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Optimum conditions for Invertase production from *Saccharomyces cerevisiae* using solid state fermentation

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Abstract

Three *Saccharomyces cerevisiae* isolates from different sources (China, Turkey and Egypt) were screened by culturing on solid state fermentation to select the most efficient isolate for invertase production. *S. cerevisiae* from China was high specific activity 34.7 U/mg. The optimum conditions for enzyme production from this isolate were determined by using a medium composed of wheat bran moisten with 1:0.5 (v:w) corn steep liquor as nitrogen source at initial pH 5.0 for 5 days at 30^{O}C.

Keywords: *Saccharomyces cerevisiae*, Invertase, Optimization, Solid state fermentation

تحديد الظروف المثلى لإنتاج انزيم الانفرتيز من خميره الخبز *Saccharomyces cerevisia* باستخدام تخمرات الحالة الصلبة

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الخلاصة

ثلاث عزل من خميره الخبز *Saccharomyces cerevisiae* المأخوذة من مصادر مختلفة (الصين، تركيا ومصر) غربلت واختبرت كفاءتها لإنتاج انزيم الانفرتيز باستخدام تخمرات الحالة الصلبة. وجد ان عزله خميره الخبز *Saccharomyces cerevisiae* المأخوذة من المصدر الصيني كانت اكثر كفاءة لإنتاج انزيم الانفرتيز مع فعالية نوعيه 34.7 وحده /ملغرام. حددت الظروف المثلى لإنتاج الانزيم من خميره الخبز وكان باستخدام نخاله الحنطة كوسط انتاجي ورطب هذا الوسط بمستخلص نقيع الذرة كمصدر نيتروجيني وبنسبه ترطيب 1:0.5 (وزن :حجم) وبرقم هيدروجيني 5 ومده حضانة 5 ايام وبدرجه حراره 30 درجه مئويه.

Introduction

Saccharomyces cerevisiae is a species of yeast. It is perhaps the most useful yeast, having been instrumental to winemaking, baking, and brewing since ancient times. It is believed that it was originally isolated from the skin of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-color fruits such as plums; it exists among the waxes of the cuticle). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model bacterium. It is the microorganism behind the most common type of fermentation [1]. *Saccharomyces cerevisiae* is currently the only yeast cell that is known to have berkeley bodies present, which are involved in particular secretory pathways [2]. *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 [3]. Expression of invertase in the *S. cerevisiae* is greatly delayed

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when derepression occurs in a medium that lacks a usable carbon source [4]. *Saccharomyces* inverts sugar but inversion is often endocellular, without enzyme released into the medium [5]. Invertase (ß-Fructofuranosidases) (EC 3.2.1.26) is enzyme that is capable of hydrolyzing substrates with terminal fructosyl groups. Most ß-fructofuranosidases have been shown to hydrolyze sucrose to release glucose and fructose and to possess fructosyltransferase activity for the synthesis of short-chain fructooligosaccharides [6]. Invertase is one of the most widely used enzymes in food industry, especially in the preparation of jams and candies [7]. The enzyme is a glycoprotein, with some residues of mannose being the major component of the carbohydrate moiety. Invertase is mainly used in the food industry, where fructose is preferred over sucrose because it is sweeter and does not crystallize easily [8]. Invertase is seriously limited because another enzyme, glucose isomerase, can be used to convert glucose to fructose at lower costs [9]. The aim of this study was determination of optimum conditions for Invertase production from *S.cerevisiae* by using solid state fermentation.

Materials and Methods

Media and chemicals

Potato-dextrose agar (PDA) was obtained from hi-media**s,** Coomassie brilliant blue, protein standards and other chemicals were supplied by BDH Chemicals.

Screening of Invertase Producing Isolates:

Isolates efficiency for invertase production were screened according to the method described by [10]. The screening was performed by culturing *S. cerevisiae* from different sources (China, Turkey and Egypt) on solid medium containing 10 gram from red carrot powder pH 5.5 with moisture ratio 1:1(v:w), and inoculated with (1%) *S. cerevisiae* 1.6×10^6 cells/ml. The cells number was estimated by direct microscopic counting using haemocytometer. After 4 days of incubation at 30 ^{o}C, enzyme activity and protein concentration were estimated, and the most efficient isolate for invertase production was selected for remaining studies.

