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Optimization of Culture Media Parameters of *Saccharomyces Cerevisiae* PTCC 5209 for Maximized Invertase Enzyme Production

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Abstract

Background and Objective: Invertase enzyme or D-fructofuranosidfructohydrolase EC (3.2.1.26) is a member of the hydrolase family and responsible for the decomposition of sucrose into fructose and glucose. In recent years, extensive research has been carried out to increase the industrial production of invertase enzyme.

Material and Methods: This study focused on maximizing invertase production in *Saccharomyces cerevisiae* by optimizing culture media conditions. Elements of the culture media were investigated using monofunctional optimization method. Moreover, basic salt culture media, containing compounds such as Na_2HPO_4, K_2HPO_4, $MgSO_4$ and $CaCl_2$, were used. Then, growth curve of the yeast was plotted and results showed that the highest growth rate occurred within 38 h and the strongest enzyme activity occurred within 18 h. Optimizing the culture conditions showed that yeast provided the most activity with 1% sucrose as a carbon source, urea and 0.5% meat peptone as nitrogen sources, pH 5, 30 °C and shaking speed of 150 rpm. In this research, 3-l fermentor was used to assess yeast growth and enzyme activity at a larger scale.

Results and Conclusion: Results of this study showed that the highest OD value was included at 48 h and the highest enzyme activity was recorded at 28 and 96 h. The difference between the time of maximum growth and peak enzyme activity indicated the need of careful control of fermentation time to prevent unnecessary biomass accumulation. Therefore, further research in the field of advanced fermentation and optimization of yeast strains can help researchers achieve the highest secretion and enzyme activity.

Keywords: Invertase, Optimization, *Saccharomyces cerevisiae* PTCC 5209, Yeast fermentation

What is "already known" on this topic:	➢ *Saccharomyces cerevisiae* is one of the most effective microorganisms used for industrial invertase production because of its capability to ferment sucrose. ➢ Previous studies have shown that culture medium composition and fermentation conditions significantly affect enzyme production efficiency. ➢ Optimizing nutritional and environmental parameters is essential for enhancing enzymatic productivity in fermentation processes.
What this article adds:	➢ This study identifies the optimal culture conditions, including sucrose concentration, nitrogen sources, pH, temperature, and agitation speed—that maximize invertase production in S. cerevisiae PTCC 5209. ➢ It highlights the time difference between peak cell growth and maximum enzyme activity, emphasizing the need for precise fermentation time control to avoid excess biomass accumulation. ➢ The research demonstrates the applicability of optimized conditions in a 3-liter fermenter, confirming scalability and providing a foundation for industrial-level production improvements.

1. Introduction

Enzymes are macromolecules that play a critical role in enabling the chemical transformations needed for sustaining biological processes. Enzymes are classified based on the types of reactions they catalyze, reflecting their diverse catalytic activities [1]. Enzymes are used in several industrial processes, including baking, brewing, detergents, fermented products, pharmaceuticals, textiles and leather processing and include a crucial role in the pharmaceutical and diagnostic industries [2]. One of the enzymes that has been most discussed in recent years is the invertase enzyme. Invertase (D-fructofuranosid fructohydrolase, EC 3.2.1.26) catalyzes the hydrolysis of the α-1,4-glycosidic bonds between D-glucose and D-fructose in sucrose and transfers the αβ-D-O-fructofuranoside residue to an acceptor substrate [3]. Thus, invertase functions under high sucrose concentrations showing transferase activity. This dual characteristic classifies it within the group of transferases, referred to as fructosyltransferases (EC 2.4.1.9) [4]. In addition, invertase can hydrolyze other oligosaccharides, including kestose, raffinose and stachyose [5]. Nowadays, invertase is widely used for commercial purposes in various industries such as foods, beverages, pharmaceuticals and biosensors. It facilitates the conversion of sucrose and linked glycosides into simple commercial carbohydrates. *Saccharomyces* sp. invertase is the most common commercial source, compared to others. Yeast invertase is a β-fructosidase, whereas the fungus produces an α-glucosidase type of invertase. These two types of invertase include various catalytic mechanisms. The β-fructosidase hydrolyzes the sucrose from the fructose end, while the α-glucosidase hydrolyzes sucrose from the glucose end. The two reactions yield a mixture named invert syrup, which consists of glucose and fructose. Due to the high sweetness of fructose, the invert syrup is much sweeter than sucrose. Fructose is more appropriate than glucose for diabetic patients and enhances iron absorption in children [6]. The generally recognized as safe (GRAS) *S. cerevisiae* is a preferred protein-production host due to its well-understood genetics, collection of molecular biology tools that enable precise strain engineering and significant tolerance to industrial and chemical stresses [7]. First, invert sugar was produced using chemical method by the hydrolysis of sucrose with acid. Before identification of the invertase enzyme this method was highly used; however, acid hydrolysis of sucrose includes several disadvantages such as byproduct generation and low efficiency, limiting its industrial uses [8]. Invertase is a glycoprotein rich in mannose residues that belongs to the glycoside hydrolase (GH) family and consists of 370 enzymes [9]. Various isoforms with distinct characteristics of invertase are located in various parts of the cell and produced in intracellular and extracellular forms [10]. The major strain for the production of the invertase enzyme for the industries is *S. cerevisiae* [11]. In addition to its ability to catalyze and hydrolyze several sugars, invertase is capable of degrading numerous chemical compounds such as rhamnose and stachyose. As the first known protein in the role of biological catalysts, this enzyme has formed one of the most fundamental principles in enzymology. This characteristic has led to suggest invertase as a basis for the development of various models used in the study of enzyme reaction kinetics [12]. Previous studies' major focus was on conventional yeast strains and standard fermentation conditions, focusing primarily on basic production and biochemical characterization [3, 4]. Optimization of culture conditions, particularly nitrogen sources, has been verified as effective in enhancing enzyme yield [13]. The goal of this research was to enhance invertase activity. Invertase production and activity highly depend on the microbial strain, culture media and environmental conditions. However, systematic assessment of *S. cerevisiae* PTCC 5209 with optimized nitrogen sources is limited. This study demonstrated that combining this strain with two nitrogen sources enhanced the enzyme yield, while Amicon ultrafiltration efficiently concentrated the enzyme. The novelty of this study was linked to the combined approach of strain selection and nutritional optimization, including use of a combination of nitrogen sources, to maximize invertase production under controlled culture conditions.

