EFFECT OF AGITATION SPEED AND TEMPERATURE ON GROWTH KINETICS AND BIOMASS PRODUCTION OF *SACCHAROMYCES CEREVISIAE* IN A STIRRED TANK BIOREACTOR

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EFECTUL VITEZEI DE AGITARE ȘI AL TEMPERATURII ASUPRA CINETICII DE CREȘTERE ȘI PRODUCERII BIOMASEI DE SACCHAROMYCES CEREVISIAE ÎNTR-UN BIOREACTOR CU AMESTECARE MECANICĂ

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ABSTRACT

This study assesses the impact of agitation speed (150, 300, 450 rpm) and temperature (25°C, 35°C) on the growth kinetics of Saccharomyces cerevisiae cultivated for 46 h in a 3 L stirred-tank bioreactor with malt extract medium. Cultures were inoculated with 2 mL fresh suspension derived from PDA (Potato Dextrose Agar) grown colonies. Cell density was monitored spectrophotometrically, and final biomass quantified gravimetrically (gDW·L $^{-1}$). The study demonstrates that the interaction between agitation and temperature governs Saccharomyces cerevisiae growth, with values for specific growth rate (μ_{max}) ranging from 0.122 h^{-1} to 0.290 h^{-1}. The condition of 450 rpm at 35°C facilitated optimal oxygen transfer, resulting in the highest biomass concentration of 4.15 gL^{-1}.

REZUMAT

Acest studiu examinează influența vitezei de agitare (150, 300, 450 rpm) și a temperaturii (25°C, 35°C) asupra cineticii de creștere și producerii biomasei de Saccharomyces cerevisiae cultivate timp de 46 h într-un bioreactor cu agitare mecanică de 3 L cu mediul extract de malt. Inocularea s-a realizat cu 2 mL suspensie proaspătă obținută din culturi pe PDA (Potato Dextrose Agar). Densitatea celulară a fost monitorizată spectofotometric, iar biomasa finală a fost determinată gravimetric. Studiul demonstrează că interacțiunea dintre agitație și temperatură guvernează creșterea Saccharomyces cerevisiae, valorile ratei specifice de creștere (μ_{max}) variind între 0.122 h^{-1} și 0.290 h^{-1}. Condiția de 450 rpm la 35°C a asigurat un transfer optim de oxigen, rezultând cea mai mare concentrație de biomasă (4,15 gL^{-1}).

INTRODUCTION

Saccharomyces cerevisiae is recognized as a highly valuable yeast species, widely employed in baking and various industrial processes. Owing to its ease of cultivation, it serves as an important eukaryotic model organism in biological and biotechnological research. This yeast performs the most prevalent form of fermentation and respiratory processes, exhibits a predominantly round to ovoid cellular morphology, and propagates through a characteristic budding mechanism (*Feldmann et al., 2012; Walker et al, 2004*). Industrial cultivation of this yeast is typically carried out in aerated stirred-tank bioreactors, which is widely used due to its low contamination risk, low cost, and food-grade safety. Its thick cell wall, composed of beta-glucans, chitin, and mannoproteins, provides controlled-release properties and mechanical strength, enabling efficient delivery of various active substances (*Roshanak et al., 2017).*

The industrial exploitation of the yeast Saccharomyces cerevisiae has traversed a remarkable evolutionary trajectory, evolving from the empirical fermentation practices of the 19th century to contemporary precision bioprocesses that are essential for the global production of biomass, recombinant proteins, and biofuels.

Although classical fed-batch and continuous cultivation protocols are well-established, modern imperatives for streamlining High-Cell-Density Cultivation (HCDC) mandate a re-evaluation of operational paradigms, necessitating a granular understanding of the physiological interplay between hydrodynamics and thermodynamics *(Rocio et al.,2011; Malairuang et al., 2020).*

Fed-batch and continuous cultivation methods in different types of bioreactors, especially stirred-tank bioreactors, are used to increase yeast cell density with fed-batch offering notable advantages such as high biomass and broad industrial applicability. In these systems, culture conditions – including temperature, pH, feed composition, and feeding rate – are carefully controlled to maximize cell growth and productivity (*Vu et al., 2009).*

