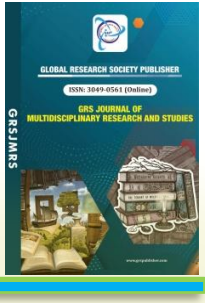
OPEN ACCESS

GRS Journal of Multidisciplinary Research and Studies
Abbreviate Tittle- GRS J Mul Res Stud
ISSN *(Online) - 3049-0561*
https://grspublisher.com/journal-details/GRSJMRS

Vol-3, Iss-11(Nov)-2026)



Microbial Growth Kinetics of *Saccharomyces* Cerevisiae from Commercial Dry Yeast: Experimental Assessment and Mathematical Modeling

Sivamani Selvaraju*

Mechanical and Chemical Engineering Unit, Department of Engineering and Technology, University of Technology and Applied Sciences, Salalah, Oman.

Article History
Received: 25.09.2026
Accepted: 17.10.2026
Published: 07.11.2026

Corresponding Author: Sivamani Selvaraju*

Abstract: *Saccharomyces cerevisiae* is an industrially important microorganism widely employed in the production of bioethanol, baker's yeast, single-cell protein, enzymes, and other value-added bioproducts. Understanding its growth characteristics under different inoculum concentrations is essential for improving microbial cultivation and fermentation performance. The present study investigated the influence of five inoculum concentrations (1%, 2%, 3%, 4%, and 5% v/v) on the growth behavior of *S. cerevisiae* in a glucose-based medium containing 20 g/L glucose. Microbial growth was assessed through absorbance measurements at 600 nm (OD_{600}), and growth profiles were constructed over a 24-day cultivation period. The results indicated that increasing inoculum concentration was associated with higher culture absorbance. The 5% inoculum exhibited the highest modeled final absorbance (1.818), followed by 4% (1.634), 3% (1.377), 2% (0.873), and 1% (0.246). The constructed growth profiles suggested an initial adaptation period of approximately 0–1 day, followed by an active growth region during days 2–8 and a gradual approach toward stationary conditions thereafter. Logistic modeling indicated that the cultures reached 95% of their assumed maximum absorbance at approximately 10.7–11.3 days. However, these phase durations and stationary-phase characteristics were model-dependent and could not be independently validated from the limited experimental observations. The findings demonstrate the influence of initial inoculum concentration on culture turbidity and highlight the potential usefulness of mathematical modeling for preliminary microbial growth assessment. Although 5% inoculum produced the highest modeled absorbance, further experimental validation is required to establish its suitability for industrial biomass and bioproduct production.

Keywords: *Saccharomyces cerevisiae; Inoculum concentration; Growth kinetics; Logistic growth model.*

Cite this Article

S. Selvaraju, (2026) Microbial Growth Kinetics of *Saccharomyces* Cerevisiae from Commercial Dry Yeast: Experimental Assessment and Mathematical Modeling *GRS Journal of Multidisciplinary Research and Studies, Vol-3 (Iss-11). 26-33*

Introduction

Saccharomyces cerevisiae is one of the most widely studied microorganisms in industrial biotechnology because of its ability to utilize different carbon sources and produce valuable fermentation products. It is commonly used in baking, brewing, bioethanol production, and other biotechnological applications [1]. The growth of *S. cerevisiae* depends on several factors, including the availability of nutrients, temperature, pH, oxygen supply, and the accumulation of metabolic products. Understanding its growth behavior is important for evaluating microbial activity, substrate consumption, biomass production, and fermentation efficiency. In a typical batch cultivation system, the growth of *S. cerevisiae* follows a characteristic pattern consisting of four main phases: lag, exponential, stationary, and death. These phases reflect changes in the physiological condition of the yeast cells as they adapt to the cultivation environment, multiply, and respond to nutrient depletion and other stresses.

