Pak. J. Biotechnol. Vol. 4 (1-2) 7-13 (2007) ISSN. 1812-1837
IBGE, University of Sindh, Jamshoro, Pakistan

BATCH CULTURE THERMO TOLERANCE OF *SACCHAROMYCES CEREVISIAE* DURING INVERTASE PRODUCTION

Sikander Ali*, Aafia Aslam and Ikram-ul-Haq

Institute of Industrial Biotechnology, GC University Lahore
*Email: alisbiotech@yahoo.com

ABSTRACT

Saccharomyces cerevisiae strains IIB-12 (wild) and NG-46 (mutant) were compared on the basis of kinetics and thermo tolerance for invertase production. NG-46 showed significantly higher ($p \leq 0.05$) values of Q_p and $Y_{p/s}$ compared to other yeast strains. Thermodynamics revealed that cell system exerted protection against thermal inactivation during product formation. The enthalpy (ΔH, KJ/mol) and entropy of activation (ΔS, J/mol/K) for Invertase production substantially improved specificity constant activation energy, free energies for transition state formation and substrate binding for sucrose fermenting. The results highlighted an improvement in thermo stability (>40°C) of microbial endogenous metabolism for enzyme secretion.

INTRODUCTION

Invertase (EC 3.2.1.26) is an intra-cellular as well as extracellular enzyme. *Saccharomyces cerevisiae* is the organism of choice for invertase production because of its characteristic high sucrose fermenting ability (Nakano *et al.,* 2004). Appropriate incubation period is critical for invertase synthesis but longer incubation can cause feedback repression of enzyme. The activity of enzyme has enhanced by mutagenesis and extensive screening (Vrabel *et al.,* 1997). Fermentation efficiency of *S. cerevisiae* at a high temperature (<35°C) is low because of increased fluidity in membranes, which changes the fatty acid composition. The increase in growth temperature usually results in the biosynthesis of heat-shock proteins that are implicated in conferring thermal cross-tolerance in various organisms. Catabolic repression by glucose and fructose does not permit formation of extracellular invertase, which is then sequentially consumed by the organism (Weber and Roitsch, 2003). There is a need to quantify the effect of cultivation conditions on the production of invertase activity in yeast, fermenting sucrose at 40°C in commercial plants. In this manuscript, we report a comparison of kinetics and thermo tolerance of wild (IIB-12) and mutant (NG-46) strains of *S. cerevisiae* for the production of invertase in shaking culture. The mutant was tested for enhanced substrate uptake and enzyme production rates. Activation enthalpy and entropy of invertase activity were determined to clarify the phenomenon involved in enzyme production and its thermal inactivation.

MATERIALS AND METHODS

The wild (IIB-12) and mutant (NG-46) strains of *S. cerevisiae* were obtained from the available culture collection of our Labs. The strains were maintained on the medium containing (g/l); sucrose 20.0, agar 20.0, peptone 5.0 and yeast extract 3.0 (SAPY medium) at pH 6.0 and stored at 4°C. Twenty-five millilitre of SAPY medium (-

agar) was autoclaved at 15 psi (121°C) for 15 min. After cooling, 1.0 ml yeast cell suspension was transferred aseptically and incubated in a rotary shaker (SANYO Gallenkamp PLC, UK) at 30°C for 24 h (160 rpm). Production of invertase was carried out in 250 ml Erlenmeyer flasks by shaking culture. Fifty millilitre of SAPY medium (-agar) was transferred to individual flasks. The cotton-plugged flasks were autoclaved and cooled. One millilitre of the inoculum was aseptically transferred and incubated in a rotary shaker at 30 °C for 48 h. The agitation rate was kept at 200 rpm.

