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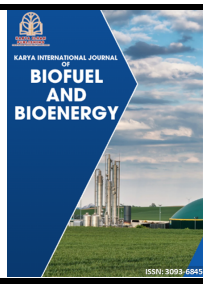

Kinetic Study and Simulation of Glucose Degradation Through Anaerobic Fermentation for Bioethanol Production

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ABSTRACT

As a renewable biofuel, bioethanol offers a sustainable alternative to fossil fuels, addressing the global demand for cleaner energy. The utilisation of sugar derived from biomass is one of the key strategies in advancing bioethanol production, which involves a catalytic reaction with yeast during the fermentation process. Although bioethanol fermentation has been widely studied, limited experimental validation of temperature-dependent kinetics constrains model reliability and process scalability. This work integrates experimental and modeling approaches to examine glucose degradation kinetics by *Saccharomyces cerevisiae* under anaerobic conditions at 30°C, 35°C, and 40°C. Results indicate that anaerobic fermentation at 30°C achieved the highest ethanol concentration of 3.11 g/L after 96 hours, with 88.5% glucose utilization. A kinetic model incorporating Monod and Hinshelwood equations was simulated using COMSOL Multiphysics software, with the Arrhenius relationship applied to describe temperature-dependent reaction rates. This study also revealed the activation energy of 9.12 kJ/mol derived from Arrhenius linear regression, which confirms the efficient kinetics at moderate temperatures. COMSOL Multiphysics® simulation validated experimental trends (R^2>0.95), highlighting the model's capability for process optimization. These findings highlight the critical role of glucose degradation kinetics in guiding the development of energy-efficient and thermally optimized fermentation processes for industrial applications.

1. Introduction

The global energy landscape is increasingly pressured by the environmental and sustainability concerns associated with the extensive use of fossil fuels. These challenges, prominently greenhouse gas emissions and the rapid depletion of finite resources, have spurred the search for renewable energy alternatives [1]. The shift towards renewable energy is crucial in the fight against climate change and the quest to reduce greenhouse gas emissions [2]. In addition, the global energy is predicted to increase by more than 28% by 2040, raising the urgency of switching to renewable energy sources to produce more biofuels, like bioethanol [3]. Bioethanol offers a more environmentally friendly alternative to fossil fuels, with lower emissions of harmful pollutants and greenhouse gases.

The production of bioethanol primarily relies on the fermentation of carbohydrates by microorganisms, notably *Saccharomyces cerevisiae*, which efficiently converts glucose into ethanol under anaerobic conditions. From a biochemical standpoint, fermentation occurs in yeasts (and

certain bacteria) when pyruvate, generated through glucose metabolism, is broken down into ethanol and carbon dioxide. Furthermore, developments in various fermentation technologies, such as batch, continuous, and fed-batch processes, have opened new avenues for improving bioethanol production efficiency, each with its distinct advantages and challenges regarding yield, time, and operational costs [4]. However, the performance of microbial fermentation is highly sensitive to process parameters, particularly temperature, which directly influences the yeast metabolism, enzyme activity and substrate conversion efficiency.

Despite the potential of bioethanol as a renewable energy source, its production efficiency during anaerobic fermentation is often hindered by factors such as incomplete glucose degradation. This inefficiency, strongly influenced by temperature variations, results in suboptimal ethanol yields [5]. A comprehensive understanding of how temperature affects glucose degradation, particularly in relation to the metabolic activity of key fermentative yeasts is therefore essential for bioethanol production. Moreover, kinetic modeling serves as a crucial tool for predicting and understanding the dynamic behavior of fermentation systems. It enables the evaluation of process efficiency and provides a foundation for process design, control, and optimization [6]. The choice of an appropriate kinetic model depends on the study objectives, microbial metabolic pathways, and operating conditions.

