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Isolation of Saccharomyces Cerevisiae and Production of Invertase Enzyme

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Abstract
In the present study industrially important yeast *Saccharomyces cerevisiae* was isolated from grape samples using dilution and plating technique. The isolated *Saccharomyces cerevisiae* was tested for the invertase production by using different carbon and nitrogen sources at 37°C temperature and 5 pH. The maximum growth of saccharomyces cerevisiae was observed when sucrose (20g/L) was used as carbon source and peptone 20g/L as nitrogen source while the higher invertase production was observed at 48h. Total sugar, reducing sugar and total protein concentrations was also checked by using spectrophotometric method. Isolated Invertase (β-fructofuranosidase) could be utilized in food, cosmetics, and pharmaceutical industries.

Introduction

Invertase [β-fructofuranosidases (EC.3.2.1.26)] is an enzyme that is widely distributed among the biosphere. It is broadly used in the food and beverage industry to produce candies, chocolates, lactic acid and glycerol etc. [1]. Invertase is produced by different strains of microorganisms. Saccharomyces cerevisiae commonly called Baker's yeast is the primary strain used to produce Invertase commercially. They are found in wild growing on the skin of grapes, oranges and other fruits. Tough plants like pineapple (Ananas comosus), oat (Avena sativa), pea (Pisum sativum), can also be used, but in common microorganisms like *A. niger, S. cerevisiae,* Candida utilis, are considered ideal for their study [2, 3]. The enzyme invertase catalyse the cleavage of the disaccharide sucrose to the monosaccharaides of glucose and fructose. Reducing sugars which yield a deep red colour when reacted with Benedict's reagent. Sucrose commonly known as alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha- 1, 4-glucosidic bond. When this bond is cleaved in a hydrolysis reaction, an equimolar mixture of glucose and fructose is generated. This mixture of monosaccharide is called as invertase (or) invert sugar and enzyme that hydrolysis is called sucrose. Commercially fructose and glucose syrups are produced from sucrose by the action of invertase [5]. Invertase which occur in higher plant tissues are mostly extracellular or in soluble form. In plant tissues invertase are usually

classified as acid, neutral or alkaline depending on the basis of the range required for their maximum activity. The acid invertase is widely distributed in plants such as beet root, carrot, potato and red beet root whereas acid neutral type of invertase have been detected in sugarcane. However both acid and alkaline invertase has been isolated from soybean nodules [4].

A strain of Saccharomyces cerevisiae (S. cerevisiae) has been selected for its ability to produce high invertase levels. This yeast is produced in the same production process as that of commercial Baker's yeast. The income generated by the sale of this specific yeast strain to the sugar industry in 1998 was approximately R600$ (Du Plessis, J., pers. Comm).Another important application for the invertase enzyme is the production of high-test molasses. In South Africa, the industry has grown significantly over recent years. About 150 tons of high-test molasses was produced daily in 1998 (Soji, C. N., pers. comm.). The main use of this high glucose/fructose syrup at present is as a raw material in the production of lysine by fermentation (Imrith, N., pers. comm.). Other possible uses for high test molasses include, usage as a substrate for ethanol and yeast biomass fermentations. Using yeast with a high invertase activity to carry out the inversion of the sucrose reduces the cost of production of high-test molasses. Invertase also has important applications in the sweet and confectionery industry. Currently, NCP Yeast (PTY) Ltd. producing high invertase activity yeast by fermentation of S. cerevisiae, using sugar cane blackstrap molasses as a substrate. There are two problems with this fermentation: a) The high invertase activity yeast produced, has an enzyme activity of 10-15% lower than the minimum acceptable limit required by the sugar refineries, b) There is little or no consistency in the invertase activity from batch to batch. In an attempt to increase the invertase activity of the yeast, different parameter of carbon and nitrogen source was used.

