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Dynamic Simulation of the Liquid-Phase Fermentation in Traditional Vietnamese Rice Wine Production

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Abstract

The production of traditional Vietnamese rice wine involves a simultaneous saccharification and fermentation (SSF) process, which is a complex biochemical system influenced by multiple factors, including pH, temperature, substrate concentration, microbial biomass, and metabolic byproducts. Among these factors, temperature plays a critical role throughout fermentation, as it strongly affects both product quality and microbial kinetics. In this study, a dynamic mathematical model based on differential equations was developed to describe the liquid-phase stage of traditional rice wine fermentation using experimental data derived from artisanal practices. The model focuses on yeast growth and ethanol formation during the predominantly ethanol-producing phase and aims to support process analysis and standardization. Dynamic simulations were implemented in MATLAB using the stiff solver ODE15s (Ordinary differential equations), with key initial conditions specified as model inputs. The model generates fermentation trajectories and kinetic profiles under defined operating conditions. Simulation results show strong agreement between predicted and experimentally measured temperature profiles, while glucose consumption and ethanol production are presented as mechanistic model predictions consistent with established fermentation theory.

Keywords: Kinetic modeling, process simulation, simultaneous saccharification and fermentation (SSF), temperature-dependent kinetics, yeast-bacterial system.

1. Introduction[*]

Traditional rice wine is a culturally significant fermented beverage widely produced across regions of Vietnam. Unlike modern industrial processes, Vietnamese rice wine is typically made through artisanal techniques using steamed rice and naturally occurring microbial consortia present in traditional fermentation starters known as "bánh men" [1-4]. These starters contain a diverse array of yeasts, molds, and bacteria, primarily *Saccharomyces cerevisiae* and *Bacillus subtilis*, which simultaneously drive saccharification and ethanol fermentation in a single process step [5, 6]. This method, known as simultaneous saccharification and fermentation (SSF), is highly complex, governed by interactions among temperature, substrate concentration, microbial growth, enzymatic activity, and metabolic byproducts.

The quality and consistency of traditional rice wine often vary due to the empirical nature of its production. Notably, temperature is a critical environmental factor that significantly influences microbial kinetics, enzymatic hydrolysis rates, and ethanol yield [7]. Previous studies have examined SSF systems involving glutinous rice and isolated enzyme systems, but these models typically address simplified systems and do not capture the unique dynamics of traditional Vietnamese rice wine fermentation, particularly during the liquid-phase stage that follows initial solid-state fermentation [8-12].

Recent kinetic modeling efforts by Liu *et al.* and Kroumov *et al.* have contributed valuable frameworks for SSF systems, however, these models often rely on controlled monoculture fermentations with exogenous enzyme addition [13-16]. In contrast, traditional Vietnamese processes rely entirely on in situ enzyme production by native microorganisms, which makes the modeling and optimization of such systems more challenging yet scientifically valuable [17].

Therefore, using existing knowledge and data to improve the simulation process, avoiding the need to redefine all parameters, can significantly reduce both time and effort. This study aims to create and simulate a temperature-sensitive dynamic model for the liquid-phase stage (Phase 2) of traditional rice wine fermentation, using MATLAB's solver. The model incorporates microbial growth, substrate breakdown, product formation, and heat transfer feedback, all within the context of authentic artisanal practices. This study

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contributes to the standardization and improvement of traditional fermentation processes by using mechanistic modeling. The results offer insights that could help with industrial adaptation while also preserving cultural integrity.

2. Materials and Methods

2.1. Materials and Experimental Setup

Raw materials were collected from the actual production facility in Xom Trong, Uy No commune, Dong Anh district, Hanoi city with specific technical parameters and characteristics. The main raw material used in the study was fragrant Tam rice from the Hai Hau region, Nam Dinh province, selected based on quality and purity criteria. In this experiment, the raw material used was 1.5 kg of broken rice grains (non-whole), with a moisture content of approximately 14%. The composition of the fermentation microorganisms included "bánh men" (traditional Vietnamese rice wine starter cake) sourced from the manual production process in the Yen Phong locality of Bac Ninh province. The microbial composition of the traditional rice wine starter mainly included *Saccharomyces cerevisiae* yeast along with various molds and bacteria, with a total microbial density of about 10^7 CFU/g (colony forming units per gram) [18, 19]. The fermentation process was done in a plastic container that held about 5 liters of liquid for the research equipment system. To ensure accurate monitoring of parameters throughout the experiment, the system was equipped with temperature sensors installed at three locations: inside and outside the tank (as detailed in Fig. 1). Temperature monitoring was employed as an indirect indicator of ongoing biochemical transformations within the fermenting medium, while simultaneously allowing the influence of external environmental temperature on the internal fermentation process to be assessed under non-isothermal, artisanal fermentation conditions [12, 20].

