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Kinetics of Invertase Synthesis by *Saccharomyces Cerevisiae* in Synthetic Medium

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Abstract

Present investigation deals with optimization of appropriate substrate concentration and incubation temperature both for growth of *Saccharomyces cerevisiae* and invertase production. Submerged fermentation technique was employed in the present study. The maximal production of invertase during the course of study was achieved after 48 h of incubation using initial sucrose concentration, 15.0 g l^{-1}. The sugar consumption and dry cell mass were also examined. Data was subjected to kinetic analysis and on the basis of kinetic parameters such as $Y_{p/x}$ (amount of enzyme produced mg^{-1} cell mass), $Y_{p/s}$ (amount of enzyme produced mg^{-1} sugar consumed), $Y_{x/s}$ (mg cells mg^{-1} substrate consumed), $Y_{s/x}$ (mg sugar consumed mg^{-1} cell mass produced), q_p (Amount of enzyme produced mg^{-1} sugar consumed h^{-1}), q_s (mg mg^{-1} cells h^{-1}), q_x (mg cells mg^{-1} sugar consumed h^{-1}), μ (mg cells produced h^{-1}), it was found that temperature had a direct influence both on substrate consumption and synthesis of enzyme. Similarly, higher concentrations of sucrose in fermentation medium induced catabolite repression of yeast invertase.

Keywords: Kinetics, fructose, invert sugar, cell mass, fermentation.

1. 0 Introduction

Saccharomyces cerevisiae produces an extracellular beta-D-fructofuranoside fructohydrolase (invertase) when grown on a medium containing beta-fructofuranosides sucrose or raffinose, indicating that synthesis is subjected to induction by the substrate. Expression of invertase in the *Saccharomyces cerevisiae* is greatly delayed when derepression occurs in a medium that lacks a usable carbon source [1]. *Saccharomyces* inverts sugar but inversion is often endocellular, without enzyme released into the medium. Kirillova [2] observed the invertase activity of sucrose tolerant and osmiophilic micromycetes. Strains with high invertase activity and capable of growing and developing on media containing 20, 30, 40 % and more of sucrose were found. Various sugars have been investigated for induction of invertase, but only the two beta-fructofuranosides were found to induce high production levels; with the other sugars, the enzyme was produced only at a low constitutive level. Sucrose is considered as the best source because yeast hydrolyses sucrose into glucose during growth to use glucose as substrate. It was found that invertase is more synthesized in medium supplemented with sucrose rather than glucose [3]. In *Saccharomyces cerevisiae*, the expression of invertase, which is the hydrolyzing enzyme of sucrose, is controlled by the presence of monosaccharides, such as glucose and fructose, and referred to as carbon catabolite repression [4]. Catabolite repression of invertase synthesis produced by glucose operates at

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the levels of transcription and translation and produces an increase in the rate of mRNA degradation [5]. Thus appropriate conditions for yeast growth are directly related with invertase secretion in the medium. Growth kinetics of *Saccharomyces cerevisiae* in glucose syrup from cassava starch and sugarcane molasses was studied using batch and fed-batch cultivation. The optimum temperature and pH required for growth were 30°C and pH 5.5, respectively [6].

Present study deals with the optimization of inducible level of sucrose and appropriate incubation temperature for enhanced production of yeast invertase.

2. 0 Materials and Methods

2.1 Organism and culture media

*Saccharomyces cer*evisiae KR_{18}, obtained from stock culture of Biotechnology Research Centre, 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 g l^{-1}, Peptone 5.0 g l^{-1}, Yeast extract 3.0 g l^{-1} and Agar 20.0 g l^{-1}) at initial pH value of 6.0.