Although numerous studies have examined the kinetics of bioethanol fermentation, few have experimentally validated temperature-dependent kinetic parameters through integrated computational modeling platforms such as COMSOL Multiphysics®. This study addresses that gap by coupling experimental kinetic data with numerical simulations to more accurately predict fermentation behavior under varying thermal conditions. Accordingly, this research aims to simulate glucose degradation using a kinetic model developed in COMSOL Multiphysics®, focusing on temperature effects and validating the model against experimental data. The study is limited to using a single yeast strain and glucose substrate in batch mode, which may not fully represent the complexity of mixed-sugar or continuous fermentation systems. Additionally, the COMSOL model assumes ideal mixing and does not account for enzyme inhibition during prolonged exposure to elevated temperatures. Nevertheless, the findings provide valuable insights for improving bioethanol production efficiency, contributing to sustainable energy development, and supporting progress toward Sustainable Development Goal 7: Affordable and Clean Energy.

2. Methodology

2.1 Materials

The materials used in this study included commercial glucose powder, which served as the primary carbon source for fermentation. The microorganism employed for fermentation was *Saccharomyces cerevisiae*, obtained in its commercial yeast form. The standards used were glucose (≥99.5% purity, Sigma Aldrich, USA) and ethanol absolute (99.9% purity, Sigma Aldrich). All reagents were of analytical grade. Standard solutions of glucose and ethanol were prepared for calibration of the HPLC before sample analysis.

2.2 Preparation of Anaerobic Fermentation

The fermentation experiments were performed by dissolving 4 g/L of glucose in 50 mL of distilled water. The glass flasks were labelled according to the intended sample collection times (24 h, 48 h, 72 h, and 96 h). Subsequently, 1% w/v of *S. cerevisiae* was inoculated into each flask. The flasks were sealed and incubated in an incubator shaker at 30°C under continuous shaking to ensure

homogeneity and proper oxygen exclusion. To evaluate the effect of temperature on fermentation kinetics, the experiments were replicated at 35°C and 40°C, as the optimal growth temperature range of *S. cerevisiae* typically lies between 28°C and 33°C [7]. Samples were collected aseptically at the designated time intervals, filtered to remove solids, and centrifuged for 15 minutes to obtain a clarified liquid for further analysis [8].

2.3 High-Performance Liquid Chromatography (HPLC)

Quantitative analysis of glucose and ethanol concentrations was performed using a HPLC system equipped with an Aminex HPX-87H column (Bio-Rad, USA) and a refractive index detector (RID). Ultra-pure water with 0.0005 M H_2SO_4 was used as the mobile phase with a flow rate of 0.7 mL/min at a temperature of 60°C.

2.4 Basis Equation of Fermentation Kinetics

The rate of glucose consumption(r_G) by yeast cells can be approximated by Monod's equation [9].

$$r_G = \mu = \mu_{max} \frac{C_G}{C_G + K_s} \quad (1)$$

Where C_G is glucose concentration (g.dm^{-3}), K_S is the Monod constant or the half-saturation constant of growth kinetics and μ_{max} maximum specific growth rate.

Previous literature has reported that the values of Monod's constant and maximum specific growth rate of yeast were 2.23 g.L^{-1} and 0.35 g L^{-1} h^{-1}, respectively [10].Since the rate of yeast growth is proportional to the rate of glucose consumption, the rate of yeast growth can be estimated by:

$$r_x = \frac{dx}{dt} = \mu X = \mu_{max} \left(\frac{C_G}{C_G + K_S}\right).X \quad (2)$$

Where C_G is glucose concentration (g.dm^{-3}), K_S is Monod constant or half saturation constant of growth kinetics, X is yeast cell growth rate (g.dm^{-3}. s^{-1}) and μ_{max} is the maximum-specific growth rate.

The rate of ethanol concentration over time (dC_E/dt), incorporating the Monod dependence on the substrate, can be written using the Hinshelwood model [11].

$$\frac{dCE}{dt} = q_{max} \left(\frac{C_G}{C_G + K_S}\right) \quad (3)$$

Where C_G is glucose concentration (g.dm^{-3}), K_S is Monod constant or half saturation constant of growth kinetics. q_{max} is the maximum-specific ethanol production rate (g.(g.h)$^{-1}$) and μ_{max} is the maximum-specific growth rate.

