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An Evaluation of Mathematical Modeling of Ethanol Fermentation with Immobilized *Saccharomyces cerevisiae* in the Presence of Different Inhibitors

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Abstract: In ethanol production processes, inhibitors are formed as by-products depending on the raw materials and pretreatments. Inhibitors negatively affect both ethanol yield and biomass growth. This study aimed to examine the influence of inhibitors, including acetic acid (AA), formic acid (FA), and phenol, on ethanol production from the glucose-based medium using immobilized *Saccharomyces cerevisiae* in a bioreactor. The results showed that the highest ethanol yields and productions were determined as 45.64% and 38.10 g/L, 44.8% and 36.67 g/L, and 44.46% and 39.07 g/L, by the addition of 2.5 g/L AA, 0.5 g/L FA, and 0.5 g/L phenol into the fermentation medium, respectively. Regarding mathematical modeling, the models MGM (AA) and Huang (FA-phenol) were the best models to predict experimental ethanol production. It was determined that the values forecasted with the models MMF (AA-FA) and Weibull (phenol) agreed with the actual biomass growth. Additionally, to forecast the observed values of the substrate consumption, the most suitable model was Weibull (AA-FA-phenol). Consequently, the immobilized-cell ethanol fermentations with inhibitors were successfully performed, and their limit values were determined.

Keywords: ethanol; acids; phenol; inhibitor; mathematical modeling

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1. Introduction

Ethanol is an energy source that is sustainable, has a high productivity, and is eco-friendly [1]. Globally, the USA (54%) and Brazil (30%) account for 84% of the world's production [2]. Additionally, ethanol has also been used as a food additive in the production of beverages, in the chemical industry, and in scientific studies. Thanks to its many positive effects, it has started to be used widely. The most important area of use is as an energy source [3]. Today, most of the energy required in the world is supplied from underground sources such as petroleum, natural gas, and coal. However, due to their high emissions, global warming is increasing day by day. Additionally, the chemicals that are used in many sectors are also derived as a result of further processing of underground resources [4]. However, the amount of fossil fuels is gradually decreasing due to increasing energy consumption, parallel to the growing world population and its needs [5]. In addition, gases released from fossil fuels cause environmental problems like acid rain

and climate change [6]. Due to these negative effects, the use of fossil fuels should be avoided as much as possible. However, considering the rapidly depleting energy resources, this situation has led developed countries to find alternative ways [7]. In such countries, ethanol production is carried out with either pure sources, or with cheap and suitable lignocellulosic raw materials. There are many types of microorganisms, especially yeast groups, used in the production of ethanol. The carbon sources that these microorganisms can ferment and the fermentation conditions in which they can work differ. *Saccharomyces cerevisiae* strains, on the other hand, are a species that can operate at 30–37 °C and use glucose, fructose, maltose, and sucrose in the environment. *Saccharomyces cerevisiae* is the most preferred microorganism among other species because of its high production rates. In addition, it can convert both glucose, which is a simple six-carbon sugar, and sucrose, a disaccharide, into ethanol [8].

Ethanol can be produced with both pure carbon sources and lignocellulosic raw materials, such as corn, wheat, straw, and herbaceous wastes with suitable content. Products containing starch, such as grains, roots, and tubers, can be used as carbon sources after gelatinization and saccharification processes, while sugary products, such as sugar cane and sugar beet, can be used as a carbon sources after extraction [9]. Ethanol fermentation includes only two stages, fermentation and distillation, in which pure carbon sources are used. However, the process becomes more complex when lignocellulosic materials are used as the carbon source. In such cases, the process steps increase to four. These are as follows: a mechanical decrease in size, acidic or enzymatic hydrolysis, fermentation, and distillation [10]. During these processes, inhibitory substances that may have negative effects on the medium are formed. Inhibitory substances can be formed in three different ways: substrate-induced, hydrolysis-induced, and fermentation-induced. The formation of inhibitory substances is affected by three main factors such as adverse hydrolysis conditions, acidity in the environment, and high temperature. Inhibitors consisting of lignocellulosic hydrolysates originate from lignin, hemicellulose, and cellulose. A lignocellulosic raw material contains 28% lignin, 28% hemicellulose, and 40% cellulose. The inhibitory substances formed are examined under three categories: weak acids, such as acetic, formic, and levulinic acid, consisting of hemicellulose; furans (furfural, HMF); and phenols, consisting of lignin (vanillin, phenol, syringaldehyde) [11]. These acids are the most abundant acids among hydrolysates. The product of the reaction between hemicelluloses, acetic acid, is a by-product that can occur not only in hydrolysis but also in the fermentation process. Formic acid can be formed not only from the breakdown of HM but also from a furfural exposed to high temperature in an acid medium. Many of the phenolic compounds are found among the dilute acid hydrolysates. These are the products formed by the breakdown of lignin during hydrolysis or by the breakdown of sugars [11].

The inhibition effect caused by weak acids on cells can be explained as intracellular anion deposition and separation. The undissociated form of weak acids can diffuse across the plasma membrane from the fermentation medium and decompose due to a high intracellular pH. Thus, a decrease in medium pH occurs. The decrease in intracellular pH is balanced by the plasma membrane ATPase, which pumps the cell's protons via the hydrolysis of ATP. As a result, the amount of ATP required for biomass formation decreases, and growth slows down [12]. At the same time, they change the ion charge in the cell wall, cause damage to the cell membrane, and denature the enzymes in the cell [12]. Phenolic substances show their effect by disrupting the selective permeability and enzymatic system of the cell membrane, thus damaging cell integrity [13]. Phenolic substances with weak acidity have the ability to break down the electrochemical gradient by returning the protons to the mitochondrial membranes [12]. Furthermore, the processes by which phenolic chemicals inhibit microorganisms remain incompletely understood, mostly

because of the group's heterogeneity and the dearth of precise qualitative-quantitative analyses [13]. These inhibitory substances not only restrict microbial growth but also reduce the ethanol yield. Due to these adverse effects, their presence in the fermentation medium is undesirable. These substances can be eliminated with detoxification methods. Moreover, immobilization methods can be an option to overcome the inhibition of fermentation so as to preserve microorganisms. The immobilization technique is used to make the cells used in biotechnological studies reusable or provide easier usability in continuous systems and be less affected by media inhibition. The immobilization process is the attachment or confinement of the cells to the targeted space in the determined arresting material without losing their activity [3].

