

Time Course Study for Yeast Invertase Production by Submerged Fermentation

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Abstract: The present investigation deals with the time course study of three different of *Saccharomyces cerevisiae* (GCB-K5, GCA-II and KR₁₈) during invertase production by submerged fermentation. GCA-II was found to be the best producer of invertase (8.35 U ml⁻¹, 48 h after inoculation) having high production yield coefficients (i.e., $Y_{p/s}$ and $Y_{p/x}$). The product is a high cost product. Thus the use of optimized strain for invertase production is economically more feasible due to shorter incubation period and optimal production.

Key words: Time course, production, submerged fermentation

Introduction

Invertase is one of the most widely used industrial enzymes. Scaling up of Invertase production broadens the field of its application, since it prevents the crystallization of sugar in food products and the assimilation of alcohol in fortified wines (Mirzarakhmetova and Abdurazakova, 1998). The organism showing greatest ability to secrete invertase is yeast because of its characteristic high sucrose fermentability. Sucrose is designated as the best carbon source in medium because availability of glucose for yeast was dependent on sucrose hydrolysis by invertase (Koo *et al.*, 1998). Time course study is one of the most critical factors, which determines the efficacy of the process along with product formation. The pattern of accumulated reducing sugar after specific incubation time is characteristic to the each species (Matrai *et al.*, 2000). Maintenance of cell viability throughout the fermentation process is an important factor that depends on medium composition and incubation conditions (Laluce *et al.*, 1991). Yusa and Enokida (1953) worked on invertase of yeast. The amount of invertase in yeast was determined every 24 h by autolysis at 25°C and amount reached a maximum in 72 h. Optimal incubation time for yeast invertase production was observed as 48 h after inoculation (Haq *et al.*, 2002). The present investigation deals with the time course study for invertase production by different *Saccharomyces cerevisiae* cultures and their comparison on kinetic basis.

Materials and Methods

Organism: In the present study, 3 different strains of *Saccharomyces cerevisiae* were used. These strains, GCB-K5 (isolated from commercial yeast), GCA-II (isolated from dates) and KR₁₈ (isolated from soil) were maintained in Biotechnology Centre, G.C. University, Lahore. The cultures were stored at 4°C in a refrigerator.

Sterilization

All the culture media were sterilized in an autoclave at 15-lbs/inch² pressure (121°C) for 15-20 minutes.

Fermentation technique

Invertase production was carried out by submerged fermentation technique, following the method of Shafiq *et al.* (2002). 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. One ml of cell suspension (1.2×10^3 cells approx.) from the slant culture was aseptically transferred in sterilized growth medium. The flask was incubated at 30°C in an incubator (Gallenkamp, UK) and shaken at 200 rpm for 12 h. The agitation rate was kept at 200 rev min⁻¹. The vegetative inoculum was transferred (1.0 ml per 250 ml) to the production medium, same as used for growth medium. Flasks were then incubated under same conditions as for vegetative inoculum. The results are sum mean of three parallel replicates.

Analysis and comparison

Dry cell mass of yeast was determined by centrifugation of fermented broth at 5000 rev min⁻¹ using weighed centrifuge tubes. Supernatant was used for further analysis. Residual sugar was estimated by DNS method (Tasun *et al.*, 1970) while 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). A scanning spectrophotometer (Cecil-700 series, UK) was used for the determination of color intensity at 420-546 nm wavelengths. Invertase activity in fermented broth (U ml⁻¹), sugar consumption (mg ml⁻¹) and dry cell mass (mg ml⁻¹) were determined with reference to time course. Statistical analysis and kinetics of time course was also under taken for data comparison (Pirt, 1975).

Results and Discussion

During growth of *Saccharomyces cerevisiae*, sucrose in the medium is hydrolyzed to glucose and fructose, suggesting the formation of an extracellular invertase. The invertase formation is repressed by the presence of glucose in the medium after hydrolysis by yeast as fermentation proceeds. Thus, fermentation time is a critical factor to define yeast's capacity to secrete optimal amount of product while not being repressed by sugar accumulation. Regular fermentation changes in the activity of the enzyme invertase of three different strains of yeast *Saccharomyces cerevisiae* were examined. Table 1 shows the synthesis of invertase at different intervals of time. Yeast cultures GCB-K5, GCA-II and KR₁₈ were incubated at 30°C for 8-96 h. Maximum production (8.35 U ml⁻¹) was obtained after 48 h of inoculation with GCA-II. Further increase in the incubation period did not enhance the production of invertase rather it was decreased. Sugar consumption and dry cell mass at this level were 20.24 and 3.02 mg ml⁻¹, respectively. KR₁₈ strain also gave enhanced units of invertase (7.53 U ml⁻¹) but not feasible due to prolonged incubation time i.e. 72 h after inoculation. Sugar consumption and cell mass for this

