 min. The cell free supernatant was used for the estimation of alpha amylase.

Enzyme Assay: Alpha amylase estimation was carried out according to the method of Rick and Stegbauer (1974). The enzyme solution at pH 5.5 was incubated at 40°C using 1% soluble starch solution. The reducing sugars were measured by adding 3,5-dinitro salicylic acid reagent, boiling for 5 min, cooling and measuring the O.D at 546 nm in the spectrophotometer (Model CECIL CE7200 Aquaris UK) against maltose as standard. One unit of activity is equivalent to that amount of enzymes, which in 30 minutes librates reducing group from 1% Lintner's soluble starch corresponding to 1 mg maltose hydrate. Treatment effects were compared by the method of Snedecor and

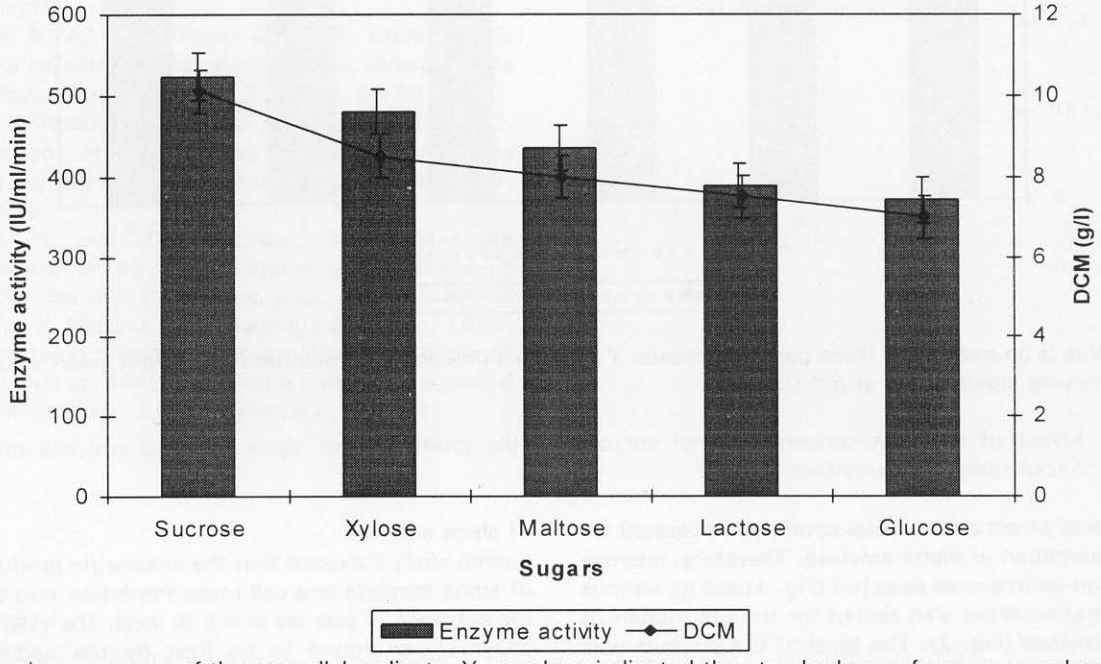
Cochrane (1980). Post Hoc Multiple Comparison tests were applied under one-way ANOVA. Significance has been presented in the form of probability ($p < 0.05$) values

Kinetic Study: Kinetic parameters for batch fermentation were determined according to the method describe by Pirt (1975) and Lawford and Rouseau (1993). The following parameters of kinetics were studied

1. The value of μ (h^{-1}) was calculated from plot of ln (x) vs time of fermentation.
2. Product yield co efficient namely Yp/x was determined by the equation: $Yp/x = dP/dx$
3. Qp (IU/ml /min) was determined from the maximum slope of enzyme produced vs time of fermentation.
4. Qx (g cell mass /l/h) was determined from the maximum slope of cell mass formation vs time of fermentation.
5. Specific rate of product formation was determined by the equation $q_p = \mu \times Y\ p/x$

Results and Discussion

The fermentation of alpha amylase is greatly effected by the addition of different carbon sources (Dubey *et al.*, 2000; Carlesen and Nielsen 2001). The carbon sources affected not only the mode of amylase formation but also the velocity with which the carbohydrates metabolized. In present study the effect of different carbon sources such as Lactose, glucose, maltose, xylose and sucrose were tested for the production of alpha amylase (Fig.1). The sugars were added to the fermentation media at the level of 1%. Of all the sugars examined, sucrose gave maximum production of alpha amylase (523.8 IU $ml^{-1}min^{-1}$). The production of enzyme, however, was greatly inhibited with the addition of glucose (371 IU $ml^{-1}min^{-1}$). The production of the enzyme by the addition of sucrose to the fermentation media was found to be highly significant and varied significantly ($P<0.05$) with other sugars. Fairbairn *et al* (1986) and Nguyen *et al.* (2000) have reported starch was the best carbon source for alpha amylase production. However, in present study sucrose was found as best source. It might be due to



