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Research Article

EFFECT OF NITROGEN SOURCES ON THE PRODUCTION OF INVERTASE BY YEAST *SACCHAROMYCES CEREVISIAE* 3090

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ABSTRACT: Invertase from *Saccharomyces cerevisiae* is high cost enzyme and primarily used in the confectionary industry. For large scale production of the enzyme, feasible synthetic medium with appropriate supplemented nutrients are required. The effect of carbon source on invertase production is well known, but little is known about the effect of different nitrogen source. The aim of the present study is to see the effect of different nitrogen sources on the production of invertase in submerged fermentation by *Saccharomyces cerevisiae*. It was found that when urea as nitrogen source is added in a little amount to the fermentation medium it showed a marked increase in Invertase production.

Key words: *Saccharomyces cerevisiae*, Invertase, Nitrogen source

INTRODUCTION

Chemohetrotrophic organisms are dependent on chemical energy sources and employ organic compounds as the principle carbon source. Carbohydrates are an excellent source of carbon, oxygen, hydrogen and metabolic energy. They are frequently present in the media at a concentration of 0.5-30%. After carbon source, the next major compound in the media is the nitrogen source. The presence of appropriate concentration of carbon and nitrogen source greatly influences the production metabolites/desirable product. Therefore, it is necessary to find out suitable carbon and nitrogen sources. Invertase (EC: 3:2:1:26) acts on 1, 4 glycoside linkage of sucrose and splits it into D-glucose and D-fructose (Kaur and Sharma, 2005). Invertases are intracellular as well as extracellular enzymes (Nakano, et al., 2000). It is one of the most widely used enzymes in food industries such as in the preparation of jams and candies (Aranda, et al., 2006),in production of confectionary with liquid or soft centre, manufacturing of invert syrups, calf feed preparations and fermentation of cane molasses into ethanol (Park and Sato, 1982; Gehlawat, 2001). On industrial scale citric acid fermentation uses molasses as a feedstock, which contains principally sucrose as a carbon source. The invertase is also used in combination with glucose isomerase for sweetening the syrups. The organism showing greatest ability to secrete invertase is yeast (Moreno, et al., 1979; Silveira, et al., 2000). Along with the different substrates containing carbon source, nitrogen source have a major effect on the yeast to synthesize invertase (Nakano, et al., 2000).

The objective of the present work was to see the effect of different nitrogen sources and concentration of urea on the growth of the yeast and invertase production. Effect of nitrogen as an inducer on the specific product rate and specific growth rate were also studied.

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MATERIALS AND METHODS

Organism and culture media: Yeast *Saccharomyces cerevisiae* (NCIM, National Collection of Industrial Microorganisms, 3090) was obtained from National Chemical Laboratory (NCL), Pune, India. Yeast slants used in this study were maintained at 4°C. The culture was maintained on YEPS agar medium (yeast extract 3.0gm/L, peptone 10.0g/L, sucrose 20.0g/L, and agar 20.0g/L) at initial pH 6.0. The strain was used for the production of invertase by submerged fermentation.

Inoculum preparation and Fermentation Condition: Yeast cell suspension was prepared from 2-3 day old yeast *Saccharomyces cerevisiae* strain no. 3090. The medium used for enzyme production under submerged fermentation comprised of (gm/L): yeast extract 3, peptone 10, and sucrose 30, pH 6.0. Cultivation was carried out in 250 ml Erlenmeyer flasks each containing 50 ml of sterile medium. After inoculation (1×10^6cells /ml), the flasks were incubated in a rotary shaker incubator (SANYO, Japan) at 30ºC for 48 hrs with shaking at 200 rev/min. The flasks were run parallel in duplicates.

Determination of Invertase activity: Invertase activity was determined using the method of Sumner and Howells (Sumner and Howell, 1935) with slight modification by incubating 0.1 ml of enzyme solution with 0.9 ml of sucrose in 0.03M acetate buffer (pH 5.0). To stop the reaction, 1ml of the dinitrosalicylic acid reagent was added and heated for 5 minutes in boiling water bath. Finally, the absorbance was read at 540 nm in spectrophotometer (Miller, 1959). One unit of enzyme activity (IU) is defined as the amount of enzyme which liberates 1micro moles of glucose/minute/ml under the assay condition.

Optimization of Nitrogen source for Invertase production: Various nitrogen sources viz. nutrient broth, peptone+yeast extract (control), urea, ammonium chloride and potassium nitrate were examined for optimum invertase production.

Determination of dry cell mass*:* Dry cell mass of yeast was determined by centrifugation of medium at 5000 rev/min. Tubes were oven dried at 105°C for one hour. The supernatant was used for further analysis.

RESULTS AND DISCUSSION

Effect of Sucrose concentration: Proper concentration of carbon source is important for the optimum production of invertase enzyme. In this study, we used Sucrose as a carbon source and to get optimum enzyme production, 20-40g/l sucrose was used (Fig.1). Maximum enzyme activity was obtained at 30g/l sucrose concentration. More than 30g/l sucrose concentration shows increase in sugar consumption and dry cell mass, however, there was no increase in invertase production. This might be due to the generation of high concentration of inverted sugar in the medium which results in glucose-induced repression of invertase (Elorza et al., 1977; Vitolo, et al., 1995). When sucrose concentration less than 30g/l was used, enzyme production was lesser than the optimum. Similar results were obtained in our previous study (Kamble and Shinde, 2011). This lower concentration of carbon source might insufficient for the proper growth of yeast which results in less invertase production (Myers, et al., 1997).

