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INVERTASE PRODUCTION FROM A HYPERPRODUCING *SACCHAROMYCES CEREVISIAE* STRAIN ISOLATED FROM DATES

IKRAM-UL-HAQ AND SIKANDER ALI

Department of Botany,
Biotechnology Research Centre,
Government College University, Lahore, Pakistan

Abstract

Invertase production by wild cultures of *Saccharomyces cerevisiae* isolated from dates available in local market is reported. Five hyperproducing yeast strains (>100 fold higher invertase activity) were kinetically analyzed for invertase production. *Saccharomyces cerevisiae* strain GCA-II was found to be a better invertase yielding strain among all other isolates. The values of Q_p and $Y_{p/s}$ for GCA-II were highly significant as compared to other *Saccharomyces* cultures. The effect of sucrose concentration, rate of invertase synthesis, initial pH of fermentation medium and different organic nitrogen sources on the production of invertase under submerged culture conditions was investigated. Optimum concentrations of sucrose, urea and pH were 3 %, 0.2 % (w/v), and 6.0 respectively. The increase in the enzyme yield obtained after optimization of the cultural conditions was 47.7 %.

Introduction

Invertases are intracellular as well as extracellular enzymes (Nakano *et al*., 2000). The enzyme has wide range of commercial applications e.g., the production of confectionary with liquid or soft centers. It also aids fermentation of cane molasses into ethanol. Microbial invertase activity is used for the manufacture of calf feed and food for honeybees (Weber & Roitsch, 2000; Sanchez *et al.*, 2001). Many organisms produce invertase such as *Neurospora crassa*, *Candida utilis, Fusarium oxysporium*, *Phytophthora meganosperma, Aspergillus niger*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Schwanniomyces occidentalis* (Silveira *et al*., 2000). *Saccharomyces cerevisiae* is the organism of choice for invertase production because of its characteristic high sucrose fermentability.

Sucrose is considered to be the best sole carbon source for invertase production as the availability of glucose for yeast is dependent on sucrose hydrolysis by invertase. Therefore, sucrose concentration markedly influences invertase biosynthesis. Appropriate incubation period is of critical importance for invertase synthesis as longer incubation can cause feedback repression of the enzyme (Gomez *et al*., 2000; Vrabel *et al.*, 1997). In the present study we report the isolation of *Saccharomyces cerevisiae* for the production of invertase and kinetic analysis of shake flask fermentation. Five strains of *S. cerevisiae* were isolated from dates (*Phoenix dactylifera*) and tested for invertase activity. The effect of sucrose concentration, incubation period, initial pH and different nitrogen sources was studied.

Materials and Methods

Saccharomyces cerevisiae was used for the production of invertase in the present study. The organism was isolated from dates (fruit of date palm, *Phoenix dactylifera)*, cultured and maintained on the medium containing g/l sucrose 20.0; agar 20.0; peptone 5.0 and yeast extract 3.0 at pH 6.0 (Dworschack & Wickerham, 1960). The cultures were stored at 4 °C. Cell suspension was prepared from 2-3 days old slant culture of *S. cerevisiae*. Twenty-five ml of seed medium was transferred to each 250 ml Erlenmeyer flask. The medium consisted of (g /l w/v) sucrose 30.0; peptone 5.0 and yeast extract 3.0 at pH 6 unless stated otherwise. The flasks were cotton plugged and autoclaved at 103.5 Pa pressure (121°C) for 15 minutes and cooled at room temperature. One ml of inoculum was transferred to each flask under sterile conditions. Flasks were then incubated in a rotary incubator shaker (SANYO Gallenkamp PLC, UK.) at 30°C for 24 h. Agitation rate was kept at 200 rev/min.

Production of invertase 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. The cotton-plugged flasks were autoclaved at 103.5 Pa pressure for 15 minutes and cooled at room temperature. One ml of vegetative inoculum was aseptically transferred to each flask; dry cell mass content of vegetative inoculum was 0.45g/l. 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.

Dry cell mass of yeast was determined by centrifugation of fermented broth in centrifuge at 5000 rev/min using weighed centrifuge tubes. The tubes were oven dried at 105°C for 2 h in an oven (Model: 1442A, Memmert, Germany). Sugar was estimated by DNS method (Tasun *et al*., 1970) using double beam UV/Vis scanning spectrophotometer for measuring colour intensity. Transmittance was measured at 546 nm using Scanning Spectrophotometer. Enzyme activity was determined according to the method of Sumner & Howell (1935). One invertase unit is defined as the amount of enzyme which releases 1 mg of inverted sugar in 5 minutes at 20°C, at pH 4.5.