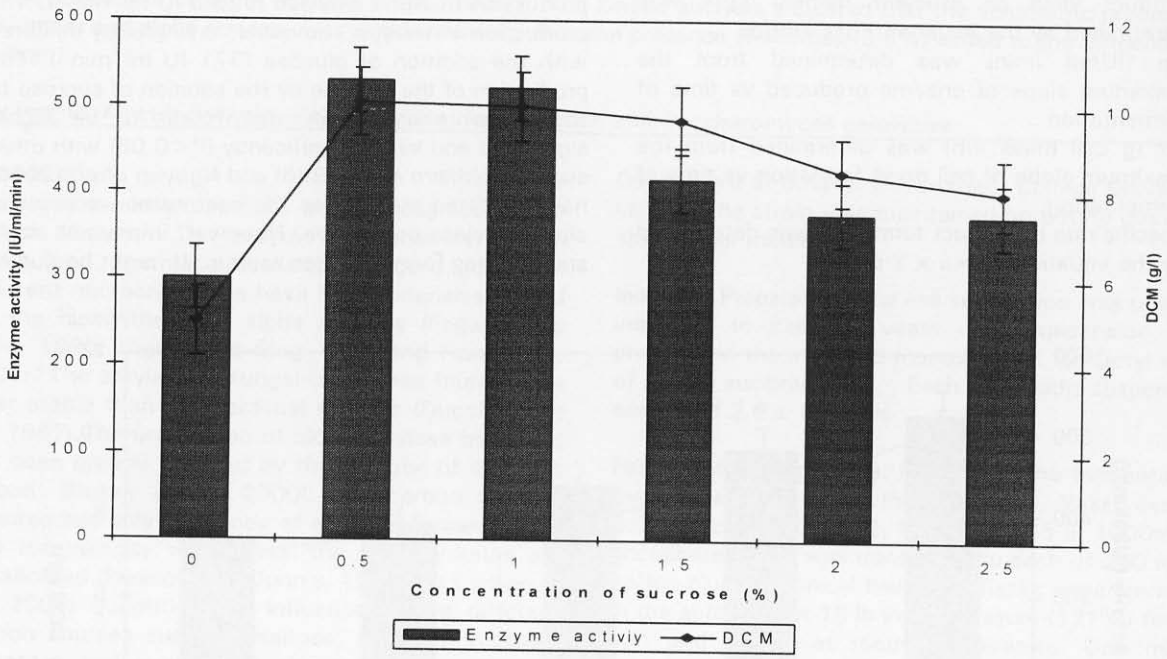
Each value is an average of three parallel replicate. Y error bars indicated the standerd error from mean value. The values in vary significantly at $p < 0.05$.

Fig. 1: Effect of replacement of starch by sugars on the production of alpha amylase by *Saccharomyces cerevisiae* GCB-20

Table 1: Kinetic parameteric study following growth of organism for effect of different concentration of sucrose on the production of alpha amylase by *Saccharomyces cerevisiae* GCB-20

Kinetic	Sucrose Concentrations (%)					
Parameters	0	0.5	1	1.5	2	2.5
Y_{px}	6000.0 ± 2	52475.2 ± 23	52100 ± 20	42886.5 ± 25	44235.2 ± 20	46750 ± 23
Q_p	5.0 ± 1.5	8.83 ± 2.6	8.68 ± 3.2	6.93 ± 2.1	6.26 ± 5	6.23 ± 4.1
Q_x	0.083 ± 0.06	0.168 ± 0.5	0.166 ± 1.4	0.161 ± . 1	0.141 ± 0.9	0.133 ± 0.08
q_p	1200.0 ± 5	10495.0 ± 25	10420 ± 20	8577.3 ± 16	8847.0 ± 15	9350.0 ± 21

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



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 $p < 0.05$.

Fig. 2: Effect of different concentration of sucrose on the production of alpha amylase and cell mass by *Saccharomyces cerevisiae* GCB-20

the type of strain and cultural condition necessary for the fermentation of alpha amylase. Therefore, sucrose as carbon source was selected (Fig. 1)and its various concentration were also tested for the production of alpha amylase (Fig. 2). The level of the sucrose was kept at 0.5-2.5%. The sucrose at the level of 0.5% was found to be the best for the production of alpha amylase (530 $IUml^{-1}min^{-1}$) following growth of organism. Further increase in the level of sucrose resulted decrease in the production of the enzyme. At 2.5 % level of sucrose the production of the enzyme was greatly inhibited. Thus, the addition of 0.5% level of sucrose was found to be optimal for the production of alpha amylase

Kinetic study indicated that the volumetric productivity of alpha amylase and cell mass formation was best in the presence of sucrose at 0.5 % level. The yield of the enzyme was found to be best by the addition of sucrose (0.5%) and varied significantly than other concentrations of sucrose. Thus, the addition of sucrose at the level of 0.5 % was selected as carbon source for the maximum accumulation of alpha amylase in the fermentation medium.

Conclusion

From the present study it was concluded that the

supplementation of sucrose as carbon source to the fermentation medium resulted the maximum accumulation of alpha amylase in the fermentation medium.

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