Yeast dry cell mass was determined after drying harvested cells at 105°C for 1 h. Sugar was estimated by DNS method (Miller 1959). The enzyme activity was determined after Akgol *et al.* (2001). One invertase unit was defined as the amount of enzyme, which released 1.0 mg of inverted sugar in 5 min at 35°C, pH 5.5. A UV/VIS double beam scanning spectrophotometer (Cecil CE 100-series, Aquarius Inc., London, UK) was used to determine colour intensity. Enzyme activity was estimated at different time intervals (8-72 h). All experiments were performed in triplicate. The empirical approach of Arrhenius was used to describe the relationship of temperature-dependent irreversible inactivation of invertase production (Aiba *et al.,* 1973). Kinetic parameters for batch fermentation were determined following Pirt (1975) and treatment effects were compared after Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Invertase production starts after a lag phase of about 8-12 h and reaches maximal at late exponential phase; afterwards it declines due to decreased nutrients availability in the medium, or carbon catabolite repression (Myers *et al.,* 1997). Figs (I, II & III) show the comparison rate of invertase production by *S. cerevisiae* IIB-12 (wild) and NG-46 (mutant). Maximum invertase production (78.96±2.0 U/ml) was observed at 48 h of incubation by the mutant. Further increase in incubation period did not enhance invertase production. The mutant has lower rate of biomass formation while in contrary higher rate of sucrose fermenting ability compared to wild-culture. Among comparison of different kinetic parameters for invertase production, the maximum value for $Y_{x/s}$ (0.19±0.02 g cells/g) was recorded with the wild-culture of *Saccharomyces cerevisiae*. However, mutant NG-46 showed a higher value for $Y_{p/s}$ (i.e., 3.85±1.3 U/g substrate utilized) compared to the wild-culture and other mutant derivatives, which showed its ability of higher substrate consumption rate (Table 1). Similarly, the highest value for $Y_{p/x}$ (52.46±2.0 U/g cells formed) was noted with the mutant culture. This value was 6.76 fold higher as compared to the wild-culture. The product yield coefficients were improved over Pirt (1975) and Elorza *et al*., (1977). The lowest values of volumetric rates for sugar consumption were obtained with the wild-culture (Table 2). The value for Q_x with the mutant NG-46 was recorded 0.036±0.01 g cells/l/h. The mutant showed highest value for Q_p (i.e., 1.57±0.2 U/ml/h), which was about 3.73 fold higher than the wild-culture. For substrate consumption parameters, the mutant strain *S. cerevisiae* NG-46 revealed that it utilized sucrose not only for growth but for enzyme formation also. Its sugar conversion rate was the highest. The maximum value for Q_p was several folds improved over the cultures studied by previous workers (Pirt 1975, Myers *et al.,* 1997, Silveira *et al.,* 2000).

NG-46 ($\Delta H = 38.26 \pm 3.0$ kJ/mol) was 1.2 fold more stable than IIB-12 ($\Delta H = 44.96 \pm 2.9$ kJ/mol) as it required lower energy of activation for growth in the production medium (Table 3). The values of the thermodynamic parameters indicated that the activation enthalpy of invertase formation by mutant (ΔH = 39.28±3.4 kJ /mol) was lower than that of its wild parent (Aiba *et al.,* 1973), but compared favourably with those estimated for many different whole-cell bioprocesses, such as cell growth (ΔS = 49.62±5.1 kJ/mol). The activation entropy value of thermal inactivation by mutant cells (ΔS= -36.50 ± 6.5 J/mol/K) was very low and reflected that the inactivation phenomenon implied a little disorderness during growth and subsequent product formation at temperature up to 42°C. Practically this value was lower than those estimated for invertase production by other systems (Converti and Dominguez, 2001). This suggested more protection exerted by mutant cell system compared with wild cells against thermal inactivation.

CONCLUSION

The mutant-derived invertase required less free energy (ΔG) for substrate binding. In addition, the putative enzyme released a higher amount of transition state formation ($\Delta G_{E\text{-}T}$), signifying that high enzyme catalytic efficiency was due to transition state stabilization.

ACKNOWLEDGEMENTS

HEC is gratefully acknowledged for financial assistance.

