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Predicting Vegetative Inoculum Performance to Maximize Invertase Production by *Saccharomyces cerevisiae* in Submerged Fermentation

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Abstract: The influence of the age and amount of the inoculum on invertase synthesis by *Saccharomyces cerevisiae* KR_{18} was studied during submerged cultivation. Fermentation was carried out at 30°C in fermentation medium containing sucrose, yeast extract and peptone at initial pH 6.0 at 200 rpm. The maximum production of invertase (6.9 U ml^{-1}) was obtained when 1.0 ml vegetative inoculum (4 %), developed for 12 h, was used per 25 ml of fermentation medium. The dry cell mass and sugar consumption were 3.0 and 16.18 mg ml^{-1}, respectively. The specific growth and product rates (h^{-1}) are more encouraging to use 4 % vegetative inoculum under optimized conditions.

Key words: Invertase, Inoculum age, *Saccharomyces cerevisiae*, Submerged fermentation, specific product, growth rate

Introduction

Invertase is enzyme that catalyzes the breakdown of sucrose, a disaccharide, into glucose and fructose, two monosaccharides (Rossi and Rocha-Leão, 2003). This equimolar mixture of fructose and glucose, known as inverted sugar, has applications in several industrial processes like confectionary industry, production of lactic acid and ethanol production from fermentation of cane sugar molasses (Acosta *et al.*, 2000).

Invertase is a high cost enzyme and its production is influenced by many factors. Inoculum quality and the influence of inoculum quality on invertase production are important factors which need in-depth investigation before scaling-up of high-yielding fermentation process. Number of yeast cells introduced in culture medium determines the extent and quality of enzyme produced (Prescott and Dunn's, 1987). So there exists a strong correlation between amount of inoculum and substrate concentration in context of invertase production by *Saccharomyces*. Neto *et al.* (1996) used 0.45-litre inoculum (0.70g l^{-1} dry cell mass) for invertase production. A significant increase in enzyme production was observed when 36-hour inoculum was used (Serova & Dobrolinskaya, 1975).

The present investigation deals with the effect of vegetative inoculum on submerged invertase production by *Saccharomyces cerevisiae* KR_{18} and its evaluation on kinetic basis. For this, sucrose was employed as the basal fermentation medium.

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 (g l^{-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 8-48 h. The agitation rate was kept at 200 $revmin^{-1}$. The vegetative inoculum was transferred (0.5-3.0 ml per 250 ml) 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 and kinetic analysis: 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 one hour. Supernatant was used for further analysis. Sugar was estimated spectrophotometrically by DNS method (Tasun *et al.*, 1970). A scanning UV/VIS spectrophotometer (Cecil-700, UK) was used for measuring % color intensity at 546 nm. 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. Kinetics of research work was studied after Pirt (1975).

Results and Discussion

Among the factors that determine morphology and the general course of yeast fermentations, the type and size of inoculum is of prime importance. Fig. 1 shows the effect of vegetative inoculum age for invertase production by *Saccharomyces cerevisiae* KR_{18}. It can be seen that after 12 h growth, vegetative cells were quite active to secrete optimal invertase in fermentation medium during 48 h fermentation while agitation speed was kept at 200 rpm. Maximum units of invertase i.e. 6.2 per ml of fermented broth were obtained under these conditions. Sugar consumption was 15.22 mg ml^{-1} while dry cell mass was 2.8 mg ml^{-1}. After 12 h, vegetative cells may be quite older to utilize available nutrients for the product secretion. Increase in number of cells often cause inhibition of production capacity because of catalytic action of yeast cells (Shafiq *et al.*, 2002). After 12 h growth in seed medium, number of yeast cells in one ml of inoculum was 1.2×10^3.

Fig. 2 shows the effect of inoculum size on invertase production by *Saccharomyces cerevisiae* KR_{18}. Inoculum size was varied from 0.5 ml to 3.0 ml per 25 ml of fermentation medium (2-12 % based on total working capacity of shake flask). Maximum invertase production (6.9 U ml^{-1}) was obtained with 1.0 ml of inoculum (4.0 %). At low concentration of vegetative inoculum, 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. Sugar consumption and dry cell mass were found to be 16.18 abd 3.0 mg ml^{-1}, respectively. The work was in accordance with that of Bokosa *et al.* (1992). Kinetic parameters studied i.e. specific product and growth rate (μ h^{-1}) were also in favor of inoculum at 4.0 % level. Further research work is required to reveal general trends in relation to morphology of the producer organism and its capacity to utilize nutrients more efficiently.