2.5 Simulation using COMSOL Multiphysics®

Numerical simulations were performed using COMSOL Multiphysics® v6.1 to model the coupled kinetics of glucose consumption and ethanol formation. Input parameters included

experimentally determined kinetic constants, temperature-dependent rate coefficients (derived from Arrhenius analysis), and initial substrate concentrations. Simulations were conducted under transient conditions, and the solver was configured using a time-dependent study with adaptive time-stepping. Model validation was achieved by comparing simulated concentration profiles with experimental data at 30°C, 35°C, and 40°C. This approach follows the modeling framework proposed by previous work emphasizing the integration of empirical and computational data for kinetic validation [12].

3. Results and Discussion

In this section, the result examines the fermentation process by analysing HPLC data and COMSOL Multiphysics simulations, highlighting the temperature-dependent kinetics of glucose and ethanol. It highlights significant gaps in simulations, such as overlooking enzyme inhibition at elevated temperatures, and suggests enhancing experimental precision and refining simulation models to improve ethanol production forecasts.

3.1 Analysis of Glucose and Ethanol Concentrations

A summary of glucose (%w/v) and ethanol concentrations (%v/v) is shown in Table 1 below. The results are based on the peak areas measured in nano Refractive Index Units per second (nRIU*s) and calculated using their respective standard calibration curves.

Table 1
Glucose and ethanol concentrations over 96h at different fermentation temperatures

Temperature (°C)	Time (h)	Glucose Concentration (%w/v)	Ethanol Concentration (g/L)
30	24	0.87	1.88
	48	0.68	2.13
	72	0.61	2.54
	96	0.59	3.11
35	24	2.30	1.10
	48	1.16	1.34
	72	0.63	2.19
	96	1.06	2.37
40	24	0.46	2.55
	48	0.49	2.77
	72	0.56	2.96
	96	1.06	2.32

The glucose concentration decreased progressively over time for all three temperatures, indicating active fermentation and sugar utilisation by *S. cerevisiae.* At 30°C, the initial glucose concentration of 4.0% (w/v) dropped sharply to 0.87% after 24 hours and continued to decline steadily, reaching 0.59% at 96 hours. This consistent decrease suggests efficient and sustained glucose consumption under moderate temperature conditions.

At 35°C, glucose consumption was slower and less consistent. After a significant drop to 2.3% at 24 hours, the concentration continued to decrease to 0.63% by 72 hours but unexpectedly increased to 1.06% at 96 hours. This rise could be due to cell lysis or reduced sugar uptake caused by stress at

this temperature, resulting in the release of intracellular carbohydrates or reduced sugar uptake efficiency. At 40 °C, the glucose concentration declined sharply to 0.46% within the first 24 hours, indicating rapid substrate utilization. However, unlike the trend observed at 30 °C, glucose levels remained relatively stable between 24 h and 72 h, followed by a slight increase to 1.06% at 96 h. This late-stage rise suggests possible glucose accumulation or inhibited uptake, potentially resulting from thermal stress impairing yeast metabolic activity.

The highest glucose consumption occurred at 40 °C, with 88.5% of the initial glucose depleted after 24 hours of fermentation. Comparable findings were reported by Sari *et al.* [13] who observed approximately 90% glucose utilization when starting from a high initial glucose concentration (22%), with a residual glucose of 2.04% after fermentation. These results collectively suggest that elevated temperatures can accelerate glucose metabolism initially but may subsequently hinder uptake due to temperature-induced metabolic inhibition.

