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Optimization of Cultural Conditions for the Biosynthesis of β -fructofuranosidase by *Saccharomyces cerevisiae*

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Abstract: The enzyme β -fructofuranosidase attacks beta-D-fructofuranoside (raffinose, stachyose and sucrose) from the fructose end. *Saccharomyces* GCA-II was used for the production of β -fructofuranosidase by submerged fermentation. The effect of sucrose concentration and incubation time was worked out for maximal enzyme production. Sucrose at the level of 30.0 g l⁻¹ in fermentation medium was found to exert an inducible effect on enzyme synthesis by *Saccharomyces* strain after 48 h of inoculation. Enzyme production increased from 107.42 U ml⁻¹ to 118.58 U ml⁻¹ after optimization of the cultural conditions. Specific product rate and specific growth rate were also determined for the production of β -fructofuranosidase by *Saccharomyces* in shake flask.

Key words: β -fructofuranosidase, *Saccharomyces*, microbial production, specific product and growth rate, sucrose concentration

Introduction

β -fructofuranosidases (invertases) are enzymes that cleave α -1, 4 glucosidic linkage between α -D-glucose and β -D-fructose molecules of sucrose by hydrolysis producing glucose and fructose. β -fructofuranosidases are intracellular as well as extracellular enzymes. This enzyme is of particular interest since it appears to have a high hydrolytic activity in 1 M sucrose solution. This fact would make the enzymatic hydrolysis process economically efficient for syrup production using by-products with high salt and sugar contents such as sugar cane molasses (Rubio *et al.*, 2002). The enzyme has wide range of commercial applications. β -fructofuranosidase is used in the production of confectionary with liquid or soft centers (Belcarz *et al.*, 2002).

Different substrates can be used in submerged fermentation for the production of β -fructofuranosidase. Sucrose is designated as the best sole carbon source for β -fructofuranosidase production because the availability of glucose for yeast is dependent on sucrose hydrolysis by β -fructofuranosidase (Gomez *et al.*, 2000). Therefore, sucrose concentration is found to exert a great influence on β -fructofuranosidase biosynthesis (Kiran *et al.*, 2002). The optimum time of incubation for maximal enzyme production varies both with the organism and fermentation conditions. Appropriate incubation period provided for β -fructofuranosidase synthesis by yeast has been evaluated very critically. Incubation period more than optimum can cause feedback repression of the enzyme (Haq *et al.*, 2002).

This work describes the propagation of *Saccharomyces* species GCA-II for the production of extracellular enzyme β -fructofuranosidase and optimization of culture conditions. The effect of sucrose concentration along with incubation time was studied.

Materials and Methods

Organism: *Saccharomyces* species, isolated from dates (*Phoenix dactylifera*) cultured and maintained on the medium containing sucrose 20.0 g l⁻¹, agar 20.0 g l⁻¹, peptone 5.0 g l⁻¹ and yeast extract 3.0 g l⁻¹ at pH 6.0 (Dworschack and Wickerham, 1960) was used for production of β -fructofuranosidase.

Preparation of vegetative inoculum: Cell suspension was prepared from 2-3 days old slant culture of *Saccharomyces* species. Twenty-five ml of medium for vegetative propagation containing (g l⁻¹ w/v) sucrose 30.0, peptone 5.0 and yeast extract 3.0 at pH 6 was transferred to each 250 ml Erlenmeyer flask. One ml of inoculum was aseptically transferred to each flask having sterilized medium. Flasks were then incubated in a rotary incubator shaker (SANYO Gallenkamp PLC, UK.) at 30°C for 24 h. The agitation rate was kept at 200 rev min⁻¹.

Fermentation technique: Production of β -fructofuranosidase was carried out by shake flask technique using 250 ml Erlenmeyer flasks. Same medium composition was used for vegetative inoculum preparation and for fermentation. Twenty-five ml of

fermentation medium was transferred to each Erlenmeyer flask. One ml of vegetative inoculum (4% v/v) was added in each flask aseptically. Flasks were then incubated in a rotary incubator shaker (SANYO Gallenkamp PLC, UK) at 30°C for 48 h. The agitation rate was kept at 200 rev min⁻¹. The flasks were run parallel in duplicates.

Analytical methods

Dry cell mass: Dry cell mass of yeast was determined by centrifugation of fermented broth at 5000 rev min⁻¹ using weighed centrifuge tubes. The tubes were oven dried at 105°C for 1 h.

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

Beta-fructofuranosidase activity: Enzyme activity was determined according to the method of Sumner and Howell (1935). The specific growth and product rate, μ (h⁻¹) were calculated as dry cell mass/enzyme produced per h of fermentation period.

Results and Discussion

Effect of sucrose concentration: Effect of sucrose concentrations (20.0-40.0 g l⁻¹) on β -fructofuranosidase production by *Saccharomyces* species GCA-II was studied (Fig. 1). Maximum enzyme activity (118.50 U ml⁻¹) was found at sucrose concentration of 30.0 g l⁻¹. Dry cell mass and sugar consumption were 1.06 and 25.27 g l⁻¹, respectively. Kiran *et al.* (2002) obtained maximal enzyme activity from *Saccharomyces cerevisiae* at sucrose concentration of 25.0 g l⁻¹.