2. Materials and Methods

2.1. Materials

Chemicals and mineral salts in this study included carbon sources of molasses (Brix 80, Jahan Alcohol, Iran) and sucrose (Merck, Germany); nitrogen sources of yeast extract (Leiber, Germany), meat peptone (Sigma-Aldrich, Germany), urea (pharmaceutical grade; Behansar, Iran) and diammonium phosphate (Merck, Germany); mineral salts of calcium chloride (Merck, Germany), magnesium sulfate (Merck, Germany), disodium hydrogen phosphate (Merck-DNA Biotech, Germany), dipotassium hydrogen phosphate (Merck, Germany), sodium potassium tartrate (Merck, Germany) and sodium acetate trihydrate (Merck, Germany); agar (Ibresco, Germany); sodium hydroxide (Merck, Germany); and glucose (Merck, Germany).

2.2. Microorganism

The yeast strain of *S. cerevisiae* PTCC 5209 was provided by the Persian Type Culture Collection (PTCC, Iran). The strain was cultivated in yeast peptone dextrose

adenine (YPDA) media (pH 5). For further experiments, glycerol stocks and slants were prepared.

2.3. Media

For enzyme production, the optimized culture media contained Na_2HPO_4 (2.5 g l^{-1}), K_2HPO_4 (2.5 g l^{-1}), meat peptone (10 g l^{-1}), $MgSO_4$ (0.05 M) and $CaCl_2$ (0.01 M). The primary pH of the media was adjusted to 5.0 before sterilization.

2.4. Invertase assay

The culture media were centrifuged at 9,000 rpm for 20 min at 4 °C and the supernatant was collected as the crude enzyme source for the invertase assay. Invertase activity was investigated by measuring the quantity of reducing sugars released from sucrose using DNS method according to Miller [14]. The reaction mixture contained 0.4 ml of 1% (w/v) sucrose as substrate, 1.2 ml of 0.1 M acetate buffer (pH 5.0) and 0.4 ml of the crude enzyme supernatant. The mixture was incubated at room temperature (RM) for 30 min. After incubation, 0.25 ml of the reaction mixture was added to 1 ml of DNS reagent to terminate the reaction and the tubes were boiled for 10 min using water bath. After cooling to RM, the absorbance was measured at 540 nm using UV-vis spectrophotometer. One unit of invertase activity was reported as the quantity of enzyme needed to release 1 µmol of glucose per minute under the assay conditions.

2.5. Invertase activity calculation

Enzyme activity (mol min^{-1} ml^{-1}) or (U ml^{-1}) $= \frac{[Product\ concentration\ (\mu mol/ml)] \times total\ reaction\ volume\ (ml)}{Reaction\ time\ (min) \times Enzyme\ volume\ (ml)}$

2.6. Carbon source optimization

Two carbon sources were assessed to investigate the optimal substrate for invertase production, including molasses with a Brix of 80 and sucrose. The culture media were supplemented with either 1% (v/v) molasses or 1% (w/v) sucrose and the pH was adjusted to 5.0. Each 250-ml flask containing 100 ml of the media was inoculated with *S. cerevisiae* and incubated at 30 °C for 24 h at 150 rpm using shaker incubator.

2.7. Sucrose concentration optimization

The culture media were prepared with various concentrations of sucrose ranging from 1 to 4% (w/v) to optimize invertase production [15]. With pH 5 in 250-ml flasks, culture media were inoculated with *S. cerevisiae* and incubated at 30 °C for 24 h at 150 rpm using shaker incubator.

2.8. Nitrogen source optimization

Four nitrogen sources were selected to investigate the best nitrogen source for invertase activity. These nitrogen sources were urea, yeast extract, meat peptone [16] and diammonium phosphate (DAP). The culture media were supplemented with 1% (w/v) of each nitrogen source and pH was adjusted to 5.0. Each 250-ml flask containing 100 ml of the media was inoculated with *S. cerevisiae* and incubated at 30 °C for 24 h at 150 rpm using shaker incubator.

2.9. Combination nitrogen source optimization

To enhance invertase production, various combinations of nitrogen sources were assessed, including 0.5% yeast extract and 0.5% meat peptone, 0.5% urea and 0.5% meat peptone, 0.5% urea and 0.5% yeast extract, and 0.5% yeast extract and 0.5% diammonium phosphate. Each combination was prepared in 100 ml of the culture media (pH adjusted to 5.0) using 250-ml flasks, inoculated with *S. cerevisiae* and incubated at 30 °C for 24 h at 150 rpm using shaker.