In aerobic or semi-aerobic bioprocesses, the accumulation of biomass or biosynthesized product results in continuous changes to the rheological properties of the medium, creating heterogeneous zones within the bioreactor. Under such conditions, a challenge is determining the optimal hydrodynamic regime for the reactor *(Cascaval et al., 2001*). Agitation ensures adequate mixing, nutrient and oxygen distribution, preventing gradients and ensuring homogeneity; insufficient agitation can lead to oxygen limitation, while excessive agitation may cause hydrodynamic stress, influencing cell viability. Indeed, agitation has been shown to significantly enhance yeast propagation: in a study on artisanal beer production, constant agitation increased cell growth by ~15% compared with static culture *(Chesca et at., 2017*). For another example, *Liu et al. (2019),* reported that increasing agitation from 100 to 300 rpm in a laboratory-scale bioreactor led to a 35-45% increase in biomass concentration of *Saccharomyces cerevisiae,* accompanied by a higher specific growth rate (μ). However, further increases above 400 rpm resulted in marginal gains in oxygen transfer and no significant improvement in biomass yield. Similar trends were observed by *Ahmed et al., (2021)*, who showed that agitation intensities above 450 rpm induced cellular stress without proportional increases in growth performance.

Temperature likewise exerts a strong influence on yeast physiology. It modulates enzymatic reaction rates, membrane fluidity, nutrient uptake, metabolic fluxes, and stress responses. In industrial fermentations (e.g., ethanol) temperature impacts sugar consumption rate, fermentation duration, and final yield, making temperature optimization a nontrivial but essential parameter for process efficiency *(Nolasco -Hipólito et al., 2023).* For *Saccharomyces cerevisiae*, optimal growth temperature is typically observed between 25 and 35°C, where enzymatic activity, membrane fluidity and nutrient uptake are balanced. At temperatures around 30 °C, maximum specific growth rates (μ_{max}) ranging from 0.30 to 0.45 h^{-1} have been reported under aerobic batch conditions *(Redon et al., 2011; Walker et al., 2016*). Lower temperatures (20–25 °C) generally reduce metabolic rates, leading to prolonged lag phases and decreased biomass productivity, while elevated temperatures (>35 °C) may trigger heat stress responses, reduce cell viability and alter metabolic fluxes toward maintenance rather than growth (*Abdel-Banat et al., 2010)*. In another comprehensive review, *Ramos et al., (2023),* highlighted that temperature variations between 25-37 °C significantly modulate oxygen demand and biomass yield, especially under aerobic or microaerobic conditions. The authors reported that at higher temperatures, yeast cultures often require increased oxygen transfer rates to sustain respiratory metabolism, making agitation intensity a problematic control variable.

Beyond individual parameter effects, the interaction between agitation and temperature jointly influence oxygen transfer rate (OTR) and dissolved oxygen availability. Experimental evidence from large bioreactors has demonstrated that pronounced substrate gradients may arise near feed zones, resulting in localized substrate excess, while other regions experience substrate limitation (*Bylund et al., 1998; Wright et al., 2018)*. They measured glucose concentrations as high as 2000 $mg \cdot L^{-1}$ near the feed zone of a 12 m^3 bioreactor, while concentrations below 2 $mg \cdot L^{-1}$ were detected in distant regions, leading to a biomass yield reduction of approximately 20% compared to laboratory-scale cultures. Also, recent studies emphasize that while high agitation rates enhance the volumetric oxygen transfer coefficient (kLa), they also induce significant fluid dynamic stress, which can alter cell morphology and metabolic flux distribution (*Rahimzadeh et al., 2024).* Therefore, an appropriate temperature and adequate agitation are required in order to maximize biomass growth throughout all regions within the bioreactor, whether at laboratory scale or at industrial scale.

Although yeast fermentation has been extensively studied, most investigations assess the effects of agitation or temperature in isolation. Factorial designs that systematically combine these parameters are rarely employed, particularly in bench-scale stirred-tank bioreactors for biomass production. Consequently, comprehensive data on the influence of specific agitation and temperature combinations on growth kinetics, biomass yield and culture behavior under controlled batch conditions remain limited.