Kinetic parameters for batch fermentation process were determined after Pirt (1975). Treatment effects were compared after Snedecor & Cochran (1980). Significance has been presented as Duncan's multiple ranges in the form of probability (p) values.

Results and Discussion

Five cultures of *Saccharomyces cerevisiae* (GCA-I, GCA-II, GCA-III, GCA-IV and GCA-V) were isolated from five different samples of dates (Pakistani, Iranian and Arabian types obtained from different areas of Lahore). Isolates were identified on the basis of characteristic features. *S. cerevisiae* is non-mycelial yeast, which reproduce mainly by budding. Budding cells show rounded, oval or elliptical buds, which are 58 m in diameter. Many bud scars are found on a single yeast cell. Young *S. cerevisiae* colonies are white in color, which become cream colored with age. The strains were screened for the production of invertase (Table 1). Enzyme production ranged from 75.7 to 107.4 U m/l. Yeast strain GCA-II gave maximum production. This strain showed above average growth yield coefficients and low specific growth rate, however, remarkable specific product rate was noted (Table 2). The selected strain was used in the subsequent kinetic studies.

Table 1. Comparison of *Saccharomyces cerevisiae* isolates for invertase activity.

Yeast strain	Dry cell mass (g/l)	Sugar consumption (g/l)	Invertase activity (U/ml)
GCA-I	1.25 ± 0.02	15.0 ± 0.2	75.7 ± 0.2
GCA-II	0.89 ± 0.01	13.7 ± 0.1	107.4 ± 0.2
GCA-III	0.86 ± 0.02	10.6 ± 0.2	96.5 ± 0.2
GCA-IV	1.00 ± 0.02	11.8 ± 0.2	82.9 ± 0.2
GCA-V	1.52 ± 0.02	16.8 ± 0.2	98.2 ± 0.2

(Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min). ± indicates standard deviation among replicates.

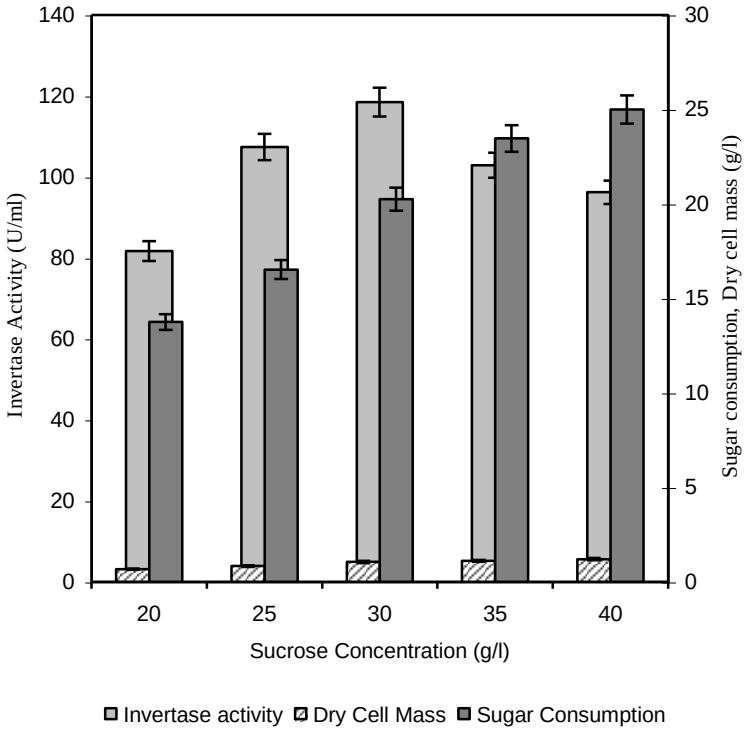

Fig. 1. Effect of Sucrose concentration on the production of invertase. (Incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

Effect of sucrose concentrations (20.0-40.0 g/l) on invertase production by *S. cerevisiae* GCA-II was studied (Fig. 1). Maximum enzyme activity was obtained at sucrose concentration of 30.0 g/l. Sucrose concentration more than 30.0 g/l caused an increase in sugar consumption and dry cell mass, however, there was no increase in invertase production. The reason might be generation of higher concentration of inverted sugar in the medium resulting in glucose-induced repression of invertase (Elorza *et al*., 1977; Vitolo *et al*., 1995). At concentrations of sucrose less than 30.0 g/l, enzyme production was lesser than optimum. As sucrose is carbon source in the medium, lower concentrations might limit proper growth of yeast, resulting in less yield of invertase (Myers *et al*., 1997).