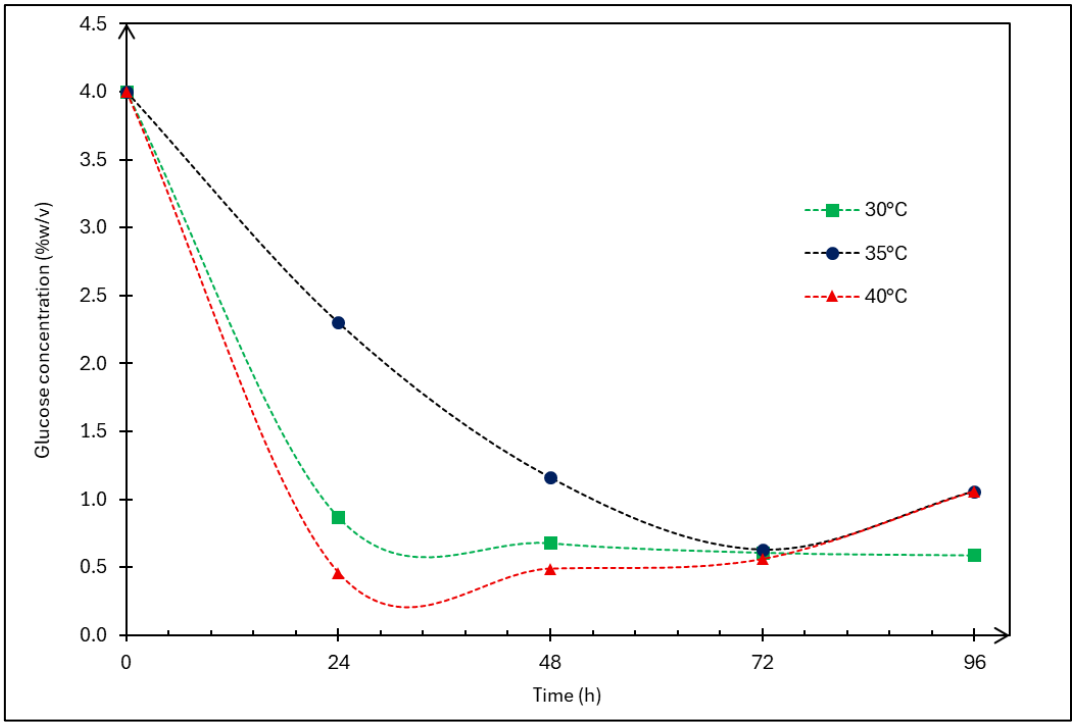

Fig. 1. Glucose concentration profiles at different temperatures (30°C, 35°C, and 40°C) over 96 h.

The effect of temperature on ethanol production was evaluated at 30°C, 35°C, and 40°C over a 96-hour fermentation period, as shown in **Figure 2** below. Ethanol production increased over time for all tested temperatures, showing that glucose was being successfully converted into ethanol. At 30°C, ethanol concentration rose steadily from 1.88 g/L at 24 hours to the highest yield of 3.11 g/L at 96 hours, reflecting efficient and continuous fermentation. This suggests that 30°C is a favorable temperature for the yeast and substrate concentration used in this study, allowing optimal metabolic activity and product formation. Compared to industrial fermentation processes, which typically produce much higher ethanol concentrations, the 3.11 g/L yield observed in this study is relatively modest. For instance, Sahu [14] achieved a peak bioethanol concentration of 29.5 g/L when fermenting glucose from rose petals at 30°C . Additionally, Kassim *et al.* [15] reported that ethanol production was lowest at 40°C, whereas higher yields were obtained at 30°C and 35°C.