In bioprocess engineering, modeling involves establishing relationships between the key components of the system under study. This can be achieved through mathematical functions, graphics, tables, or even cause-and-effect relationships that are not fully understood. The system itself could be a bioreactor, a strain of microbe, or an enzyme. The key components of interest might include factors such as temperature, pH, substrate formulation, and operating cost. The primary goal of modeling is to predict system responses to environmental changes or modifications, facilitating improvements in the bioprocess design and operation. Modeling can be used to build on the knowledge gained from previous batch fermentations and can be used to predict the growth rate of cells, for example, over time in response to changes in their environment. This knowledge can be used to create new designs, equipment, and bioprocesses. Modeling can also be used to predict the amount of inoculum required and when it should be added. By understanding the relationship between different elements of the system, new knowledge can be gained, and more effective processes can be developed [14,15]. Models simplify the understanding of the process and help in experimental design. In addition, they are also used in control, training, and process optimization. Due to these positive aspects, they are important for studies [16].

Sensitivity analysis is used to predict the impact of small changes in input parameters or control variables on a system over time. This can be used to determine the best way to optimize the system, and to identify the most effective procedures for achieving the desired outputs.

Finally, ensuring the cost-effectiveness of bioprocesses requires economic analysis. The cost of the nutrients and other elements used in the process plays a critical role in determining the overall feasibility. Researchers are therefore working to find cost-effective alternatives to the traditional materials and bioprocess technologies used in the industry. By finding new, more cost-effective ways to produce the necessary substrates for fermentation, the overall economics of the process can be improved [14].

In this study, we aimed to produce ethanol in the presence of different inhibitors by increasing the endurance of *Saccharomyces cerevisiae* yeasts by immobilizing them with calcium alginate. This was carried out by using different amounts of acetic acid, formic acid, and phenol inhibitors in the fermentations that took place on a bioreactor scale. The growth of immobilized cells and ethanol production were evaluated throughout the fermentation. Different mathematical modeling approaches (Table 1) were applied to the data obtained in the study, and the most suitable model was identified using different model evaluation metrics [17]. Additionally, sensitivity analysis and a techno-economic feasibility assessment were performed using the most appropriate model. Compared to previous literature, it was seen that the immobilization process expanded the tolerance range and protected the cells. Furthermore, it was observed that the determined tolerance ranges contributed positively to process efficiency in terms of time, cost, and energy.

Table 1. The equations of the mathematical models.

Model	Model Equation	Reference
MGM	$A_t = A_o + (A_m - A_o) \times exp\left[-exp\left\{\frac{Q \times e \times (\lambda - t)}{A_m} + 1\right\}\right]$	[18]
MLM	$A_t = A_o + \frac{A_m - A_o}{1 + \left(\frac{4 \times Q \times (\lambda - t)}{A_m} + 2\right)}$	[18]
MRM	$A_t = A_o + \frac{A_m - A_o}{\left\{1 + v_1 \times exp(1 + v_1) \times exp\left(\frac{Q \times (1 + v_1)^{\left(1 + \frac{1}{v_1}\right)} \times (\lambda - t)}{A_m}\right)\right\}^{\left(\frac{1}{v_1}\right)}}$	[18]
Stannard	$A_t = A_o + \frac{A_m - A_o}{\left[1 + \left(-\frac{\beta + Q \times t}{v_2}\right)\right]^{v_2}}$	[19]
Weibull	$A_t = A_m - (A_m - A_o) \times exp\left[-\left(k_1 \times t\right)^{\delta_1}\right]$	[20]
MMF	$A_t = A_m - \frac{(A_m - A_o)}{1 + (k_2 \times t)^{\delta_2}}$	[21]
Asymmetric	$A_t = A_o + (A_m - A_o) \times \left[1 - \frac{1}{1 + \left(\frac{t}{T_L}\right)^d}\right]$	[22]
Baranyi	$A_t = A_o + Q \times B_t - ln\left[1 + \frac{exp(Q \times B_t) - 1}{exp(A_m - A_0)}\right]$ $B_t = t + \frac{1}{Q} \times ln[(-Q \times t) + (-h_o) - exp(-Q \times t - h_o)]$	[23]
Huang	$A_t = A_0 + A_m - ln[(A_0) + [(A_m) - (A_0)] \times (-Q \times H_t)]$ $H_t = t + \frac{1}{a} \times ln\left[\frac{1 + exp(-a \times (t - \lambda))}{1 + exp(a \times \lambda)}\right]$	[24]
Fitzhugh	$A_t = A_o + (A_m - A_o) \times [1 - exp(-k_3 \times t)]^{v_3}$	[25]

2. Materials and Methods

2.1. Microorganism and Fermentation Medium

Saccharomyces cerevisiae (ATCC 36858) was utilized for fermentations [3]. The microorganism was incubated at 30 °C for 48 h and then stored at +4 °C as a stock culture form. For pre-culture, the microorganism was grown in a shaking incubator at 30 °C and 150 rpm for 24 h. Stock cultures were renewed every month. A fermentation medium was selected based on a previous study [1].