This knowledge gap hinders the development of predictable operational protocols, which are critical for yeast biomass production and scale-up applications (*Sica et al., 2025).* Moreover, the current trend toward Industry 4.0 and digital twins requires metabolic shifts, such as the transition from respiratory–fermentative to purely respiratory growth, generating accurate primary data regarding parameter improvement for establishing the operational baselines required for high cell density cultivation *(Valencia-Velásquez et al., 2025).*

Moreover, industrial and high gravity fermentation are increasingly common, demanding perfecting of multiple parameters simultaneously: high sugar concentration, osmotic stress, ethanol accumulation and variable oxygen transfer (*Sica et al., 2025).*

MATERIALS AND METHODS

Bioreactor and cultivation conditions

All experiments were performed using a Minifors 2 bench-top stirred-tank bioreactor, illustrated in Fig. 1, featuring a total volume of 5 L and a working volume of 3 L. The bioreactor was equipped with two Rushton impellers and different types of sensors for temperature, dissolved oxygen, pH, agitation. Cultures were aerated using an oil-free air compressor with continuous monitoring of dissolved oxygen (DO). Temperature and pH were maintained at set values during the cultivation process. The system enabled precise adjustment of agitation speed (150, 300, and 450 rpm) and temperature (25°C and 35°C). These parameters were combined in a factorial design to assess their impact on yeast growth and biomass accumulation.

Fig. 1 – Macroscopic view of the *Saccharomyces cerevisiae* culture broth in the Minifors 2 bioreactor

A commercially malt extract broth (Merck, 17 g/L) served as the growth medium. Sterilization was achieved by autoclaving at 121°C for 15 minutes. All glassware and instruments contacting the medium were sterilized before inoculation to reduce contamination risk.

The yeast strain used was *Saccharomyces cerevisiae* obtained from a local supplier. Cells were initially propagated on Potato Dextrose Agar (PDA) and subsequently suspended in sterile distilled water to prepare the inoculum. For each bioreactor condition, 2 mL of cell suspension was inoculated into the working volume. The initial optical density (OD_{660}) was measured as 0.189.

A factorial combination of agitation speed (150, 300, 450 rpm) and temperature (25°C, 35°C) was investigated. Each cultivation was conducted for 46 hours. Samples were collected periodically to monitor cell growth, biomass accumulation, pH, and DO.

These thermal regimes were selected to compare the physiological response at the standard mesophilic optimum (35°C) versus a lower temperature (25°C), which is often investigated to mitigate metabolic stress and byproduct formation in high-density cultures.

Optical density measurement

Yeast growth was monitored by measuring **optical density (OD) at 660 nm** using a UV-VIS spectrophotometer.

The bioprocess was initiated with inoculation at 16:00 on the first day (t=0h). To capture the kinetic profile with high resolution, samples were withdrawn at hourly intervals throughout the entire 46 hour cultivation period.

Samples exceeding an OD of 1.8 were diluted with sterile water, typically at a 1:1 ratio (1 mL sample + 1 mL water). Other dilutions (1:2, 1:3, 1:4, or 1:5) were applied as needed to ensure readings within the linear range of the spectrophotometer.

To quantitatively assess the physiological fitness and metabolic efficiency of the culture under the varying hydrodynamic and thermal regimes, the kinetics of biomass accumulation were analyzed. The specific growth rate (μ) serves as a fundamental metric of cellular proliferation during the exponential phase, reflecting the dynamic response of the population to environmental constraints. The specific growth rate (μ) was calculated using:

$$\mu = \frac{\ln(OD_t) - \ln(OD_0)}{t} \quad (1)$$

where: OD_0 and OD_t represent the optical density at the initial and subsequent time points, respectively, and t is the elapsed time in hours.

Biomass properties

Biomass accumulation was determined as both wet weight and dry weight. Cells were collected by filtration through a 0.8 µm membrane filter. To quantify biomass accumulation, the dry weight was measured gravimetrically. The samples were processed using a KERN DBS60-3 moisture analyzer (Fig. 2), operating at temperatures between 90–110 °C until a constant weight was achieved. The dry biomass concentration (X, gDW/L) was calculated as:

$$X = \frac{m_{dry}}{V_{culture}} \quad (2)$$

where: m_{dry} is the dry biomass (g) and $V_{culture}$ is the working volume of the culture (3L).

Fig. 2 – KERN DBS60-3 moisture analyzer

pH was monitored throughout cultivation, with initial values around 7.0 decreasing to approximately 5.5. Dissolved oxygen (DO) was continuously measured to monitor aerobic conditions and selected samples were evaluated for cell density using a TOMA counting chamber, complementing OD based measurements.

Foam formation was observed during the exponential growth phase, particularly at higher agitation speeds. To control excessive foaming, a small amount of sterile vegetable oil was added manually as an antifoaming agent. The addition effectively reduced foam formation without visibly affecting yeast growth.