2.10. Optimization pH

Briefly, 100 ml of the production media were distributed into each 250-ml flask and pH was adjusted to 3, 4, 5, 6, 7 and 8 [17]. These were inoculated with *S. cerevisiae* and incubated at 30 °C for 24 h at 150 rpm using shaker incubator. Then, the supernatant was used for enzyme assay and investigation of invertase activity.

2.11. Acetate buffer pH optimization

To investigate that at what pH the enzyme was most active, acetate buffer was prepared at various pH levels between 4 and 7 and enzyme activity was assessed using enzyme assay with the acetate buffer at various pH levels.

2.12. Shaker incubator rpm optimization

To investigate the effect of the rpm of the shaker incubator on enzyme activity, the culture media containing microorganisms were incubated at 30 °C for 24 h at 150 and 160 rpm after inoculation, and enzyme activity was assessed, as described in the previous steps.

2.13. Shaker incubator temperature optimization

To investigate the Optimal shaker temperature to achieve higher enzyme activity, the culture media containing microorganisms were transferred into shakers at 25, 30 and 32 °C and 150 rpm after inoculation. After 24 h, enzyme assay was used as previously described.

2.14. Growth curve and enzyme activity

Yeast growth and invertase activity were monitored over 48 h. Cell growth was assessed spectrophotometrically at 600 nm every 2 h and the growth curve was plotted. Enzyme activity was investigated approximately every 4 h using standard invertase assay with each measurement carried out in duplicate (n = 2). Results were reported as mean ±SD (standard deviation).

2.15. Effects of temperature on enzyme activity

The crude enzyme from the supernatant was incubated at 30 to 90 °C for 30 min using water bath [18]. Enzyme

activity was then assessed using standard invertase assay and absorbance of the samples was read at 540 nm.

2.16. Effects of substrate concentration and kinetic parameters (Michaelis-Menten equation)

The effect of substrate concentration on invertase activity was assessed using sucrose at final concentrations ranging from 0 to 300 mM under standard assay conditions. The reactions were carried out at constant temperatures of 30 and 50 °C. Kinetic parameters, including the Michaelis-Menten constant (Km) and the maximum reaction rate (Vmax), were calculated using non-linear regression analysis and Michaelis-Menten model.

2.17. The SMF fermentor

To assess enzyme production at a larger scale, fermentation was carried out using 3-l laboratory-scale bioreactor (working volume of 2.0 l; Zist Fan Sanat Iranian, Iran). The bioreactor was equipped with a Rushton-type impeller, an air sparger and automatic control systems for temperature, pH and dissolved oxygen (DO). Fermentation was carried out at 30 °C with pH 5.0. The culture was agitated at 150 rpm and continuously aerated with sterile air, while DO was set at approximately 5 mg l^{-1} throughout the process to ensure adequate oxygen transfer and homogeneous mixing. To minimize the risk of contamination, no sampling was carried out during the first 24 h after inoculation. Then, samples were collected at regular intervals to investigate yeast growth (OD_{600}) and invertase activity and growth and enzyme activity profiles were recorded. For each sample, OD_{600} was measured in duplicate (technical replicates) to monitor cell growth and invertase activity was assessed in duplicate. Data were present as mean ±SD and error bars in figures represented SD.

2.18. Amicon (ultra centrifugal filter of 10 kDa)

Briefly, 10-kDa Amicon ultrafiltration was used to concentrate the crude invertase enzyme and simultaneously remove low-molecular-weight (LMW) salts and other solutes in the supernatant. The enzyme solution was processed according to the manufacturer's instructions and the concentrated enzyme was collected for activity assays.

2.19. Statistical analysis

All experiments, except fermentation studies, were carried out in duplicate as independent biological replicates (n = 2). Enzyme activity for each replicate was calculated individually and results were present as mean ±SD. For fermentation samples, only one fermentor was used; each sample was assessed in duplicate (technical replicates) and OD_{600} and enzyme activity were reported as mean ±SD. Michaelis-Menten plots were generated using GraphPad Prism 8 (GraphPad, USA) and the mean values of duplicate measurements. Standard deviations were not included in the fitting analysis since averaged values were used for each substrate concentration.

3. Results and Discussion

3.1. Carbon source optimization

After 24 h of yeast incubation in culture media containing 1% molasses or 1% sucrose as carbon sources, enzyme activity was assessed to investigate the most suitable substrate. Sucrose was selected as the optimal carbon source since molasses interfered with the DNS assay due to its strong reaction with the reagent, making accurate quantification of enzyme activity unreliable.

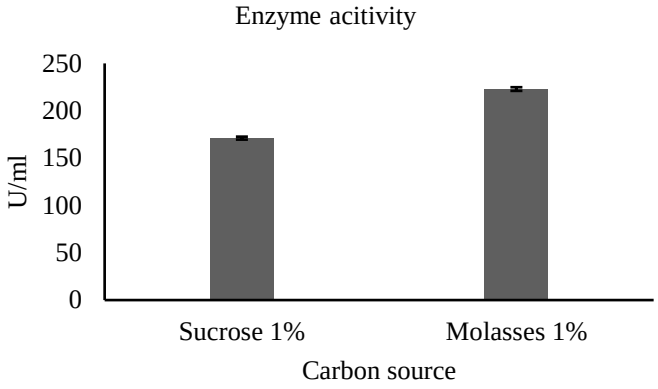

Figure 1. Effects of various carbon sources on invertase production from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.2. Sucrose concentration optimization

After selecting sucrose as the preferred carbon source for enzyme production, various concentrations of sucrose (1–4% w/v) were assessed to investigate the optimal level for maximum enzyme activity. The results showed that the highest enzyme activity was achieved at 1% sucrose.