2.2. Immobilization Process

The immobilization process of the microorganism, incubated at 30 °C for 24 h with 150 mL of working volume, was as follows: After the centrifugation process of the yeast at 3800× *g* and 4 °C for 20 min in 50 mL sterile tubes, the supernatant was removed. Subsequently, 20 mL of 2% Ca-alginate was added to the tube and immediately mixed using a vortex. Then, the yeast suspension was transferred into a sterile syringe under aseptic conditions, and the solution was inserted drop-wise in a constantly stirred sterile 0.1 M $CaCl_2.2H_2O$ solution to form the beads. Afterward, to enhance the gel strength, the beads were kept in 0.05 M sterile $CaCl_2.2H_2O$ solution. After 12 h, the beads were treated with a 0.85% NaCl solution [3,26].

2.3. Batch Fermentation Medium and Conditions

Ethanol fermentation was carried out according to the previous research by Erkan et al., 2022 [8]. The fermentation volume was chosen as 1.5 L. The concentrations of inhibitory substances were determined by reviewing the literature. Acetic acid (AA), formic acid (FA), and phenol at different concentrations were used as inhibitors in the fermentations. AA was used in the amounts of 2.5, 5, 7.5, 10, and 12.5 g/L. FA was added at concentrations

of 0.5, 1, 1.5, 2, and 2.5 g/L. Lastly, the phenol inhibitor concentrations are 0.5, 1, 5, 7, and 9 g/L. The pH was adjusted to 5.5 using 4N NaOH and 2N HCl. After inoculation at a rate of 1%, the conditions of fermentation were set at 30 °C and 150 rpm. Additionally, two different control fermentations were carried out. In the first, immobilized cells in an inhibitor-free medium performed the fermentation. In the second, suspended cells were used to carry out fermentation in the presence of inhibitory chemicals. Samples were collected periodically (0, 2, 4, 8, 11, 14, 24, 30, 36, and 48 h) from all fermentations and stored at 4 °C until analysis. Additionally, a control fermentation was conducted in an inhibitor-free fermentation medium.

2.4. The Residual Sugar, Ethanol, and Biomass Analyses

A spectrophotometric method was applied to determine the residual sugar amount [26,27]. To quantify the ethanol, high-performance liquid chromatography with a RefractoMax 520 refractive index detector, autosampler, column oven, and computer control were employed. The analysis was performed at a flow rate of 0.05 mL per min and a temperature of 70 °C using a Transgenomic ICSep ORH-801 column [26,28]. Biomass analysis was spectrophotometrically determined, where absorbance values measured at 620 nm were converted into the biomass concentration using a standard curve [1].

2.5. Kinetic Parameters

The following kinetic parameters were determined: biomass production (ΔX, g/L), biomass yield ($Y_{X/S}$, %), sugar consumption (ΔS, g/L), ethanol production (ΔP, g/L), ethanol yield ($Y_{P/S}$, %), sugar utilization yield (SUY, %), maximum consumption rate (Q_S, g/L/h), maximum production rate (Q_P, g/L/h), maximum growth rate (Q_X, g/L/h), and ethanol efficiency (TY, %) [8].

2.6. Mathematical Modeling

For mathematical modeling, the fermentation data with the highest ethanol production rate were analyzed using the Microsoft Excel program. Some parameters, such as v, β, k, and δ, were determined using nonlinear least squares regression analysis. The predicted data by the models were plotted against the experimental data, and the R^2 values were determined according to the drawn-line equation. To minimize the error and achieve an R^2 value closest to 1, the nonlinear least squares regression procedure was applied [8,29]. Additionally, metrics such as *MAE*, R^2, *RMSE*, *AF*, and *BF* were used. The values of *BF*, *AF*, and R^2 are desired to be 1 or the closest to 1 for the success of the model. Another success criterion was maintaining a low error [30]. Model parameters such as v, β, k, and δ were recorded and represented in Table 2. The *RMSE*, *MAE*, *BF*, and *AF* were calculated as follows:

$$\mathrm{RMSE} = \sqrt{\left[\sum_{i=1}^{n}(x_i - y_i)^2\right]/n}, i = 1, 2, \ldots\ldots\ldots\ldots\ldots\ldots, n$$

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}|x_i - y_i|, i = 1, 2, \ldots\ldots\ldots\ldots\ldots.., n$$

$$\mathrm{AF} = 10^{\sum_{i=1}^{n}\frac{|\log(y_i/x_i)|}{n}}, i = 1, 2, \ldots\ldots\ldots\ldots\ldots\ldots, n$$

$$\mathrm{BF} = 10^{\sum_{i=1}^{n}\frac{\log(x_i/y_i)}{n}}, i = 1, 2, \ldots\ldots\ldots\ldots\ldots\ldots\ldots, n$$

Table 2. The model parameters calculated from experimental data.

Model Parameters	Acetic Acid			Formic Acid			Phenol		
	S	**X**	**P**	**S**	**X**	**P**	**S**	**X**	**P**
A_0 (g/L)	1.76	1.07	0.04	1.8	1.06	0	2.73	1.06	0
A_m (g/L)	85.22	4.89	38.73	83.64	3.7	36.67	90.62	6.94	39.09
Q (g/L/h)	3.58	0.18	1.45	2.93	0.13	1.26	5.21	0.37	1.37
λ (h)	28.56	1.73	6.83	29.8	1.05	5.81	24.79	6.1	3.68
v_1	316	0.95	1.04	340	0.98	1.05	286	0.98	1.1
v_2	81.9	67.4	79.6	79.7	65.6	78.5	87	68	78
v_3	49	1.5	40	41	1.6	36	52	1.3	25
v_4	2	2	2	2	2	2	2	2	2
β	319	304	319	315	302	316	323	300	319
h_0 (g/L)	102.17	0.31	9.93	87.32	0.14	7.3	129	2.27	5.05
d	3	0.26	17	1.3	0.22	17	1	0.2	3.7
T_L	26.18	1.48	26.9	30.27	0.83	123.83	32.26	0.56	13.47
k_1	0.06	0.04	0.05	0.06	0.04	0.05	0.06	0.05	0.06
k_2	0.07	0.05	0.06	0.07	0.05	0.07	0.07	0.06	0.07
k_3	0.29	0.05	0.25	0.29	0.06	0.24	0.3	0.06	0.25
k_4	1	1	1	1	1	1	1	1	1
δ_1	3	3	3	3	3	3	3	3	3
δ_2	4.1	2.9	3.7	4.1	3.7	3	5	5	3.6
e	2.718	2.718	2.718	2.718	2.718	2.718	2.718	2.718	2.718
a	4	4	4	4	4	4	4	4	4

