Pak. J. Biotech. 1(1) 25-30 (2004)

KINETRIC ANALYSIS F NUTRITIONAL STRATEGIES FOR NVERTASE PRODUCTION BY *SACCHAROMYCES CEREVISIAE* KR_{18}

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ABSTRACT: Fermentation conditions for invertase secretion by *Saccharomyces cerevisiae* strain were optimized. Submerged fermentation technique was employed for present investigation. 6.76±0.5 U of invertase ml^{-1} of fermented broth were obtained by *Saccharomyces cerevisiae* KR_{18} when fermentation medium was supplemented by 15.0 mg sucrose ml^{-1} and incubated at 25°C at initial pH 6.0. Sugar consumption and cell mass production (mg ml^{-1}) at this pH level were 11.21±0.5 and 4.67±0.5, respectively. One ml of vegetative inoculum (1.2×10^3 cells) developed for 12 h was used. Kinetic analysis of fermentation results was made to check out the feasibility of fermentation process. All the kinetic parameters i.e. product ($Y_{p/x}$, $Y_{p/s}$) and growth rate ($Y_{x/s}$) coefficients as well as specific rates (μ h^{-1}) were favorable for optimized conditions.

INTRODUCTION: Invertase is an important industrial enzyme with applications in the production of noncrystallizable invert sugars and soft-centered chocolates. Invertase hydrolyses sucrose into a mixture of glucose and fructose under sucrose concentrations lower than 10 % (Rubio *et al*., 2002). β-D- fructosyltransferase obtained from plants and fungi has a similar activity (Chen and Liu, 1996). Invertase has been widely studied, especially in the yeasts *Saccharomyces cerevisiae* and *Schwanniomyces occidentalis* (Shafiq *et al*., 2002; Costaglioli *et al*, 1997). Invertase is a high cost enzyme and its production is influenced by many factors. Hydrolysis side reaction experiments indicate that the reactions by invertase are uncompetitively inhibited by glucose, which accumulates in the fermentation medium as yeast cells grow. Suitable pH and temperature, both for growth and productivity of organism, are critical factors. It is based on pH changes for regulating metabolic activities of the yeast population. Experimental results have shown that it enables the attaining of high cell density with both high productivity and high yields (Porro *et al*., 1991).

MATERIAL AND METHODS:

*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 (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. Sterilized medium was then inoculated with one ml of cell suspension (1.2×10^3 cells) from the slant culture and incubated for 48 h. The agitation rate was kept at 200 revmin^{-1}. The vegetative inoculum was transferred (1.0 ml per 250 ml) to the production medium, same as used for growth medium.

***Assay method*:** 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 according to Sumner and Howell (1935) method based on dinitrosalicylic acid test for reducing sugar: 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 and statistical studies: The kinetics of the research work was studied after Pirt (1975). Statistical analyses of the data were determined following the procedures of Snedecor & Cochran (1980). Standard deviation among the replicates was presented in the form of probability (< p >) values.

RESULTS and DISCUSSION

Effect of initial sucrose concentration: Initial sucrose concentration was investigated and sugar level was varied from 5.0-30.0 mg ml^{-1} (Table 1). Maximum production of invertase (5.63±0.5 U ml^{-1}) was obtained when 15.0 mg ml^{-1} sucrose was used in the medium. At this level sugar consumption and cell mass (mg ml^{-1}) were 9.65±1.1 and 2.62±1.1, respectively. Kinetic parameters i.e. product and growth yield coefficients fare also significant for 15.0 mg ml^{-1} level of sucrose in fermentation medium (Table 2). Figure 1 shows the comparison of specific rates of product and cell mass for this parameter. Further increase in the sucrose concentration causes decrease in invertase synthesis by yeast. It might be due to substrate-induced repression both for yeast cells and product secretion. Elorza *et al*. (1977) reported that *S. cerevisiae*-136ts synthesized invertase in media containing maltose and sucrose. In the presence of glucose, synthesis of enzyme took place when the sugar concentration was lower than 1%. At higher concentrations enzyme formation was repressed. Pejin and Razmovski (1993) investigated the influence of sugar concentration in nutrient media on the specific growth rate and biomass yield in the course of continuous fermentation of *S. cerevisiae*. It was found that an increase of sugar content in media decreased the specific growth rate and the biomass yield. Haq *et al*., (2002) reported 2.5 g L^{-1} sucrose for optimal production of invertase by yeast.