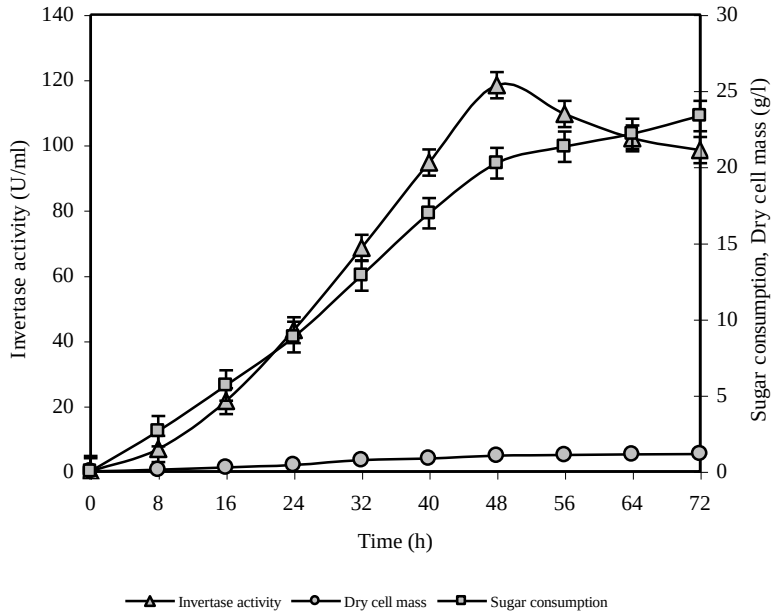

Fig. 2. Rate of invertase production by *Saccharomyces cerevisiae.* (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

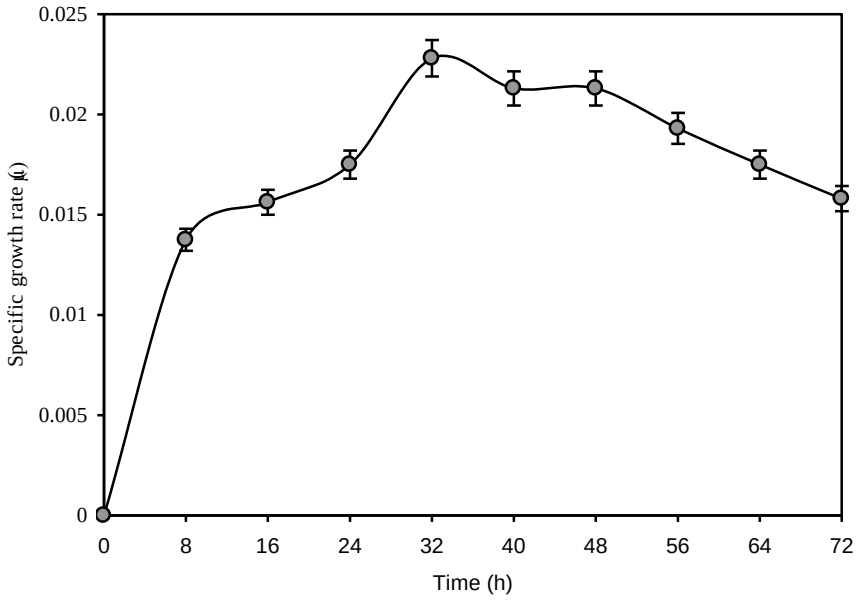

Fig. 3. Effect of incubation period on the specific growth rate □ (h^{-1}) of *Saccharomyces cerevisiae.* (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