2.7. Sensitivity Analysis

A sensitivity analysis was conducted to assess the validity of the MGM and Huang models. This analysis involved evaluating the behavior of the asymmetric model when a 5% escalation or lower in each parameter was performed [14]. For this objective, the endpoint deviation, integral deviation, and integral absolute deviation were calculated as follows:

$$ED = 100 \times \left(\frac{P_{end\ 5\%}}{P_{end\ control}} - 1 \right)$$

$$MFP_t = \frac{(P_t + P_{t+x}).(t + x - t)}{2}$$

$$ID = 100 \times \sum_{t=0}^{n} \left(\frac{[MFP]\ t, 5\%}{[MFP]tcontrol} - 1 \right)$$

$$IAD = 100 \times \sum_{t=0}^{n} \left(\left| \frac{[MFP]t, 5\%}{[MFP]t\ control} - 1 \right| \right)$$

where [*P*] is the biomass (g/L), ethanol (g/L), and substrate (g/L) as the fermentation concludes offered a 5% enhance or minimize, [*P*] is the biomass (g/L), ethanol (g/L), and substrate (g/L) as the fermentation concludes by the MGM and Huang models, [*MFP*] is [*MFP*] offering a 5% enhancement or minimization in each parameter of the models, and [*MFP*] is [*MFP*] in the MGM and Huang models. [MFP] is the mean area of the biomass (g/L), ethanol (g/L), and substrate (g/L) at time *t* (h) and *t* + *x* (h), and [P] and [P] are the biomass (g/L), ethanol (g/L), and substrate (g/L) at time *t* (h) and time *t* + *x* (h) [14].

2.8. Economic Analysis of Ethanol Fermentation

An economic analysis of the fermentation with inhibitors was examined by estimating the economic yield (EY) and productivity (EP). The EY and EP of ethanol production were estimated using the following mathematical statements.

$$EY = \frac{P.I}{\sum(CN)}$$

$$EP = \frac{P.I}{t.\sum(CN)}$$

where P is the ethanol amount (g/L), I is the selling price of ethanol (EUR/g), C is the cost of the nutrients deployed in the fermentation broth (EUR/g), N is the nutrient amount (g/L), and t is the fermentation period (h) [14].

2.9. Statistical Analysis

In order to statistically evaluate the results ($Y_{P/S}$ and Q_P), the Duncan multiple comparison test was employed at a confidence level of 95% (p = 0.05). SAS Statistical software (Version 9) was used to conduct this analysis.

3. Result and Discussion

The present study examined the effect of different levels of inhibitors, including AA, FA, and phenol, on ethanol production from the glucose-based medium by using *Saccharomyces cerevisiae* in the immobilized-cell-stirred tank bioreactor. Following the determination of the limit values of inhibitors, the fermentation data carried out at these limit values were estimated using mathematical models.

3.1. Control Fermentation

The results of the control fermentations carried out in the medium without inhibitors are shown in Table 3 (mentioned as 0 g/L of inhibitor). Based on the results, the ΔS, ΔP, ΔX, $Y_{P/S}$, $Y_{X/S}$, Q_S, Q_P, SUY, and TY values were 72.54 g/L, 35.71 g/L, 6.10 g/L, 49.24%, 8.41%, 3.38 g/L/h, 1.80 g/L/h, 96.10%, and 92.98%, respectively [8]. These values results were then utilized for comparison with the data from the fermentations with inhibitors in the medium. In the control fermentation, the sugar in the medium was used by the cells without any problems.

Table 3. Kinetic parameters in fermentations at different inhibitory concentrations.