Effect of incubation temperature: Incubation temperature is one of the critical factors that have a profound effect on the production of invertase. Temperature maintenance of fermentation process is very important factor both for production and activity of enzyme (Caville and Combes, 1995). Optimal incubation temperature for yeast invertase production was worked out (Table 1). Temperature of fermentation process was varied from 25 to 40°C. Invertase secretion in fermentation medium was maximum (6.25±15 U ml^{-1}) when

fermentation preceded at 25°C. Higher temperature not only decreases the enzyme synthesis by yeast but also restrict the budding capacity of yeast cells. Favorable values of product and growth rate coefficients were also observed at this temperature i.e. 3.0, 0.56 and 0.28, respectively. High temperature induces thermal inactivation of yeast cells as well as secreted enzyme. Catabolite repression induced by high temperature resulted in less expression of invertase (Vrabel *et. al*., 1997). *A. japonicus* NTU-1249 can produce 83.5 units of transfructosylating activity per ml broth when cultivated in a shaking flask at 28 °C for 72 hours (Su *et. al*., 1991). Temperature factor is also very important for specific rates of product and cell mass formation (Figure 2). Cell mass production as well as product formation rate at this temperature is much pronounced as compared to others.

Effect of initial pH of fermentation medium: Synthesis of invertase depends largely on initial pH of fermentation medium. The maximum production of invertase occurs at pH favorable for yeast growth. Optimum pH for invertase production also seems to correspond with that of sucrose fermentation (Mirzara-khmetova and Abdurazakova, 1998). Initial pH was worked out for optimal invertase synthesis in fermentation medium by invertase (4.5-7.0). Maximum invertase (6.76±0.5 U ml^{-1}) by *Saccharomyces cerevisiae* strain was observed at pH 6.0 (Table 1). Increase as well as decrease in pH other than optimum also resulted in marked decrease in cell mass. Therefore, pH 6.0 was found optimum for further studies. Sugar consumption and cell mass production ($mgml^{-1}$) at this pH level were 11.21±0.5 and 4.67±0.5, respectively. Values of all kinetic parameters are also significant at this pH. Figure 3 shows effect of pH on specific rates for growth and product formation. It can be observed that optimized pH not only supports cell mass formation but also enhance the capacity of yeast cell to secrete invertase in the medium (Vitolo *et. al*., 1995).

Table 1: Optimization of sucrose concentration level, incubation temperature and initial pH for Invertase production by *Saccharomyces cerevisiae* KR_{18}.

Fermentation conditions	*Final pH*	Dry cell mass (mg ml^{-1})	Sugar consumption (mg ml^{-1})	Invertase activity (U ml^{-1})
Sucrose concentration (mg ml^{-1})				
5.00	6.0	1.98±0.3	3.70±0.5	1.80±1.2
10.0	5.3	2.35±1.0	8.10±1.3	2.10±0.1
15.0	5.3	2.62±1.1	9.65±1.1	5.63±0.5
20.0	4.8	2.91±0.2	13.27±0.1	3.40±0.4
25.0	6.4	4.01±0.4	20.44±0.5	3.02±1.0
30.0	6.1	4.43±0.1	25.11±0.2	2.15±2.2

Incubation temperature (°C)				
25	5.2	2.08±0.5	11.01±0.1	6.25±15
30	5.3	2.65±0.1	10.18±0.2	5.70±02
35	6.0	2.45±0.4	9.76±0.4	5.31±05
40	6.2	2.01±0.2	9.54±1.0	4.22±05
Initial pH				
4.5	3.9	3.84±0.3	10.33±1.5	3.33±0.1
5.0	4.8	4.13±15	10.26±0.5	4.51±1.2
5.5	5.4	4.43±0.2	11.10±0.3	5.61±1.0
6.0	5.5	4.67±0.5	11.21±0.5	6.76±0.5
6.5	6.4	4.58±0.8	10.18±0.1	5.07±0.2
7.0	7.3	4.47±0.1	10.11±0.4	4.92±0.1

Incubation period, 72 h; agitation rate, 200 rev.min^{-1}.