2.2. Methodology

2.2.1. Fermentation method

The preparation and fermentation process followed a traditional method with several defined steps. Briefly, 1.5 kg of pre-treated rice was cooked at 100 °C with a rice-to-water ratio of 1:1, yielding approximately 2.7 kg of cooked rice. After cooling to ambient temperature (~30 °C), the rice was loosened in a clean container.

During the yeast preparation stage, the traditional rice wine starter cake was finely crushed and mixed with the rice at a starter-to-rice ratio of 1:50 (w/w). The mixture was then transferred to a fermentation tank and fermented under covered conditions for approximately 120 hours. The initial stage was conducted under open conditions to allow oxygen availability for microbial biomass growth, followed by a closed stage to promote anaerobic fermentation.

The fermentation process was divided into two main stages. The semi-solid phase fermentation stage (Phase 1) was carried out at room temperature of approximately 30 °C, during which molds, bacteria primarily produced hydrolytic enzymes for starch degradation, while yeast underwent cell proliferation. Due to the relatively low temperature, saccharification occurred slowly and gradually. This stage involves multiple simultaneous biochemical reactions within a heterogeneous solid–liquid system and is therefore described here only for process completeness, but was not included in the kinetic modeling and simulation. Accordingly, Phase 1 will be investigated separately to elucidate its reaction mechanisms. After completion of Phase 1, 2.25 L of distilled water was added and the fermentation tank was sealed to initiate the liquid-phase fermentation stage (Phase 2), during which yeast actively converted fermentable sugars into ethanol. The entire natural fermentation process lasted approximately 336 h, following traditional practices in the Bac Ninh region [13,14,18]. The present study and all subsequent simulations focus exclusively on Phase 2, spanning 216 h (from 120 to 336 h of the total process), which represents the predominantly ethanol-producing period and allows meaningful analysis of temperature dynamics, heat release, and fermentation kinetics.

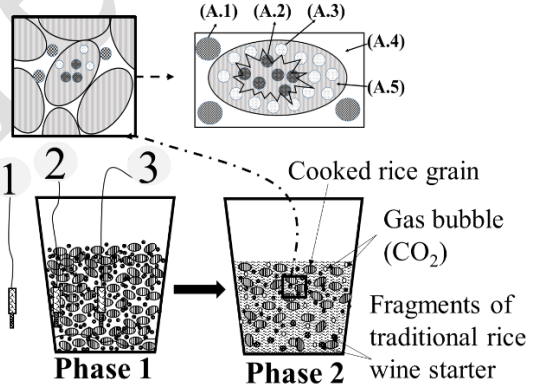

Fig. 1. Model describing the traditional rice wine fermentation process in semi-solid and liquid phases

(A.1) – Traditional rice wine starter fragment containing microorganisms; (A.2) – Residual starch granule within the cooked rice; (A.3) – Microorganisms embedded in the rice matrix; (A.4) Extracellular liquid phase surrounding the rice grain; (A.5) – Starch hydrolysate liquid inside the rice grain); 1, 2, 3 – Temperature sensor at ambient environment, inner wall of the vessel and the center of the vessel.

2.2.2. Simulation methods

The simulation process is built on the theoretical knowledge of the wine fermentation process, combined with technical parameters collected from the actual craft wine production process [17]. The differential equation system in the model describes the variation of substrate concentrations over time, considered as an Ordinary

Differential Function (ODE), in which spatial variation is ignored to simplify the model. Due to this characteristic, measurement values need to be taken at many different locations at the same time, then averaged to ensure high reliability of the research results.

The applied numerical method for Ordinary Differential Equations (ODEs) in this study needs to handle the constraints and algebraic relations because the process model lead to a system of Differential Algebraic Equations (DAEs) [21, 22]. For numerical implementation, the ODE15s solver was used in MATLAB R2024b. The computations were performed on a Windows 10 system with 16 GB RAM, exceeding the minimum requirement (Windows 7 or higher, 4 GB RAM) and ensuring stable performance. ODE15s, based on Numerical Differentiation Formulas (NDF), is well suited for stiff and dynamically complex differential systems [21].

2.2.3. Experiment setup

For experimental verification, an equivalent setup was constructed to validate the simulation model. Appropriate sensors and measurement points were arranged to collect data for comparison with theoretical predictions. The primary parameters measured were temperatures throughout the fermentation process, including the tank center, inner wall, outer wall, and ambient temperature. Temperature was selected due to its ease of measurement, rapid acquisition, and relevance for monitoring and assessing process behavior [7, 20].

To obtain representative data and reduce errors caused by spatial heterogeneity, tank temperature values were averaged. Ambient temperature was continuously monitored to approximate traditional natural fermentation conditions in Vietnam, thereby enhancing the model's practical applicability. The measurement system employed Pt-100 sensors at four corresponding locations. Pt–100, a widely used Resistance Temperature Detector (RTD), provides high accuracy, reliability, wide measurement range, and good stability (Fig. 2).