The lag phase begins immediately after the yeast cells are introduced into a fresh growth medium. During this period, the cells adjust to their new surroundings and prepare for

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active growth. Although there may be little noticeable increase in cell number, several metabolic processes take place within the cells. These include the synthesis of enzymes, activation of nutrient transport systems, and production of cellular components required for growth and reproduction [2]. The length of the lag phase depends largely on the condition of the inoculum and the difference between its previous environment and the fresh cultivation medium. Factors such as temperature, pH, nutrient composition, and initial cell concentration may also influence the adaptation period. Cells transferred from an actively growing culture generally adapt more rapidly than those obtained from an aged or nutrient-depleted culture. A shorter lag phase is usually preferred in industrial fermentation because it allows the microorganisms to begin active growth sooner and can reduce the overall cultivation time.

Once the yeast cells have adapted to the medium, they enter the exponential or logarithmic growth phase. This phase is characterized by a rapid increase in cell number and biomass concentration. *S. cerevisiae* reproduces mainly through budding, in which a small daughter cell develops from the parent cell and eventually separates after completing the cell division process [3]. When sufficient nutrients are available and environmental conditions remain favorable, the cells multiply at an approximately constant specific growth rate. During this period, the yeast actively consumes available sugars and other nutrients to meet its energy requirements and support the synthesis of new cellular material. The exponential phase is particularly important for evaluating microbial growth kinetics because parameters such as specific growth rate and doubling time can be determined from experimental biomass measurements. However, the relationship between sugar consumption and biomass formation depends on the metabolic conditions. For example, *S. cerevisiae* can produce ethanol even in the presence of oxygen when glucose is abundant. This behavior, known as the Crabtree effect, influences the distribution of substrate carbon between biomass formation and fermentation products [4,5].

As cultivation continues, the growth rate gradually decreases, and the culture enters the stationary phase. This transition commonly occurs when essential nutrients become limited or when metabolic products accumulate to concentrations that interfere with further growth. During this phase, the net increase in the microbial population becomes negligible, although individual cells may remain metabolically active [6]. The yeast cells respond to these conditions by adjusting their metabolism and directing more resources toward cellular maintenance and survival. They may also accumulate storage compounds such as glycogen and trehalose, which help them withstand unfavorable conditions. In addition, changes in cell-wall composition and the activation of stress-response mechanisms can improve resistance to environmental stress. The stationary phase should therefore not be considered a period of complete inactivity. Instead, it represents a stage in which cellular activity shifts from rapid growth toward maintenance and survival. These physiological changes are important for understanding yeast performance during prolonged fermentation and storage [5].

The death or decline phase develops when unfavorable conditions continue for an extended period. At this stage, the number of viable yeast cells begins to decrease because the rate of cell death exceeds the formation of new cells [7]. Nutrient exhaustion, ethanol accumulation, changes in pH, and other environmental stresses may contribute to the loss of cell viability. Prolonged exposure to these conditions can damage cellular membranes, disrupt metabolic processes, and reduce the ability of yeast cells to reproduce. In some cases, cell autolysis may occur, resulting in the release of intracellular materials into the surrounding medium. However, a reduction in viable cell count may not be immediately reflected in biomass concentration or optical density because dead cells can remain suspended in the culture. For this reason, measurements of cell viability are useful when studying the decline phase, particularly in experiments involving extended cultivation periods.

An important feature of *S. cerevisiae* growth is its ability to change metabolic pathways according to the availability of carbon sources and oxygen. In aerobic batch cultures containing high initial glucose concentrations, the yeast may initially rely heavily on fermentation, leading to ethanol accumulation in the medium. As glucose becomes depleted, the cells can gradually shift toward respiratory metabolism and begin utilizing the ethanol produced during the earlier stage. This change is commonly referred to as the diauxic shift [8]. Depending on the cultivation conditions, a second period of slower growth may occur before the culture reaches the stationary phase. Therefore, changes in the slope of the microbial growth curve should be interpreted carefully, particularly when glucose and ethanol concentrations are not measured alongside biomass. The presence of a temporary reduction in growth rate does not always indicate that the cells have entered the stationary phase.