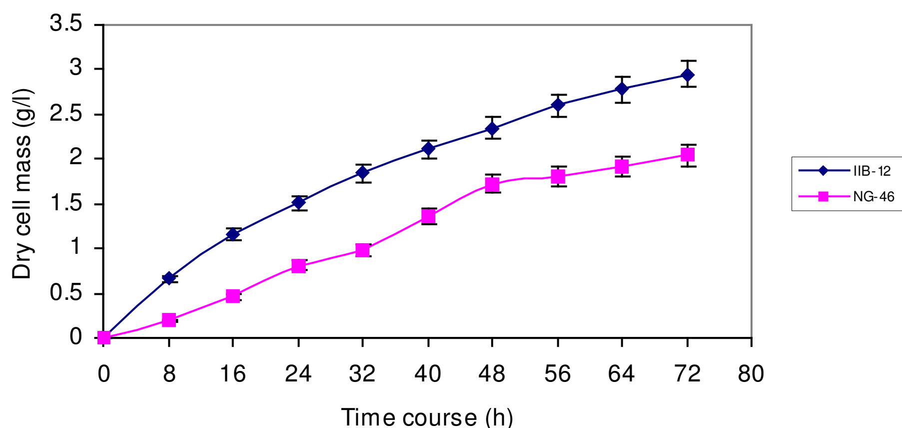

Fig-1: Rate of dry cell mass formation by wild and mutant *S. cerevisiae*
Sucrose concentration 30.0 g/l, temperature 28°C, initial pH 6.5, agitation rate 200 rpm.
Y-error bars indicate standard deviation among the three parallel replicates.

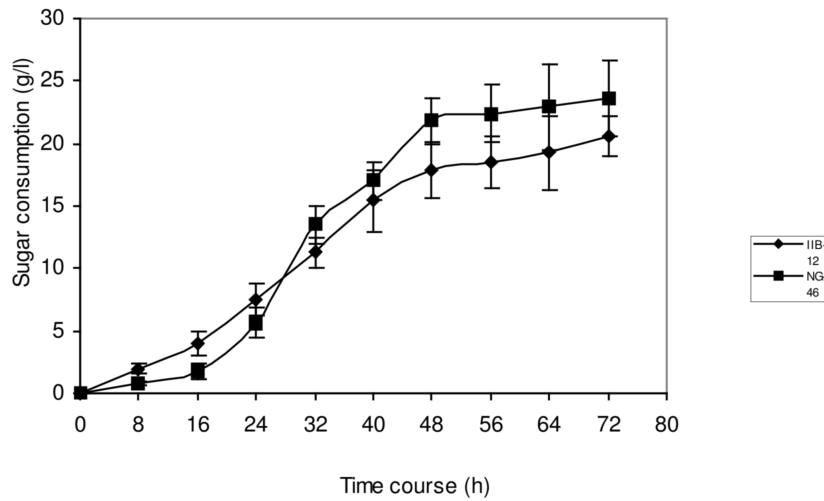

Fig-2: Rate of sugar consumption by wild and mutant *S. cerevisiae*
Sucrose concentration 30.0 g/l, temperature 28°C, initial pH 6.5, agitation rate 200 rpm. Y-error bars indicate standard deviation among the three parallel replicates.

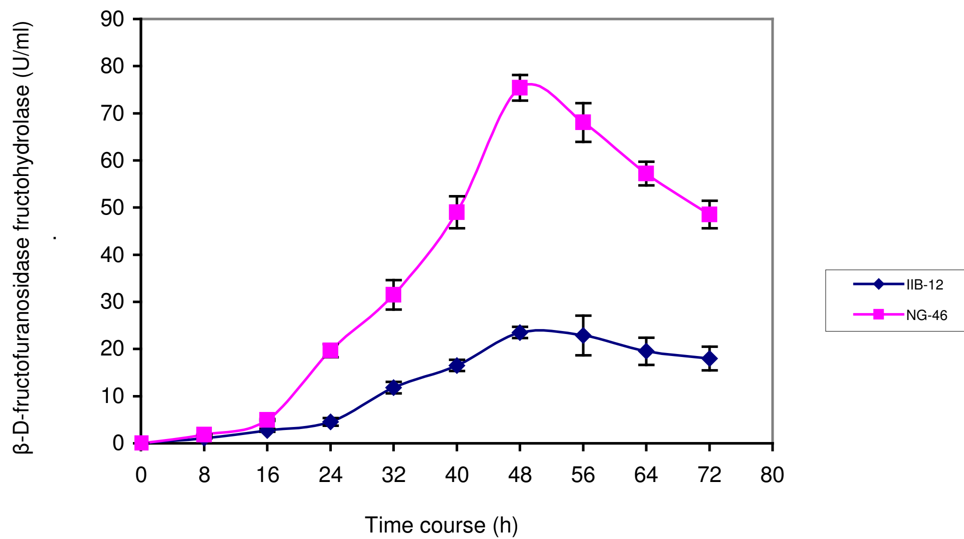

Fig-3: Rate of invertase production by wild and mutant *S. cerevisiae*
Sucrose concentration 30.0 g/l, temperature 28°C, initial pH 6.5, agitation rate 200 rpm.
Y-error bars indicate standard deviation among the three parallel replicates.