Initial characterization measurements were performed immediately after water addition, marking the initiation of Phase 2 fermentation, and were used solely to define the initial conditions required for model simulation. Yeast concentration was determined by direct hemocytometric counting under light microscopy (Nikon YS100) using a standard cell counting chamber. Bacterial density was estimated via serial dilution plating on Luria–Bertani agar, followed by incubation at 37 °C for 24 hours and colony enumeration. Microbial concentrations were subsequently converted to mass-based units (g/g of fermentation broth) for consistency with the model formulation, accounting for density and volume variations induced by temperature changes during fermentation [18, 19].

To minimize operational intervention and maintain the naturalness similar to the traditional fermentation process, the entire sensor system is connected to the PLC (Programmable Logic Controller) system, allowing data to be collected and stored continuously throughout the experiment. The PLC system ensures automatic, accurate and stable data recording over a long period of time. In addition, a real-time measurement data display is also prepared to check the parameter values at any time during the measurement process, supporting timely monitoring and adjustment if necessary.

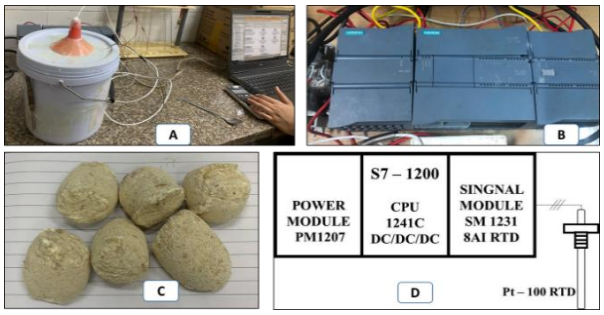

Fig. 2. Experimental equipment and materials

(A – Fermentation vessel with sensor system; B, D – SM 1231 8AI RTD Module of the S7-1200 system; C – Traditional rice wine starter cake)

At the onset of Phase 2, the substrate consisted of residual starch and free sugars. Free glucose was quantified directly using a standard enzymatic glucose oxidase–peroxidase (GOD–POD) assay [23, 24]. Starch content was indirectly estimated by acid hydrolysis (2% w/v HCl), followed by quantification of the resulting reducing sugars expressed as glucose equivalents, according to established methods [24, 25]. Time-resolved biochemical sampling during Phase 2 was not the primary objective of this study, as the focus was placed on temperature evolution and fermentation heat release for dynamic model validation rather than detailed biochemical kinetics. Accordingly, parameters such as pH, dissolved oxygen, and enzyme activities were not explicitly modeled in the present framework.

2.3. Mathematical Model and Error Estimation

2.3.1. Mathematical model

A mathematical model will be developed from a basic understanding of the fermentation process, utilizing classical theories and established theorems by previously published scientists [21, 26]. This model tracks the temporal evolution of substance concentrations as detailed in subsequent sections.

The conversion of the concentration of substances to the unit of mass percentage is carried out to optimize the monitoring of the concentration of ethanol at the end of the process, especially in the context of traditional artisanal wine fermentation, which always gives a concentration that does not exceed a certain limit value [12]. This approach also allows for the appearance of unwanted volatiles during the fermentation process, changing their volume concentration. However, if the amount of these volatiles is considered to be negligible compared to the total mass of the mixture, the use of the

unit of mass percentage of substances is completely reasonable and scientific.

Table 1. Variable parameters during fermentation

Parameter	Symbol
Glucose Concentration	G
Yeast Cell Concentration	X_1
Initial oxygen concentration in the headspace	O_2
Bacterial Cell Concentration	X_2
Product Concentration	P
Starch Content	S

In this simplified model, the assumption is made that during the entire fermentation process, glucose is the only substrate for fermentation, and the entire amount of starch also produces only glucose as the sole product, which is consistent with the hypothesis of many other studies. This simplification contributes to bringing the model to the minimum possible form, helping to reduce the complexity of calculations while still ensuring the accuracy of the research results [14, 15, 27].

Regarding the microbial composition, traditional rice wine starter is known to contain a diverse consortium of microorganisms, including *Saccharomyces cerevisiae*, filamentous molds, and various bacteria. In order to ensure model tractability, the microbial consortium other than yeast was represented by a single bacterial surrogate, *Bacillus subtilis*, which was selected based on its well-documented biochemical characteristics and its representative functional role in starch hydrolysis and fermentation-related processes. This simplification allows the essential fermentation dynamics to be captured without introducing excessive model complexity.

The microbial community diversity within the traditional rice wine starter was represented through inclusion of two representative microorganism classes: the yeast *Saccharomyces cerevisiae* and a composite bacterial category (represented by *Bacillus subtilis*) encompassing molds and bacteria that exhibit similar biochemical characteristics and metabolic roles during fermentation [1, 2, 5, 6]. This consolidation reflects the similar enzymatic capabilities and functional roles of molds and bacteria in the early fermentation stage [18].