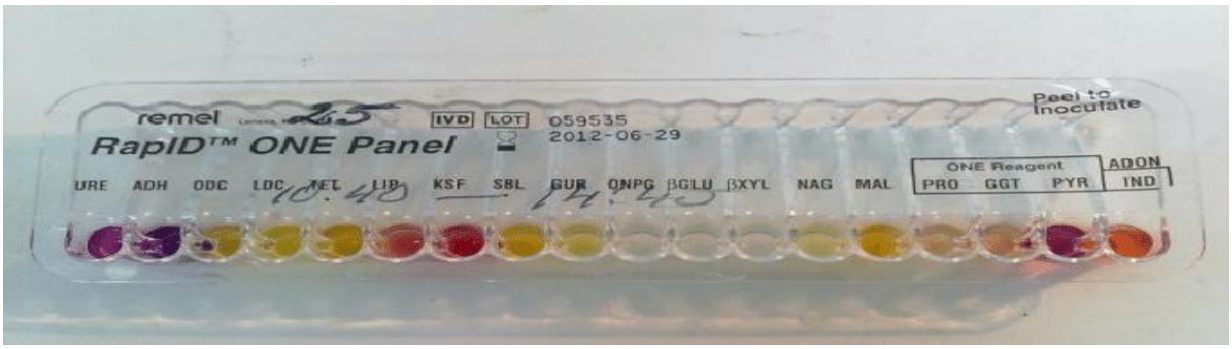
Materials and methods

Organisms and inoculum preparation

Saccharomyces cerevisea was isolated from the grapes at Institute of Biotechnology and Genetics Engineering university of Sindh Jamshoro Pakistan. Culture was maintained on yeast extract peptone and dextrose agar medium (YEPD) and sub cultured every week on YEPD agar plates. The YEPD agar media contained following: dextrose 20g, yeast extract 10g, peptone 20g and agar 20g(per litter of double distilled water).

Biochemical analysis of *saccharomyces cerevisea*

Biochemical analysis of *Saccharomyces cerevisea* was carried out by RapID ONE cavities panel system at Institute of Biotechnology and Genetic Engineering university of Sindh Jamshoro Pakistan.



Invertase production

The medium used for enzyme production under submerged fermentation contained (g/L of distilled water): sucrose 20, yeast extract 10, ammonium sulphate 1.0, magnesium sulphate 0.75, potassium dihydrogen phosphate 3.5, pH 6.5. Cultivation was carried out in250 ml Erlenmeyer flasks each containing 50 ml of sterile medium and inoculated with 0.5ml of culture. After 24h of incubation period fermented media was centrifuge at 8000rpm for 20minutes, the invertase containing supernatant was collected by filtration and ready to use further analysis.

Effect of carbon source

Effect of carbon source (20g/L) on microbial growth and invertase production was determined by replacing sucrose in the above mentioned fermentation medium with date syrup, maltose, galactose and glucose. Culture was incubated at 37°C for 60 h.

Effect of nitrogen source

Various nitrogen sources, including yeast extract, peptone, urea, meat extract, and ammonium sulphate at a concentration of 10 g/L were supplemented in synthetic medium.

Effect of pH and temperature

The pH and temperature was fixed according to (Qureshi et al., 2012b).

Invertase assay

Invertase activity was assayed from culture supernatant collected after centrifugation 8000rpm for 10 min, according to method adapted from (Qureshi et al., 2012b). 1 mL of crude sample was taken, and then 1 mL of substrate (sucrose solution prepared in 20 mM acetate buffer pH 5.5) was added. Reaction contents were mixed thoroughly and incubated at 37°C for 15 min. Then, 2 mL of dinitrosalicylic acid (DNS) reagent was added for color formation and to stop the reaction, and then reaction mixture was boiled for 5 min. The absorbance of samples was read at 540 nm against blank, according to reducing sugar determination method (Miller, 1959). Enzyme and substrate blank were prepared by replacing substrate or enzyme with 1 mL of double distilled water. One unit of invertase activity was defined as, the amount of crude enzyme required for

releasing 1 µg of reducing sugar under assay conditions. All experiments were performed in triplicate and averages of results are shown in figures as well in text.

Results and discussions

Saccharomyces cerevisea was isolated from grapes at Institute of Biotechnology and Genetic Engineering university of Sindh Jamshoro Pakistan Isolation was confirmed by staining showed oval shaped cells of saccharomyces cerevise shown in figure 1 and by biochemical analysis through RAPID ONE panel system, saccharomyces cerevisea has an ability to metabolize urea, lysine, fatty acids, suger aldehyde, adonitol the same results are shown in table 1, and screened for invertase activity.