2.2 Vegetative inoculum and fermentation

Cell suspension was prepared from 2-3 days old slant culture of yeast strain. Fifty ml of the medium containing (gl^{-1}, wv^{-1}) sucrose 30.0; peptone 5.0 and yeast extract 3.0 at pH 6.0, 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 (1.2×10^7 cells) from the slant culture was aseptically transferred into the growth medium. The flask was incubated at 30°C in an incubator (Gallenkamp, UK) for 12 h. The agitation rate was kept at 200 rev min^{-1}. The vegetative inoculum was transferred (1.0 ml per 50 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 at an agitation rate of 200 rev min^{-1}. The flasks were run parallel in triplicates.

2.3 Assay protocol

Dry cell mass of yeast was determined by centrifugation of fermented broth at 5000 rev min^{-1} using pre-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 [7]. 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 [8] 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.

2.4 Kinetics and statistical studies

The kinetics of the research work was studied after Pirt [9]. Statistical analyses of the data were determined following the procedures of Snedecor and Cochran [10]. Following parameters were studied:

$Y_{p/x}$ (amount of enzyme produced mg^{-1} cell mass), $Y_{p/s}$ (amount of enzyme produced mg^{-1} sugar consumed), $Y_{x/s}$ (mg cells mg^{-1} substrate consumed), $Y_{s/x}$ (mg sugar consumed mg^{-1} cells mass), q_p (Amount of enzyme produced mg^{-1} sugar consumed h^{-1}), q_s (mg mg^{-1} cells h^{-1}), q_x (mg cells mg^{-1} sugar consumed h^{-1}), μ (mg cells produced h^{-1}).

3.0 Results and Discussion

3.1 Effect of sucrose concentration

The initial sugar concentration plays an important role in determining the maximum amount of enzyme produced and residual sugars produced after hydrolysis by *Saccharomyces cerevisiae* [11]. Initial sucrose concentration was varied from 5.0 mg ml^{-1} to 40.0 mg ml^{-1}. *Saccharomyces cerevisiae* strain KR_{18} secreted optimal amount of invertase (15.83±0.9 U ml^{-1}) in the medium containing 15.0 g l^{-1} sucrose (Figure 1). The sugar consumption and mycelial dry weight were 9.65±1.3 and 5.70±0.3 mg ml^{-1}, respectively. Further increase in concentration of sugar resulted in the gradual decrease in product formation. It might be due to repeated budding of yeast cells, which resulted in more viscous medium and less efficient mineral availability. A concentration higher than 15.0 mg ml^{-1}, however, leads to greater amount of residual sugars, making the process uneconomical, while a lower concentration of sugar leads to lower enzyme secretion due to accumulation of hydrolysed monosaccharides. Accumulation of monosaccharides in medium causes glucose repression of invertase enzyme. The values for kinetic parameters i.e. $Y_{p/s}$, $Y_{p/x}$, and $Y_{x/s}$ were more significant at 15.0 mg ml^{-1} initial sucrose level than all other concentrations (Figure 2). It is evident from the results that increase in sucrose concentration has a repressive effect both on product formation and cell mass production in relation to sugar consumption. Glucose, hydrolysis product of sucrose, plays an important regulatory role in the yeast *Saccharomyces cerevisiae*, which is mostly reflected at the transcriptional level by glucose repression. The signal that initiates glucose repression is unknown, but data indicate that it is located at or above the level of glucose 6-phosphate, suggesting the involvement of either the intracellular or extracellular glucose concentration or the glucose flux in triggering glucose repression [12]. Figure 3 shows the comparison of specific rate constants at different concentrations of sucrose. The more significant value of the specific formation rate of product and cell mass (q_p, q_x) was calculated at 15.0 mg ml^{-1} sucrose level. Specific growth rate μ (h^{-1}) was maximal at 20.0 mg ml^{-1} sucrose level, beyond which decrease in growth rate was observed. Pejin & Razmovski [13] investigated the influence of sugar concentration in nutrient media on the specific growth rate and biomass yield in the course of continuous fermentation of *Saccharomyces cerevisiae*. It was found that an increase of sugar content in media decreased the specific growth rate and the biomass yield. *Saccharomyces cerevisiae* KR_{18} is industrially more feasible strain based on the fact that use of lower amount of sugar in fermentation medium makes the process more economical.