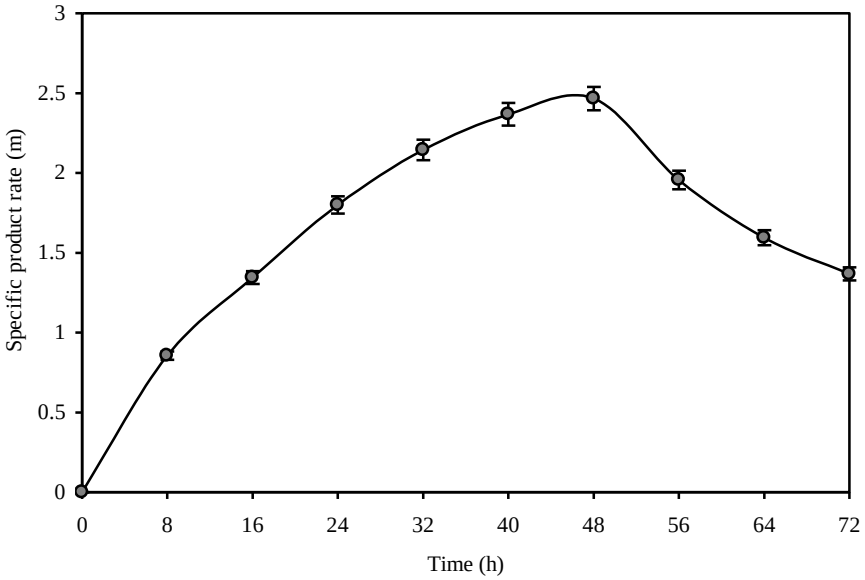

Fig. 4. Effect of incubation period on the specific product rate (h^{-1}). (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

In batch wise fermentation, the enzyme production starts after a lag phase of 8 h and reaches maximum at the onset of stationery phase. Afterwards, enzyme activity declined due to decrease in nutrients availability in the medium, or carbon catabolite repression, as the expression of invertase in *Saccharomyces* is checked by the presence of monosaccharides like glucose and fructose (Herwig *et al.*, 2001). Thus proper incubation time is very important and critical for maximal enzyme production. Fig. 2 shows the rate of invertase production by *S. cerevisiae* GCA-II. Total incubation time was 72 h. Enzyme activity was estimated for different time intervals (8-72 h). Maximum invertase production was observed at 48 h of incubation. At 48 h incubation time, specific growth and product rates also supported the observed results indicating significant enzyme yield (Fig. 3 and 4). Further increase in incubation period did not enhance invertase production. It might be due to decrease in amount of available nitrogen in fermentation medium, the age of organism, the addition of inhibitors produced by yeast itself and the protease production characteristic of decline phase. Other workers have reported invertase production by *S. cerevisiae* culture medium incubated for 24-48 h (Dworschack & Wickerham, 1960).

Production of invertase is largely dependent on initial pH of the fermentation medium. Fig. 5 shows the effect of initial pH on enzyme production by *Saccharomyces cerevisiae* GCA-II. Maximum production of invertase was obtained when initial pH of the fermentation medium was kept at 6.0. Similarly, dry cell mass and sugar consumption were maximal at pH 6.0 i.e., 1.05 and 25.53 g /l, respectively. Final pH of the medium was 6.7. Less enzyme activity, accompanied by a decrease in dry cell mass and sugar consumption, was noticed at pH other than optimum. Persike *et al*., (2002) also reported similar results. Significant growth rate was observed at pH 5.5, however maximum product rate was noted at initial pH 6. It means that although growth is more favoured at

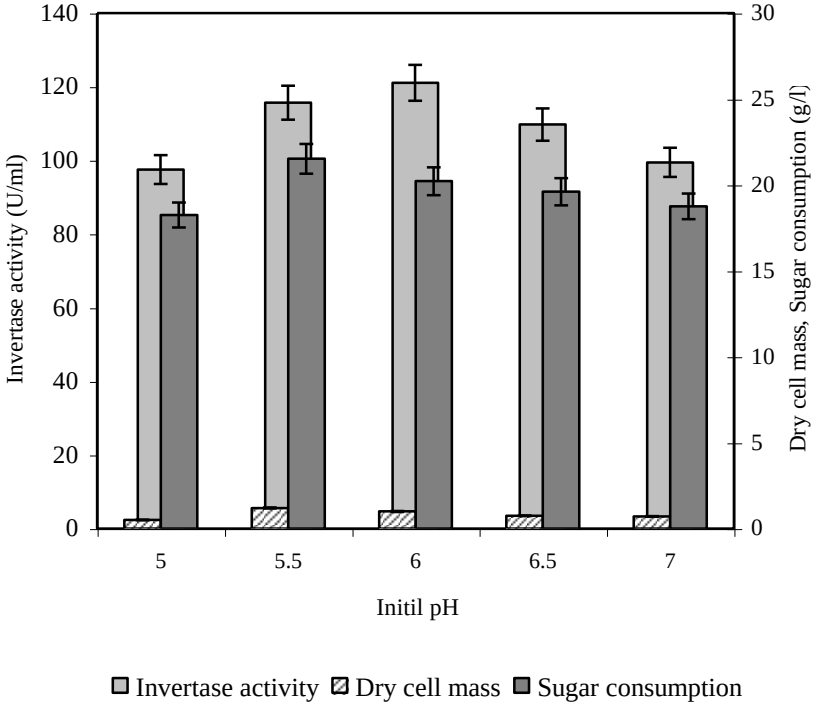

Fig. 5. Effect of initial pH on invertase production from *Saccharomyces cerevisiae* GCA-II. (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