Effect of incubation time on growth and production of invertase enzyme by *Saccharomyces cerevisea* is shown in Figure 5.1. The growth of *saccharomyces cerevisea* and production of invertase enzyme was increased with passage of time, maximum invertase production (4.212U/ml) was observed at 72 h, but on further incubation, enzyme production decreased. This decreasing in enzyme production may be because of change in pH, production of inhabiting metabolites or denaturation of enzyme. Similar reports have been reported by (Qureshi et al., 2012b).

Effect of carbon sources (glucose and sucrose) on invertase production was analyzed. It is shown in Figure 3 , *Saccharomyces cerevisea* grows better and produced maximum invertase enzyme when 2% sucrose with magnesium sulphate(0.75g/L)in medium after 48h of incubation as compare to glucose. This may be due to more activeness of *Saccharomyces cerevisea* to sucrose rather than glucose.

Cavity No	Biochemical test code	Reaction
1	Urea	+
2	Arginine	_
3	Omithine	_
4	Lysine	+
5	Aliphatic thiole	_
6	Fatty acid ester	+
7	Sugar aldehyde	+
8	Sorbitol	_
9	p-Nitrophenyl-β D-glucuronide	_
10	£-Nitrophenyl-β D-galactoside	_
11	p-Nitrophenyl-β D-glucuside	_
12	p-Nitrophenyl-β D-xyloside	_
13	NAG	_
14	Melonate	_
15	Proline	_
16	Glutamine	_
17	Adonitole	+

Table1: Biochemical analysis confirmed utilization of urea, lysine, fatty acid, sugar aldehyde and adonitole.

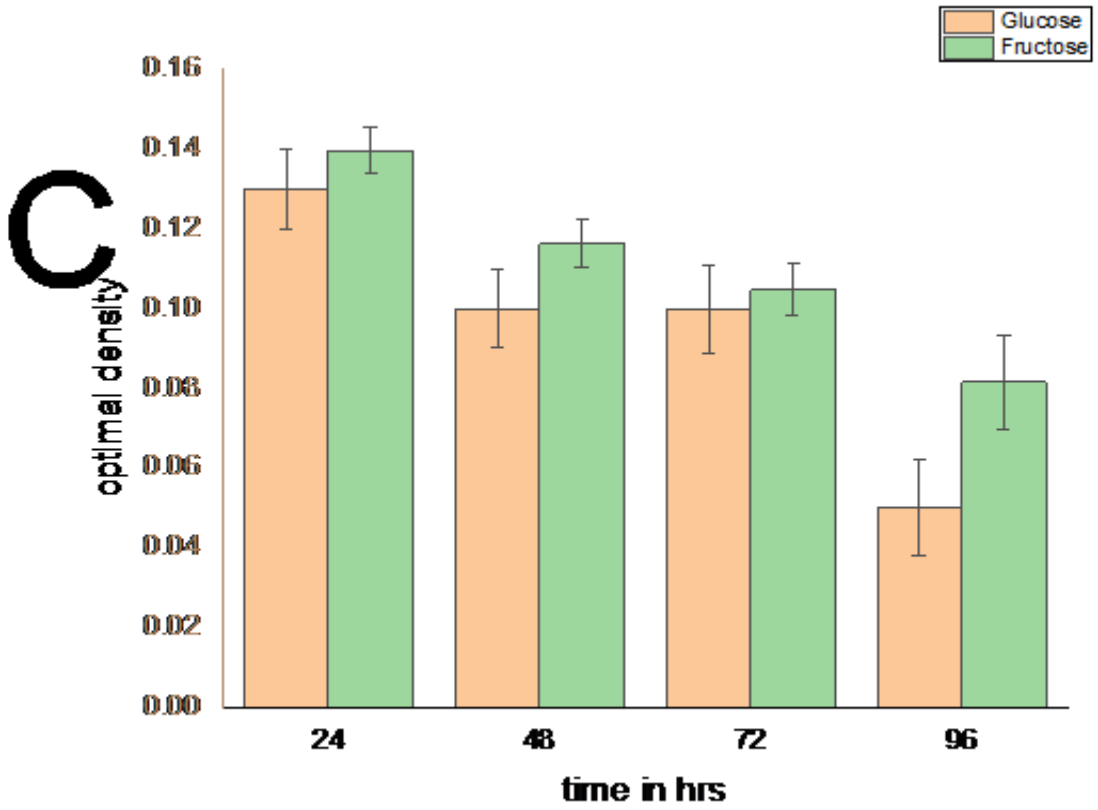


Figure 3: Effect of carbon source of growth of *Saccharomyces cerevisea*, maximum growth occurred after 24h incubation when 2% sucrose used as carbon source

Figure 4 shown the effect of nitrogen sources (yeast extract and peptone) on growth of *Saccharomyces cerevisea*. Maximum growth (.268g/ml) occurred when peptone was used as nitrogen source, this may be due to metabolic activity of saccharomyces cerevisea much better to words peptone as compared to yeast extract.