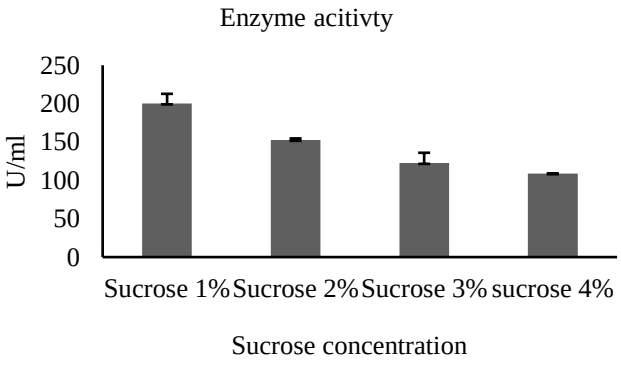

Figure 2. Effects of various sucrose concentrations on invertase production from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.3. Nitrogen source optimization

Culture media containing yeast extract, meat peptone, urea and diammonium phosphate (DAP) were assessed as nitrogen sources. After 24 h of incubation, the enzyme assay results showed that the media containing pharmaceutical-grade urea as the nitrogen source included the highest invertase activity.

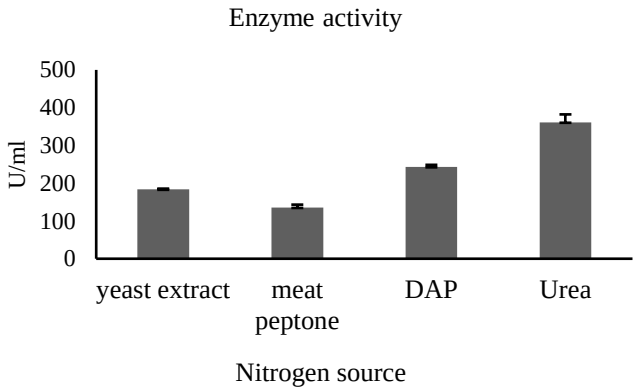

Figure 3. Effects of various nitrogen sources on invertase production from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.4. Combination of nitrogen sources

To enhance enzyme production, various combinations of nitrogen sources were assessed. After 24 h of incubation, the results indicated that the media containing a combination of meat peptone and pharmaceutical-grade urea included the highest invertase activity.

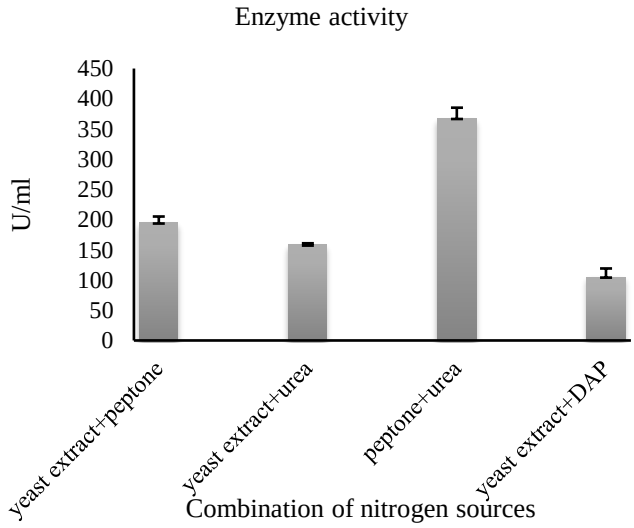

Figure 4. Effects of the combination of nitrogen sources on invertase production from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.5. Media pH optimization

Media of various pH levels between 3 and 8 were prepared. After 24 h of yeast inoculation and enzyme assay, the culture media with pH of 5 included the highest enzyme activity. At pH 7 and pH 8, enzyme activity could not be assessed due to the precipitation in the culture media.

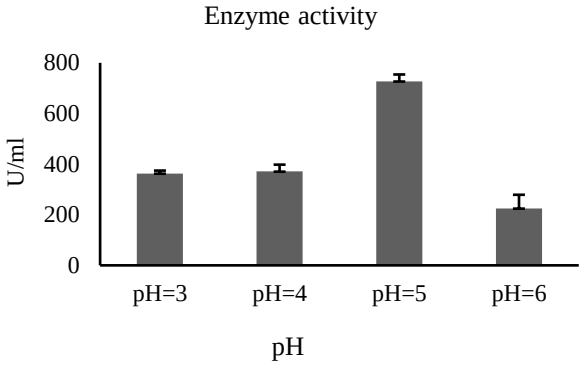

Figure 5. Effects of various pH on invertase production from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.6. The pH acetate buffer

The buffer used in the assay included acetate buffer. To achieve and ensure the appropriate pH for the invertase enzyme, buffers with various pH levels between 3 and 7 were prepared. Then, the enzyme assay was carried out. The invertase enzyme showed the highest activity at pH 5.

