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Optimization of Assay Conditions and Inoculum Size with Kinetic Analysis for Biosynthesis of Invertase by *Saccharomyces cerevisiae* GCB-K5

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Abstract: The present study concerns with the optimization of inoculum level for invertase production by *Saccharomyces cerevisiae* GCB-K5. The thermal inactivation of yeast invertase and effect of pH, utilizing the measurements of enzyme activity, was also estimated. Nutritional studies were carried out in 250 ml Erlenmeyer flasks by submerged fermentation. The fermentation medium (pH 6.0) was kept at 30°C for 48 h after inoculation and agitation rate was 200 rpm. Enzyme production was reached maximum (7.1 U ml^{-1}) at 4% inoculum (v v^{-1}). Sugar consumption and dry cell mass were 21.13 and 5.93 mg ml^{-1}, respectively. Comparison of product and growth yield coefficients was also significant for invertase biosynthesis.

Key words: Assay conditions, inoculum, invertase, *Saccharomyces cerevisiae*

Introduction

Baker's yeast invertase catalyses transfructosylation reactions in aqueous and anhydrous organic media with sucrose as a substrate, leading to release of D-glucose and D-fructose (Farine ***et al.***, 2001). High fructose syrup containing fructose and glucose in roughly equal proportions is greater in demand than pure glucose as food and drink sweeteners, because fructose is sweeter than glucose. Thus commercial value of inverted syrup is greater.

There exists a strong correlation among inoculum amount and substrate concentration in the context of invertase production by *Saccharomyces cerevisiae* (Shafiq ***et al.***, 2002). The critical problem in the identification of enzyme inactivation is to properly relate the phenomenon of the loss of enzyme activity with the undergoing changes in the structure of enzyme molecule (Maiko ***et al.***, 2000). Invertase exhibits marked stability towards temperature, pH changes and denaturants. Temperature of the reaction mixture determines the rate of sucrose inversion by the active enzyme (Vrábel ***et al.***, 1997).

Materials and Methods

Organism and inoculum preparation

Yeast strain *Saccharomyces cerevisiae* GCB-K5 was used for the production of invertase.

Inoculum was prepared from 2-3 days old slant culture of Saccharomyces cerevisiae GCB-K5. The number of cells (1.0×107 cells ml^{-1}) was counted with the help of Haemocytometer Slide Bridge.

Fermentation conditions

Production of yeast invertase was studied by shake flask technique using 250 ml Erlenmeyer flasks. Twenty-five ml of fermentation medium (sucrose 30.0 gl^{-1}, peptone 5.0 gl^{-1} and yeast extract 3.0 gl^{-1}) was transferred to each cotton wool plugged Erlenmeyer flask. After sterilization at 15 lbs per $inch^2$ pressure (121°C) for 15 minutes, inoculum was aseptically transferred to each flask. Flasks were then rotated in a rotary incubator shaker (Model: GLSC 051.HR.196-11) at 30°C for 48 h. The agitation rate was kept at 200 rev/min.

Assay methods

"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". Reducing-sugar-releasing activity was assayed with sucrose as substrate by measuring the amount of reducing sugar released by the dinitrosalicylic acid method (Sumner and Howell, 1935).

Dry cell mass

Dry cell mass of yeast was determined by centrifugation of fermented broth at 5000 rev. min^{-1}. using weighed centrifuge tubes.

Sugar

Sugar was estimated spectrophotometrically by DNS method (Tasun ***et al.***, 1970) using Scanning Spectrophotometer (CECIL CE-7200, UK) for measuring color intensity. Transmittance was measured at 546 nm using Scanning Spectrophotometer.

Results and Discussion

Effect of inoculum size

Inoculum level for optimal production of invertase by ***Saccharomyces cerevisiae*** GCB-K5 was worked out (Fig. 1). Enzyme production reached maximum (7.1 U ml^{-1}) when the level of inoculum was kept at 1.0 ml per 25 ml of basal medium. At low concentration, the number of yeast cells was not well enough to utilize essential amount of substrate to produce enzyme. At high concentrations of inoculum, viscosity of fermentation medium increased due to tremendous growth of yeast resulting in nutritional imbalance in medium (Shafiq ***et al.***, 2002). The work was in accordance with that of Haq ***et al.*** (2002).