During the initial semi-solid fermentation phase (Phase 1), molds and bacteria functioned as the primary enzyme-producing organisms, hydrolyzing insoluble rice starch into fermentable sugars that subsequently supported yeast biomass accumulation [2, 19]. In contrast, during the aqueous liquid-phase fermentation (Phase 2), the role of molds and bacteria became substantially diminished. As the starch concentration declined to minimal levels and yeast biomass accumulated to high concentrations, coupled with vigorous ethanol biosynthesis, the enzymatic contribution of molds and bacteria declined significantly [13, 14].

Notwithstanding their reduced contribution to ethanol production in Phase 2, the continued presence of molds and bacteria maintained ecological significance. These organisms continued consuming glucose substrate and producing hydrolytic enzymes that processed residual starch. Consequently, they engaged in substrate competition with yeast for available glucose, thereby reducing the overall efficiency of ethanol yield relative to theoretical maximum. The kinetics of bacterial populations, particularly under conditions of stressed growth or oxygen limitation, have been documented for alternative species such as *Acetobacter* species, providing comparative frameworks for understanding microbial interactions in fermentation systems [27,28].

During the alcohol fermentation process, particularly at the initiation of the liquid phase (Phase 2), yeast cells initially utilized dissolved molecular oxygen in conjunction with glucose to support biomass accumulation through aerobic respiratory metabolism. Upon depletion of dissolved oxygen reserves, anaerobic alcoholic fermentation became predominant, simultaneously cessation of aerobic biomass synthesis and commencement of the dominant ethanol production phase. This metabolic transition clearly exemplifies the classical Pasteur effect [20, 22], whereby microorganisms systematically switch from oxygen-dependent respiration to oxygen-independent fermentation under hypoxic conditions [26, 29, 30].

Based on known theoretical knowledge and assumptions, the transformation of substances during fermentation is visually shown in Figure 3, showing the complex kinetic relationship between the components in the system. In this model, the glucose substrate source is considered the main and only substrate for the formation of yeast (X_1) and bacterial (X_2) biomass with a growth rate of μ, which is affected by the concentration of dissolved oxygen (O) according to the extended Monod equation [28-30].

Although significantly simplified, this is still a complex dynamic system with many different variables, requiring the application of advanced mathematical methods to solve the system of DAEs. This complexity reflects the multicomponent and multistage nature of traditional fermentation, where factors such as temperature, pH, substrate concentration, and microbial interactions all play important roles in determining the yield and quality of the final product.

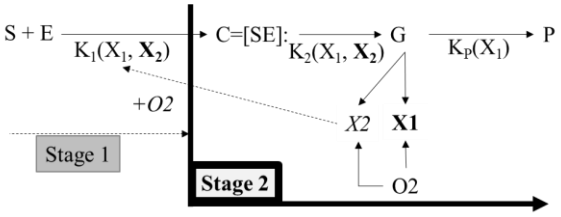

Fig. 3. Diagram illustrating substance transformations in the fermentation process

Table 2. Parameters of the model (M.c: Monod constant)

Parameter	Symbol	Value	Unit	Refer-ence
Pre-exponential constant in the Arrhenius equation for yeast	$A_{1,1}$	$2.82.10^8$		[17]
Pre-exponential constant in the Arrhenius equation for bacteria	$A_{2,1}$	0.5		[28]
Pre-exponential constant in the Arrhenius equation for bacteria	$A_{2,2}$	$8.97.10^7$		[28]
Heat transfer surface area	A_T	0.155	m^2	
Specific heat capacity of glucose	$C_{p,G}$	1.55	kJ/kg.K	
Specific heat capacity of water	C_{p,H_2O}	4.18	kJ/kg.K	
Specific heat capacity of ethanol	$C_{p,P}$	2.44	kJ/kg.K	
Apparent activation energy for yeast growth and associated denaturation reaction	E_1	52200	J/mol	
Apparent activation energy for bacterial growth and associated denaturation reaction	E_{21}	417.2	J/mol	[28]
	E_{22}	5974	J/mol	[28]
Heat of metabolism from glucose to yeast	ΔH_{GX_1}	-2870	kJ/kg	
Heat of metabolism from glucose to bacteria	ΔH_{GX_2}	-2870	kJ/kg	
Heat transfer coefficient of the fermentation vessel	K	0.75	W/m^2K	
Yeast decay coefficient	k_{d1}	0.0059		
Bacterial decay coefficient	k_{d2}	0.00043		
Oxygen mass transfer coefficient	k_{La}	25	h^{-1}	
M.c for the effect of glucose on ethanol product formation	K_P	0.01	g/g	
M.c of oxygen's effect on yeast biomass production	k_{S1}	17.25	g/g	[17]
Monod coefficient of oxygen's effect on bacteria	k_{S3}	69	g/g	
M.c for the effect of glucose on yeast biomass production	k_{S2}	24.54	g/g	
M.c for the effect of glucose on bacteria	k_{S4}	98.16	g/g	
M.c for the effect of ethanol on yeast	k_{S5}	1.15	g/g	
Correlation coefficient: glucose conversion into yeast biomass	Y_{X1}	1		
Oxygen conversion coefficient to yeast biomass	Y_{O_2}	0.97		[17]
Oxygen conversion coefficient to bacterial biomass	Y'_{O2}	0.485		
Glucose conversion coefficient to ethanol	Y_P	0.06		
Fermentation vessel temperature	T		°C	
Ambient temperature surrounding the fermentation vessel	T_r		°C	
Ethanol formation coefficient from glucose	V_m	2		
Oxygen influence coefficient on yeast growth rate	μ_{1,O_2}	0.001		
Oxygen influence coefficient on bacterial growth rate	μ_{2,O_2}	0.65		

The parameters and symbols of the differential equation system describing the kinetic system are shown in detail in Table 2, in which these parameters play an important role in determining the dynamics of the fermentation process.