Table-1: Comparison of yield coefficients for invertase production by wild and mutant cultures of *S. cerevisiae* in batch culture

Cultivation conditions	Yield coefficients					
	Product yield coefficients				Growth yield coefficients	
	$Y_{p/x}$ (Enzyme units/g cells formed)		$Y_{p/s}$ (Enzyme units/g substrate utilized)		$Y_{x/s}$ (g cells formed/g substrate utilized)	
	IIB-12	NG-46	IIB-12	NG-46	IIB-12	NG-46
Sugar conc. (g/l)						
25	7.98±1.2	46.31±2.5	1.56±0.4	3.34±1.1	0.19±0.02	0.07±0.01
30	5.60±1.0	40.75±2.8	0.70±0.02	2.97±1.0	0.12±0.02	0.07±0.01
35	4.02±0.6	38.59±3.4	0.41±0.02	1.87±0.3	0.13±0.01	0.05±0.02
Incubation temperature (°C)						
24	2.28±0.2	19.22±1.3	0.98±0.02	1.98±0.2	0.09±0.03	0.06±0.02
26	5.80±1.10	31.45±2.3	1.12±0.03	2.06±0.06	0.10±0.02	0.06±0.02
28	9.16±2.1	43.94±2.5	1.40±0.5	2.79±0.4	0.15±0.01	0.06±0.02
30	8.20±1.4	38.31±2.0	1.00±0.3	2.99±0.9	0.12±0.01	0.08±0.01
32	8.12±1.0	37.07±3.12	0.98±0.1	2.78±0.3	0.12±0.02	0.09±0.03
34	8.07±1.1	34.56±3.76	0.91±0.07	2.69±0.4	0.12±0.02	0.09±0.03
36	8.00±0.08	32.29±4.18	0.90±0.03	2.66±0.3	0.11±0.01	0.10±0.02
38	7.86±1.28	31.48±2.14	0.86±0.2	2.61±0.07	0.09±0.03	0.11±0.03
40	7.49±0.8	29.95±1.5	0.85±0.11	2.60±0.2	0.09±0.03	0.09±0.01
42	6.78±1.12	24.62±2.1	0.68±0.02	1.97±0.3	0.05±0.01	0.06±0.02
Initial pH						
6.0	11.67±2.2	44.36±1.8	1.63±0.6	2.82±1.0	0.14±0.02	0.06±0.02
6.5	8.19±2.5	44.96±2.0	1.04±0.2	3.41±1.0	0.13±0.02	0.08±0.01
7.0	7.76±1.0	52.46±2.0	0.91±0.02	3.85±1.3	0.11±0.02	0.07±0.02

$Y_{p/s}$ = U of invertase produced/g substrate consumed, $Y_{p/x}$ = U of invertase produced/g cells formed, $Y_{x/s}$ = g cells/g substrate utilized. ± Indicates standard deviation among the three parallel replicates.

Table 2: Comparison of volumetric rates for invertase production by wild and mutant cultures of *S. cerevisiae* in batch culture

Cultivation conditions	Volumetric rates					
	Q_p (Enzyme units/ml/h)		Q_s (g substrate consumed/l/h)		Q_x (g cells formed/l/h)	
	IIB-12	NG-46	IIB-12	NG-46	IIB-12	NG-46
Sugar conc. (g/l)						
25	0.33±0.02	1.56±0.3	0.21±0.02	0.35±0.02	0.042±0.01	0.025±0.01
30	0.25±0.02	1.43±0.3	0.36±0.03	0.48±0.01	0.045±0.02	0.035±0.02
35	0.19±0.01	1.23±0.2	0.46±0.02	0.66±0.01	0.059±0.02	0.032±0.01
Incubation temperature (°C)						
24	0.15±0.01	0.46±0.02	0.28±0.02	0.42±0.02	0.025±0.01	0.012±0.01
26	0.28±0.04	0.79±0.01	0.31±0.03	0.48±0.02	0.031±0.02	0.023±0.01
28	0.45±0.02	1.48±0.2	0.32±0.02	0.53±0.01	0.049±0.02	0.034±0.02
30	0.36±0.02	1.40±0.4	0.36±0.01	0.47±0.01	0.044±0.02	0.036±0.02
32	0.35±0.01	1.38±0.01	0.37±0.08	0.46±0.02	0.043±0.01	0.038±0.01
34	0.32±0.07	1.29±0.02	0.41±0.02	0.42±0.02	0.041±0.01	0.039±0.01
36	0.31±0.02	1.26±0.02	0.40±0.02	0.40±0.13	0.038±0.02	0.042±0.02
38	0.31±0.02	1.25±0.05	0.35±0.02	0.38±0.01	0.036±0.02	0.045±0.02
40	0.27±0.02	1.17±0.02	0.29±0.02	0.35±0.05	0.035±0.02	0.046±0.02
42	0.18±0.02	0.94±0.06	0.23±0.11	0.29±0.02	0.026±0.02	0.041±0.01
Initial pH						
6.0	0.56±0.01	1.44±0.6	0.34±0.02	0.51±0.01	0.047±0.02	0.032±0.01
6.5	0.42±0.02	1.57±0.2	0.39±0.03	0.46±0.03	0.051±0.01	0.035±0.02