pH 5.5, but as far as invertase production is concerned, pH 6 is best. It was noted that during submerged fermentation of *S. cerevisiae*, final pH of the reaction mixture was more than initial pH; besides, extent of the increase in pH was proportional to the invertase activity. This relationship between change in pH and invertase activity is shown in Fig. 6. The reason for this relationship may be that invertase production might accompany secretion of some anions and basic proteins, or selective uptake of cations.

Nitrogen sources and their concentrations have major effect on enzyme yield because sucrose metabolism shows a specific physiological response to the presence of nitrogen source (Silveira *et al*., 2000). Effect of different organic nitrogen sources (nutrient broth, peptone + yeast extract (control), urea + yeast extract and yeast extract only) on the production of invertase by *S. cerevisiae* was studied (Fig. 7). Application of appropriate nitrogen source is very important for optimal production of invertase. Significant invertase activity and dry cell mass was obtained when peptone + yeast extract was used as nitrogen source. Least dry cell mass was obtained when urea was used in the medium (0.77 g/l) however enzyme production was maximum. Reduced cell mass might be due to denaturing effect of urea on yeast cells (Pitombo *et al*., 1994). The reason for high enzyme yield might be positive influence of urease and invertase on each other's secretion into the culture medium (Egorov *et al*., 2000).

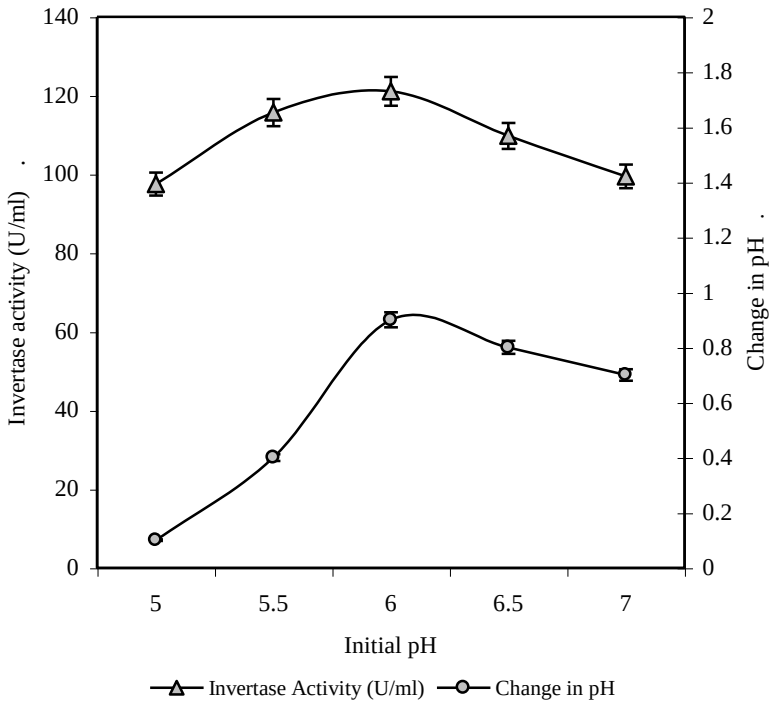

Fig. 6. Comparison of change in pH during fermentation and the production of invertase. (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

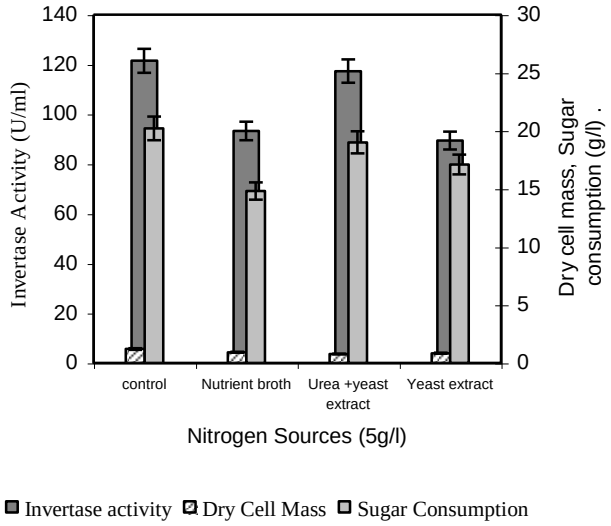

Fig. 7. Effect of organic nitrogen sources on the production of invertase. (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