Figure 5.1 shows the production of invertase enzyme by *Saccharomyces cerevisea.* Maximum production (4.212U/ml) of invertase enzyme was observed after 48h of incubation time when sucrose was used as carbon source and peptone used as nitrogen source. After 72 h of incubation enzyme production was decreased this may be due to denaturation of enzyme, depletion of nutrients or production inhibitor's in fermentation media. Figure 5.2 shows the production of reducing suger and total sugar. Maximum reducing sugar (5.261mg/ml) at 24h and maximum total sugar observed (8.067 mg/ml) at 24h.

Figure 6.1 shows the effect of carbon sources (glucose and sucrose). Maximum production of invertase enzyme was observed at 72h of incubation time at 37C^{o} at 5.5pH sucrose was used as carbon source this is may be due to metabolic activity of *Saccharomyces cerevisea* to sucrose, *Saccharomyces cerevisea* is less capable to utilized glucose. Figure 6.2 shows production of reducing sugar and total sugar at different time of incubation. Maximum reducing sugar (5.261mg/ml at) 24h and maximum total sugar observed (8.067 mg/ml) at 24h.

Figure 7.1 shows effect of nitrogen source on production of invertase enzyme by *Saccharomyces cerevisea.* Maximum invertase(4.212U/mg) production observed when 2% peptone used as nitrogen source. Figure 7.2 shows the production of total sugar and reducing sugar. Maximum total sugar observed (8.067 mg/ml) at 24h, Maximum reducing sugar (5.261mg/ml) at 24h when 2% peptone used as nitrogen source.