Table 1: Time course study during invertase synthesis by *Saccharomyces cerevisiae*

| Incubation period (h) | Dry cell mass (mg ml ⁻¹) | | | Sugar consumption (mg ml ⁻¹) | | | Invertase activity (U ml ⁻¹) | | |
|-----------------------|--------------------------------------|--------|------------------|--|--------|------------------|--|--------|------------------|
| | GCB-K5 | GCA-II | KR ₁₈ | GCB-K5 | GCA-II | KR ₁₈ | GCB-K5 | GCA-II | KR ₁₈ |
| 8 | 1.03 | 1.21 | 1.00 | 10.67 | 8.64 | 8.50 | 0.80 | 1.28 | 0.67 |
| 16 | 1.50 | 1.45 | 1.50 | 13.06 | 9.64 | 9.5 | 2.34 | 1.92 | 1.26 |
| 24 | 2.11 | 1.62 | 2.51 | 16.33 | 10.82 | 11.20 | 3.76 | 2.87 | 2.50 |
| 32 | 3.14 | 1.93 | 3.52 | 17.75 | 12.86 | 14.03 | 4.98 | 3.51 | 3.80 |
| 40 | 4.02 | 2.15 | 3.62 | 18.64 | 16.95 | 14.70 | 5.51 | 5.67 | 4.04 |
| 48 | 4.62 | 3.02 | 3.91 | 20.01 | 20.24 | 15.55 | 6.88 | 8.35 | 5.55 |
| 56 | 4.89 | 4.08 | 4.20 | 21.22 | 21.32 | 17.0 | 5.23 | 6.56 | 6.22 |
| 64 | 5.02 | 4.72 | 4.50 | 23.01 | 22.16 | 19.6 | 4.55 | 5.05 | 6.90 |
| 72 | 5.23 | 5.14 | 4.80 | 23.35 | 23.34 | 20.20 | 4.21 | 4.43 | 7.53 |
| 80 | 5.3 | 5.01 | 5.32 | 23.40 | 23.30 | 21.5 | 4.0 | 4.01 | 6.62 |
| 88 | 5.32 | 4.93 | 5.50 | 23.42 | 23.25 | 22.5 | 3.5 | 3.92 | 5.40 |
| 96 | 5.35 | 4.85 | 5.45 | 23.45 | 23.23 | 22.8 | 3.2 | 3.5 | 5.23 |

Sugar concentration 30 mg ml⁻¹, Initial pH 6.0, Temperature 30° C

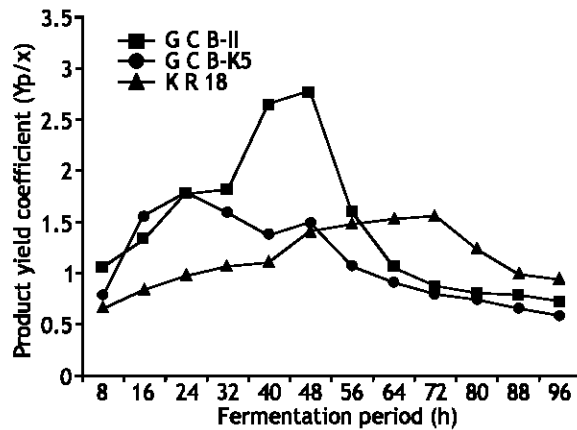


Fig. 1: Comparative study of product yield coefficient ($Y_{p/x}$) during invertase fermentation by *Saccharomyces cerevisiae* strains (GCB-K5, GCA-II and KR₁₈). The value of $Y_{p/x}$ was determined by product (U ml⁻¹) / cell mass produced (mg ml⁻¹)

strain were 20.20 and 4.80 mg ml⁻¹, respectively. GCB-K5 gave 6.88 units of invertase per ml of fermented broth after 48 h. Production of invertase in shake flask started after a lag phase of less than 8 hours and reached maximum at the onset of stationary or late exponential phase. Further increase in incubation period gave less invertase production. It might be due to decrease in the availability of nutrients in medium, budding capacity of yeast or depletion of sugar contents (Vitolo *et al.*, 1995).

The maximum activity of the enzymes occurs in the stationary phase of the yeast growth (Abdurazakova and Salomov, 1975). The secretion of this invertase requires protein synthesis, but was found to be independent of RNA formation. The level of mRNA accumulated and translated is inversely proportional to the glucose present in the growth medium (Mormeneo and Sentandreu, 1982). The kinetic time course study of invertase fermentation by different strains

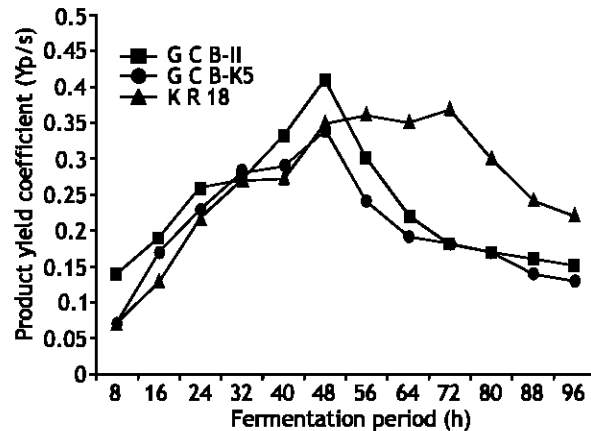


Fig. 2: Comparative study of product yield coefficient ($Y_{p/s}$) during invertase fermentation by *Saccharomyces cerevisiae* strains (GCB-K5, GCA-II and KR₁₈). The value of $Y_{p/s}$ was determined by product ($U\ ml^{-1}$) / substrate utilized ($mg\ ml^{-1}$)

of *Saccharomyces cerevisiae* was also worked out (Fig. 1 and 2). There was a marked difference of product yield coefficients ($Y_{p/s}$ and $Y_{p/x}$) among different strains i.e., maximum $Y_{p/s}$ value in case of GCA-II was much higher as compared to GCB-K5 and KR18 and same is the case with values of $Y_{p/x}$. So, GCA-II is better producer of invertase enzyme as compared to others. It might be due to its isolation from dates. This strain was obtained from dates having natural sucrose contents, thus invertase-producing capacity of this strain is more pronounced than others.

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