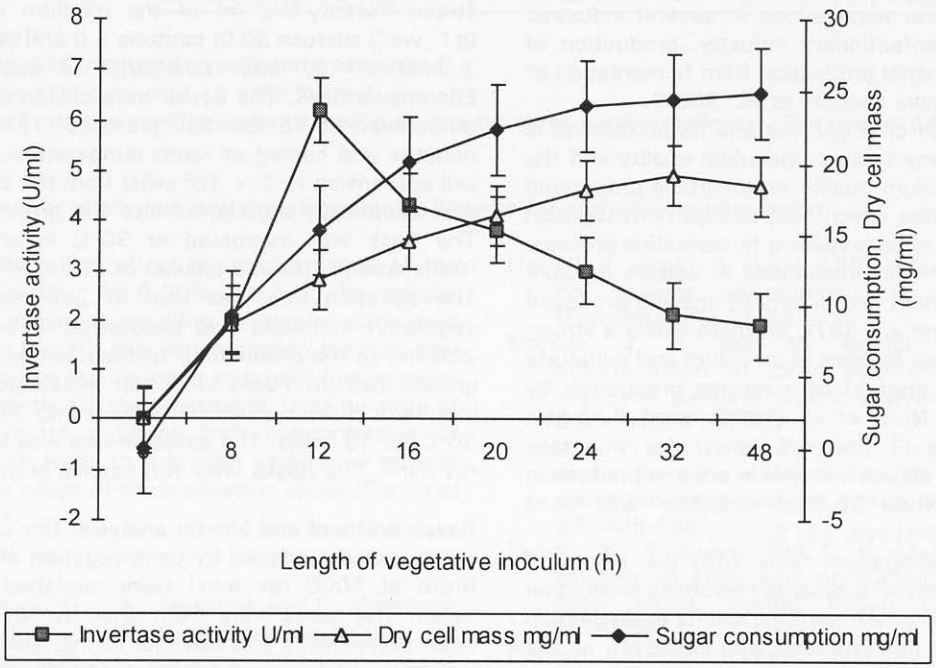

Fig. 1: Development of vegetative inoculum for optimal invertase production by *Saccharomyces cerevisiae* KR_{18}.

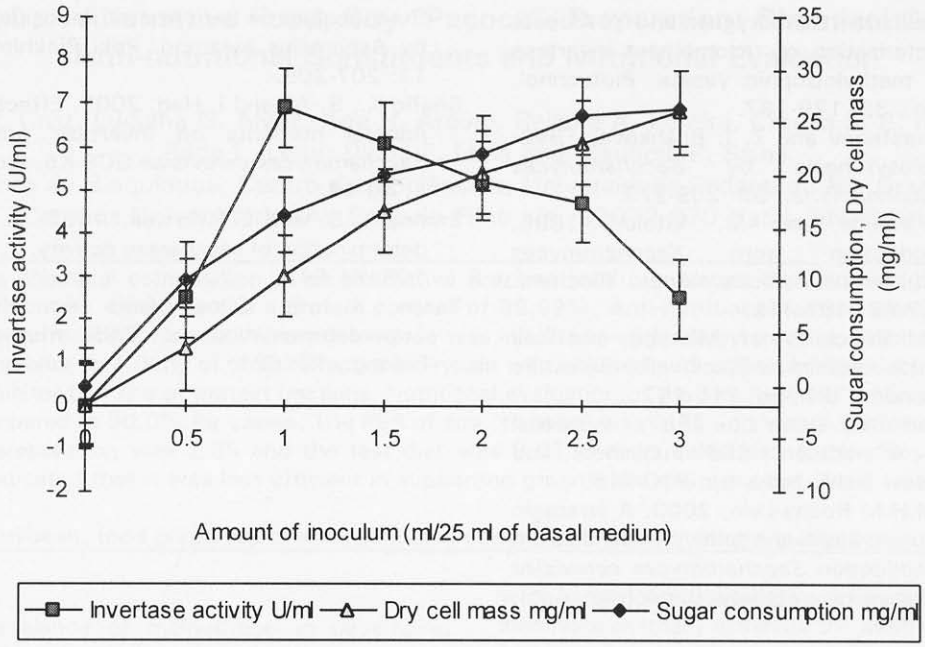

Fig. 2: Vegetative inoculum size for invertase production by *Saccharomyces cerevisiae* KR_{18}

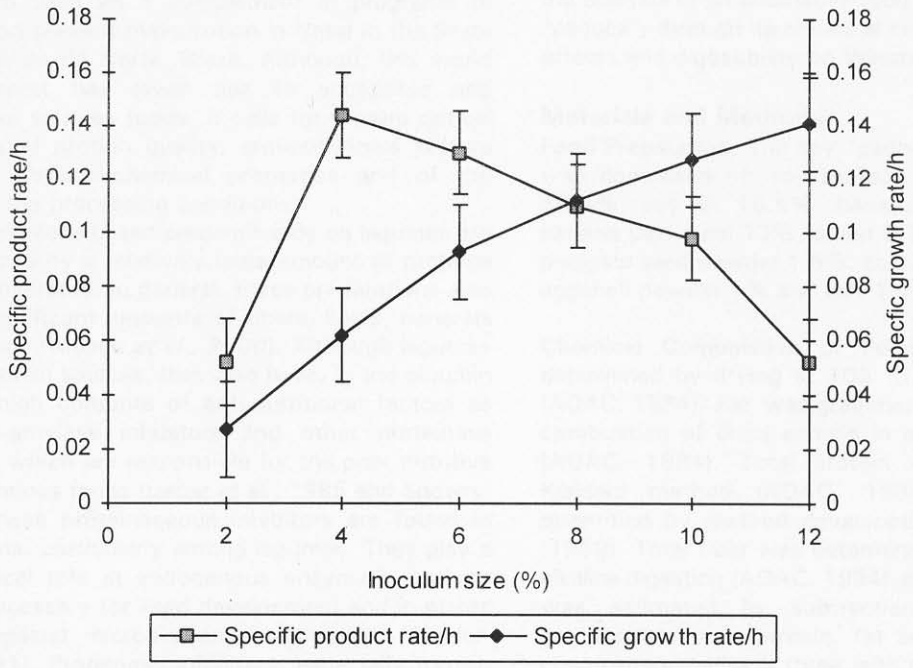

Fig. 3: Comparison of product and growth yield coefficients for invertase production by *Saccharomyces cerevisiae* KR_{18}.

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