The establishment of the notation system must follow consistent scientific principles, ensuring the clarity and reproducibility of the mathematical model. principle of glucose mass balance in the system is built on the classical model, in which the amount of glucose lost is equal to the total amount consumed by the growth of two types of microorganisms and the product formation process.

This reflects the basic principle of mass conservation in a closed biological system, described through the material balance equation based on the classical Monod model and its extended equations.

The amount of glucose used by the yeast: G_{X1}– to create biomass ($X1$); G_P – to create the main product (ethanol). The amount of glucose used by the bacteria; G_{X2} – to create biomass ($X2$). The product formation yield coefficient is denoted such as:

$$\frac{dG_{X1}}{dt} = -\mu_{X_1} \frac{1}{Y_{X1}} \frac{G}{k_{S2}+G} \frac{O}{k_{S1}+O} X_1 \quad (1)$$

$$\frac{dG_{X2}}{dt} = -\mu_{X_2} \frac{1}{Y_{X2}} . \frac{G}{k_{S4}+G} \frac{O}{k_{S3}+O} X_2 \quad (2)$$

In addition, a substantial amount of glucose is metabolized by yeast to produce ethanol, as represented by following:

$$\frac{dG_P}{dt} = \frac{-1}{Y_P} V_m \frac{G}{k_{S5}+G} \frac{P}{K_P+P} X_1 \quad (3)$$

Combining (1) through (3), the resulting equation for the change in glucose concentration is as follows:

$$\frac{dG}{dt} = \frac{dG_{X1}}{dt} + \frac{dG_{X2}}{dt} + \frac{dG_P}{dt} = -\mu_{X_1} \frac{1}{Y_G} \frac{G}{k_{S2}+G} \frac{O_2}{k_{S1}+O_2} X_1 - \mu_{X_2} \frac{1}{Y_G} . \frac{G}{k_{S4}+G} \frac{O_2}{k_{S3}+O_2} X_2 - \frac{1}{Y_P} V_m \frac{G}{k_{S5}+G} \frac{P}{K_P+P} X_1 \quad (4)$$

In addition to their growth rates, the density of bacteria and yeast is also influenced by the glucose concentration and oxygen concentration specific to each type. When the production follows the Monod equation, it can be expressed as follows:

$$\frac{dX_1}{dt} = \mu_{X_1} \frac{G}{k_{s2}+G} \frac{O_2}{k_{s1}+O_2} X_1 \quad (5)$$

$$\frac{dX_{-1}}{dt} = -k_{d1} X_1 \quad (6)$$

$$\frac{dX_2}{dt} = \mu_{X_2} . \frac{G}{k_{S4}+G} \frac{O_2}{k_{S3}+O_2} X_2 \quad (7)$$

$$\frac{dX_{-2}}{dt} = -k_{d2} X_2 \quad (8)$$

The concentration of ethanol (*P*) produced primarily depends on the amount of yeast present; however, it is also influenced by its precursor, glucose:

$$\frac{dP}{dt} = V_m \frac{G}{k_{S5}+G} \frac{P}{K_P+P} X_1 \quad (9)$$

To increase biomass, microbial cells require oxygen for respiration. This amount of oxygen depends on the dissolved oxygen available in the fermentation broth and the mass transfer coefficient (k_{La}) from the gas phase to the liquid phase, and it is consumed by the growth of microbial cells:

$$\frac{dO_2}{dt} = -\mu_{1,O_2} . \frac{1}{Y_{O_2}} . \frac{O_2}{k_{S1}+O_2} . X_1 + + k_{La}(O_2^* - O_2) - \mu_{2,O_2} . \frac{1}{Y'_{O_2}} . \frac{O_2}{k_{S3}+O_2} . X_2 \quad (10)$$

This mathematical model is constructed from the aforementioned assumptions and developed based on the research of many previous researchers as following.