Inhibitor	Inhibitor Concentration (g/L)	Total Sugar Consumption (ΔS, g/L)	Total Ethanol Production (ΔP, g/L)	Biomass Growth (ΔX, g/L)	Biomass Yield ($Y_{X/S}$, %)	Ethanol Yield ($Y_{P/S}$, %)	Ethanol Efficiency (%)	Sugar Utilization Yield (%)	Maximum Ethanol Production Rate (Q_P, g/L/h)	Maximum Sugar Consumption Rate (Q_S, g/L/h)	Maximum Biomass Growth Rate (Q_X, g/L/h)
Acetic Acid	0	$72.54^{b} \pm 2.54$	$35.71^{ba} \pm 0.05$	$6.10^{a} \pm 0.00$	$8.41^{a} \pm 0.27$	$49.23^{a} \pm 1.52$	$92.98^{a} \pm 2.98$	$96.09^{a} \pm 0.34$	$1.80^{a} \pm 0.15$	$3.38^{a} \pm 0.17$	$0.31^{a} \pm 0.07$
	2.5	$83.46^{a} \pm 2.39$	$38.10^{a} \pm 1.51$	$3.82^{b} \pm 0.02$	$4.58^{b} \pm 0.09$	$45.64^{a} \pm 0.50$	$89.33^{a} \pm 0.98$	$97.94^{a} \pm 0.36$	$1.78^{a} \pm 0.19$	$3.57^{a} \pm 0.03$	$0.17^{b} \pm 0.01$
	5	$75.01^{ba} \pm 0.01$	$33.46^{b} \pm 0.15$	$3.20^{c} \pm 0.05$	$4.26^{b} \pm 0.07$	$44.60^{a} \pm 0.20$	$87.28^{a} \pm 0.40$	$98.28^{a} \pm 0.15$	$1.34^{b} \pm 0.00$	$2.99^{a} \pm 0.00$	$0.11^{cb} \pm 0.00$
	7.5	$6.65^{c} \pm 2.22$	$0.72^{c} \pm 0.06$	$0.15^{d} \pm 0.01$	$2.32^{c} \pm 0.68$	$10.95^{cb} \pm 1.38$	$21.43^{cb} \pm 2.70$	$9.06^{cb} \pm 3.57$	$0.01^{c} \pm 0.00$	$0.24^{c} \pm 0.07$	$0.02^{c} \pm 0.01$
	10	$14.11^{c} \pm 5.37$	$1.97^{c} \pm 0.04$	$0.14^{d} \pm 0.16$	$1.02^{d} \pm 0.39$	$13.99^{b} \pm 2.92$	$27.38^{b} \pm 5.71$	$16.11^{b} \pm 4.74$	$0.09^{c} \pm 0.00$	$0.98^{cb} \pm 0.04$	$0.04^{c} \pm 0.00$
	12.5	$4.36^{c} \pm 0.63$	$0.32^{c} \pm 0.00$	$0.19^{d} \pm 0.00$	$4.50^{b} \pm 0.09$	$7.44^{c} \pm 0.21$	$14.56^{c} \pm 0.41$	$4.94^{c} \pm 0.62$	$0.01^{c} \pm 0.00$	$1.58^{b} \pm 0.78$	$0.02^{c} \pm 0.01$
Formic Acid	0	$72.54^{b} \pm 2.54$	$35.71^{ba} \pm 0.05$	$6.10^{a} \pm 0.00$	$8.41^{a} \pm 0.27$	$49.23^{a} \pm 1.52$	$92.98^{a} \pm 2.98$	$96.09^{a} \pm 0.34$	$1.80^{a} \pm 0.15$	$3.38^{a} \pm 0.17$	$0.31^{a} \pm 0.07$
	0.5	$81.83^{a} \pm 3.87$	$36.67^{a} \pm 1.50$	$2.63^{b} \pm 0.69$	$3.21^{b} \pm 0.68$	$44.80^{a} \pm 3.98$	$87.68^{a} \pm 7.79$	$97.84^{a} \pm 0.44$	$1.87^{a} \pm 0.11$	$4.11^{a} \pm 0.37$	$0.13^{b} \pm 0.09$
	1	$80.35^{a} \pm 5.47$	$33.47^{a} \pm 0.07$	$0.94^{c} \pm 0.03$	$1.17^{c} \pm 0.06$	$41.65^{a} \pm 2.76$	$81.51^{a} \pm 5.41$	$98.27^{a} \pm 2.71$	$0.94^{b} \pm 0.00$	$2.47^{ba} \pm 0.23$	$0.05^{cb} \pm 0.01$
	1.5	$16.49^{b} \pm 8.93$	$9.82^{b} \pm 1.67$	$0.59^{c} \pm 0.29$	$3.59^{b} \pm 0.35$	$15.59^{b} \pm 4.12$	$30.52^{b} \pm 8.07$	$20.85^{b} \pm 10.25$	$0.11^{c} \pm 0.02$	$0.49^{b} \pm 0.18$	$0.002^{c} \pm 0.01$
	2	$23.29^{b} \pm 12.04$	$0.02^{b} \pm 0.01$	$0.07^{c} \pm 0.00$	$0.32^{c} \pm 0.43$	$0.10^{c} \pm 0.22$	$0.19^{c} \pm 0.44$	$29.62^{b} \pm 14.59$	$0.00^{c} \pm 0.00$	$0.44^{b} \pm 0.18$	$0.008^{c} \pm 0.02$
	2.5	$34.10^{b} \pm 15.02$	$0.31^{b} \pm 0.01$	$0.06^{c} \pm 0.01$	$0.17^{c} \pm 0.03$	$0.93^{c} \pm 0.04$	$1.82^{c} \pm 0.08$	$43.30^{b} \pm 18.58$	$0.01^{c} \pm 0.01$	$2.55^{ba} \pm 1.62$	$0.005^{c} \pm 0.02$
Phenol	0	$72.54^{c} \pm 2.54$	$35.71^{a} \pm 0.05$	$6.10^{a} \pm 0.00$	$8.41^{ba} \pm 0.27$	$49.23^{a} \pm 1.52$	$92.98^{a} \pm 2.98$	$96.09^{a} \pm 0.34$	$1.80^{b} \pm 0.15$	$3.38^{a} \pm 0.17$	$0.31^{ba} \pm 0.07$
	0.5	$87.88^{b} \pm 6.14$	$39.07^{a} \pm 0.01$	$5.87^{a} \pm 0.24$	$6.68^{ba} \pm 0.73$	$44.46^{ba} \pm 3.01$	$87.00^{ba} \pm 5.90$	$96.98^{a} \pm 0.29$	$0.16^{c} \pm 0.01$	$0.06^{b} \pm 0.19$	$0.37^{a} \pm 0.09$
	1	$94.90^{a} \pm 9.20$	$37.78^{a} \pm 0.28$	$3.30^{b} \pm 0.40$	$3.47^{bc} \pm 0.07$	$39.80^{ba} \pm 3.57$	$77.90^{b} \pm 6.99$	$97.93^{a} \pm 0.12$	$2.36^{a} \pm 0.13$	$2.41^{ba} \pm 0.04$	$0.15^{bc} \pm 0.03$
	5	$19.49^{e} \pm 0.01$	$0.80^{b} \pm 0.00$	$0.09^{d} \pm 0.02$	$0.50^{c} \pm 0.10$	$4.12^{c} \pm 0.00$	$8.08^{c} \pm 0.00$	$13.01^{b} \pm 1.49$	$0.01^{c} \pm 0.00$	$2.23^{ba} \pm 0.05$	$0.004^{c} \pm 0.01$
	7	$24.27^{d} \pm 0.34$	$0.25^{b} \pm 0.03$	$2.40^{c} \pm 0.00$	$9.91^{a} \pm 0.10$	$1.05^{c} \pm 0.16$	$2.05^{c} \pm 0.31$	$15.19^{b} \pm 0.44$	$0.01^{c} \pm 0.00$	$1.14^{ba} \pm 1.18$	$0.15^{bc} \pm 0.01$
	9	$20.26^{d} \pm 8.92$	$0.35^{b} \pm 0.00$	$2.44^{c} \pm 0.00$	$12.07^{a} \pm 3.78$	$1.74^{c} \pm 0.52$	$3.42^{c} \pm 1.03$	$10.89^{b} \pm 4.91$	$0.09^{c} \pm 0.01$	$0.75^{ba} \pm 1.28$	$0.13^{bc} \pm 0.02$