Optimization for Invertase Production:

Many factors that influence invertase production from selected *S.cerevisiae* had been studied; these factors included type of carbon source, moisture ratio, and initial pH of the medium, nitrogen source, incubation temperature and period of incubation.

Optimum Carbon Source:

Eight carbon sources were tested to determine the optimum carbon source for invertase production from selected isolate; these sources were apple pomace, grape juice residue, red carrot, wheat bran, corn, lemon pomace, orange pomace and sugarcane bagasse. All sources were washed with tap water then sliced to small pieces and dried. These dried parts were grinded until they became powder. 10 gm of each one was moistened with 10 ml (w:v) distilled water pH 5.5 in 250 ml flask, and inoculated with (1%) *Saccharomyces cerevisiae* 1.6×10^6 cells/ml, then incubated at 30^{o}C for four days.

Moisture Ratio:

Ten gram of wheat bran was moistened with different volumes of distilled water. Different moisture ratios were tested 1:0.5, 1:0.75, 1:1, 1:1.25 and 1:1.5 (w:v) to select the optimum moisture for invertase production.

Incubation Temperature:

The culture which consist of the medium contained to wheat bran (10 gm), corn steep liquor (5 ml) and pH 5.0, inoculated with (1%) 1.6×10^6 cells/ml of isolate *S.cerevisiae* was incubated in different temperature ranged (30, 35, 40 , 45 and 50) ^{O}C to find the optimum incubation temperature for enzyme production.

Optimum pH:

Production media was distributed into flasks, the pH of the medium was then adjusted to 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 by using diluted NaOH and HCl, then inoculated with *S. cerevisiae*, and it was then incubated at 30 ^{O}C for 4 days. The invertase activity was determined after incubation to determine the optimum pH for invertase production.

Nitrogen Sources:

Different nitrogen sources described by [11], were examined to determine the best nitrogen source for invertase production from selected *S. cerevisiae*. These nitrogen sources are: ammonium chloride, urea, Yeast extract, ammonium sulphate, peptone, gelatin, potassium nitrate, calcium nitrate and corn steep liquor. They were tested individually at the concentration of 0.5% dissolvent in distilled water. Distilled water used as control treatment. Five ml of each solution was added separately to 10 gm of

wheat bran (v:w) in 250 ml flask, and inoculated with (1%) *S. cerevisiae* 1.6×10^6 cells/ml, then incubated at 30°C for four days.

Incubation Period:

After inoculation the medium wheat bran (10 gm), corn steep liquor (5 ml) and pH 5.0 with (1%) 1.6×10^6 cells/ml of isolate *S. cerevisiae*, the culture was incubated at 30 °C and checked every day for 7 days to estimate enzyme activity, protein concentration and specific activity for invertase.

Estimation of Invertase Activity and Protein Concentration:

Invertase activity was estimated in solutions resulted after extraction of the enzyme by (0.1 M) sodium acetate at pH 4.8, using the method described by [12] which depends on sucrose as substrate (substrate concentration 1% in sodium acetate). One unit of enzyme activity (IU) is defined as the amount of enzyme which liberates 1 µm of glucose/ ml /minute under the assay condition. Protein concentration was estimated according to the method described by [13] depending on bovine serum albumin standard curve using coomassie blue G-250, measured at 595 nm. The specific activity was determined by using following equation:

$$\textbf{Specific activity U/mg} = \frac{\textbf{Enzyme activity U/ml}}{\textbf{Protein concentration mg/ml}}$$

Results and Discussions

Screening of Invertase Producing Isolates:

In order to examine the most efficient isolate for invertase production, all yeast isolates of *S. cerevisiae* were recultured on solid state fermentation. The culture was incubated for 4 days, at 30 °C. Results showed different efficiencies in invertase production. *S. cerevisiae* from China was appears high specific activity 34.7 U/mg, while *Saccharomyces cerevisiae* from Turkey and Egypt with 15.5 U/mg and 26.7 U/mg respectively Figure-1. Therefore, this isolate was selected for further study. Differnce between isolates for invertase production reverting to isolate source, genetic content and yeast producing methods [14].

