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Kinetics of Phosphate Ions Induced Invertase Synthesis by *Saccharomyces cerevisiae*

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ABSTRACT

Invertase (β-fructofuranosidase) is a high cost enzyme. Aim of present study was to develop an economically feasible synthetic fermentation medium with appropriate supplemented nutrients. *Saccharomyces cerevisiae* GCB-K5 was used for investigation. Phosphate ions in fermentation medium greatly influence the invertase secretion capacity of yeast cells. By comparing the kinetic parameters, namely product yield coefficient ($Y_{p/x}$, $Y_{p/s}$ in Amount of enzyme produced mg^{-1} substrate consumed & mg^{-1} cell mass produced respectively), growth yield coefficient ($Y_{x/s}$ in mg mg^{-1}) and specific product and growth rate μ (h^{-1}), it was found that when K_2HPO_4 at the level of 0.020 % was added in medium invertase secretion was increased. Thus it is favorable to use K_2HPO_4 as a readily available phosphate source for optimal invertase synthesis.

Key Words: Invertase; Phosphate ions; *Saccharomyces cerevisiae;* Product yield coefficient; Specific growth rate

INTRODUCTION

Invertase is an enzyme of food grade and has major implications in confectionery industry. Function of invertase is directed towards the hydrolysis of sucrose and production of inverted syrup with almost equal proportions of reducing sugars. The secreted invertase (EC 3.2.1.26) of *Saccharomyces cerevisiae* is a glycoenzyme that contains N- and O-linked mannoses in 40/1 proportions (Mormeno *et al*., 1989). Two different forms of yeast invertase have been observed. One form is intracellular that is non-glycosylated and the extracellular one containing approximately 50% carbohydrate of the high mannose type (Li *et al*., 1998). Present study deals with the phosphate ions effect on the synthesis of extracellular invertase by *Saccharomyces cerevisiae*. Appropriate source for phosphate ions and its concentration in fermentation medium is a determining factor for invertase production by microorganism (Gines *et al*., 2000). Results show that phosphate ion concentration in fermentation medium has a correlation with enzyme secretion by microorganism. At high concentrations, phosphate reduces cell mass and increases the alkalinity of medium, which is highly unfavorable for yeast growth and enzyme stability (Shafiq *et al.,* 2002).

Kinetic analysis of fermentation process determines the efficacy and feasibility of product yield in relation to growth of microorganism and substrate uptake (Walsh *et al*., 1994). Pejin and Razmovski (1993) investigated the influence of glucose concentration in nutrient media on the specific growth rate and biomass yield in the course of continuous fermentation of *Saccharomyces cerevisiae*. An increase of glucose content in media decreased the specific growth rate and the biomass yield. Pilgrim *et al*. (2001) studied the reaction kinetics of the enzyme-induced hydrolysis of sucrose. In the present study, different kinetic parameters like comparison of growth and product rates and comparison of specific product and specific growth rates were worked out.

MATERIALS AND METHODS

Saccharomyces cerevisiae strain GCB-K5 was employed for the present study. Culture was obtained from a commercial yeast brand and activated in seed medium for nutritional investigations.

Preparation of vegetative inoculum. Cell suspension was prepared from 2-3 days old slant culture of *Saccharomyces cerevisiae* GCB-K5. Twenty-five mL of the medium containing (g L^{-1}, w/v) sucrose 10.0; peptone 5.0 and yeast extract 3.0 at pH 6, 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 (1.0×10^7 cells) from the slant culture was aseptically transferred into the growth medium. The flask was incubated at 30°C in an incubator shaker (Gallenkamp, UK) and shaken at 200 rpm for 12 h.