The kinetic parameters were also calculated for inoculum level (0.5-3.5 ml per 25 ml of basal medium) and the course of reaction was predicted (Pilgrim ***et al.***, 2001). The product yield coefficient values $Y_{p/x}$, $Y_{p/s}$ (enzyme produced mg^{-1} of substrate consumed or per mg of cell mass

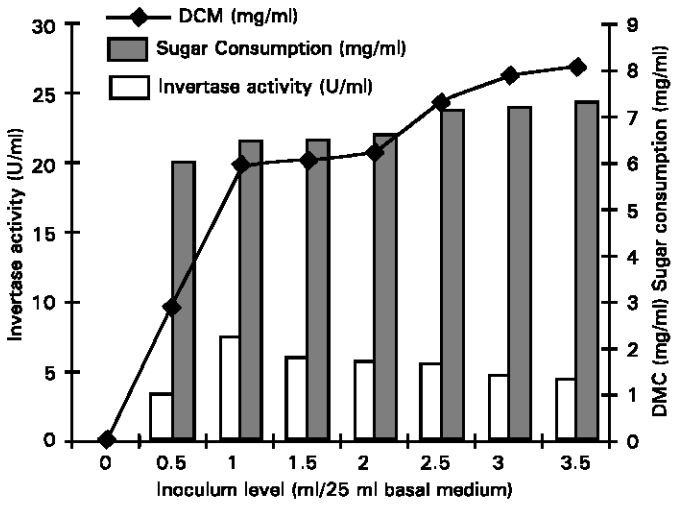

Fig. 1: Effect of inoculum size on the production of invertase by *Saccharomyces cerevisiae* GCB-K5. Sucrose concentration, 25 mg ml^{-1}; incubation period, 48 h; temperature, 30°C; initial pH, 6.0; agitation rate, 200 rev. min^{-1}. per 25 ml of basal medium

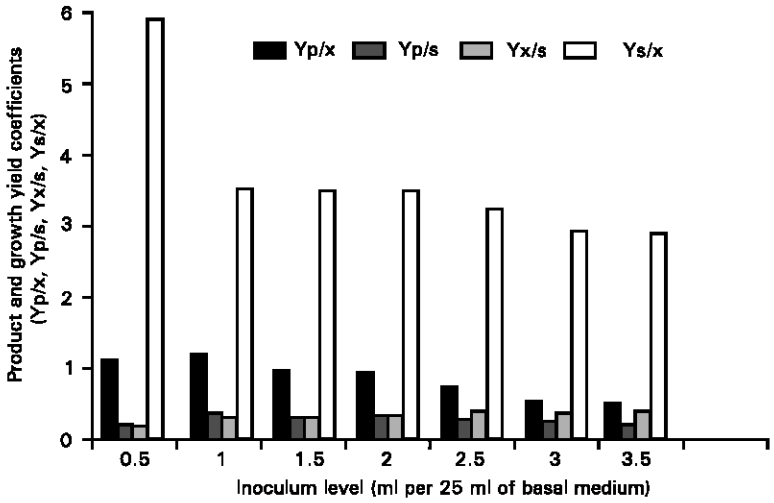

Fig. 2: Comparison of product and growth yield coefficients for invertase production by *Saccharomyces cerevisiae* GCB-K5. Kinetic parameters: $Y_{p/s}$ = Amount of enzyme produced mg^{-1} substrate consumed. $Y_{p/x}$ = Amount of enzyme produced mg^{-1} cell formed. $Y_{x/s}$= mg cell formed mg^{-1} substrate consumed. $Y_{s/x}$ = mg substrate consumed mg^{-1} cell formed