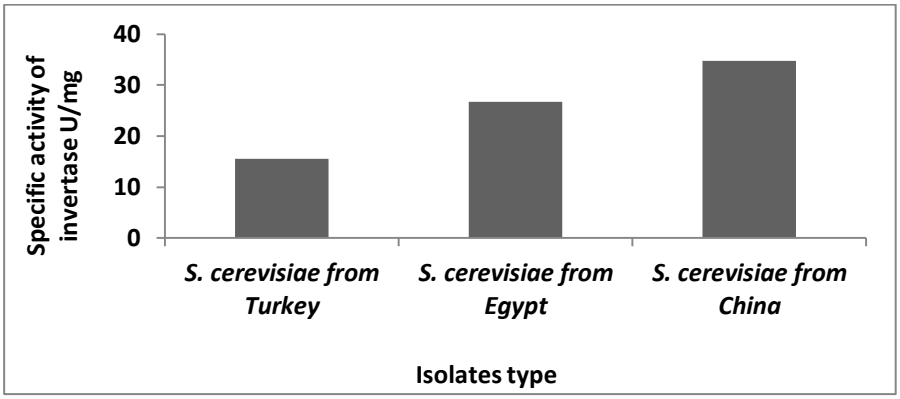

Figure 1- Efficiency of *S. cerevisiae* isolates for invertase production using red carrot pH 5.5, incubation for 4 days at 30 °C.

Optimum Carbon Source:

Eight carbon sources were tested for their efficiency in invertase production. These sources were apple pomace, grape juice residue, red carrot, wheat bran, corn, lemon pomace, orange pomace and sugarcane bagasse, Figure-2. The highest activity was shown in wheat bran with specific activity 121.5 U/mg, while apple pomace, grape juice residue, red carrot, corn, lemon pomace, orange pomace and sugarcane bagasse showed specific activities as follows 18 U/mg, 35.37 U/mg, 33.7 U/mg, 102.2 U/mg, 61.4 U/mg, 110 U/mg and 49.7 U/mg, respectively. This indicates that wheat bran was the most efficient source for invertase production from *S. cerevisiae*, because wheat bran has been mentioned as a good carbon source owing to its nutritional characteristics, such as 14% proteins, 27% carbohydrates, 5% minerals, 6% fatty acids, vitamin B and 64% nitrogen. There for, wheat bran was chosen as the best carbon source for remaining study. These results were similar to the results of Giraldo, *et al.*, [14], who proved that production of invertase from *Paecylomyces variotii* depends on changing the media components, and found that wheat bran was efficient medium for invertase production from same isolate. While Mazutii *et al.*, [15] found that the best medium for invertase production from *kluyveromyces marixianus* was sugar cane bagasse with corn steep liquor as nitrogen source.

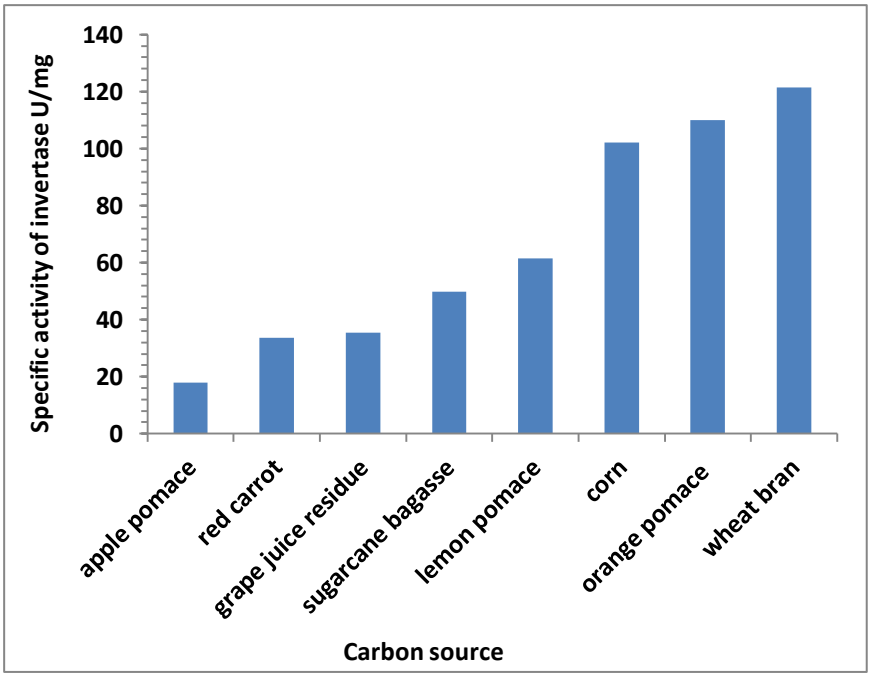

Figure 2- Effect of different carbon source on invertase production from *S. cerevisiae*, pH 5.5, incubation for 4 days at 30 °C.