The difference between values bearing different letters in the same column is statistically significant ($p < 0.05$).

3.2. Effect of Inhibitory Concentration on Sugar Consumption

3.2.1. Acetic Acid

Sugar consumption was observed in the fermentations made with the addition of 2.5 and 5.0 g/L of AA, as in the control fermentation. In these two fermentations, the initial sugar contents decreased from 84.95 and 75.27 g/L to 1.76 and 1.31 g/L, respectively. The inhibition started in the medium with the addition of 7.5 g/L of AA into the medium. With the increase in the concentration of 10 and 12.5 g/L of AA, the sugar consumption decreased considerably because of absolute inhibition. Therefore, the sugar content, which was 88.19 g/L with the addition of 12.5 g/L of AA, which is the maximum concentration, decreased to 79.65 g/L after 48 h (Figure 1).

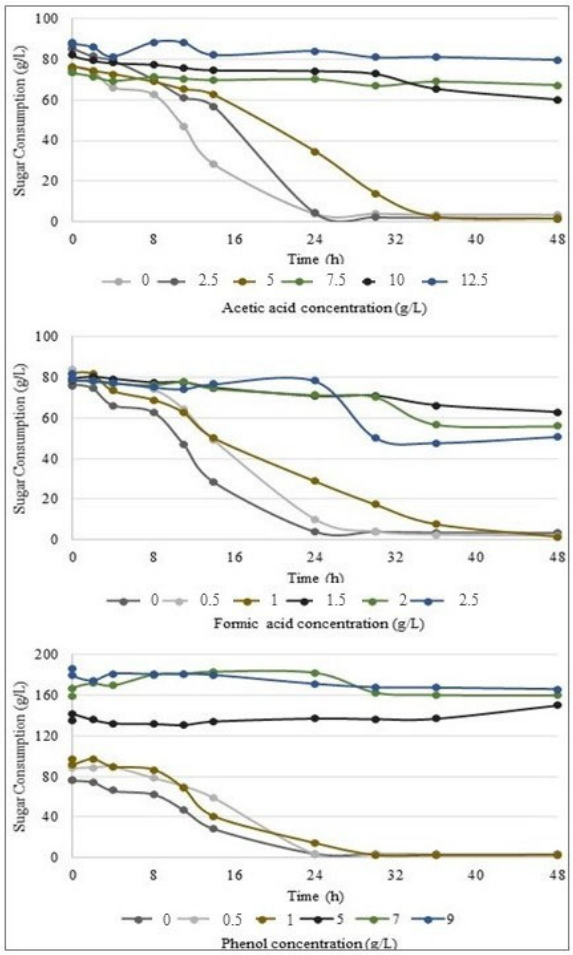


Figure 1. Sugar consumption at different inhibitor concentrations.

3.2.2. Formic Acid

Sugar consumption was observed in the fermentations performed with the addition of 0.5 and 1 g/L of FA as an inhibitor. At the end of these fermentations, the amount of sugar in the medium decreased from 83.64 and 78.53 g/L to 1.80 and 1.41 g/L, respectively. These results were highly similar to the control fermentation. With the addition of 1.5 g/L of FA to the medium, inhibition started and sugar consumption decreased. It was determined that higher concentrations of FA (2 and 2.5 g/L) caused a greater inhibitory effect. At the end of the fermentations consisting of 1.5 g/L, 2 g/L, and 2.5 g/L of FA, the sugar in the medium decreased to 62.62 g/L, 55.88 g/L, and 50.55 g/L, respectively. The amount of sugar remaining in the medium shows that the sugar cannot be used by the immobilized cells by inhibition (Figure 1).

3.2.3. Phenol

The fermentations were performed by adding 0.5 and 1 g/L of phenol into the medium; the sugar in the medium was used by the cells, as it is in the control fermentation (Figure 1). The sugar consumption determined in these two fermentations is too close to each other. However, with the addition of 5 g/L of phenol to the medium, inhibition started in the medium. Due to the inhibitory effect of phenol, immobilized *Saccharomyces cerevisiae* cells could not use the sugar in the medium. Therefore, microbial growth was inhibited and sugar consumption was not observed (Figure 1). Similarly, the same inhibitions were in media with 7 and 9 g/L of phenol. Additionally, an increase was found in the amount of sugar obtained from the analysis results, depending on the phenol concentration added to the medium. The reason for this is that in the sugar analysis method with DNSA, the phenol and DNSA compounds react and darken the color of the sample to be analyzed [27]. These changes in sugar consumption and amount over time are given in Figure 1.