Sucrose concentration more than 30.0 g l⁻¹ causes an increase in sugar consumption and dry cell mass, however, there is no increase in β -fructofuranosidase production. The reason might be generation of higher concentration of inverted sugar in the medium resulting in glucose-induced repression of β -fructofuranosidase (Elorza *et al.*, 1977 and Vitolo *et al.*, 1995). At concentrations of sucrose less than 30.0 g l⁻¹, enzyme production is lesser than optimum. As sucrose is carbon source in the medium, lower concentrations might limit proper growth of yeast, resulting in less yield of β -fructofuranosidase (Myers *et al.*, 1997).

Rate of β -fructofuranosidase production: Fig. 2 shows the rate of β -fructofuranosidase production by *Saccharomyces* species GCA-II. Total incubation time was 72 h. Enzyme activity was estimated for different time intervals (8-72 h). Maximum β -fructofuranosidase production was observed at 48 h of incubation

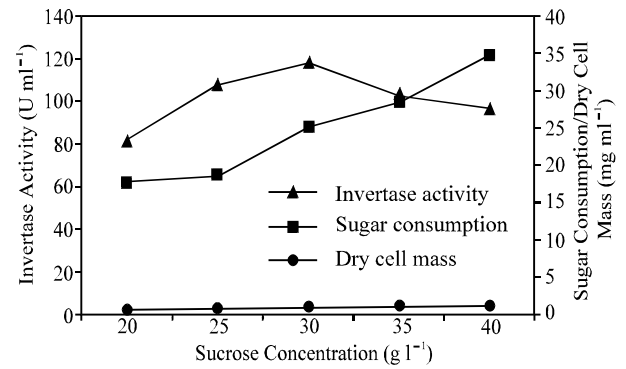


Fig. 1: Effect of Sucrose concentration on the production of β -fructofuranosidase

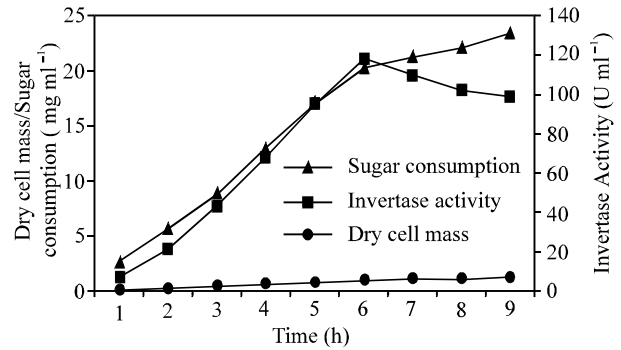


Fig. 2: Rate of β -fructofuranosidase production by *Saccharomyces* species

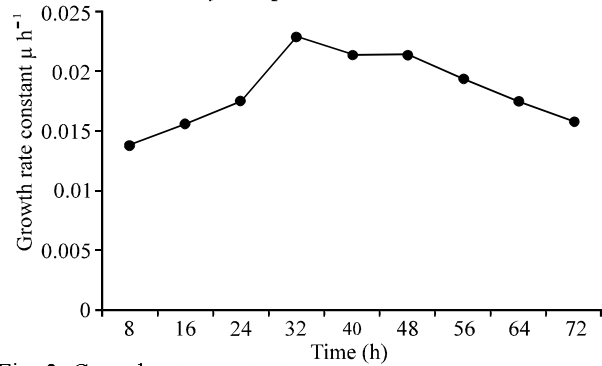


Fig. 3: Growth rate constant

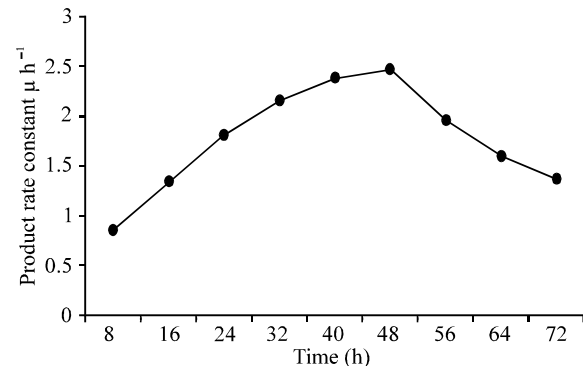


Fig. 4: Product rate constant

(118.35 U ml⁻¹). Dry cell mass and sugar consumption were 1.02 and 20.24 g l⁻¹, respectively. After 48 h of inoculation, specific growth and product rate were also in the favor of observed results (Fig. 3 and 4).

Further increase in incubation period did not enhance β -fructofuranosidase production. It might be due to decrease in amount of available nitrogen in fermentation medium, the age of organism, the presence of inhibitors produced by yeast itself and the depletion of sugar contents. In batch wise fermentation of enzyme, the production starts after a lag phase of 8 h and reaches maximum at the on set of stationery phase. Afterwards, enzyme activity declined either due to decrease in nutrients availability in the medium, or carbon catabolite repression of enzyme, as the expression of β -fructofuranosidase in *Saccharomyces* is checked by the presence of monosaccharides like glucose and fructose (Herwig *et al.*, 2001; Haq *et al.*, 2002).

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