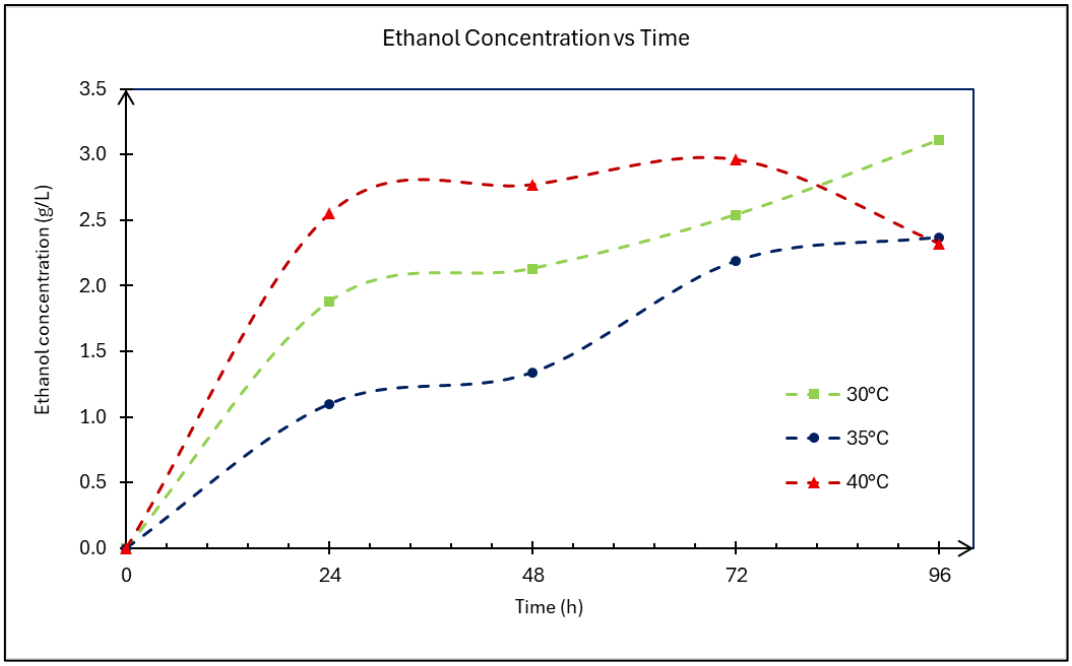

Fig. 2. Ethanol concentration profiles at different temperatures (30°C, 35°C, and 40°C) over 96 h.

At 35°C, ethanol production was slower and less efficient, reaching only 2.37 g/L by 96 hours. The low initial ethanol yield (1.10 g/L at 24 h) and gradual increase may reflect suboptimal enzymatic or microbial activity at this temperature. Interestingly, fermentation at 40°C showed the fastest initial ethanol production, peaking at 2.96 g/L at 72 hours, followed by a decline to 2.32% at 96 hours. This suggests that while higher temperatures can enhance early fermentation rates, prolonged exposure may negatively affect yeast viability and reduce ethanol accumulation in the later phase. the ethanol productivity was calculated for each temperature condition. The estimated rates were 0.278, 0.319, and 0.236 g L^{-1} h^{-1} at 30, 35, and 40°C, respectively, corresponding to 0.00604, 0.00693, and 0.00512 mol L^{-1} h^{-1}. The maximum productivity observed at 35°C indicates the optimal condition for yeast metabolic activity and enzymatic catalysis. In contrast, the decrease at 40 °C suggests the onset of thermal inhibition, consistent with previous findings on temperature effects in ethanol fermentation. This result aligns with the observations of previous studies that also reported that ethanol production reached its maximum at a fermentation temperature of 35 °C [16], [17]. The decrease in ethanol productivity observed at 40 °C can be attributed to yeast cell denaturation, as *S. cerevisiae* exhibits limited tolerance to stress induced by elevated temperatures [18].

3.2 Linear Regression on Arrhenius

A linear regression analysis was conducted to examine the relationship between temperature and the rate of glucose consumption during anaerobic fermentation, following the Arrhenius kinetic model. The reaction rate (r) was evaluated based on the molar rate of glucose depletion (in M/s) over the initial 24-hour period at three different temperatures (30°C, 35°C, and 40°C). These rates were then logarithmically transformed (ln (r)) and plotted against the inverse of absolute temperature (1/T in K^{-1}). **Table 2** presents a set of kinetic data collected to perform a linear regression analysis.

Table 2
Temperature-dependent kinetic data used for linear regression analysis of glucose consumption rate

Temp (K)	r (M/h)	r (M/s)	ln (r)	1/T (K^{-1})
303.15	0.00724	2.011×10^{-6}	-13.122	0.003298
308.15	0.00393	1.092×10^{-6}	-13.728	0.003244
313.15	0.00819	2.275×10^{-6}	-12.994	0.003193