Moisture Ratio:

To determine the best moisturizing ratio for invertase production of *S. cerevisiae*, five proportions were used. These results prove that the highest specific activity of invertase produced from *S. cerevisiae*, was obtained from the moisture ratio 1:0.5 (w:v), with specific activity 124 U/mg figure-3. While the ratio 1:0.75, 1:1, 1:1.25 and 1:1.5 (w:v), gave 110, 107, 104 and 100 U/mg respectively. Rashad and Nooman [16], found that the optimum moisture ratio for invertase produced from *S. cerevisiae* was 90 % (0.9:1 w:v) using red carrot sides as carbon source. While Bansal *et al.*, [17], found that the optimum moisture ratio for invertase produced from *Saccharomyces uvarum* was 1:0.5 (w:v).

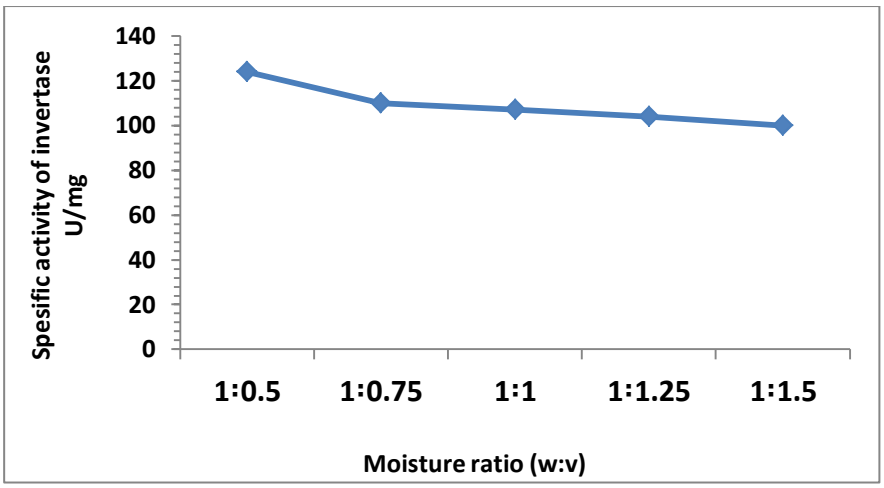

Figure 3- Effect of moisture ratio on invertase production from *S. cerevisiae*, using wheat bran, pH 5.5 and incubation for 4 days at 30 °C.

Most of solid substrates used in solid state fermentation are insoluble in water; therefore water will have to be absorbed onto the substrate particles, which can be used by the microorganisms for growth and metabolic activity [18]. Thus, it is concluded that the degree of hydration of the substrate plays an important role in the growth of the fungi and subsequently the enzyme production. Water causes the swelling of the substrate and facilitates good utilization of substrates by the microorganisms. Increasing moisture level is believed to have reduced the porosity of substrate, thus limiting the oxygen trans-

fer into the substrate [19, 20]. Likewise, a lower moisture ratio leads to reduced solubility of the nutrients of the solid substrate, lower degree of swelling and a higher water tension [21].

Incubation Temperature:

The culture which consist of the medium (wheat bran) with pH 5.5, inoculated with (1% v:v) 1.6×10^6 cells of *S. cerevisiae* was incubated in different temperature degrees (30, 35, 40, 45 and 50) ^oC to find the optimum incubation temperature for enzyme productivity. The result in Figure-4 shows that the optimum incubation temperature is 30 ^oC which gave the specific activity of 123 U/mg. Lower and higher temperatures decreases the specific activities because of the thermal effects of these temperatures on the microorganism growth and on the enzymatic reaction rate inside the cells which reflects on the vital creation of the enzyme. These results were in agreement with Sivakumar *et.al*, [22], who observed that optimum temperature for invertase productions from *S. cerevisiae* by using submerge fermentation was 30 ^oC.