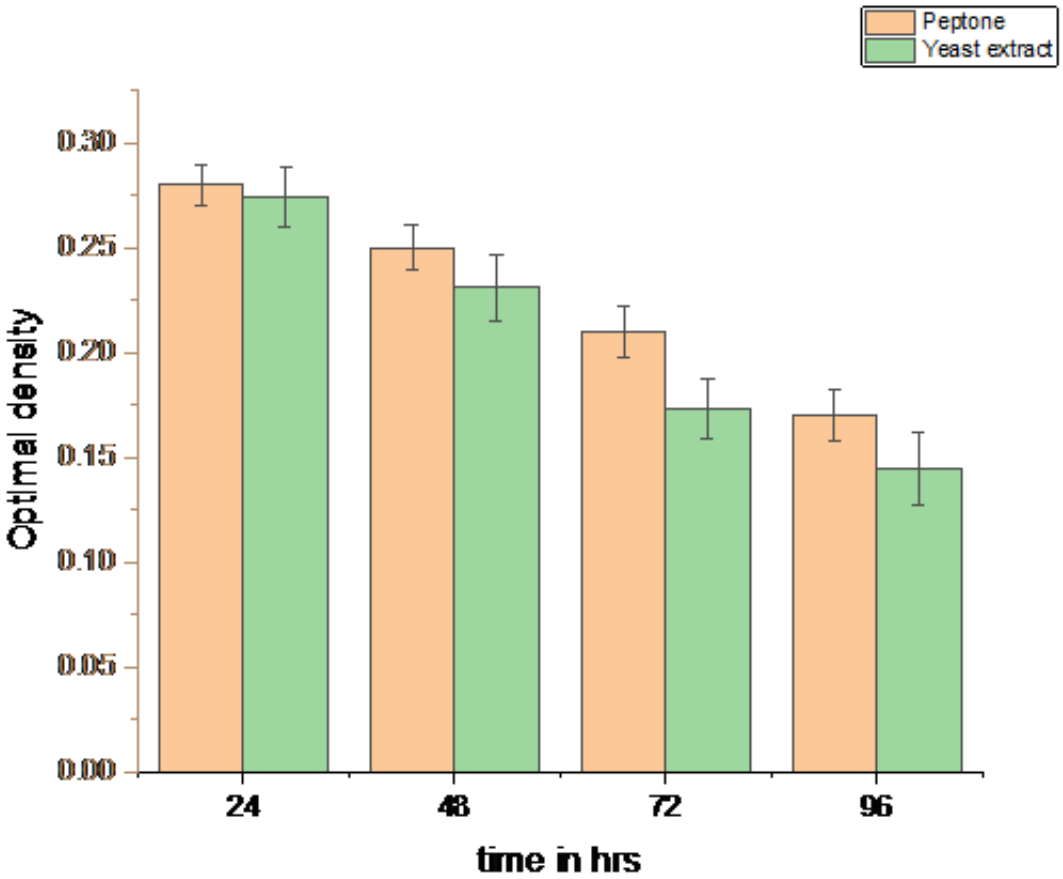


Figure 4: Effect of nitrogen sources (peptone and yeast extract) on growth of *Saccharomyces cerevisea,* maximum growth occured after 24h incubation when peptone was used as nitrogen source

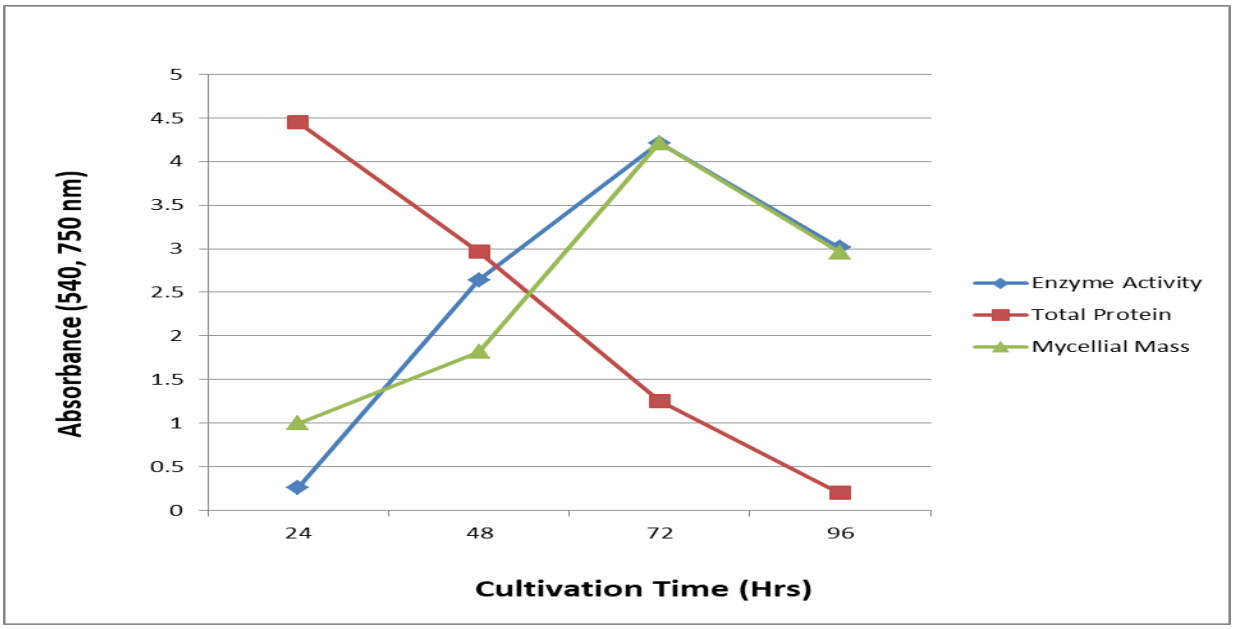


Figure 5.1: shown the production of invertase enzyme by *Saccharomyces cerevisea* was maximum (4.212U/ml) at 72h incubation time, 5.5pH and 37C⁰ temperature.