3.2 Effect of incubation temperature

Incubation temperature is one of the critical factors that have a profound effect on the production of invertase. Effect of different incubation temperatures (25-40°C) were studied (Figure 4). Maximum production of invertase (16.10±1.1 U ml^{-1}) was obtained when incubation temperature was maintained at 30°C. The dry cell mass and sugar consumption were 5.68±0.5 and 20.18±0.23 mg ml^{-1}, respectively. Further increase in incubation

temperature resulted in marked decline in invertase production. Values of all kinetic parameters were highly significant.

Below 25°C, enzyme production was insignificant which might be due to fact that the temperature was not suitable for yeast growth. At high temperature, less enzyme production was due to thermal inactivation of yeast cells as well as enzyme. Catabolite repression induced by high temperature resulted in less production of invertase [14, 15]. It might also be due to that at high temperature the moisture contents of the fermentation medium was reduced that was very essential for the fermentation of invertase by the yeast. Similar temperature, optimum for invertase production, was also reported by previous workers [16, 17]. Abrahao-Neto *et al*. [18] studied effects of pH (4.0, 4.5, or 5.0), temperature (30, 35, or 40 degrees C) and dissolved oxygen (0.2, 2.0, 4.0,or 6.0 mg O_2/L) on invertase formation by yeast.

The kinetic parametric study (Figure 5) indicated that the production of invertase at this temperature is significant as compared to other incubation temperatures. Values of growth yield coefficients and rate constants were highly encouraging at this variable. Values of specific product rates were significant at 25 ^oC incubation temperature, while values of substrate consumption in relation to cell mass formation were found to be slightly constant at high temperature. Figure 6 shows the comparison of specific rates of product and cell mass formation under different temperatures. Values of each parameter were significant when fermentation was preceded at 30^oC temperature. Thus the optimized temperature is favourable both for yeast growth and enzyme secretion by yeast. Fermentation process with high incubation temperature requires thermo-tolerant strain otherwise thermal denaturation of cells and product is induced.

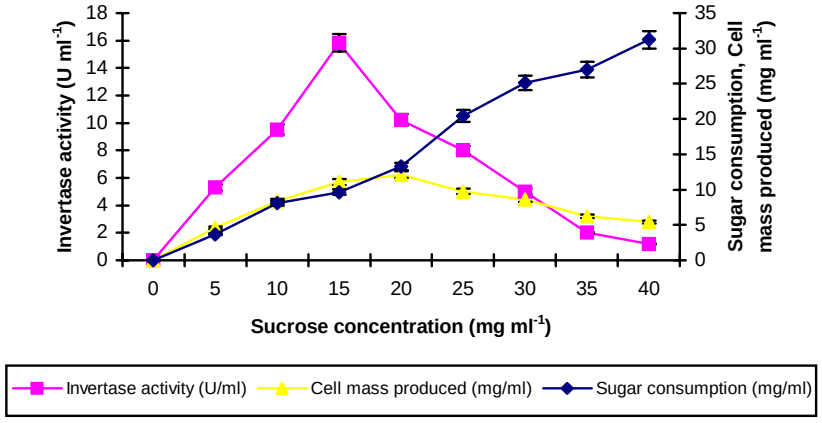

Figure 1 Inductive effect of sucrose concentration on invertase production by *Saccharomyces cerevisiae* KR_{18} at different surose concentrations.

Y error bars indicate the standard error of means among the three parallel replicates. The values differ significantly at $p \leq 0.05$.

Incubation period, 48 hours; initial pH, 6.0; incubation temperature, 30 ^oC; agitation rate, 200 rev min^{-1}.