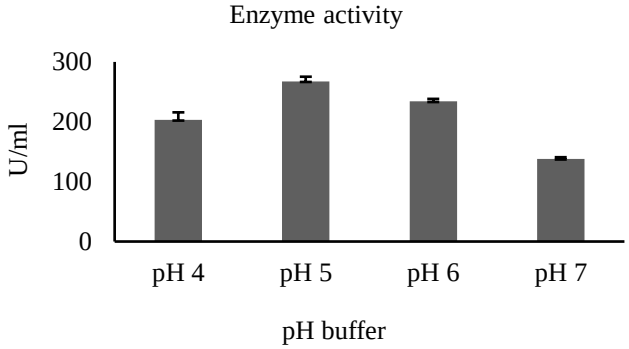

Figure 6. Effects of various pH buffers on invertase activity from *Saccharomyces cerevisiae*. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.7. The rpm optimization

To assess the effects of agitation speed on enzyme activity, shaking rates of 150 and 160 rpm were assessed. The results showed that the culture agitated at 150 rpm included the highest invertase activity.

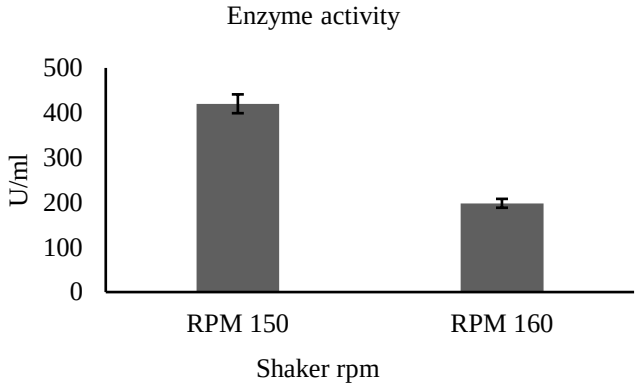

Figure 7. Effects of shaker incubator rotation per minute (rpm) on invertase activity from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (*n* = 2). Error bars show standard deviation (SD)

3.8. Shaker incubator temperature optimization

After inoculation, the culture flasks were incubated at 25, 30 and 35 °C for 24 h. Enzyme assay results showed that the highest invertase activity was achieved at 30 °C.

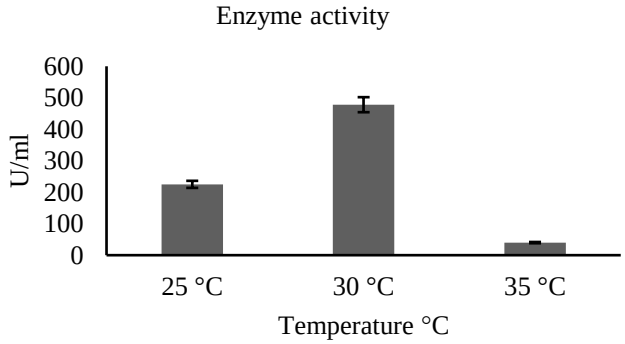

Figure 8. Effects of shaker incubator temperature on invertase production from *Saccharomyces cerevisiae*, pH 5, incubated for 24 h. Data were represented as mean ±SD from duplicate experiments (*n* = 2). Error bars show standard deviation (SD)

3.9. Growth curve and enzyme activity curve

The growth and enzyme activity curves were monitored over 48 h by measuring cell growth (OD_{600}) every 2 h and invertase activity approximately every 4 h, except during the early stage of inoculation, when enzyme secretion did not begin. The growth curve showed two distinct logarithmic phases, likely due to the presence of dual nitrogen sources in the media. The highest enzyme activity was observed at 18 and 48 h, while the maximum optical density (OD_{600}) occurred at 38 h. These results indicate that the peak enzyme secretion did not coincide with the time of maximum yeast growth (biomass accumulation).

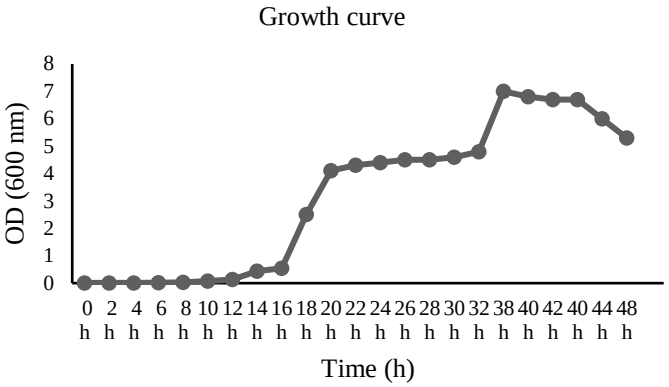

Figure 9. Growth curve OD of *Saccharomyces cerevisiae*, pH 5, incubated for 48 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (*n* = 2). Error bars show standard deviation (SD)

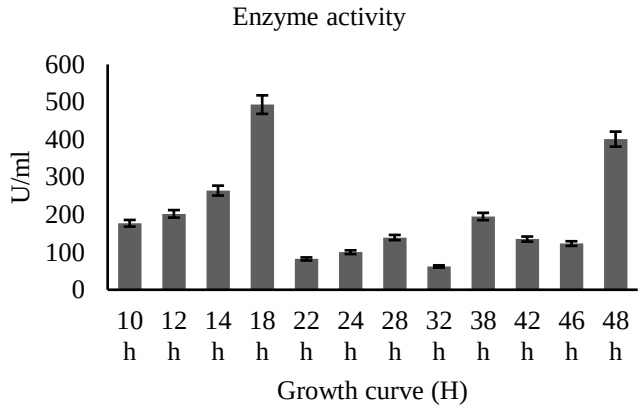

Figure 10. Enzyme activity of *Saccharomyces cerevisiae*, pH 5, incubated for 48 h at 30 °C. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.10. Fermentor

During the first 24 h of fermentation, no sampling was carried out to minimize the risk of contamination. After this time, samples were collected at regular intervals to monitor cell growth and enzyme activity. The highest optical density (OD_{600}) was recorded at 48 h, while the maximum invertase activity occurred at 28 and 96 h. Similar to the shake-flask experiments, the peak of enzyme activity did not match with the maximum cell growth, indicating that invertase secretion was not directly correlated with the biomass accumulation.