The growth of *S. cerevisiae* can also be evaluated using mathematical models that describe changes in biomass concentration with time. During exponential growth, the increase in biomass is commonly expressed as a function of the specific growth rate and the existing biomass concentration [9]. The specific growth rate provides an indication of how rapidly the microbial population increases under the prevailing cultivation conditions, while the doubling time represents the time required for biomass concentration to increase twofold. When microbial growth is limited by the availability of a particular substrate, the Monod model can be used to describe the relationship between substrate concentration and specific growth rate. Nevertheless, the Monod model may not fully represent

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yeast growth under all fermentation conditions, especially when high sugar concentrations, ethanol inhibition, or oxygen limitations influence microbial activity. In such situations, modified kinetic models may be required to describe the observed growth behavior more accurately.

The growth pattern of *S. cerevisiae* is closely linked to substrate utilization and the formation of fermentation products. Glucose and other fermentable sugars provide the carbon and energy required for cell maintenance, reproduction, and metabolic activity. During fermentation, part of the consumed substrate is converted into cellular biomass, while the remaining fraction is used for energy generation and the formation of products such as ethanol, carbon dioxide, and glycerol [10]. The relative distribution of these products depends on cultivation conditions and the metabolic state of the yeast. Consequently, a high rate of sugar consumption does not necessarily indicate a high rate of biomass production. Evaluating microbial growth together with substrate concentration and product formation can therefore provide a more complete understanding of fermentation performance. Overall, the growth curve of *S. cerevisiae* provides useful information about how yeast cells respond to changes in their cultivation environment. Each growth phase reflects a different physiological condition, from initial adaptation and rapid multiplication to nutrient limitation and eventual loss of viability. Examining these phases helps identify the conditions that support microbial growth and the factors responsible for growth inhibition. Furthermore, determining growth parameters such as specific growth rate, doubling time and maximum biomass concentration can support comparisons between different cultivation conditions. A clear understanding of these characteristics is important for improving fermentation efficiency, selecting suitable operating conditions, and developing reliable microbial cultivation processes.

Materials and Methods

Microorganism and Experimental Design

The present study investigated the influence of inoculum concentration on the growth characteristics of *Saccharomyces cerevisiae* under batch cultivation conditions. Five inoculum concentrations, namely 1%, 2%, 3%, 4%, and 5%, were considered to evaluate their effects on microbial growth and biomass accumulation. The growth behavior was assessed through changes in culture absorbance, which served as an indirect indicator of microbial biomass concentration.

The experimental design aimed to compare the growth profiles obtained at different inoculum concentrations and examine the relationship between initial inoculum concentration, microbial proliferation, and the development of the stationary growth phase. The cultivation period considered in the growth-curve analysis extended from day 0 to day 24.

Preparation of Inoculum and Cultivation Conditions

The growth of *S. cerevisiae* was investigated using a glucose-based culture medium under batch cultivation conditions. The growth medium was prepared by dissolving 2 g of glucose in 100 mL of distilled water in a 250 mL Erlenmeyer flask, corresponding to an initial glucose concentration of 20 g/L. The yeast inoculum was prepared separately by suspending 1 g of commercially available DCL active dried yeast in 50 mL of warm distilled water maintained at approximately 38°C. The suspension was mixed thoroughly to ensure uniform dispersion and rehydration of the yeast cells before inoculation. Five separate 250 mL Erlenmeyer flasks containing the glucose-based culture medium were prepared to investigate the effect of inoculum concentration on microbial growth. The flasks were inoculated with different concentrations of yeast suspension, corresponding to 1%, 2%, 3%, 4%, and 5% (v/v). The contents of each flask were mixed thoroughly to obtain a uniform distribution of yeast cells throughout the culture medium. The initial absorbance of each culture was measured at a wavelength of 600 nm (OD_{600}) using a spectrophotometer, with uninoculated glucose medium serving as the blank.