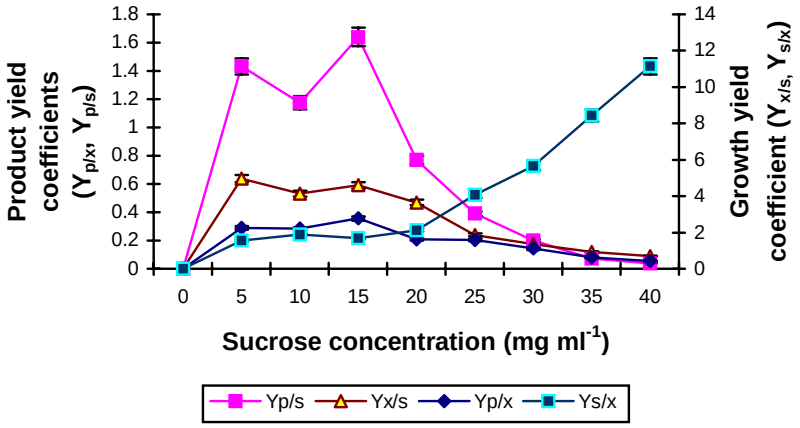

Figure 2 Comparison of product and growth yield coefficients for invertase production by *Saccharomyces cerevisiae* KR_{18} at different sucrose concentrations.

Y error bars indicate the standard error of means among the three parallel replicates. The values differ significantly at $p \leq 0.05$.

Kinetic parameters

Yp/s = Amount of enzyme produced mg^{-1} substrate consumed;
Yp/x = amount of enzyme produced mg^{-1} cell mass;
Yx/s = mg cell mass formed mg^{-1} substrate consumed;
$Y_{s/x}$ = mg substrate consumed mg^{-1} cell mass formed.

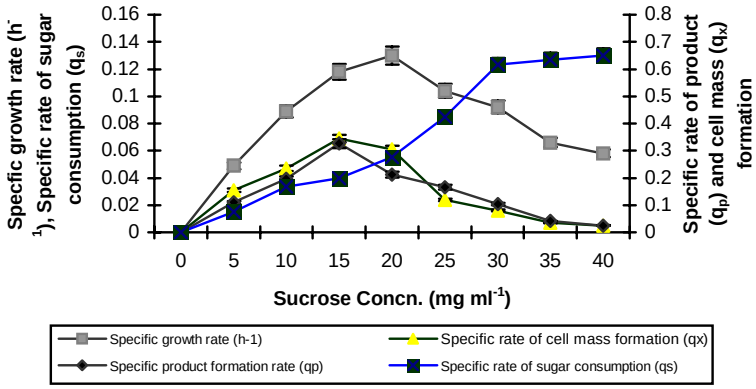

Figure 3 Comparison of specific rate constants for invertase production.

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at $p \leq 0.05$.

Kinetic parameter

Specific growth rate, μ (h^{-1}) = g cell mass produced ml^{-1};
Specific product formation rate, q_p = Amount of enzyme produced mg^{-1} sugar consumed h^{-1}
Specific cell mass formation rate, q_x = mg cells mg^{-1} sugar consumed h^{-1};
Specific rate of sugar consumption, q_s= mg mg^{-1} cells h^{-1}.

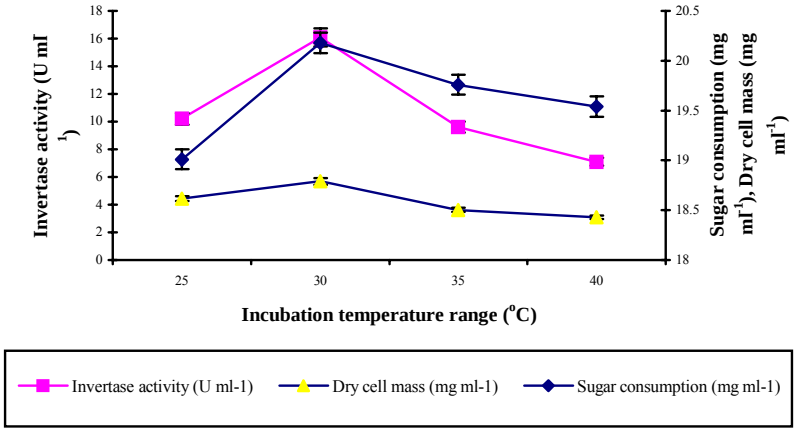

Figure 4 Production of invertase by *Saccharomyces cerevisiae* KR_{18} influenced under different incubation temperature (°C)

Sucrose concentration, 15 mg ml^{-1}; incubation period, 48 hours; initial pH, 6.0; agitation rate, 200 rev min^{-1}.