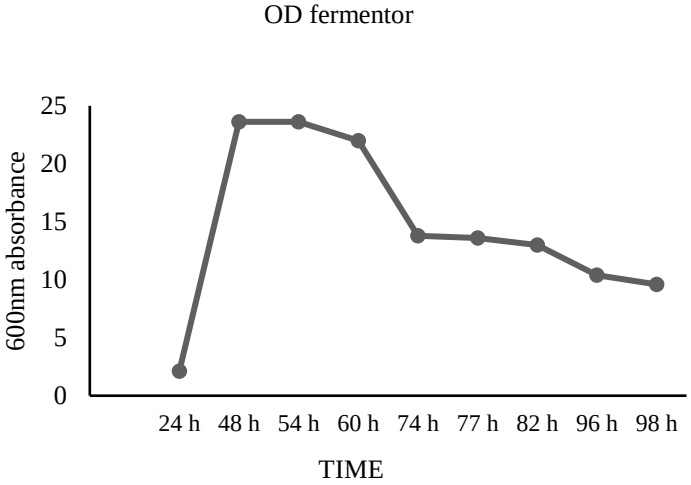

Figure 11. Growth curve of *Saccharomyces cerevisiae* PTCC 5209 using 3-l fermentor. The OD_{600} was measured at various time points. Each point represented the mean of duplicate measurements (technical replicates) from the same fermentor. Error bars indicate standard deviation (SD)

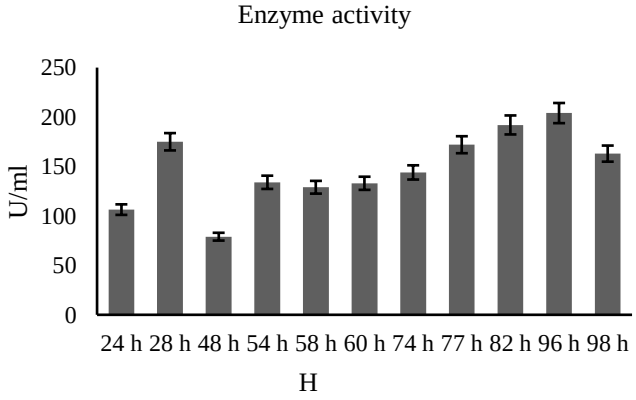

Figure 12. Time course of invertase activity in *Saccharomyces cerevisiae* PTCC 5209 during fermentation using 3-l fermentor. Samples were collected at the indicated time points and enzyme activity was assessed in duplicate (technical replicates) for each sample. Data were represented as mean ±SD (n = 2). Error bars indicate standard deviation (SD)

3.11. Effects of temperature

The crude enzyme was incubated at 30, 40, 60, 70 and 90 °C for 30 min, followed by activity measurement using standard assay. The highest invertase activity was observed within the temperature range of 40–60 °C, with similar activity levels at 40 and 60 °C, indicating that the enzyme preserved its substantial stability and catalytic efficiency across this range.

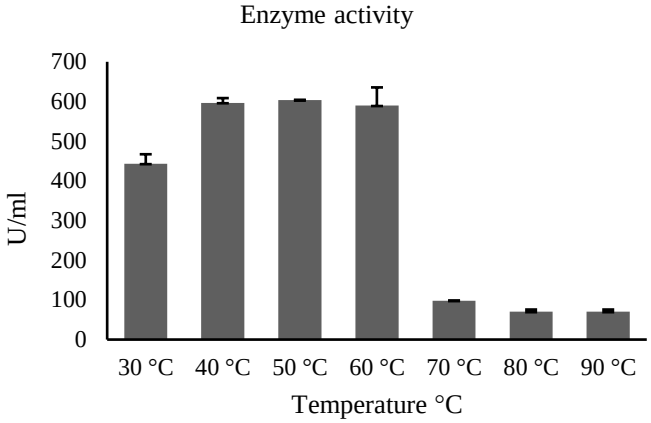

Figure 13. Enzyme activity at various temperatures. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

3.12. Michaelis-Menten equation

The Michaelis–Menten parameters of invertase were investigated through non-linear regression at 30 and 50 °C, as summarized in Table 1. At 30 °C, the Km and Vmax values were 63.35 mM and 0.8557 µmol min^{-1}, respectively. At 50 °C, Km increased to 124.2 mM and Vmax to 2.283 µmol min^{-1}. The higher Km at 50 °C indicated decreased affinity of invertase for sucrose at increased temperatures, suggesting that structural changes or increased flexibility of the enzyme at higher temperatures might affect substrate binding. The increase in Vmax at 50 °C showed that the catalytic rate could increase at higher temperatures; however, the overall efficiency was moderated by the decreased substrate affinity.