The inoculated cultures were maintained under batch cultivation conditions for 24 days. During the cultivation period, the flasks were shaken manually at regular intervals each day to promote uniform cell suspension and facilitate contact between the microorganisms and the available nutrients. The growth of *S. cerevisiae* was monitored by measuring the absorbance of the culture at 600 nm [11]. Absorbance readings were taken periodically throughout the cultivation period to evaluate changes in culture turbidity associated with microbial proliferation. Particular attention was given to identifying periods of substantial absorbance increase and subsequent stabilization, which could indicate changes in the microbial growth pattern. The recorded absorbance values were plotted against cultivation time to obtain microbial growth curves for the different inoculum concentrations. The resulting growth profiles were compared to evaluate the influence of initial inoculum concentration on the apparent growth behavior of *S. cerevisiae*. Changes in absorbance were used as an indirect measure of microbial growth, while the progression toward a relatively constant absorbance was examined as a possible indication of the stationary growth phase.

Identification of Growth Phases

The microbial growth profiles were examined with reference to the characteristic growth phases of *S. cerevisiae*, including lag, exponential, and stationary phases. The initial period of limited absorbance increase was considered potentially consistent with microbial adaptation, while the subsequent period of increasing absorbance was examined as an indication of biomass accumulation. The stationary

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phase was assessed operationally using the modeled approach toward the asymptotic absorbance.

Data Visualization and Comparative Analysis

The microbial growth data were presented using scatter plots, with cultivation time on the horizontal axis and absorbance on the vertical axis. Separate data series were prepared for the five inoculum concentrations to facilitate visual comparison of their growth behavior. The growth profiles were examined to identify differences in initial absorbance, the magnitude of absorbance increase, and the predicted approach toward a plateau. The change in absorbance between two cultivation times was calculated as:

$$\Delta A = A(t_2) - A(t_1)$$

The average rate of absorbance increase was expressed as:

$$R_a = [A(t_2) - A(t_1)]/(t_2 - t_1)$$

Where R_a represents the average rate of absorbance change per day.

These parameters were used to describe the relative changes in culture turbidity. They were not interpreted as specific microbial growth rates because the absorbance measurements were not calibrated against biomass concentration and the available observations did not establish a verified exponential growth interval.

Statistical Analysis and Limitations

The available dataset was evaluated descriptively to compare absorbance values among the five inoculum concentrations. Changes in absorbance over time and differences among inoculum treatments were summarized using numerical comparisons and graphical representations. Formal inferential statistical analyses, including analysis of variance, significance testing, and confidence interval estimation, were not performed because the available dataset did not contain independent experimental replicates or estimates of measurement variability. In addition, interpolated and extrapolated values were not treated as independent observations for statistical testing. The mathematical estimates were used to explore possible growth trajectories and support preliminary interpretation of the effect of inoculum concentration. The analysis was therefore considered exploratory, and the predicted stationary-phase characteristics require confirmation through additional experimental measurements.

Results and Discussion

Effect of Inoculum Concentration on the Growth of *S. cerevisiae*

The growth characteristics of *S. cerevisiae* were evaluated at five inoculum concentrations ranging from 1% to 5% (v/v) over a cultivation period of 24 days. Changes in microbial growth were assessed using absorbance measurements at 600 nm (OD_{600}), which served as an indirect indicator of culture turbidity and biomass accumulation [12]. Figure 1 presents the scatter plot of absorbance against cultivation time for the different inoculum concentrations. The growth profiles showed an increasing trend in absorbance during the initial cultivation period, followed by a gradual reduction in the rate of increase and an eventual approach toward relatively constant values. Although the overall profiles resembled the characteristic sigmoidal pattern of microbial growth, the dataset included mathematically interpolated and extrapolated values. Therefore, the observed trends should be interpreted as a combination of available experimental measurements and estimated growth behavior rather than as fully experimentally established growth curves.