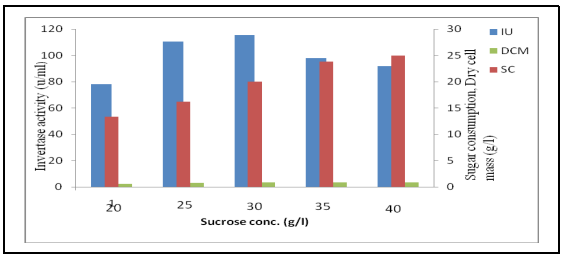

Figure 1. Optimization of sucrose concentration on invertase production by *Saccharomyces cerevisiae* 3090 (Incubation period 48hr., Temp. 30°C, initial pH 6.0, agitation rate 200rpm/min.). (IU- Enzyme activity; DCM- Dry cell mass; SC- Sugar consumption).

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Effect of different Nitrogen sources: Nitrogen sources and their concentrations have major biological effect on enzyme yield because sucrose metabolism shows a specific physiological response to the presence of nitrogen source (Nakano, et al., 2000). Effect of different organic nitrogen sources (nutrient broth, peptone+yeast extract, urea+yeast extract, and yeast extract only) on invertase production by *Saccharomyces cerevisiae* 3090 was studied. (Fig. 2). Application of appropriate nitrogen source shows a major effect on the production of invertase because of the strong correlation between nitrogen equilibrium and productivity of yeast cells in the culture medium (Rouwenhorst, et al., 1991; Neto, et al., 1996). Significant invertase activity and dry cell mass (115.82 U/ml and 1.25 g/l respectively) was obtained when peptone+yeast extract was used as nitrogen source. Least cell dry cell mass (0.81 g/l) was obtained when urea was used in the medium, however enzyme production was near to maximum i.e. 111.15 U/ml. Reduced cell mass might be due to denaturing effect of urea on yeast cell (Pitombo, et al., 1994). The reason for high enzyme yield might be positive influence of urease and invertase on each others secretion into the culture medium (Egorov, et al., 2000).

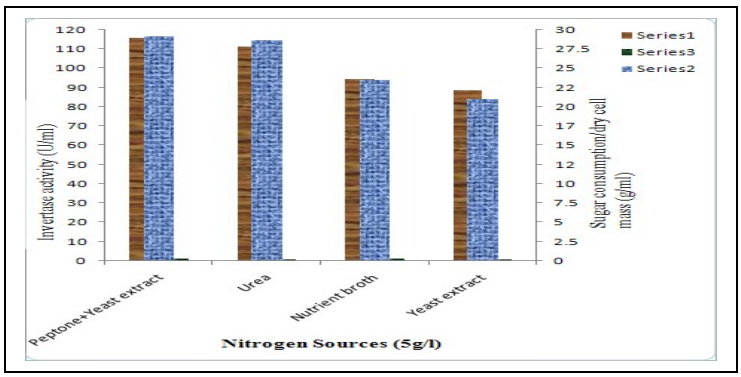

Figure 2. Effect of different Nitrogen sources on invertase production by *Saccharomyces cerevisiae* 3090. (Series1- Invertase activity; Series2- Sugar consumption; Series3-Dry cell mass)

Effect of Urea concentration on Invertase activity: The effect of urea concentration in the fermentation medium on the production of invertase by *S. cerevisiae* 3090 was studied (Fig.3). Maximum enzyme activity (154.2 U/ml) was observed in urea concentration of 2.0 g/l. Sugar consumption and dry cell mass were 23.72g/l, and 1.025 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 Qp and Yx/s (Fig. 3), indicating a reduction in cell mass with an increase in urea concentration, while increasing enzyme yield at optimal concentration of urea.

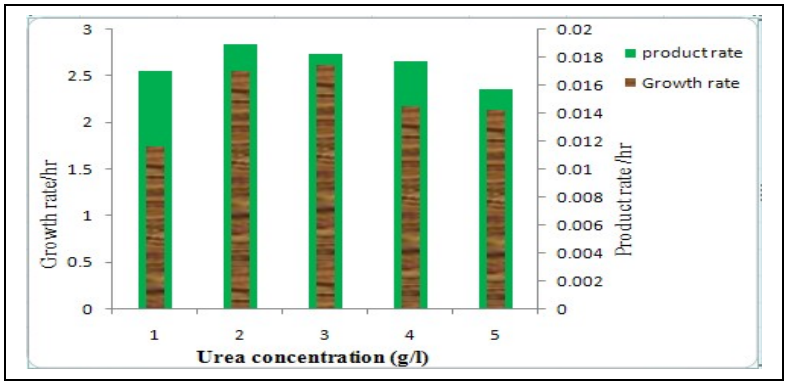

Figure 3. Effect of Urea concentration on Specific growth rate and Product rate.

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