$$\frac{dG}{dt} = -\mu_{X_1} \frac{1}{Y_G} \frac{G}{k_{s2}+G} \frac{O_2}{k_{s1}+O_2} X_1 - \mu_{X_2} \frac{1}{Y_G} . \frac{G}{k_{S4}+G} \frac{O_2}{k_{S3}+O_2} X_2 - \frac{1}{Y_P} V_m \frac{G}{k_{S5}+G} \frac{P}{K_P+P} X_1 \quad (11)$$

$$\frac{dX_1}{dt} = \mu_{X_1} \frac{G}{k_{s2}+G} \frac{O_2}{k_{s1}+O_2} X_1 - k_{d1} X_1 \quad (12)$$

$$\frac{dX_2}{dt} = \mu_{X_2} . \frac{G}{k_{S4}+G} \frac{O_2}{k_{S3}+O_2} X_2 - k_{d2} X_2 \quad (13)$$

$$\frac{dP}{dt} = V_m \frac{G}{k_{S5}+G} \frac{P}{K_P+P} X_1 \quad (14)$$

$$\frac{dO_2}{dt} = -\mu_{1,O_2} . \frac{1}{Y_{O_2}} . \frac{O_2}{k_{S1}+O_2} . X_1 + k_{La}(O_2^* - O_2) - \mu_{2,O_2} . \frac{1}{Y'_{O_2}} . \frac{O_2}{k_{S3}+O_2} . X_2 \quad (15)$$

$$\sum m_i C_{p,i} . \frac{dT}{dt} = \sum \Delta H_i . \frac{dm_i}{dt} - K . A . (T - T_r) \quad (16)$$

The dissolved oxygen solubility in the broth (O_2^*), growth rate coefficient of *Saccharomyces cerevisiae* μ_{X1}, growth rate coefficient of *Bacillus subtilis* – μ_{X2}, oxygen mass transfer coefficient (k_{La}) are expressed below [11, 21]:

$$O_2^* = (14{,}6 - 0{,}3934T + 0{,}007714T^2 - - 0{,}0000646T^3)10^{HI} \quad (17)$$

$$\mu_{X_1} = A_1 . e^{\frac{-E_1}{R}\left(\frac{1}{T+273} - \frac{1}{306}\right)} \quad (18)$$

$$\mu_{X_2} = A_{2,1} . e^{\frac{-E_{21}}{RT}} - A_{2,2} . e^{\frac{-E_{22}}{RT}} \quad (19)$$

$$k_{La} = k_{La,0} . 1{,}024^{T-20} \quad (20)$$

The phenomenological rate coefficients (k_i) incorporated in the model represent intrinsic conversion rates for each biochemical process, expressed as explicit temperature-dependent functions. These parameters were determined experimentally and described using published empirical equations appropriate to rice wine fermentation [12, 18]. Additionally, the Monod saturation constants (Ks_i) represent affinity coefficients reflecting the influence of substrate concentration upon the velocity of product formation [26, 29, 30]. Finally, the heat exchange surface area (*A*) was calculated directly from the geometric dimensions of the laboratory fermentation vessel, permitting accurate characterization of the thermal energy transfer capacity of the system [12, 20].

All state variables in the kinetic model (glucose, ethanol, starch, yeast biomass, and bacterial biomass) are consistently expressed on a mass basis, either as mass fractions (g/g) or percentage by mass (% w/w). No volume-based units (e.g., g/L or % v/v) are used within the governing equations.

This mass-based formulation was deliberately chosen to ensure strict mass conservation and to avoid ambiguities arising from volume changes during fermentation, which may occur due to CO_2 evolution, ethanol formation, and temperature-dependent density variations. In this context, % w/w values are reported solely for convenience and correspond directly to the same mass fractions expressed in g/g; they do not introduce additional independent state variables into the model.

2.3.2. Error estimation

The model reliability is determined via traditional criteria, which are model performance was evaluated using the sum of squared errors (*SSE*), mean squared error (*MSE*), and root mean squared error (*RMSE*).

$$RMSE = \sqrt{MSE} = \sqrt{\frac{SSE}{N}} = \sqrt{\frac{\sum_{i=1}^{N}(T_M - T_S)^2}{N}} \quad (21)$$

The error at each sampling time is the temperature difference between the experimental measurement (T_M) versus simulation (T_S) [15].

3. Results and Discussion

3.1. Results

The SSF model was used to simulate a 5-liter fermentation vessel. The entire process was conducted without stirring, allowing for natural fermentation to proceed from room temperature (approximately 30 °C). The simulation duration was 216 hours.

With the initial concentrations of substances calculated from actual production data, the simulation yielded the following.

The input parameters of the fermentation process are detailed in Table 3, reflecting the two-stage characteristics of the traditional winemaking process. For the semi-solid fermentation stage (phase 1), the main parameters include a raw rice mass of 1.5 kg with an initial starch concentration of 72.49% w/w, reflecting a high reserve substrate content that can be hydrolyzed into glucose during fermentation. Regarding the initial microbial composition, the yeast concentration of *Saccharomyces cerevisiae* was set at 0.98% w/w, while the bacterial concentration (represented by *Bacillus subtilis*) was 0.59% w/w, creating a suitable balance ratio for starch hydrolysis and creating a favorable environment for yeast growth in the next stage.