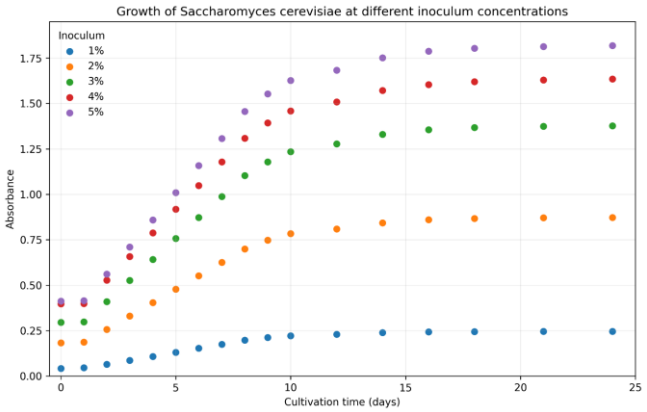


Figure 1. Growth curve of S. cerevisiae

The initial absorbance increased progressively with inoculum concentration, ranging from 0.042 for the 1% inoculum to 0.412 for the 5% inoculum. The corresponding initial values for the 2%, 3%, and 4% inoculum concentrations were 0.183, 0.295, and 0.397, respectively. These differences were consistent with the greater initial quantity of yeast suspension introduced at higher inoculum concentrations. By day 8, the absorbance values had increased to 0.197, 0.699, 1.103, 1.309, and 1.456 for the 1%, 2%, 3%, 4%, and 5% inoculum concentrations, respectively. The increasing absorbance values indicated greater culture turbidity during cultivation, although the contribution of viable biomass could not be quantified directly without an appropriate absorbance-to-biomass calibration.

The relative increase in absorbance between day 0 and day 8 was approximately 369.0%, 282.0%, 273.9%, 229.7%, and 253.4% for the 1%, 2%, 3%, 4%, and 5% inoculum concentrations, respectively. Interestingly, the 1% inoculum exhibited the highest percentage increase despite having the lowest absolute absorbance throughout the cultivation period. This difference highlights the importance of distinguishing relative growth responses from absolute culture turbidity. A lower initial absorbance can produce a comparatively large percentage increase even when the final absorbance remains substantially below that of cultures prepared with higher inoculum concentrations. Consequently, the percentage increase alone cannot be used

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to identify the inoculum concentration that provides the highest microbial biomass productivity.

The 5% inoculum consistently exhibited the highest absorbance throughout the cultivation period, followed by the 4%, 3%, 2%, and 1% inoculum treatments. This pattern suggests that the initial inoculum concentration strongly influenced the magnitude of culture turbidity. Higher inoculum concentrations introduce more yeast cells into the cultivation medium, potentially allowing the culture to establish a larger biomass population during the early stages of growth. However, the greater absorbance observed at higher inoculum concentrations should not be interpreted as evidence of a higher intrinsic specific growth rate. Since the initial cell concentrations differed among treatments, comparisons based solely on absolute absorbance may reflect differences in inoculum loading rather than differences in the physiological growth capacity of the yeast cells.

Identification of Microbial Growth Phases

The microbial growth profiles were examined to identify the characteristic phases associated with batch cultivation of *S. cerevisiae*. The initial cultivation period between day 0 and day 1 showed relatively small changes in absorbance across all inoculum concentrations. For example, absorbance increased from 0.042 to 0.046 for the 1% inoculum and from 0.412 to 0.415 for the 5% inoculum. These limited changes were consistent with a possible initial adaptation period, during which yeast cells adjust to the cultivation environment before undergoing substantial proliferation. During such a period, cellular activities may include rehydration, enzyme synthesis, nutrient uptake, and activation of metabolic pathways required for growth. Nevertheless, the apparent lag-phase behavior cannot be confirmed from the present dataset because the day-1 values were not established as independent experimental measurements.

A more pronounced increase in absorbance was represented between approximately days 2 and 8. During this interval, the culture profiles showed substantial increases in turbidity for all inoculum concentrations [13]. The absorbance of the 5% inoculum increased from 0.561 on day 2 to 1.456 on day 8, while the 4% inoculum increased from 0.527 to 1.309 over the same period. Similar trends were represented for the remaining inoculum concentrations. These increases were consistent with the expected biomass accumulation during active microbial growth. In *S. cerevisiae*, this stage is generally associated with budding, substrate assimilation, cellular biosynthesis, and the production of metabolic energy required for cell division. However, the interval from day 2 to day 8 should not automatically be classified as a confirmed exponential growth phase.