Q_p = U of invertase produced/ml/h, Q_s = g substrate consumed/l/h, Q_x = g cells formed/litre/h.
± Indicates standard deviation among the three parallel replicates.

Table-3: Thermodynamics of invertase production by *S. cerevisiae* in batch culture

Thermodynamic parameters	Enzyme formation	Thermal inactivation
ΔH (kJ/mol)		
IIB-12	44.96±2.9	49.62±5.1
NG-46	38.26±3.0	39.28±3.4
ΔS (J/mol/K)		
IIB-12	56.15±2.8	(-) 218.21±11
NG-46	(-) 36.50±6.5	(-) 279.36±15

± Indicates standard deviation among three parallel replicates. The values in each set differ significantly from each other at p≤0.05. ΔH (kJ/mol) = Activation enthalpy, ΔS (J/mol/K) = Activation entropy.

REFERENCES

Aiba, S., A.E. Humphrey and N.F. Millis, Biochemical Engineering, 2nd Edition, New York Academic Press, NY, pp. 92-127 (1973).

Akgol, S., Y. Kacarb, A. Denizlia and M.Y. Arıcab, Hydrolysis of sucrose by invertase immobilized onto novel magnetic polyvinylalcohol micro-spheres. *Food Chemist*. **74**: 281-288 (2001).

Converti, A. and J. M. Dominguez, Influence of temperature and pH on xylitol production from xylose by *Debarryomyces hansenii*. *Biotechnol. Bioengin.* 75: 39-45 (2001).

Elorza, M., R. Villanuena and R. Sentandreu, The mechanism of catabolite inhibition of invertase by glucose in *Saccharomyces cerevisiae*. *Biochimie Biophysics Acta* 475: 103-112 (1977).

Myers, D.K., D.T. Lawlor and P.V. Attfield, Influence of invertase activity and glycerol synthesis and retention on fermentation of media with a high sugar concentration by *Saccharomyces cerevisiae. Appl. Environ. Microbiol.* 63: 145-150 (1997).

Nakano, H., H. Murakami, M. Shizuma, T.Kiso, T.L. Dearaujo and S. Kitahata, Transfructosylation of thiol group by beta-fructofuranosidases. *Biosci. Bio-technol.* 64: 1472-1476 (2004).

Pirt, S.J., Principles of Microbes and Cell Cultivation. 2nd Edition, Blackwell's Scientific Corporation, London, UK, pp. 112-135 (1975).

Silveira, M.C., E.M. Oliveira, E. Carvajal and E.P. Bon, Nitrogen regulation of *Saccharomyces cerevisiae* invertase. *Appl. Biochem. Biotechnol.* 84: 247-254 (2000).

Snedecor, G.W. and W.G. Cochran. Statistical Methods, 7th edition, Iowa State University, USA, Pp. 32-43 (1980).

Miller, G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. 31:426-428 (1959).

Vrabel, P., M. Polakovic, V. Stefuca and V. Bales, Analysis of mechanisms and kinetics of thermal inactivation of enzymes: evaluation of multi temp-erature data applied to inactivation of yeast invertase. *Enz. Microbial. Technol.* 20: 348-354 (1997).

Weber, H. and T. Roitsch, Invertases and life beyond sucrose cleavage. *Trends Plant Science* 5: 47-48 (2003).