3.3. Effect of Inhibitory Concentration on Ethanol Production

3.3.1. Acetic Acid

The observed changes in ethanol production are given in Figure 2. At the end of the fermentations, 37.73 g/L and 33.46 g/L of ethanol were produced in the mediums, including 2.5 and 5 g/L of AA, respectively. In the control fermentations, ethanol was found to be 35.20 g/L. On the other hand, 0.83 g/L of ethanol was calculated from the fermentation with 7.5 g/L of AA, where inhibition started. Regarding the medium consisting of 12.5 g/L of AA, which is its maximum concentration, 0.27 g/L of ethanol was obtained after 48 h.

3.3.2. Formic Acid

Ethanol production was observed in the fermentations with 0.5 and 1 g/L of FA, as in the control fermentation (Figure 2). In both the control fermentation and 0.5 and 1 g/L of FA-added fermentation, 35.20, 36.67, and 33.47 g/L of ethanol were obtained, respectively. Ethanol production decreased with the initiation of inhibition in the medium containing 1.5 g/L of FA. As a result of the fermentations carried out by adding 1.5, 2, and 2.5 g/L of FA to the medium, 9.83 g/L, 0.043 g/L, and 0.163 g/L of ethanol were produced, respectively. These changes that were observed in ethanol production are given in Figure 2.

3.3.3. Phenol

The ethanol production of the medium, including phenol (0.5 and 1 g/L), was determined as 35.20 and 39.04, respectively. In the control fermentation, it was calculated as 37.73 g/L. Since microbial growth was not inhibited in these fermentations, ethanol production was observed (Figure 2). In fermentations with phenol (5, 7, and 9 g/L), ethanol production was completely limited by the inhibitory effect of phenol, which was in the amounts of 0.81, 0.16, and 0.21 g/L. It was observed that the inhibitory effect of phenol on ethanol production was maximum at concentrations of 5 g/L and above. This change in ethanol production is given in Figure 2.

3.4. Effect of Inhibitory Concentration on Free Cell Biomass Growth

3.4.1. Acetic Acid

The microbial growth of the fermentation with AA is presented in Figure 3. Biomass formation in the control fermentation was 6.10 g/L; this was at the rate of 4.89 and 4.26 g/L of the biomass measured in the fermentations, including AA of 2.5 and 5 g/L. It limited the growth of immobilized *Saccharomyces cerevisiae* cells due to the inhibition that started with 7.5 g/L of AA. AA in the amounts of 7.5, 10, and 12.5 g/L caused a limited biomass of 1.15, 1.14, and 1.17 g/L, respectively.

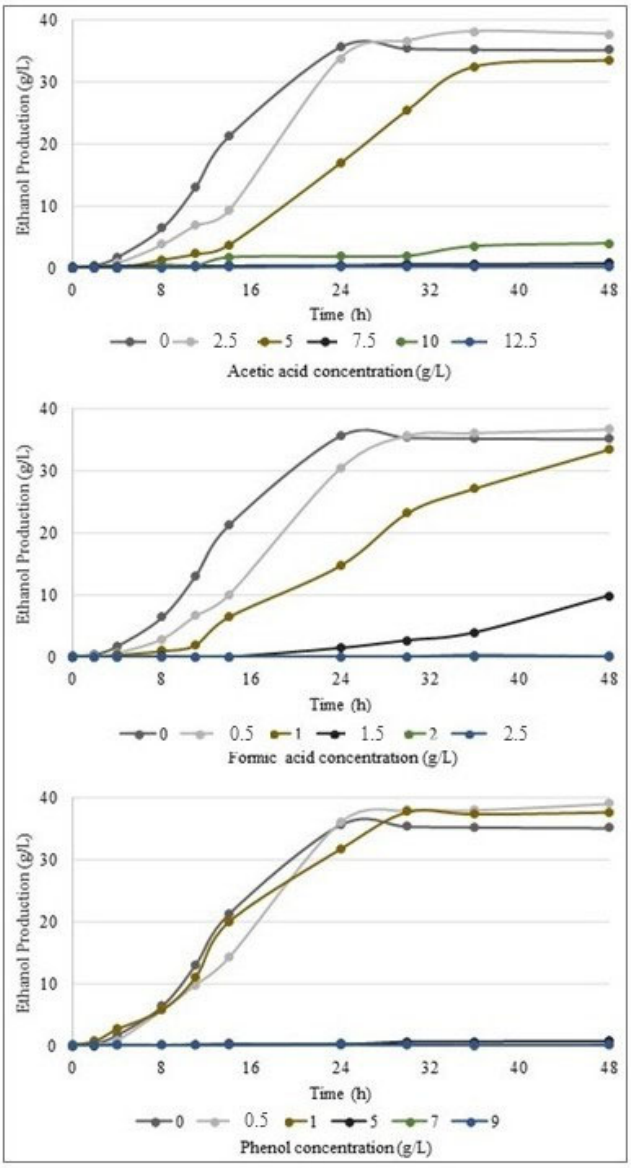


Figure 2. Ethanol production at different inhibitor concentrations.

3.4.2. Formic Acid

In the control fermentation, the biomass growth was measured as 6.1 g/L at the end of 48 h of fermentation. Biomass growths of 3.70 and 2.01 g/L were observed in media with 0.5 and 1 g/L of FA, respectively, indicating that sugar consumption and ethanol production were effectively suppressed. In contrast, the utilization of 1.5 g/L of FA resulted in the growth of immobilized *Saccharomyces cerevisiae* cells. With this limitation in growth, the biomasses were limited to 1.66, 1.10, and 1.10 g/L by an FA of 1.5, 2, and 2.5 g/L, respectively. Changes in the biomass growth are indicated in Figure 3.