Exponential growth requires an approximately linear relationship between the natural logarithm of biomass concentration and cultivation time. In the present analysis, the intermediate values were generated mathematically, and absorbance was not calibrated against dry cell weight. Consequently, the available information was insufficient to establish a biologically meaningful exponential growth interval or determine an experimentally validated maximum specific growth rate.

After approximately day 8, the growth profiles exhibited a progressively smaller increase in absorbance. For the 5% inoculum, absorbance increased from 1.456 on day 8 to 1.752 on day 14, followed by a comparatively small increase to 1.818 on day 24. Similarly, the 4% inoculum increased from 1.309 on day 8 to 1.572 on day 14 and subsequently approached 1.634 on day 24. The remaining inoculum concentrations showed comparable trends, with progressively smaller changes in absorbance toward the end of the cultivation period.

The gradual flattening of the growth profiles was consistent with the stationary-phase behavior represented by the logistic extrapolation model. In an actual batch culture, stationary-phase development may result from glucose depletion, nitrogen limitation, accumulation of ethanol and other metabolites, changes in pH, or reduced availability of essential nutrients. These factors can limit cell division and redirect cellular metabolism toward maintenance and stress tolerance.

Comparative Analysis of Absorbance and Growth Performance

A comparative evaluation of the absorbance profiles was performed to examine the influence of inoculum concentration on apparent microbial growth. The net increase in absorbance was calculated as the difference between the initial and final absorbance values. Over the 24-day cultivation period, the net absorbance increases were 0.204, 0.690, 1.082, 1.237, and 1.406 for the 1%, 2%, 3%, 4%, and 5% inoculum concentrations, respectively. These results indicated that the absolute increase in culture turbidity became progressively greater as the inoculum concentration increased.

The average rate of absorbance change over the 24-day period was calculated by dividing the net increase in absorbance by the cultivation time. The estimated average rates were 0.0085, 0.0288, 0.0451, 0.0515, and 0.0586 absorbance units per day for the 1%, 2%, 3%, 4%, and 5% inoculum concentrations, respectively. The highest average rate was associated with the 5% inoculum, whereas the lowest was obtained for the 1% inoculum. However, these values represent average changes in optical density over the complete cultivation period rather than specific microbial growth rates. They are also influenced by the model-generated final absorbance values and therefore should be interpreted as descriptive indicators of the constructed growth profiles.

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An additional comparison was made using the final absorbance values on day 24. The highest absorbance of 1.818 was obtained for the 5% inoculum, followed by 1.634 for the 4% inoculum, 1.377 for the 3% inoculum, 0.873 for the 2% inoculum, and 0.246 for the 1% inoculum. The modeled final absorbance at 5% inoculum was approximately 11.3% higher than that at 4% inoculum and 32.0% higher than that at 3% inoculum. These differences suggest that increasing the inoculum concentration was associated with higher predicted culture turbidity, although the incremental benefit became smaller between the higher inoculum treatments.

The comparison between the 4% and 5% inoculum concentrations is particularly relevant from a process engineering perspective. Increasing the inoculum concentration from 4% to 5% represented a 25% increase in inoculum loading, whereas the corresponding modeled final absorbance increased by approximately 11.3%. This difference indicates that a higher inoculum concentration did not produce a proportional increase in final culture turbidity. Nevertheless, a reliable assessment of inoculum efficiency would require experimental biomass measurements, substrate utilization data, and consideration of inoculum preparation costs. Therefore, the 5% inoculum can be identified as the treatment with the highest modeled final absorbance, but it cannot be established as the optimal inoculum concentration for biomass productivity or fermentation efficiency based on the available data alone.

Mathematical Interpretation of Growth Profiles

The growth profiles were further examined using the logistic growth model described in the methodology. The logistic model provides a mathematical representation of population growth in which the rate of biomass accumulation decreases as the population approaches an assumed upper limit. Unlike unrestricted exponential growth, the logistic equation incorporates a maximum asymptotic value that produces a gradual flattening of the growth curve.