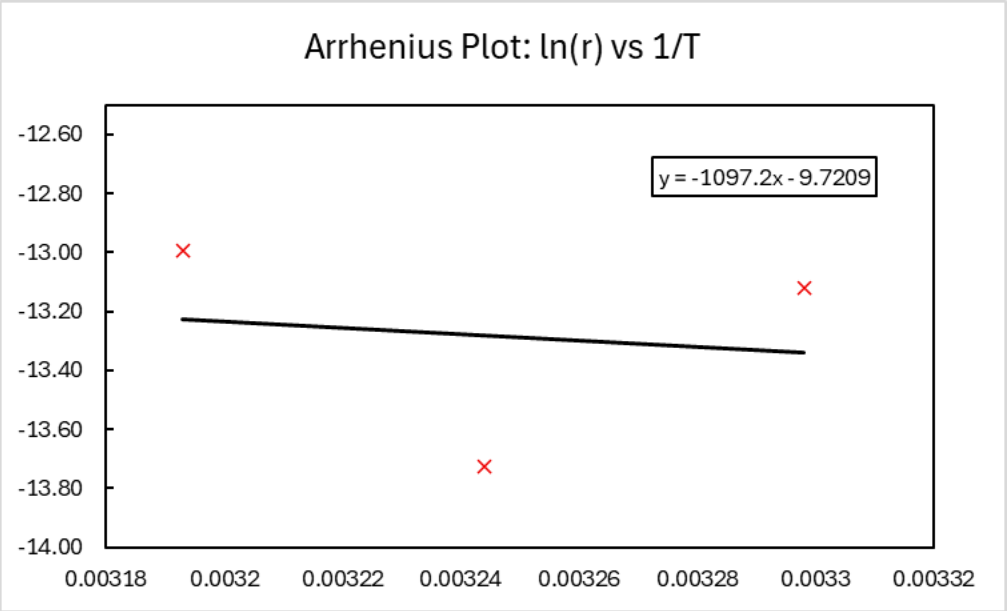

Fig. 3. The Arrhenius plot showing the linear relationship between the natural logarithm of the reaction rate (ln (r)) and the inverse of temperature (1/T)

The resulting Arrhenius plot as shown in **Figure 3** yielded a linear relationship with the regression equation $y = -1097.2x - 9.7209$ indicating a clear temperature dependence of the reaction rate. From the slope of this linear fit, the activation energy (Ea) was calculated to be approximately 9.12 kJ/mol, indicating a relatively low energy barrier for the reaction. In the mesophilic region, where temperature has little effect, the activation energy is quite low at 8.9 kJ/mol, while in the thermophilic region, which is more temperature-dependent, it rises to around 117 kJ/mol [19]. This suggests that the fermentation process proceeds efficiently even at lower temperatures, aligning with observations from glucose consumption and ethanol production trends. The strong linearity of the plot supports the validity of the kinetic model used and implies consistent reaction behaviour across the tested temperature range.

3.3 Simulation using COMSOL Multiphysics

The simulation section using COMSOL Multiphysics addresses the complex interplay between temperature and biochemical reaction rates in the fermentation process. It employs mathematical models to simulate and visualize how varying temperatures affect glucose consumption and ethanol

production over time, providing a dynamic representation of the fermentation kinetics. Model validation was performed by comparing simulated glucose and ethanol concentration profiles with experimental data, showing strong correlations (R^2 = 0.95–0.97). This confirms that the experimentally derived temperature-dependent kinetic parameters were accurately captured by the COMSOL model. Such simulations are essential for scaling up industrial fermentation processes, enabling optimization of operational conditions to achieve targeted biofuel yields.

3.3.1 Effect of Temperature on Glucose Consumption and Ethanol Production

The simulation graph in Figure 4 below shows glucose levels declining over time during fermentation at 30°C, 35°C, and 40°C, with faster consumption at higher temperatures, reflecting the typical temperature-dependent increase in biological activity until a peak is reached, where activity may decrease due to factors like enzyme denaturation.