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at $p \leq 0.05$.

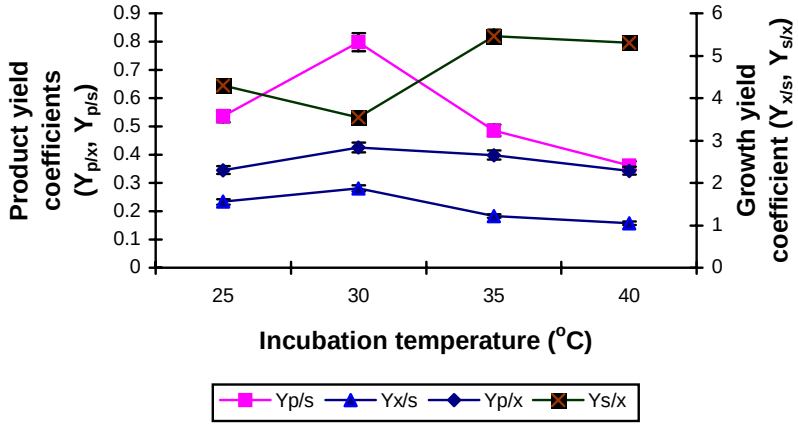

Figure 5 Comparison of product and growth yield coefficients for invertase production by *Saccharomyces cerevisiae* KR_{18} influenced under different incubation temperatures (°C).

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at $p \leq 0.05$.

Kinetic parameters

Yp/s = Amount of enzyme produced mg^{-1} substrate consumed;
Yp/x = amount of enzyme produced mg^{-1} cell mass;
Yx/s = mg cell mass formed mg^{-1} substrate consumed.

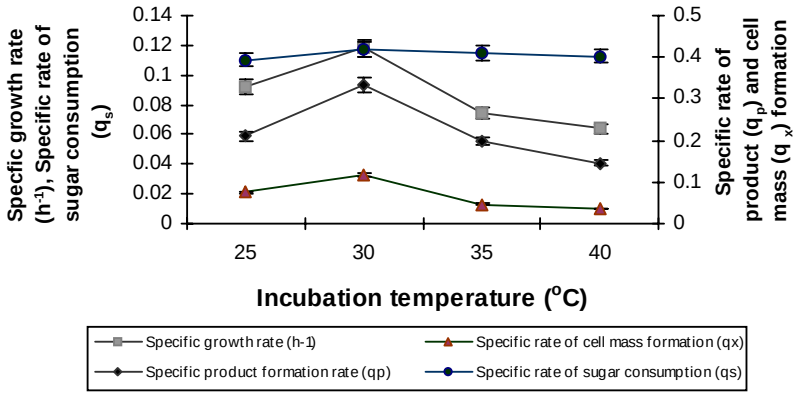

Figure 6 Comparison of specific rate constants for invertase production influenced under different incubation temperatures (°C).

Y error bars indicate the standard error of means among the three parallel replicates. The value differs significantly at $p \leq 0.05$.

Kinetic parameter

Specific growth rate, μ (h^{-1}) = g cell mass produced ml^{-1};
Specific product formation rate, q_p = Amount of enzyme produced mg^{-1} sugar consumed h^{-1}
Specific cell mass formation rate, q_x = mg cells mg^{-1} sugar consumed h^{-1};
Specific rate of sugar consumption, q_s = mg mg^{-1} cells h^{-1}

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