In the present study, the maximum absorbance was assumed to be 25% higher than the measured absorbance on the seventh day. The resulting model was used to estimate the subsequent changes in absorbance and illustrate the possible progression toward stationary growth. The assumed maximum absorbance values were 0.246, 0.874, 1.379, 1.636, and 1.820 for the 1%, 2%, 3%, 4%, and 5% inoculum concentrations, respectively, after rounding to three decimal places.

The modeled growth profiles approached these asymptotic values gradually, with relatively small changes in absorbance toward the end of the 24-day period. This behavior is consistent with the mathematical properties of logistic growth, in which the rate of increase decreases as the predicted population approaches its upper limit. However, because the asymptotic values were assumed rather than independently estimated from repeated experimental measurements, the resulting plateaus cannot be considered validated maximum biomass concentrations.

For comparative purposes, the time required to reach 95% of the assumed maximum absorbance was used as an operational indicator of the approach toward stationary growth. The estimated times were approximately 10.7, 11.0, 11.1, 11.3, and 11.2 days for the 1%, 2%, 3%, 4%, and 5% inoculum concentrations, respectively. The relatively small differences among these values suggest that the assumed logistic growth scenarios produced broadly comparable times to approach their respective asymptotic absorbances. However, these estimates depend directly on the selected plateau assumption and should not be interpreted as experimentally determined stationary-phase onset times.

The logistic model was useful for visualizing possible growth trajectories beyond the measured cultivation period. Nevertheless, the model does not explicitly account for changes in substrate concentration, ethanol inhibition, oxygen availability, or shifts between respiratory and fermentative metabolism. These factors are particularly relevant to *S. cerevisiae*, which can exhibit complex growth behavior, including diauxic transitions under suitable aerobic cultivation conditions. Therefore, future investigations incorporating substrate and metabolite measurements would allow the application of more mechanistic growth models and provide a stronger basis for understanding the physiological factors controlling yeast growth.

Statistical Interpretation and Limitations of the Growth Data

The influence of inoculum concentration on absorbance was evaluated primarily through descriptive comparisons of the available measurements and modeled growth profiles. The scatter plot provided a clear representation of the differences in absorbance among inoculum treatments and the general progression of the modeled curves toward their assumed upper limits. However, the available dataset did not contain independent replicate measurements or estimates of experimental variability. Consequently, statistical significance testing was not considered appropriate.

Although the modeled profiles showed a consistent increase in absorbance with inoculum concentration, this relationship should not be interpreted as statistically significant without experimental replication. Similarly, the interpolated and extrapolated values were mathematically dependent on the initial observations and modeling assumptions. Treating these estimated values as independent experimental observations would artificially increase the apparent sample size and could produce misleading statistical conclusions.

The absence of independent measurements at intermediate cultivation times also restricted the reliability of growth-phase identification. Linear interpolation does not capture the nonlinear behavior normally associated with microbial growth, while logistic extrapolation depends on assumptions concerning the maximum attainable absorbance.

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Furthermore, optical density measurements provide information about culture turbidity rather than direct measurements of viable biomass. At relatively high absorbance values, the relationship between optical density and cell concentration may become nonlinear because of multiple light scattering, making appropriate dilution and calibration important for accurate biomass estimation.

Another limitation concerns the composition of the cultivation medium. The cultures were prepared using glucose dissolved in distilled water, without additional specified nitrogen, mineral, or vitamin supplementation. Although commercially available dried yeast may contain intracellular nutrient reserves and residual components from its preparation, the absence of a nutritionally complete medium could influence microbial growth and limit the attainable biomass concentration. Consequently, the observed or predicted growth behavior may reflect both the initial inoculum concentration and the nutritional limitations of the cultivation system.