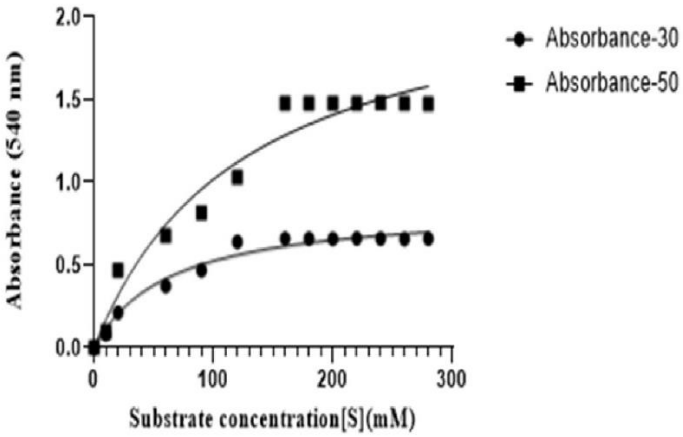

Figure 14. The Michaelis-Menten equation at 30 and 50 °C

Table 1. Michaelis-Menten kinetic parameters (Km, Vmax) for invertase from *Saccharomyces cerevisiae* PTCC 5209 at 30 and 50 °C. Data were analyzed using non-linear regression and GraphPad Prism 8. Results were represented as the mean values from duplicate experiments (n = 2)

Michaelis-Menten	Absorbance 30°C	Absorbance 50°C
Best-fit values		
Vmax	0.8557	2.283
Km	63.35	124.2
95% CI		
Vmax	0.7711 to 0.9701	1.855 to 3.069
Km	42.94 to 94.06	69.72 to 233.2
Goodness of Fit		
Degrees of Freedom	11	11
R squared	0.9777	0.9640
Sum of Squares	0.01604	0.1377
Sy. X	0.03818	0.1119
Constraints		
Km	Km ≻ 0	Km ≻ 0
Number of points		
# of X values	13	13
# Y values analyzed	13	13

3.13. Amicon ultra centrifugal filter

In this study, Amicon 10-kDa filter was used to concentrate the enzyme. Before centrifugation, the volume in the tube was 3.5 ml and after centrifugation, the rest of volume in the Amicon filter was 1.4 ml; hence, this was 2.5 times further concentrated.

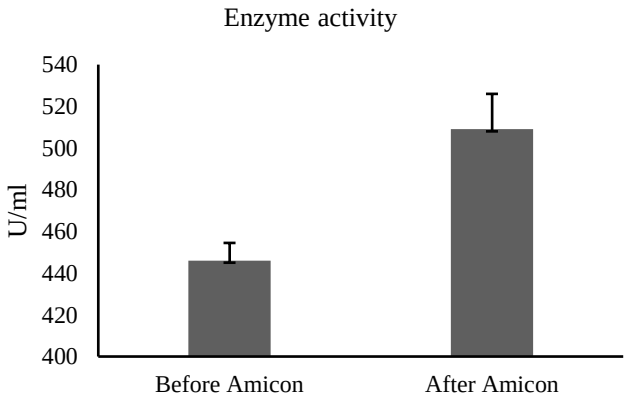

Figure 15. Effects of Amicon ultra centrifugal filter on enzyme activity. Data were represented as mean ±SD from duplicate experiments (n = 2). Error bars show standard deviation (SD)

The results of this study showed that the yeast reached its highest growth rate within 38 h, while the best enzyme activity was seen within 18 h, highlighting the fact that enzyme secretion was not strictly coupled to growth, a phenomenon reported for *S. cerevisiae* MK, where the maximum invertase production occurred at 48 h while biomass increased up to 96 h [19]. These observations highlighted the importance of optimizing harvest time to achieve maximal enzyme yield. Such differences highlighted the importance of selecting the optimal harvest time to maximize enzyme activity. The present results, including that the invertase enzyme pH optimization was 5, were similar to those of Al-Saady's study [16] but differed from Shankar's study [18], where maximum activity was observed at pH 6 for invertase from *S. cerevisiae* MTCC 170, suggesting that strain-specific variations in invertase isoforms or differences in post-translational modifications and culture conditions such as media composition, temperature and aeration might further affect the pH profile. In 2024, Dokuzparmak investigated the effect of pulp on the activity of the invertase enzyme in *S. cerevisiae* and the results showed that the invertase enzyme activity increased by 2.5 times. In the current study, a combination of 1% sucrose, 0.5% urea and meat peptone at pH 5 and 30 °C and 150 rpm yielded the highest enzyme activity, aligning with Dokuzparmak's findings under comparable conditions [20]. However, it is noteworthy that the optimization was carried out using one-factor-at-a-time (OFAT) approach, which did not account for potential interactions between the factors. For example, the optimal sucrose concentration investigated by one nitrogen source may shift, when a various nitrogen source is used. Future studies can use factorial design or response surface methodology (RSM) to investigate such interactions and achieve further precise optimization.

In this investigation, the invertase from *S. cerevisiae* PTCC 5209 showed high activity over a broad temperature range (40–60 °C), which was similar to that of Du's study [21], who reported the maximum activity of the enzyme at 45 °C. This suggested that the enzyme preserved substantial activity within a wide range of temperatures. In this study, 3-l fermentor was used to investigate the growth and activity of yeast at a large scale, which showed the highest OD value within 48 h. In general, the achieved results showed that use of *S. cerevisiae* bacteria with the optimization of culture media parameters included a positive effect on increasing the production of invertase enzyme, similar to previous studies [22–24]. In addition, analysis of the fermentor growth curve revealed differences between the maximum biomass growth and the maximum enzyme activity. This highlighted the necessity of careful monitoring of fermentation time to avoid unnecessary accumulation of biomass in conditions, where enzyme efficiency reached its optimal level. Therefore, further research can focus on developing advanced fermentation methods or optimizing strains to achieve further optimal enzyme secretion and activity.