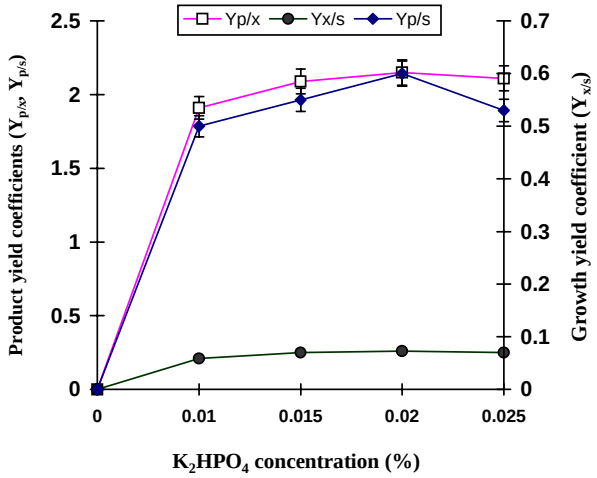
Cultivation method. Invertase production by yeast culture was carried out by shake flask technique using 250 mL Erlenmeyer flasks. Same medium composition was used for vegetative inoculum preparation and for fermentation. 25 mL of fermentation medium was transferred to each Erlenmeyer flask. The vegetative inoculum was transferred to the production medium at a level of 4% (v/v) based on total working volume of sterilized fermentation medium. 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^{-1}. Different phosphate

sources were introduced in the medium in different concentrations. The flasks were run parallel in duplicates.

***In vitro* assays.** "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". Invertase activity was determined according to the method of Sumner and Howell (1935). 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. Sugar was estimated calorimetrically by DNS method (Tasun *et al*., 1970).

Kinetic and statistical analysis of data. Data was statistically analyzed using Duncan's multiple range tests (SPSS-10, version 4.0) following the methods of Kubicek and Rohr (1985). Kinetic parameters for batch fermentation process were described by following the procedures of Pirt (1975) and Lawford and Rouseau (1993). Kinetic parameters i.e. product yield coefficients ($Y_{p/s}$, $Y_{p/x}$) and growth yield coefficient ($Y_{x/s}$) were considered for present study. The specific product and growth rate, μ (h^{-1}) were calculated as invertase or dry cell mass-produced per hour of fermentation period.

Fig. 1. Comparison of product and growth yield coefficients in relation with different concentrations of K_2HPO_4



Kinetic parameters: $Y_{p/x}$ = 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 bars indicate standard error of means among the three parallel replicates.

RESULTS AND DISCUSSION

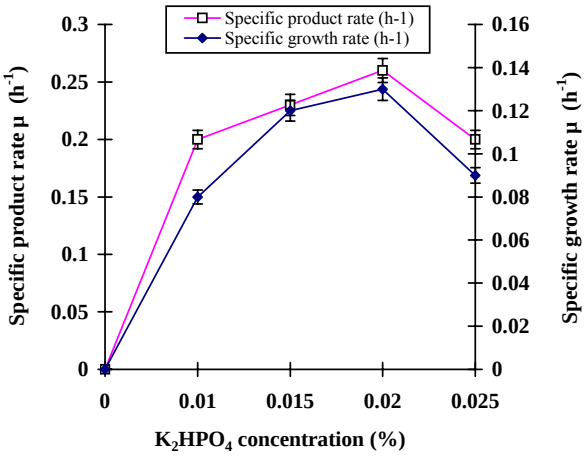
Phosphate source in fermentation medium plays a key role in enhancing the invertase secretion capacity of budding yeast cells. Different phosphate sources like NaH_2PO_4, K_2HPO_4, KH_2PO_4 and Na_2HPO_4 were tested in varied concentrations (0.010-0.025%) for their effect on invertase synthesis by *Saccharomyces cerevisiae* (Table I). Maximal extracellular invertase activity (12.68 U mL^{-1}) was observed when K_2HPO_4 (0.020 %) supplemented medium was used for production. Sugar consumption and dry cell mass (mg mL^{-1}) at this level were 21.08 and 5.88, respectively. Maximum production of invertase by using K_2HPO_4 as phosphate source might be due to fact that phosphate was readily available to yeast (Gomez *et al*., 2000). Least synthesis of enzyme (3.50 U mL^{-1}) was observed when Na_2HPO_4 was used in medium as phosphate source. It might be due to toxicity of sodium ions in combination with phosphate ions that inhibited the yeast

Table I. Effect of different phosphate sources in varied concentrations on invertase synthesis by *Saccharomyces cerevisiae* GCB-K5.