The use of periodic manual shaking may also have influenced oxygen availability and culture homogeneity. Unlike controlled orbital shaking or mechanically agitated bioreactors, manual shaking does not provide a precisely defined oxygen transfer rate. Since oxygen availability can affect the metabolic activity and biomass yield of *S. cerevisiae*, variations in aeration may contribute to differences in growth behavior. Future experiments should therefore consider controlled agitation, defined cultivation conditions, and appropriate monitoring of substrate and product concentrations.

Implications for Yeast Cultivation and Fermentation Performance

The present analysis illustrates the importance of initial inoculum concentration in determining the absorbance profile of *S. cerevisiae* cultures. Higher inoculum concentrations were associated with greater initial absorbance and higher modeled absorbance values throughout the cultivation period. The 5% inoculum produced the highest predicted final absorbance, whereas the 1% inoculum showed the greatest relative increase from its initial value. These contrasting observations demonstrate that the interpretation of microbial growth depends on the performance indicator selected.

From an industrial biotechnology perspective, inoculum concentration influences the initial microbial population, adaptation requirements, nutrient consumption, and the time required to establish an active fermentation culture. An insufficient inoculum may prolong the cultivation period, whereas an excessively high inoculum can increase seed preparation requirements and intensify competition for available nutrients. Therefore, the selection of an appropriate inoculum concentration should consider not only the final biomass concentration but also substrate conversion efficiency, product yield, cultivation time, and overall process economics.

The present findings provide a preliminary basis for understanding inoculum-dependent changes in yeast culture turbidity. However, identifying an optimal inoculum concentration requires additional experimental evidence, particularly independent biomass measurements, replicate cultivation trials, and measurements of residual glucose and fermentation products. Integrating these measurements with suitable kinetic models would provide a more comprehensive understanding of microbial growth and support the development of reliable cultivation strategies for *S. cerevisiae*.

CONCLUSION

The present study examined the influence of inoculum concentration (1–5% v/v) on the growth characteristics of *Saccharomyces cerevisiae* over a 24-day cultivation period using absorbance at 600 nm as an indirect indicator of microbial biomass. The growth profiles indicated that higher inoculum concentrations were associated with greater culture turbidity, with the 5% inoculum exhibiting the highest final modeled absorbance (1.818), followed by 4% (1.634), 3% (1.377), 2% (0.873), and 1% (0.246). The growth characteristics of *S. cerevisiae* have considerable industrial importance because this microorganism is widely employed in the production of bioethanol, carbon dioxide, baker's yeast biomass, single-cell protein, glycerol, organic acids, enzymes, and recombinant proteins. In addition, metabolically engineered strains can produce higher-value bioproducts, including terpenoids, carotenoids, pharmaceuticals, and other specialty chemicals. The suitability of a particular growth phase for bioproduct formation depends on the relationship between cellular growth and product biosynthesis. Primary metabolites, including ethanol and glycerol, are commonly associated with active substrate metabolism and may be produced during the exponential growth phase, although ethanol formation can continue under conditions of limited net biomass growth. The exponential phase is also particularly suitable for producing yeast biomass, including baker's yeast and single-cell protein, because active cell proliferation supports rapid biomass accumulation. In contrast, some non-growth-associated or growth-decoupled products, including selected engineered secondary metabolites and recombinant compounds, may be produced or accumulated during the late exponential and stationary phases. Nevertheless, secondary metabolite production in *S. cerevisiae* is highly dependent on strain engineering, metabolic regulation, nutrient availability, and fermentation strategy; therefore, the stationary phase cannot be considered universally optimal for all secondary metabolites. Overall, the study provides a preliminary assessment of the relationship between inoculum concentration and the apparent growth behavior of *S. cerevisiae*. The results emphasize the

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importance of distinguishing biomass accumulation from product formation when selecting cultivation conditions for industrial biotechnology applications. Although the mathematical growth profiles offer useful insights into possible lag, active growth, and stationary-phase behavior, additional experimental measurements are required to validate the proposed phase durations and determine the optimum inoculum concentration for specific bio products. Future investigations should incorporate replicate cultivation experiments, calibrated biomass measurements, substrate consumption analysis, and product quantification to establish reliable growth kinetics and develop efficient fermentation strategies for industrial applications.

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