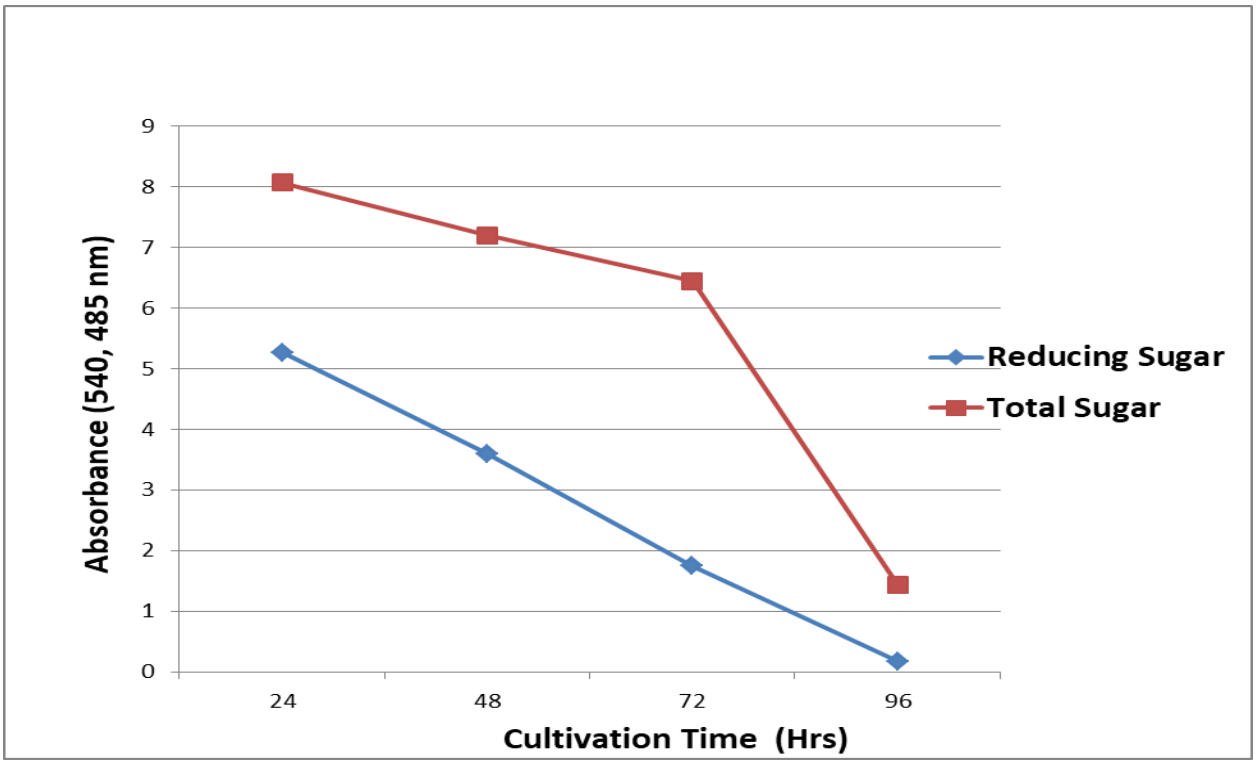


Figure 5.2: shown production of reducing sugar and total sugar at different incubation time. Maximum reducing sugar (5.261mg/ml at) 24h and maximum total sugar observed (8.067 mg/ml) at 24h