RESULTS

Growth kinetics of *Saccharomyces cerevisiae*

The growth behavior of *Saccharomyces cerevisiae* during batch cultivation was evaluated under six different combinations of temperature and agitation speed.

The kinetic profiles, illustrated in Figure 3 (25°C) and Figure 4 (35°C), demonstrate that the hydrodynamic environment within the bioreactor significantly influence the duration of the lag phase and the metabolic efficiency during the exponential growth phase.

At 25°C, the cultures reached a stationary phase after approximately 40 hours, with 450 rpm variant exhibiting the most consistent progression. However, at 35°C, the synergy between optimal metabolic temperature and high intensity agitation (450 rpm) allowed the culture to reach a peak OD_{660} of 10.56, suggesting that increased oxygen transfer rates (OTR) at higher rpm are essential to support the accelerated respiration of *Saccharomyces cerevisiae*.

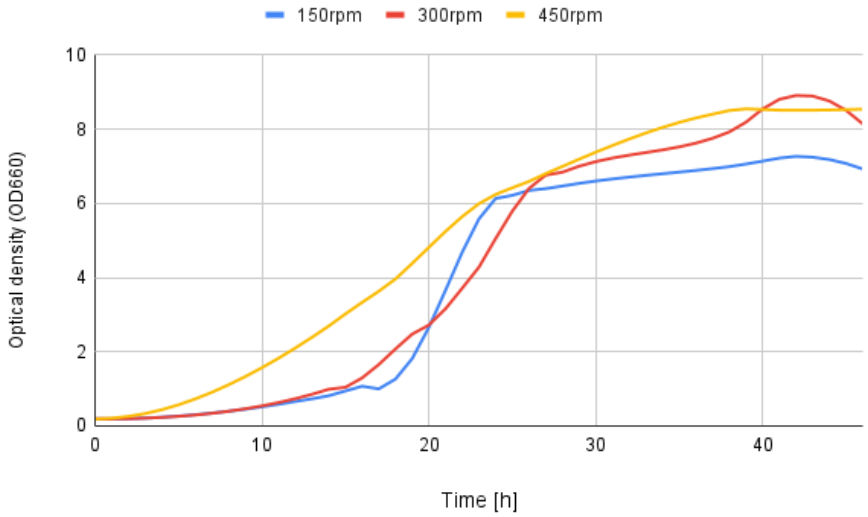

Fig. 3 - Growth kinetics of *S. cerevisiae* at 25°C

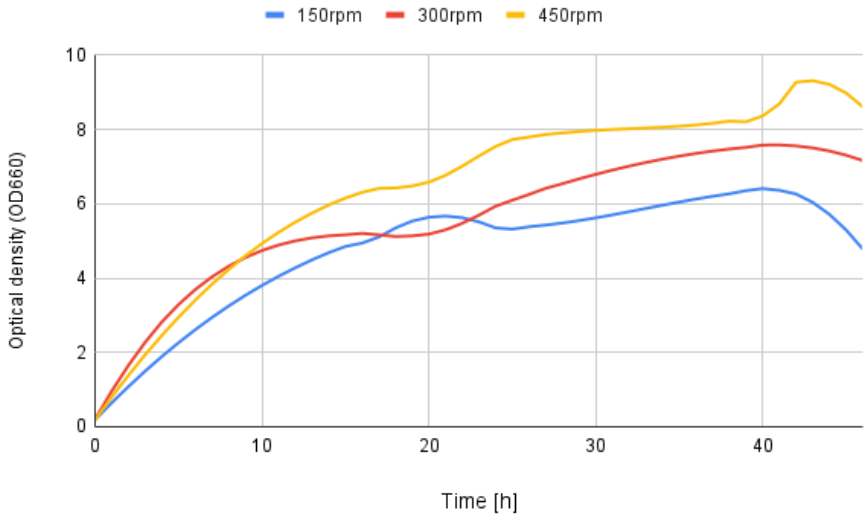

Fig. 4 – Growth kinetics of S. cerevisiae at 35°C

The maximum growth rate (μ_{max}), calculated according to Equation (1), yielded values ranging from 0.122 to 0.290 h^{-1} (Table 1).