4. Conclusion

Invertase is found in nature in various isoforms. This enzyme is widely distributed in plants and microorganisms. In plants, three isoforms of invertase differ in biochemical

characteristics and subcellular locations. The role of invertase in plants extends beyond metabolism, as it is effective in osmotic regulation, growth and immune system strengthening. In the human body, invertase is addressed as an immune system booster, antioxidant and disinfectant agent and is useful in certain cases of patients with bone and stomach cancers. The importance of this enzyme in these reactions led the current authors to investigate the structure of the enzyme and optimize conditions for its maximum production. Regarding that *S. cerevisiae*, commonly known as baker's yeast, is the major strain used for the production and purification of invertase, *S. cerevisiae* was used under optimal conditions to maximize the production and activity of this enzyme. In yeasts, this enzyme can be detected extracellularly or intracellularly. In this study, invertase production by *S. cerevisiae* PTCC 5209 was maximized under optimized nutritional and culture conditions. The study detected that invertase production peaked at 18 h under 1% sucrose, 0.5% urea and meat peptone, pH 5, 30 °C and 150 rpm conditions. Combination of urea and meat peptone resulted in the highest invertase activity, likely due to the complementary nature of these nitrogen sources as urea provided a rapidly metabolizable form of nitrogen and meat peptone supplied complex peptides and amino acids that supported enzyme synthesis. Moreover, urea represented an economically advantageous nitrogen source, being an inexpensive petrochemical product that is readily available in Iran, which made this combination particularly appropriate for industrial-scale enzyme production. The process was successfully scaled up in 3-l fermentor, demonstrating its potential for industrial enzyme production. Although experiments were carried out under specific laboratory conditions, findings included broader uses for the global biotechnology and food industries, where invertase plays a vital role in processes such as sucrose hydrolysis, prebiotic synthesis and fermentation. Further studies could focus on optimizing fermentation techniques or improving the yeast strain to increase enzyme production.

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6. Declaration of competing interest

The authors report no conflict of interest.

7. Authors' Contributions

All authors participated in project administration and writing of the primary draft of the manuscript, providing critical revision and editing.

8. Using Artificial Intelligent Chatbots

This manuscript was written entirely by the authors. Artificial intelligence tools were used only to improve the English language and enhance readability.

9. Ethical Consideration

The authors confirm that this research involved only microbial (yeast) experiments and did not include human or animal subjects. Accordingly, ethical approval was not applicable for this study.

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بهینه‌سازی پارامترهای محیط کشت *ساکارومایسس سرویزیه* PTCC 5209 برای حداکثر تولید آنزیم اینورتاز

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چکیده

سابقه و هدف: آنزیم اینورتاز، یا D-فروکتوفورانوزیدفروکتوهیدرولاز، با شماره EC (۳.۲.۱.۲۶)، عضو خانواده هیدرولازها در نظر گرفته می‌شود و مسئول تجزیه ساکارز به فروکتوز و گلوکز است. در سال‌های اخیر، تحقیقات گسترده‌ای برای افزایش تولید صنعتی این آنزیم اینورتاز انجام شده است.

مواد و روش ها: این مطالعه بر حداکثر تولید آنزیم اینورتاز از طریق بهینه‌سازی شرایط محیط کشت توسط *Saccharomyces cerevisiae* تمرکز داشت. در این پژوهش از محیط پایه نمکی متشکل از نمک های Na2HPO4و K2HPO4 و mgSO4 و CaCl2 استفاده شد . اجزای محیط براساس یک فاکتور در یک زمان بهینه سازی شدند. و با بررسی منحنی رشد مخمر بیشترین میزان رشد در ساعت ۳۸ و بیشترین فعالیت آنزیم در ساعت ۱۸ بود.نتایج بهینه سازی نشان داد که مخمر در منبع کربن ساکارز ۱٪ و منابع نیتروژن اوره و پپتون گوشت ۰.۵٪ و pH=5 در دمای ۳۰ و دور شیکر ۱۵۰ بیشترین فعالیت را داراست.به منظور رشد مخمر و بررسی فعالیت آن در حجم بالا از فرمانتور ۳ لیتری استفاده شد.

یافته‌ها و نتیجه‌گیری: نتایج این مطالعه نشان داد که بالاترین مقدار OD در ۴۸ ساعت است و بیشترین فعالیت آنزیم در ۲۸ و ۹۶ ساعت ثبت گردید. تفاوت بین زمان حداکثر رشد و بیشترین فعالیت آنزیم نشان‌دهنده ضرورت کنترل دقیق زمان تخمیر برای جلوگیری از تجمع بیومس است. بنابراین، تحقیقات بیشتر در زمینه تخمیر پیشرفته و بهینه‌سازی سویه‌های مخمر می‌تواند مسیر دستیابی به بالاترین میزان ترشح و فعالیت آنزیم را هموار کند.

واژگان کلیدی: اینورتاز، بهینه‌سازی، *ساکارومایسس سرویزیه* **PTCC 5209**، تخمیر مخمری