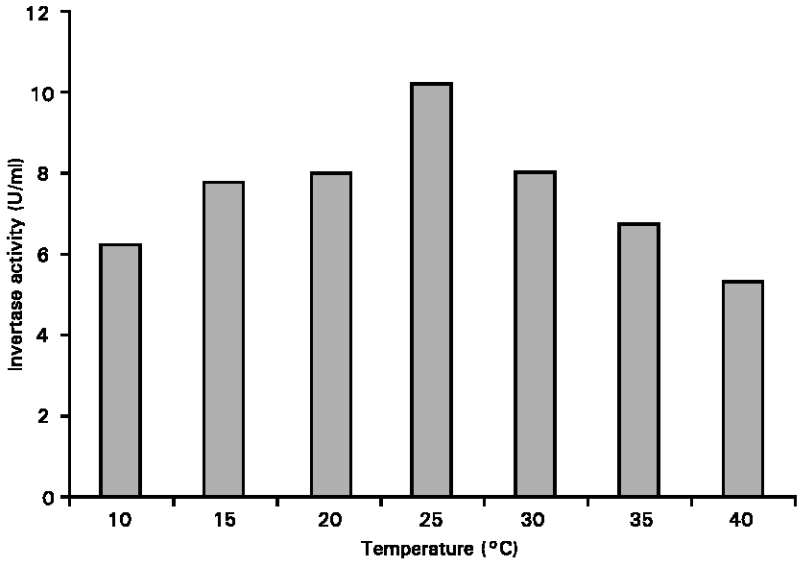

Fig. 3: Effect of temperature on invertase activity

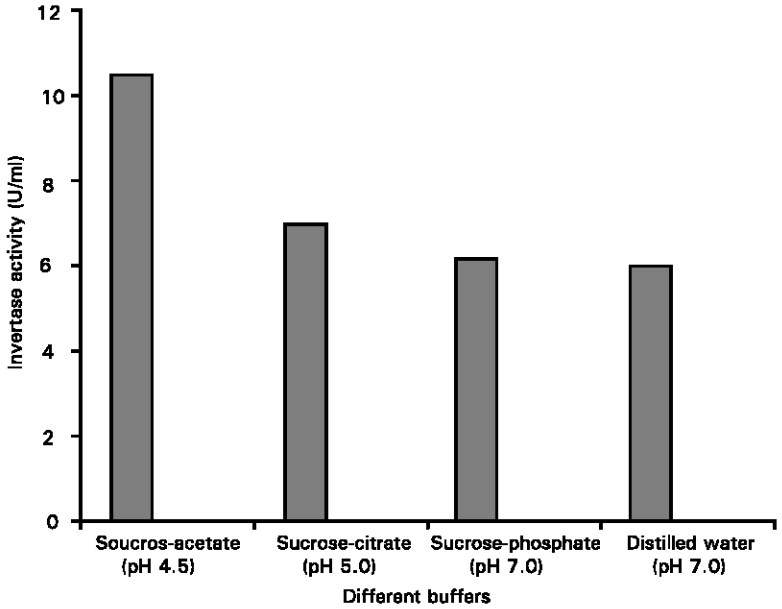

Fig. 4: Effect of buffers with different pH on invertase activity

produced) were also significant at 4% level of inoculum (Fig. 2). The growth yield coefficients $Y_{x/s}$, $Y_{s/x}$ (mg mg^{-1}) were calculated as the dry cell mass per mass of carbohydrate utilized and carbohydrate utilized per cell mass-produced, respectively.

Effect of temperature and buffers of different pH on invertase activity

The determination of the invertase activity of yeast cells presents a critical point, that is, the blockage of the enzyme action at a given moment (Vitolo ***et al.***, 1995). The behavior of the enzyme invertase, from baker's-yeast cells was examined under different conditions of temperature and buffer composition of reaction mixture. The reaction rate linearly increased with the applied temperature gradient (10-40°C), with reference to the average temperature (Fig. 3). Enzyme was most active when temperature of reaction mixture was kept at 25°C (10.2 U ml^{-1}). At high temperature, enzyme activity was not significant, because of high temperature denaturation of enzyme active site (Russo ***et al.***, 1996).

A range of buffers Sucrose-acetate (pH 4.5), Sucrose-phosphate (pH 7.0), Sucrose-citrate (pH 5.0) and Sucrose-distilled water (pH 7.0) was used to determine the effect of buffer type and pH on enzyme activity (Fig. 4). Maximal sucrose conversion activity of invertase (10.50 U ml^{-1}) was measured in sucrose-acetate buffer of pH 4.5. A marked decline in invertase activity (6.2 Um l^{-1}) was found in phosphate buffer (pH 7.0). It might be due to alkalinity induced by high pH of phosphate buffer (Ahmad ***et al.***, 2001). Enzyme is not stable towards alkaline conditions so the sucrose inversion efficiency is also affected in direct way (Balasundaram and Pandit, 2001).

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