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Temperature Optima for Invertase Secretion by Yeast in Synthetic Medium

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ABSTRACT

Present investigation deals with optimisation of appropriate temperature both for growth of yeast and invertase production. Maximal enzyme production (16.10 U ml^{-1}) was observed at 300°C incubation temperature. Data has also been subjected to kinetics analysis and on the basis of kinetic parameters such as $Y_{p/x}$ (amount of enzyme produced mg^{-1} cell mass), $Y_{p/s}$ (amount of enzyme produced mg^{-1} sugar consumed), $Y_{x/s}$ (mg cells mg^{-1} substrate consumed), $Y_{s/x}$ (mg sugar consumed mg^{-1} cell mass produced), qp (Amount of enzyme produced mg^{-1} sugar consumed h^{-1}), qs (mg mg^{-1} cells h^{-1}), qx (mg cells mg^{-1} sugar consumed h^{-1}), μ (mg cells produced h^{-1}), it was found that temperature had a direct influence both on substrate consumption and synthesis of enzyme.

Keywords: Yeast, Invertase, Temperature, Optimisation

Introduction

Microbial invertase (EC 3.2.1.26) with transfructosylating activity can catalyse the transfructosylation of sucrose and synthesize fructooligosaccharides (Su *et al.*, 1991). Monosaccharides produced by microbial enzyme hydrolysis are more preferred because of high functionalities like similar taste as of sucrose and good bulking properties. Growth conditions have a great influence on invertase production capacity of *Saccharomyces cerevisiae*. The production of the extracellular invertase shows a cyclic behaviour that coincides with the budding cycle. The invertase activity increases during bud development and ceases at bud maturation and cell scission (Rouwenhorst *et al.*, 1991). Fructose and glucose are competitive and non-competitive inhibitors of the enzyme, respectively. Percentage of these monosaccharides varies with cultural conditions (Sayago *et al.*, 2002). Thus appropriate conditions for yeast growth are directly related with invertase secretion in the medium. Growth kinetics of *Saccharomyces cerevisiae* in glucose syrup from cassava starch and sugarcane molasses were studied using batch and fed-batch cultivation. The optimum temperature and pH required for growth were 30°C and pH 5.5, respectively (Win *et al.*, 1996). Present work deals with optimisation of appropriate incubation temperature both for yeast growth and invertase secretion in medium.

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Materials and Methods

Organism and culture media

*Saccharomyces cer*evisiae KR_{18}, isolated locally at G.C. University, Lahore, Pakistan, was used for invertase production by submerged fermentation. Yeast culture was maintained on sucrose-yeast extract-peptone-agar medium (Sucrose 20.0 gl^{-1}, Peptone 5.0 gl^{-1}, Yeast extract 3.0 g l^{-1} and Agar 20.0 g l^{-1}) at initial pH 6.0.

Vegetative inoculum and fermentation

Cell suspension was prepared from 2-3 days old slant culture of yeast strain. Twenty-five ml of the medium containing gl^{-1}, (wv^{-1}) sucrose 30.0; peptone 5.0 and yeast extract 3.0 at pH 6, was transferred to each 250 ml Erlenmeyer flask. The flasks were cotton plugged and autoclaved at 15 lbs/$inch^2$ pressure (121°C) for 15 minutes and cooled at room temperature. One ml of cell suspension (1.2×10^3 cells) from the slant culture was aseptically transferred into the growth medium. The flask was incubated at 30°C in an incubator (Gallenkamp, UK) and shaken at 200 rpm for 12 h. One ml of vegetative inoculum was transferred to the production medium, same as used for growth medium. Flasks were then incubated in a rotary incubator shaker (SANYO Gallenkamp PLC, UK) at 30°C for 48 hours. The agitation rate was kept at 200 rev min^{-1}. The flasks were run parallel in duplicates.

Assay protocol

Dry cell mass of yeast was determined by centrifugation of fermented broth at 5000 rev min^{-1}. The tubes were oven dried at 105°C for one hour. Supernatant was used for further analysis. Sugar was estimated spectrophotometrically by DNS method at 546 nm (Miller, 1959). Invertase activity (saccharolytic) in supernatant was assayed as described by Sumner and Howell (1935) based on dinitrosalicylic acid method test for reducing sugar determination: One invertase unit is defined as the amount of enzyme, which releases one milligram of inverted sugar in 5 minutes at 20°C, at pH 4.5.

Kinetic study & Statistical analysis

Kinetic parameters for batch fermentation were determined after Pirt (1975). Treatment effects were compared by the method of Snedecor & Cochrane (1980). Significance has been presented as Duncan multiple range tests in the form of probability (*P*) values.