In the liquid fermentation stage (Phase 2), the input parameters were set based on the results of the previous stage after dilution. The initial glucose concentration reached 18.886% w/w, demonstrating the efficiency of starch hydrolysis in phase 1 and providing sufficient substrate for ethanol production. The microbial composition in this phase changed significantly, with the yeast concentration decreasing to 0.4535% w/w and bacteria to 0.2539% w/w due to the dilution effect when adding 2.25 liters of water. It is worth noting that the appearance of ethanol at a concentration of 3.08% w/w showed that fermentation had begun in the semi-solid phase, consistent with the traditional fermentation mechanism in Vietnam.

The dissolved oxygen concentration was set at 2.35% w/w, which plays an important role in the early stages of the liquid phase when the yeast still requires oxygen for biomass growth before switching to anaerobic fermentation. This precise control of oxygen concentration allows for accurate simulation of the transition from aerobic respiration to anaerobic fermentation, a key feature of yeast ethanol production.

Table 3. Input state of the process

Parameter	Symbol	Unit	Value
Phase 1			
Mass of rice	m	kg	1.5
Cooked rice mass		kg	2.7
Cooked rice moisture content		%w/w	52.2
Starch content	S	%w/w	72.49
Yeast concentration	$X_1(1)$	%w/w	0.98
Bacteria concentration	$X_2(1)$	%w/w	0.59
Phase 2			
Glucose concentration	G_0	%w/w	18.886
Yeast concentration	X_1	%w/w	0.4535
Bacteria concentration	X_2	%w/w	0.2539
Ethanol concentration	P_0	%w/w	3.08
Initial oxygen concentration in the headspace*	O_2	%w/w	2.35
Average temperature of the mixture	T	°C	30

**The headspace refers to the gas-filled space above the liquid phase inside the fermentation vessel.*

The results of the fermentation simulation are presented through a series of graphs as shown in Fig. 4, providing a visual view of the kinetics of the main components during the fermentation process. These graphs allow monitoring the variations of glucose, ethanol, yeast and bacterial biomass, and the temperature of the fermentation mixture over time, thereby validating the accuracy of the developed mathematical model.

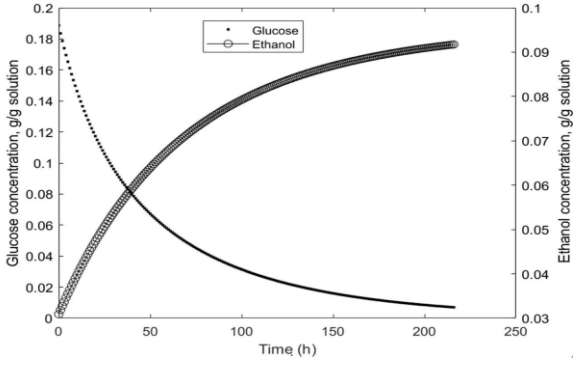

Fig. 4. Glucose and ethanol concentrations over time

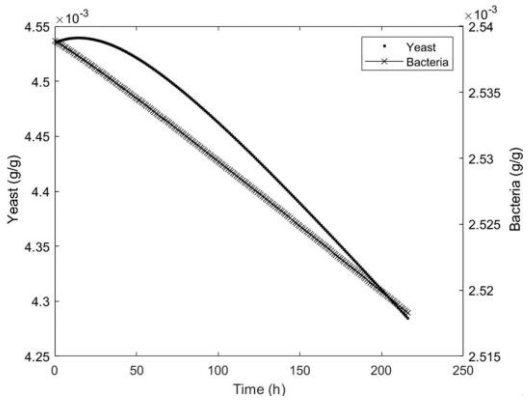

Fig. 5. Yeast and bacteria concentrations over time

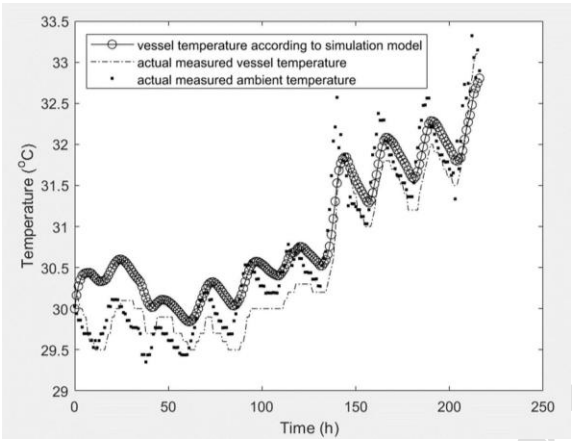

Fig. 6. Fermentation mixture temperature over time

3.2. Discussion

The fundamental difference between traditional and industrial fermentation processes is clearly shown through the mechanism of operation of the microbial system. While the industrial scale often applies a single-strain fermentation process with pure microbial strains or directly adds purified enzyme preparations, the traditional craft wine production process is based on a complex multi-microbial fermentation system. In this system, metabolic and biochemical processes are intertwined with a symbiotic relationship, in which the product of one microbial species can become a substrate for another, creating a complex network of biological interactions. At the same time, substrate hydrolysis and product biosynthesis undergo multi-stage kinetics. It is the diversity and complexity of the microbial community in yeast that creates the sensory and flavor characteristics typical of wine products in each region, depending on the geographical origin and different yeast purchasing conditions.