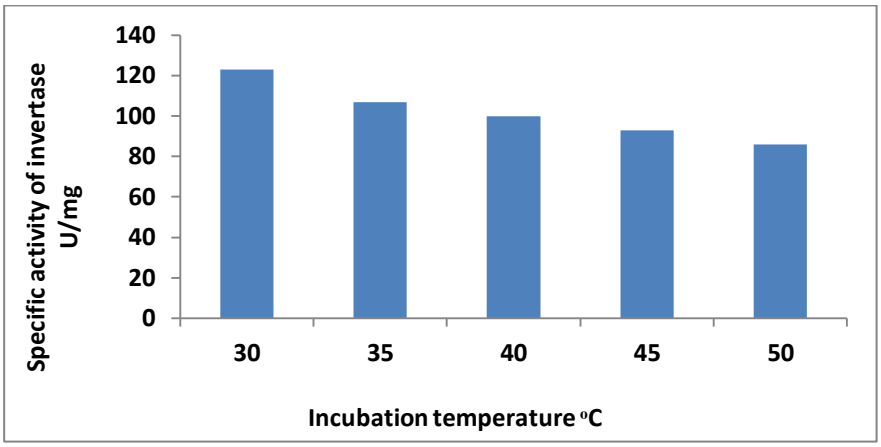

Figure 4- Effect of Incubation temperature on invertase production from *S. cerevisiae*, using wheat bran pH 5.5, incubation for 4 days.

Optimum pH:

The specific activity of invertase was estimated after incubation to determine the optimum pH and the results were illustrated in figure-5, the optimum pH for enzyme activity was 5.0 because its gave highest specific activity 216 U/mg, while pH 3.5, 4, 4.5, 5.5, 6, 6.5, 7, 7.5 and 8, gave 48, 80, 108, 181.3, 162, 120, 69, 52 and 51 U/mg respectively. Uma *et al.*, [5], found the optimum pH for invertase production from *Aspergillus flavus* was 6.0, while Uma *et al.*, [23], found the optimum pH for invertase production from *Cladosporium cladosporioides* was 5.0. Fungi generally prefer slightly acid conditions and therefore tend to dominate bacteria when these prevail. The reason for the growth rate falling away either side of the optimum value was again due to alterations in three-dimensional protein structure [20]. The pH affects in enzyme production because of its role in the solubility of medium substrates and its effect on the ionization of the substrate and it's availability for the fungal growth. Moreover the pH affects the productivity and enzyme stability.

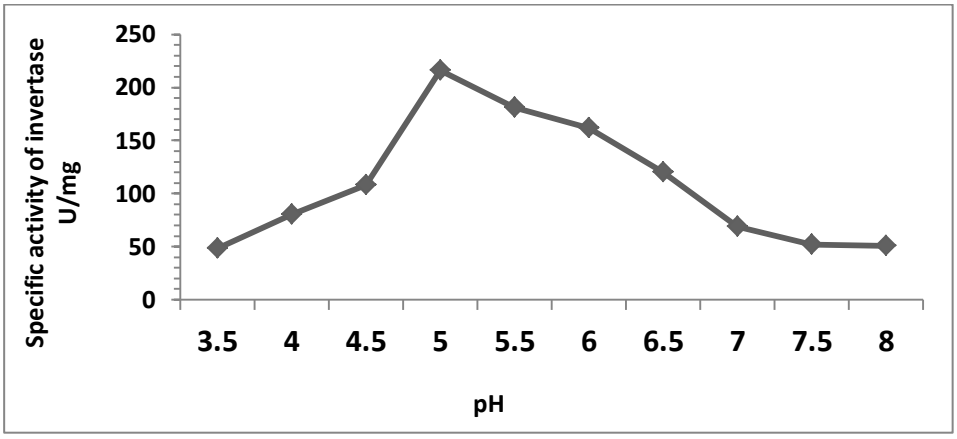

Figure 5- Effect of pH on invertase production from *S. cerevisiae*, using wheat bran, incubation for 4 days at 30 ^oC.

Nitrogen Sources:

To determine the best nitrogen sources for invertase production, nine different solutions of ammonium chloride, urea, yeast extract, ammonium sulphate, peptone, gelatin, potassium nitrate, calcium nitrate and corn steep liquor were used. From the results it was found that corn steep liquor gave the highest activity 389 U/mg, while ammonium chloride, urea, yeast extract, ammonium sulfate, peptone, gelatin, potassium nitrate and calcium nitrate gave 293, 334.5, 282.9, 114, 148, 240, 227, 200 U/mg respectively figure-6. Aslam [24], found that the best moisturizing solution for invertase production from *S. cerevisiae* was peptone.