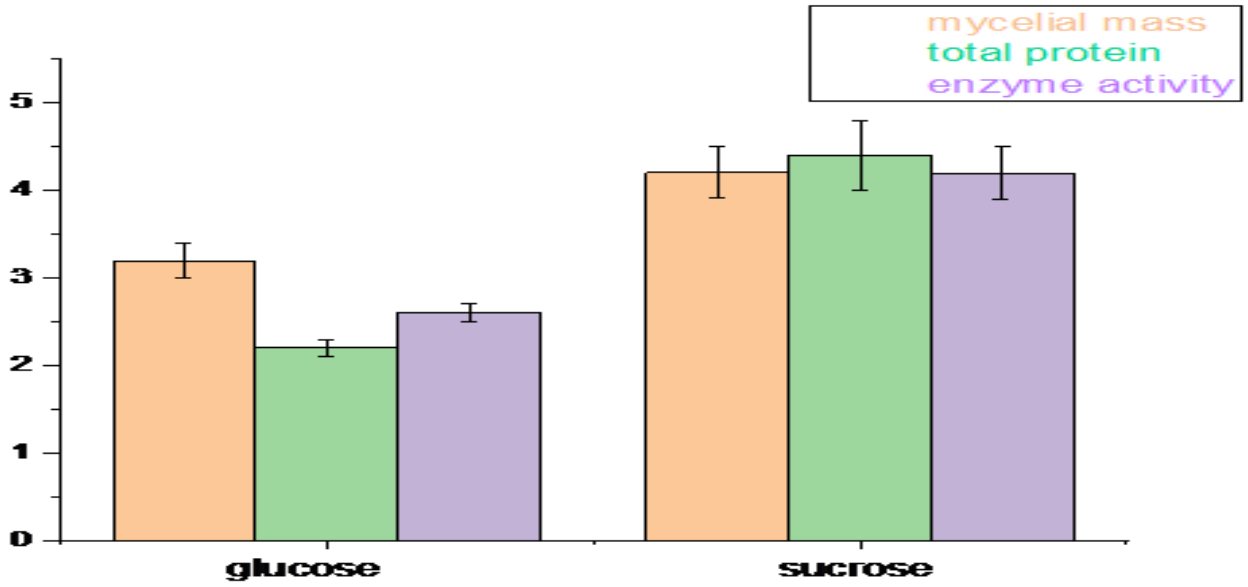


Figure 6.1: shown effect of carbon source on production of invertase by *Saccharomyces cerevisea*. Maximum production was (4.212U/ml) at 37Cº, 5.5pH and sucrose2% as carbon source.

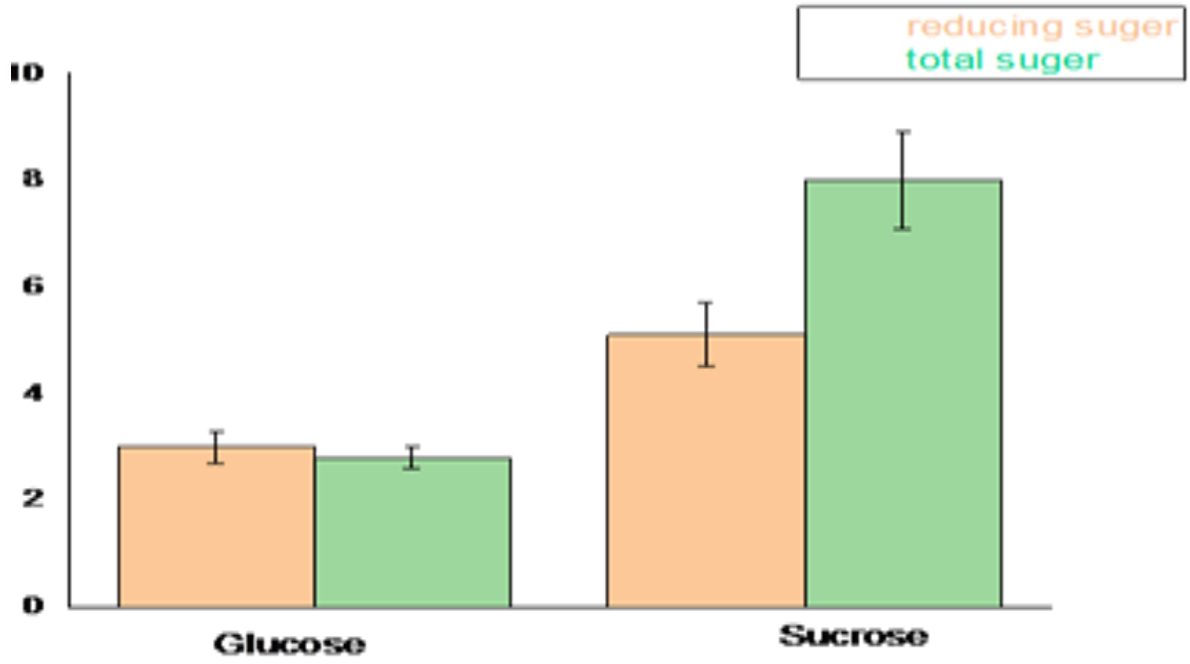
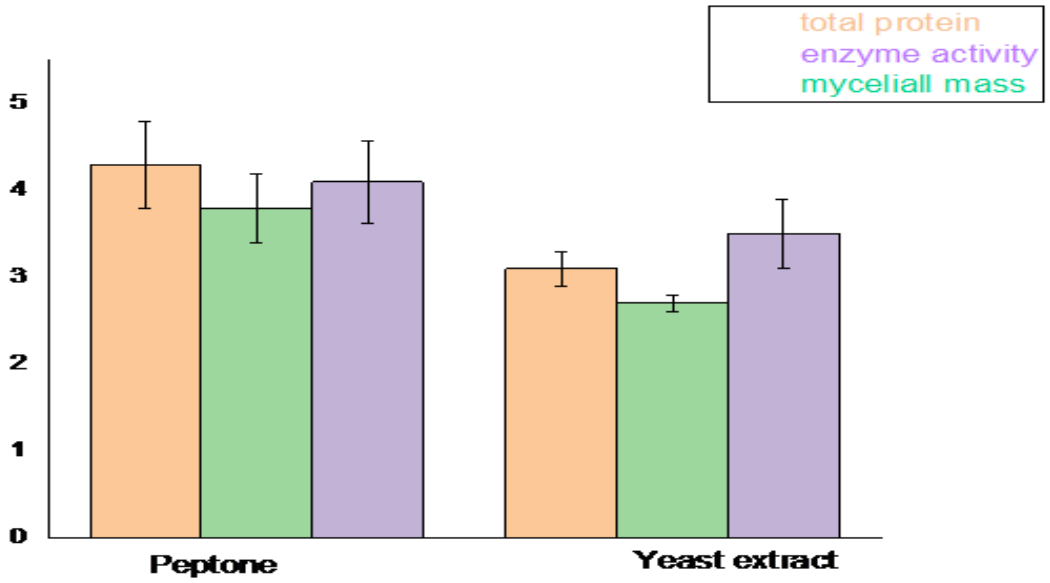