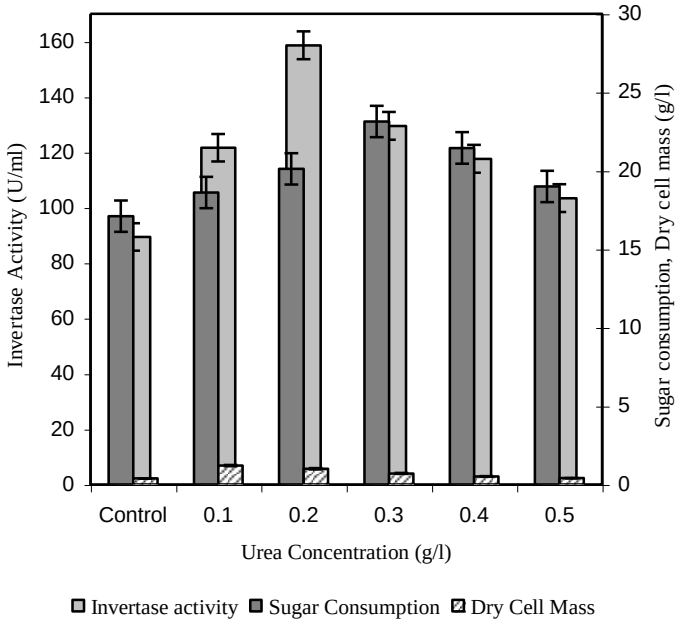

Fig. 8. Effect of urea concentration on the production of invertase. (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

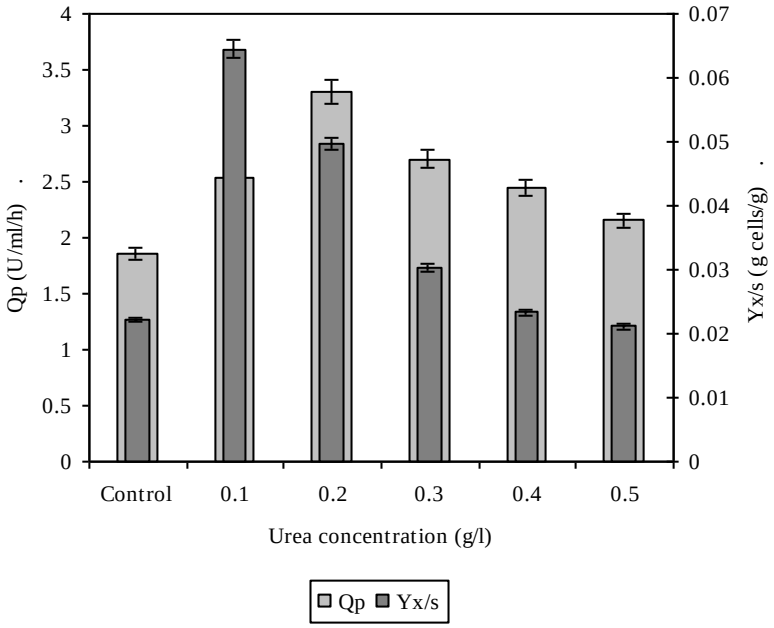

Fig. 9. Effect of urea concentration on the Q_p (U of invertase produced/ml/h) and $Y_{x/s}$ (g cells/g sugar consumed). (Sucrose concentration 20.0g/l, incubation period 48 h, temperature 30°C, initial pH 6.0, agitation rate 200 rev/min).

The effect of urea concentration in the fermentation medium on the production of invertase by *S. cerevisiae* GCA-II was studied (Fig. 8). Maximum enzyme activity was observed at urea concentration of 0.2 g/l. Sugar consumption and dry cell mass were 24.72 and 1.02 g/l, respectively. Lesser urea concentration is not enough to induce urease in amount sufficient to promote invertase production, and it does not fulfill nitrogen requirement of the yeast thus yielding lesser enzyme. Concentration of urea higher than optimum also produce less amount of invertase, as it induces denaturation of yeast cells (Pitombo *et al*., 1994), this is also supported by Q_p and $Y_{x/s}$ (Fig. 9), indicating reduction in cell mass with an increase in urea concentration, while increased enzyme yield at optimal concentration of urea.

Acknowledgements

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