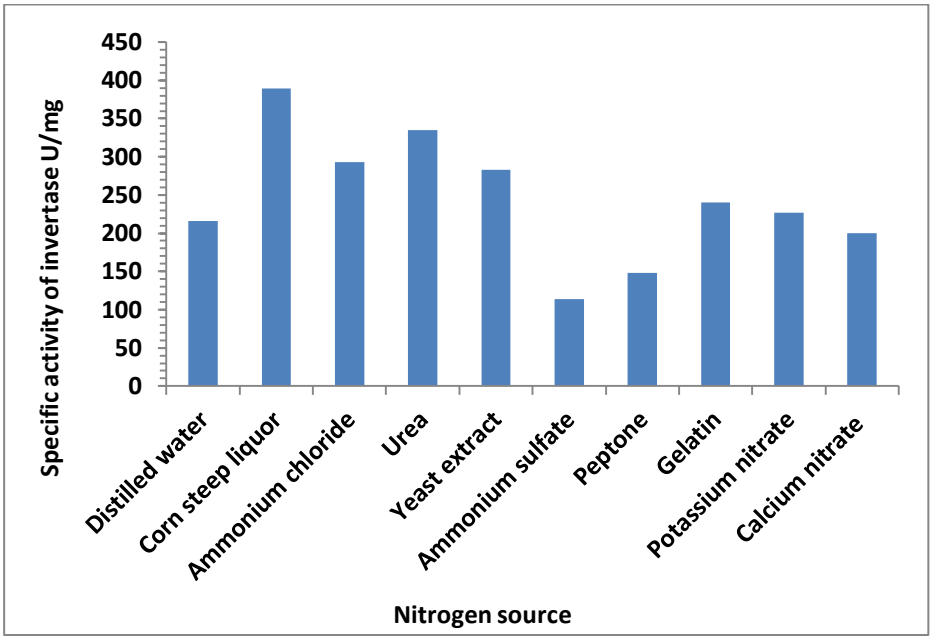

Figure 6- Effect of different nitrogen sources on invertase production from *S. cerevisiae*, using wheat bran, pH 5.0, incubation for 4 days at 30 °C.

Incubation Period:

The results in figure-7 show the effect of incubation period 1-7 days on invertase production from *S.cerevisiae*. The highest specific activity was at 5 days of incubation 393 U/mg. This result was agreed with the result obtained by Aslam [24]. While Sivakumar *et.al*, [22], found that best Incubation period for invertase production from *Saccharomyces cerevisiae* was 2 days.

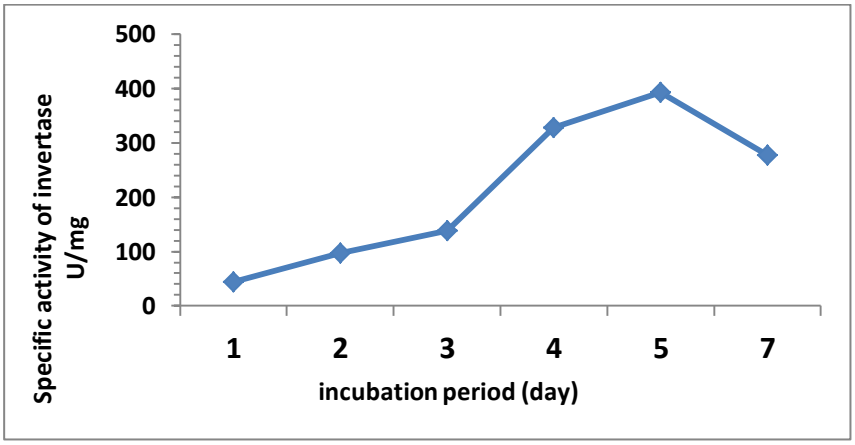

Figure 7- Effect of Incubation period on invertase production from *S. cerevisiae*, using wheat bran pH 5.0, incubation at 30 °C.

The enzyme production decreases after 5 days of incubation, was due to the production of reducing sugar such as glucose and fructose in culture medium which may lead to repression of invertase promduction, because these sugars were more readily carbon source than sucrose. The decrease in enzyme

production occurred as a result of the reduce in nutrients of the medium and as a result of accumulation the catabolic repression of enzyme [16].

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