Table 2: Comparison of kinetic (product and growth yield coefficients) and nutritional (sucrose concentration, temperature, initial pH) parameters for invertase production by *Saccharomyces cerevisiae* KR_{18}.

Fermentation conditions	**Kinetic parameters**		
	Product yield coefficients		**Growth yield coefficient**
	$Y_{p/x}$	$Y_{p/s}$	$Y_{x/s}$
Sucrose concentration (mg ml^{-1})			
5.00	0.96	0.48	0.53
10.0	0.89	0.25	0.27
15.0	2.14	0.58	0.29
20.0	1.16	0.25	0.21
25.0	0.75	0.14	0.19
30.0	0.48	0.08	0.17
Incubation temperature (°C)			
25	3.00	0.56	0.28
30	2.15	0.55	0.26
35	2.16	0.54	0.25
40	2.09	0.44	0.21
Initial pH			
4.5	0.86	0.32	0.37
5.0	1.09	0.43	0.40
5.5	1.26	0.50	0.39
6.0	1.44	0.60	0.41
6.5	1.10	0.49	0.40
7.0	1.10	0.48	0.40

Kinetic parameters:
$Y_{p/s}$ = Amount of enzyme produced mg^{-1} substrate consumed, $Y_{p/x}$ = Amount of enzyme produced mg^{-1} cell formed, $Y_{x/s}$ = mg cell formed mg^{-1} substrate consumed.

Figure 1: Comparison of specific rate constants for invertase production under different concentrations of sucrose.

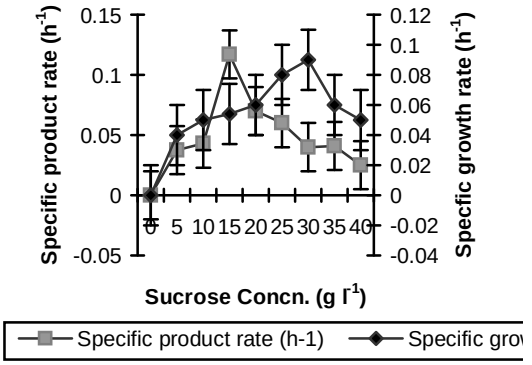


Figure 2: Comparison of specific rate constants for invertase production influenced by different incubation temperature.

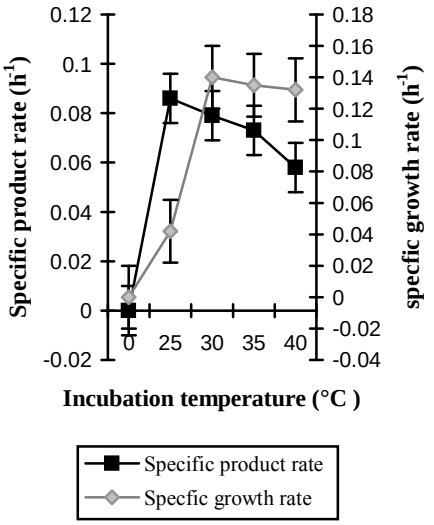
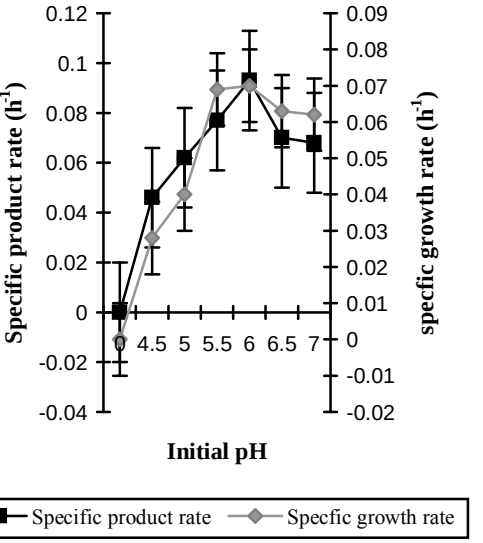


Figure 3: Comparison of specific rate constants for invertase production influenced by different initial pH.



Kinetic parameters

Specific growth rate, μ (h^{-1}) = g cell mass produced ml^{-1} min^{-1}

Specific product rate, μ (h^{-1}) = amount of enzyme produced ml^{-1} min^{-1}

Y error bars indicate the standard error of means among the three parallel replicates.

The value differs significantly at $p \leq 0.05$.

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