Fermentation process kinetic analysis showed that after 216 hours from the time of water addition and the initiation of the liquid fermentation phase (phase 2), the ethanol concentration gradually increased and reached a maximum of 0.092 g ethanol/g solution (9.2% w/w) at the end of the process. This result is relatively consistent with studies on the characteristics of traditional wine fermentation from rice, although it is somewhat higher than some reference values. Notably, the ethanol concentration curve did not clearly show the saturation stage, indicating the potential for higher concentrations if the fermentation time was extended. However, this result is still within a reasonable range and is acceptable for the research model.

In the ethanol production process, the glucose content decreased continuously throughout the fermentation process and remained at 0.64% w/w after the completion of the liquid phase. The glucose conversion efficiency in phase 2 reached 96.7%, a value that can be considered acceptable although somewhat higher than initially expected. This can be explained by the fact that the starting substrate is starch in rice, which requires a complex hydrolysis process, so the actual yield may be lower than that of the theoretical model considering only glucose as the sole substrate. It should be acknowledged that this represents a simplified assumption of the current model, which only describes the kinetics of glucose–ethanol–biomass–temperature for Phase 2. Other by-products such as glycerol, organic acids, esters, and higher alcohols have not been individually modeled at this stage, and their inclusion will be considered in future model refinements. In addition, this study does not investigate the kinetics of starch itself but assumes that it is completely hydrolyzed into glucose. Glucose thus serves as the substrate driving the subsequent processes.

With the fermentation vessel temperature, there was almost no deviation from the measured temperature data for the first 50 hours. However, in the following hours, there was a similarity in the degree of variation, although the variation was slower in phase compared to the measured dataset. This temperature variation is cyclical and correlates with the ambient temperature day and night, indicating the impact and influence of ambient temperature on the fermentation vessel temperature under conditions where the fermentation vessel is not insulated. Therefore, from the 140th hour onwards, due to the free fermentation process, as the room air temperature increases due to the weather, the temperature of the container also tends to increase significantly. This will also be a factor affecting the quality and flavor of the wine product.

The concentrations of both yeast and bacteria tended to decrease during the liquid fermentation phase. However, the two populations exhibited distinctly different kinetics: yeast showed a tendency to approach equilibrium, while bacterial concentrations decreased continuously, indicating that the population had entered a period of decline. This phenomenon is considered to be completely reasonable when considering that this phase had undergone 120 hours of semi-solid fermentation (phase 1) and was in the process of intense

ethanol production. Under these conditions, the number and growth state of yeast directly affected the ethanol production efficiency, while the continuous decrease of substrate and the anaerobic state due to the water flooding the entire tank became the main factors causing the decline of both microorganisms. The final concentrations of yeast and bacteria after 216 hours were 0.00421% w/w and 0.00252% w/w, respectively.

The error assessment between simulation results and experimental data is synthesized through the statistical indicators as shown in Table 4.

Table 4. Model error metrics for temperature prediction

SSE_{216}	39.8622	SSE_{75}	17.7984
$RMSE_{216}$	0.1837	$RMSE_{75}$	0.2342

An important limitation of the current model is the assumption that glucose is the sole substrate throughout the entire fermentation process. This simplification makes the fermentation process almost continuous throughout the study period, leading to difficulties in distinguishing clearly the different biochemical stages and may be the cause of the deviation between simulation and reality.

A larger discrepancy between the simulated and measured temperatures was observed during the first 75 h of Phase 2, resulting in a higher RMSE (0.2342) compared with that calculated for the entire simulation period (0.1837).

The research results confirm that the temperature of the tank is an important parameter and directly affects the kinetics of the fermentation process, in addition to the concentration parameters of the substances. Controlling and optimizing the temperature can help achieve higher productivity and better wine quality by determining the optimal temperature zone for each stage of the process. This is not only of scientific significance but also contributes significantly to the preservation and promotion of the value of traditional Vietnamese wine products. Therefore, the process optimization problem based on this kinetic model and temperature conditions will be the research direction implemented in the following publications.

4. Conclusion

Initial simulation results show close agreement between the simulated process temperature changes and experimental data, and the final concentrations of the substances are relatively consistent with known parameters. However, it was observed that there was still a certain degree of temperature deviation. The content of a scientific article still leaves some issues unspecifically analyzed and the model does not address the interaction of other substances during fermentation. To apply and enhance the model's practicality in factual condition, it is necessary to further study to reduce the error between the model and experimental results. Further in-depth knowledge and analysis of the fields of fermentation and biotransformation are necessary to achieve this target. A more detailed and specific model of the conversion process during ethanol fermentation needs to be developed.

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