Results and Discussion

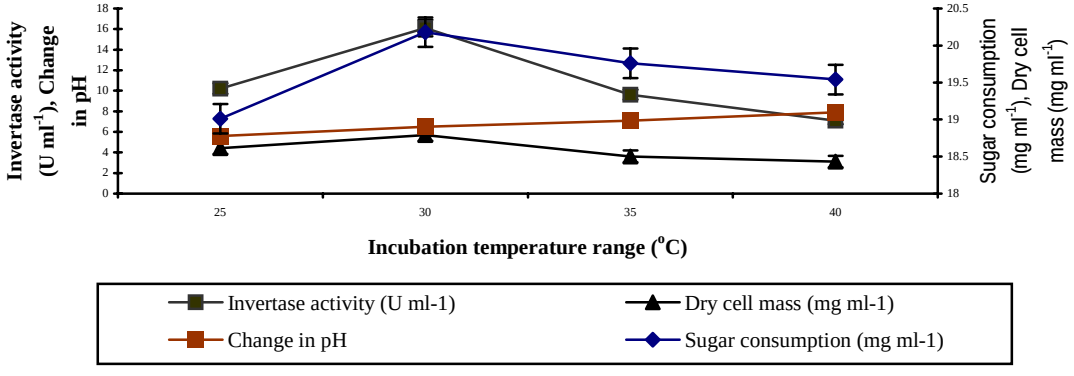
Effect of incubation temperature

Incubation temperature is one of the critical factors that have a profound effect on the production of invertase. Effect of different incubation temperatures (25-40°C) was studied (Figure 1). When temperature of fermentation medium was kept at 25°C, total invertase activity was 10.20 U ml^{-1}. Maximum production of invertase (16.10 U ml^{-1}) was obtained when incubation temperature was maintained at 30°C. The dry cell mass and sugar consumption were 5.68 and 20.18 mg ml^{-1}, respectively. Final pH of medium was 6.5. Further increase in incubation temperature resulted in marked decline in invertase production. Values of all kinetic parameters were highly significant.

Below 25°C, enzyme production was non-significant which might be due to fact that the temperature was not suitable for yeast growth. At high temperature, less enzyme production was probably due to thermal inactivation of yeast cells as well as enzyme. Catabolite repression induced by high temperature resulted in less production of invertase (Mizunaga *et al.*, 1981; Nam *et al.*, 1997). It might also be due to that at high temperature the moisture contents of the fermentation medium were reduced as moisture is essential for the fermentation of invertase by the yeast. Similar temperature, optimum for invertase production, was also reported by previous workers (Park and Sato, 1982; Vrabel *et al.*, 1997). Abrahao-Neto *et al.* (1996) studied effects of pH (4.0, 4.5, or 5.0), temperature (T) (30, 35, or 40°C) and dissolved oxygen (0.2, 2.0, 4.0, or 6.0 mg O_2/L) on invertase formation by yeast.

The kinetic parametric study (Table 1) indicated that the production of at 30°C was significant as compared to other incubation temperatures. Values of growth yield coefficients and rate constants were highly encouraging at this variable. Values of specific product rates were significant at 25°C incubation temperature, while values of substrate consumption in relation to cell mass formation were found to be slightly constant at high temperature. Table 1 also depicts the comparison of specific rates of product and cell mass formation under different temperatures. Values of each parameter were significant when fermentation was preceded at 30°C temperature. Thus the optimized temperature was favourable both for yeast growth and enzyme secretion by yeast. Fermentation process with high incubation temperature requires thermo-tolerant strain, otherwise thermal denaturation of cells and product is induced (Vrabel *et al.*, 1997).

Figure 1: Production of invertase by *Saccharomyces cerevisia*e KR_{18} influenced under different incubation temperatures (°C).



Sucrose concentration, 25 g l^{-1}; incubation period, 48 hours; initial pH, 6.0; agitation rate, 200 rev min^{-1}.

Table 1: Comparison of kinetic parameters for invertase production by *Saccharomyces cerevisia*e KR_{18}.

Kinetic parameters	Incubation temperature range (°C)			
	25°C	30°C	35°C	40°C
Product and Growth yield coefficients				
$Y_{p/x}$	2.302±0.02	2.834±0.03	2.657±0.01	2.287±0.02
$Y_{p/s}$	0.536±0.01	0.797±0.01	0.486±0.04	0.362±0.04
$Y_{x/s}$	0.233±0.04	0.281±0.02	0.183±0.03	0.158±0.04
$Y_{s/x}$	4.291±0.02	3.552±0.02	5.458±0.03	5.301±0.03
Specific rate constants				
q_p	0.211±0.01	0.334±0.02	0.199±0.03	0.146±0.02
q_s	0.394±0.01	0.419±0.02	0.410±0.01	0.403±0.02
q_x	0.021±0.03	0.033±0.04	0.013±0.03	0.010±0.01
μ (h^{-1})	0.092±0.02	0.118±0.01	0.075±0.02	0.064±0.04

Each value is the mean of three replicates. ± Indicates standard deviation among replicates. The values differ significantly by $P<0.05$. $Y_{p/x}$ (amount of enzyme produced mg^{-1} cell mass), $Y_{p/s}$ (amount of enzyme produced mg^{-1} sugar consumed), $Y_{x/s}$ (mg cells mg^{-1} substrate consumed), $Y_{s/x}$ (mg sugar consumed mg^{-1} cells mass), q_p (Amount of enzyme

produced mg^{-1} sugar consumed h^{-1}), q_s (mg mg^{-1} cells h^{-1}), q_x (mg cells mg^{-1} sugar consumed h^{-1}), μ (mg cells produced h^{-1})

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