Phosphate sources	Concentration (g 100 mL^{-1})	Dry cell mass (mg mL^{-1})	*Final pH	Sugar consumption (mg mL^{-1})	Invertase activity (U mL^{-1})
NaH_2PO_4	0.010	4.86±0.41	6.0	18.96±0.11	9.37±0.47
	0.015	5.21±0.34	5.5	20.42±0.24	10.25±0.31
	0.020	5.10±0.23	5.8	19.45±0.32	9.04±0.50
	0.025	4.92±0.40	5.8	19.32±0.22	8.20±0.52
K_2HPO_4	0.010	4.03±0.51	5.6	19.16±0.23	9.66±0.44
	0.015	5.78±0.33	5.8	20.50±0.33	11.08±0.54
	0.020	5.88±0.32	5.9	21.08±0.15	12.68±0.32
	0.025	4.63±0.40	6.0	18.34±0.62	9.82±0.33
KH_2PO_4	0.010	5.84±0.21	5.2	19.01±0.31	8.82±0.62
	0.015	4.92±0.12	5.4	16.28±0.42	5.51±0.12
	0.020	4.50±0.11	5.1	17.83±0.30	6.88±0.32
	0.025	4.19±0.09	5.6	17.35±0.40	5.23±0.11
Na_2HPO_4	0.010	5.02±0.41	5.0	19.64±0.22	4.55±0.20
	0.015	5.23±0.52	5.3	19.75±0.42	4.21±0.22
	0.020	4.02±0.32	5.9	16.33±0.32	3.76±0.15
	0.025	4.12±0.30	5.8	17.75±0.41	3.50±0.36

Sucrose concentration, 25 g L^{-1}; incubation period, 48 hours; temperature, 30°C; initial pH, 6.0; agitation rate, 200 rev min^{-1}. ± indicates standard error of means among the three parallel replicates. The values differ significantly at $p \leq 0.05$.*Final pH is the pH of fermented broth after completion of incubation hours

Fig. 2: Influence of different concentrations of K_2HPO_4 on specific product and growth rates (h^{-1})



Kinetic parameters: Specific growth rate = mg cells produced h^{-1}; Specific product rate = Amount of enzyme produced h^{-1}; Y bars indicate standard error of means among the three parallel replicates.

cells to secrete invertase in the medium.

The synthesis of β-fructofuranosidase in synchronously dividing cells of *Saccharomyces* continues throughout the budding cycle and follows the increase in cell mass. Similar patterns for cell mass and enzyme increases can be observed even in phosphate-deprived cells, which do not divide (Arnold, 1982). Thus, appropriate concentration of phosphate is a critical factor to obtain maximal enzyme secretion. Effect of phosphate ions on cell mass production and sugar uptake in relation with enzyme secretion was worked out. Product and growth yield coefficients were quite feasible at this point of K_2HPO_4 in the medium (Fig. 1). Concentration of readily available phosphate ions in the medium also affects the enzyme productivity and cell mass formation per hour of fermentation period (Fig. 2). As concentration of K_2HPO_4 was increased than optimum, cell mass per hour was reduced and same for invertase secretion in the fermentation medium. At high concentrations, phosphate reduces cell mass and increases the alkalinity of medium, which is highly unfavorable for yeast growth and enzyme stability (Underkofler & Hickey, 1954).

CONCLUSIONS

It can be concluded from present study that dipotassium hydrogen phosphate (0.02 %) as phosphate source in the fermentation medium gives the optimum invertase secretion by selected strain of *Saccharomyces cerevisiae*.

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