Figure 6.2: Concentration of Total Sugar and Reducing Sugar from culture broth during the production of enzyme by *Saccharomyces cerevisiae* at 72h, Temperature 37Cº and pH 5.5 using various carbon sources (glucose and sucrose).



7.1: Effect of different Nitrogen source on Biosynthesis of enzyme by *Saccharomyces cerevisiae* at 72h, Temperature 37C⁰ and pH 5.5.

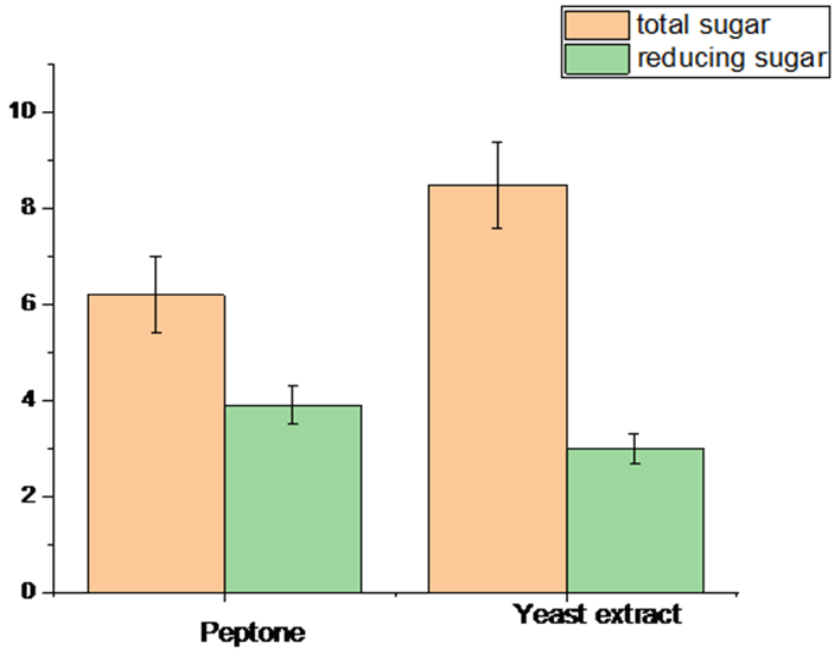


Figure 7.2: Total Sugar and Reducing Sugar from culture broth during the production of enzyme by Saccharomyces cerevisiae at 72h, Temperature 37C0 and pH 5.5 using various Nitrogen source.

Conclusion

According to the characterization and identification techniques saccharomyces cerevisiae was well identified and characterized by microscopy and sporulation, galactose fermenting ability and RapID ONE panel system. For the production of invertase enzyme it was concluded that the maximum production was observed 4.212u/ml at 72h at fixed pH and temperature. Effect of various carbon sources was studied like glucose and sucrose the maximum growth 0.224g/ml was recorded in sucrose fermentation broth.

The influence of different nitrogen sources like (beef extract and peptone) on growth and production was also studied. It is noted that the maximum growth and production was observed in peptone o.286u/ml was observed.

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