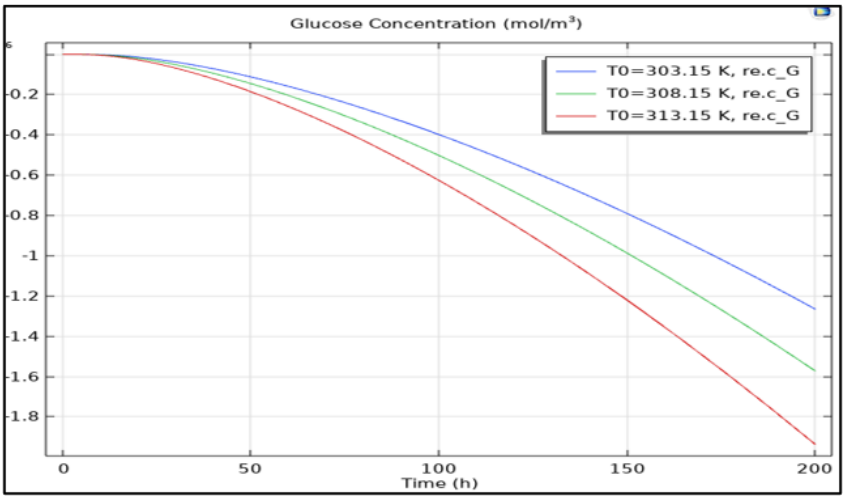

Fig. 4. COMSOL Simulations of glucose degradation kinetics under varying temperature conditions

For ethanol production, the graph in **Figure 5** reveals an increase in concentration with time at all observed temperatures, with a notably quicker production rate at elevated temperatures. This trend aligns with the Arrhenius principle, where reaction rates generally increase with temperature due to a higher frequency of effective molecular collisions. The curves are characteristic of biological processes, showing a phase of exponential growth followed by a plateau as the system approaches equilibrium or limiting factors become predominant, illustrating the balance between yeast metabolism and environmental conditions in the fermentation process.

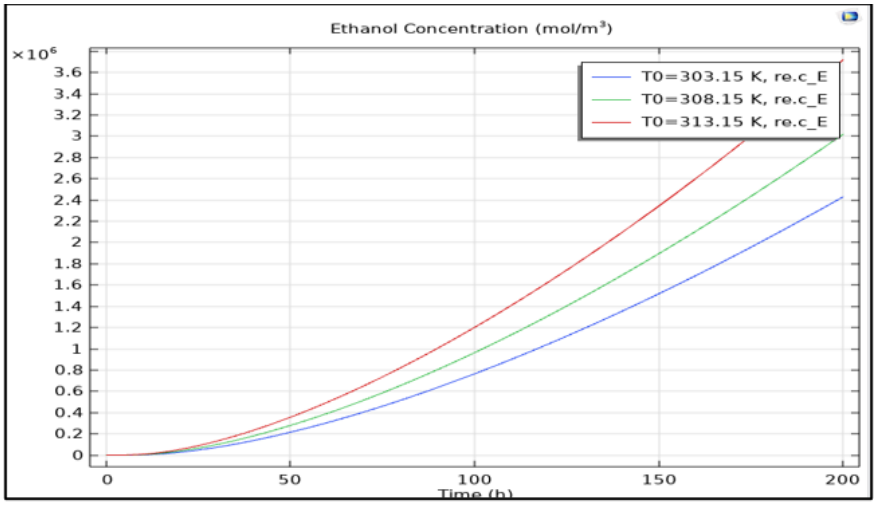

Fig. 5. COMSOL Simulations of ethanol degradation kinetics under varying temperature conditions

4. Conclusions

In conclusion, the comprehensive analysis revealed that at 30°C, a 96-hour fermentation period yielded the highest ethanol concentration, demonstrating an optimal balance between temperature and time for bioethanol production under the study's conditions. At elevated temperatures, particularly 40°C, enzyme inhibition likely hindered fermentation efficiency, as evidenced by reduced ethanol yields and altered glucose consumption patterns. These findings underscore the delicate balance between enzymatic activity and thermal stress, highlighting the importance of temperature control in optimizing fermentation processes. Despite being limited to three temperatures and a single yeast strain, the study provides a validated kinetic framework that enhances understanding of temperature effects on fermentation and supports future scale-up. The findings are especially relevant for mesophilic yeast-based bioethanol production and provide a foundation for extending the kinetic model to other feedstocks and process configurations in subsequent studies.

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