Table 1

Kinetic parameters of *S. cerevisiae* under different conditions

Culture conditions	μ_{max} [h^{-1}]	Max OD_{660}
150 rpm, 25°C	0.211	7.40
300 rpm, 25°C	0.186	9.66
450 rpm, 25°C	0.122	8.75
150 rpm, 35°C	0.194	6.85
300 rpm, 35°C	0.185	7.60
450 rpm,35 °C	0.290	10.56

The peak μ_{max} of 0.290 h^{-1} achieved at 450 rpm and 35°C aligns with literature values for aerobic batch fermentations in complex media, where values typically range between 0.25 and 0.35 h^{-1}. The decrease in the specific growth rate observed at 450 rpm and 25 °C (0.122 h^{-1}) suggests a sensitivity to hydrodynamic shear stress during the early exponential phase. However, despite this initial inhibition, the final dry biomass concentration at 450 rpm was substantially higher than that obtained at 300 rpm. This indicates that while shear stress affected the kinetics of cell division, the enhanced oxygen transfer maintained cell viability and allowed for prolonged accumulation of biomass towards the end of the cultivation. It is also possible that these behaviors are accentuated by the specific hydrodynamics of the 5-L laboratory-scale vessel, where turbulence intensity can differ from larger industrial tanks.

The efficiency of substrate conversion into biomass was directly proportional to agitation intensity, a factor that governs the volumetric oxygen transfer coefficient (kLa). According to the gravimetric data (Table 2), the dry biomass concentration reached a maximum of 4.15 g/L in the 450 rpm/35°C regime.

Table 2

Final biomass concentration and dry weight values

Culture conditions	Dry weight [g]	Biomass concentration [g/L]
150 rpm, 25°C	7.94	2.60
300 rpm, 25°C	6.08	2.00
450 rpm, 25°C	9.60	3.20
150 rpm, 35°C	8.85	2.95
300 rpm, 35°C	10.07	3.35
450 rpm,35 °C	12.51	4.15

At 150 rpm, the culture likely enters a dissolved oxygen limitation regime, forcing *Saccharomyces cerevisiae* to shift from purely oxidative metabolism to fermentative pathways (Crabtree effect), even under aeration (*Vlăduț et al., 2025).* This transition explains the lower biomass yield at low agitation speeds. Increasing the speed to 450 rpm ensures fine air bubble dispersion and continuous renewal of the liquid film at the cell surface. This aligns with the findings of *Yaroshevsky et al. (2020)*, who emphasized that the efficiency of a bioreactor bioprocesses is defined by its ability to balance through the energy dissipation and the hydrodynamic structure of the liquid, which can directly influence the fermentation performance, highlighting that inadequate agitation may lead to stagnant zones or limited mass transfer.

Continuous monitoring of dissolved oxygen (DO) confirmed a sharp decline during period of rapid biomass accumulation, underscoring the necessity of a robust hydrodynamic regime to prevent metabolic shifts and ensure process stability.

CONCLUSIONS

The experimental investigation conducted in this study demonstrated that achieving peak performance in *Saccharomyces cerevisiae* biomass production within a stirred-tank bioreactor is governed by the interaction between agitation and thermal conditions. It was established that mechanical agitation serves as the primary driver, as evidenced by the performance of the 450 rpm and 35°C regime, which emerged as the superior window by delivering a maximum biomass concentration of 4.15 g/L and a peak specific growth of 0.290 h^{-1}.

An analysis of the data reveals that at 35°C, the process is strictly limited by the oxygen transfer rate, whereas at 25°C, the growth is primarily constrained by reduced enzymatic velocity. This distinction is further supported by the observation that lower agitation speeds favor the 25°C regime, where higher oxygen solubility and lower metabolic requirements prevent the culture from entering oxygen-limiting stress. Consequently, the results indicate that any increase in operating temperature must be coupled with a proportional enhancement of the volumetric mass transfer coefficient (kLa) to avoid shifts toward less efficient fermentative pathways.

Looking forward, this study highlights the need for more granular investigations into the metabolic trade-offs of hydrodynamics shear stress, particularly the paradoxical growth inhibition observed at high agitation and lower temperatures. It is important to note that the correlation between temperature and shear sensitivity observed here is influenced by the unique hydrodynamic profile and impeller design of the bench-top bioreactor. Consequently, scale-up to larger volumes will require careful validation of these parameters. Future research should prioritize the evaluation of metabolic flux changes under varying dissolved oxygen profiles to further refine the efficiency of aerobic batch processes.

Furthermore, exploring the transition from batch to fed-batch cultivation strategies could provide deeper insights into overcoming the nutrient limitations encountered in the final stages of growth, thereby pushing the productivity boundaries of the current bioreactor configuration.

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