3.4.3. Phenol

In the fermentation, realized by adding 0.5 g/L of phenol into the medium, 6.48 g/L of biomass was produced. Regarding the addition of 1 g/L of phenol, which is another concentration without inhibition, the biomass production decreased relatively and was detected as 4.04 g/L. In the fermented broths with 5, 7, and 9 g/L of phenol, the biomass productions were 1.16, 3.47, and 3.51 g/L, respectively. At phenol concentrations above 1 g/L, the growth of immobilized *Saccharomyces cerevisiae* cells was limited and reduced. These observed changes in biomass development are displayed in Figure 3.

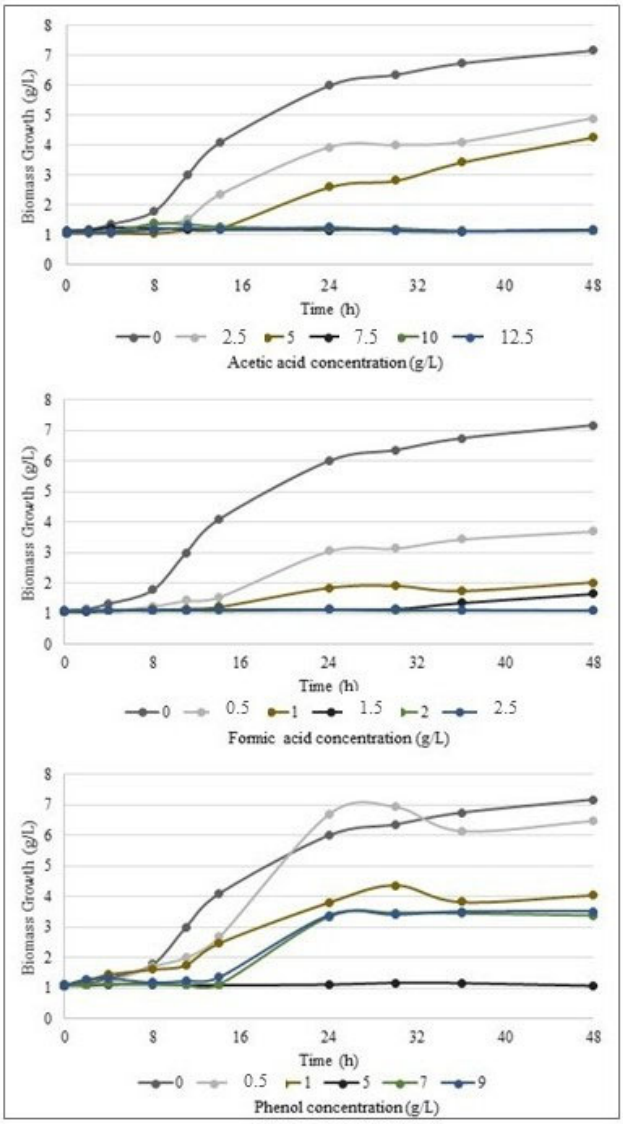


Figure 3. Biomass growth at different inhibitor concentrations.

3.5. Effect of Inhibitory Concentration on Kinetic Parameters

The kinetic parameters of the fermentations carried out with the addition of AA, FA, and P are presented in Table 3.

3.5.1. Acetic Acid

The control fermentation showed the highest $Y_{P/S}$ ratio, which was 49.23%. Among all the fermentations consisting of 2.5 g/L of AA, it produced the highest $Y_{P/S}$, 45.65%, which was quite close to the control fermentation. The results showed that there was no statistically significant difference in the ethanol yields of the control fermentation and the 2.5 and 5 g/L AA additions ($p > 0.05$). AA at the amount of 7.5 g/L was the minimum inhibition content in the medium. The fermentation carried out with 12.5 g/L of AA had the lowest $Y_{P/S}$, which was found to be 7.44%. The lowest and highest values of $Y_{P/S}$ had a significant difference ($p < 0.05$). The amounts of sugar consumed in the control fermentation and the fermentations that included 2.5 and 5 g/L of AA were comparable, and there was no statistically significant difference between them ($p > 0.05$). Additionally, the lowest SUY values were determined to be 9.07, 16.12, and 4.94%, respectively, from the fermentations performed with 5, 7, and 9 g/L of AA. There was a significant difference in maximum sugar consumption when comparing the fermentations with 5 and 12 g/L of

AA ($p < 0.05$). Furthermore, as the inhibitor concentration increased, the maximum amount of ethanol was produced, the amount of sugar was consumed, and the biomass similarly decreased (Table 3). In a previous study, acetic acid, furfural, and HMF were utilized as inhibitors in the fermentation of suspended *Pichia stipities* [31]. The research reported that fermentation was inhibited by the addition of AA at the lowest concentration of 0.25 g/L.

3.5.2. Formic Acid

The fermentation with 0.5 g/L of FA showed the highest $Y_{P/S}$ of 44.81%, which closely resembled the yield of the control fermentation. Moreover, in the fermentation process that included 1 g/L of FA without inhibition, the ethanol yield was determined to be 41.65%. There was no statistically significant difference seen between the yields of the control fermentation and the addition of 0.5 and 1 g/L of FA ($p > 0.05$). When 1.5 g/L of FA was placed into the medium, inhibition was seen to begin, and when 2 g/L of FA was added to the fermentation, the lowest $Y_{P/S}$ was determined to be 0.10%. Although the addition of 0.5 g/L of FA resulted in a slight increase in the Q_P, Q_S, and Q_X values, the data from other fermentations showed an obvious decrease. The yeast used for fermentation containing 1 g/L of FA consumed the majority of the sugar in the medium when the fermentations were evaluated in terms of the SUY. There was no statistically significant difference in the SUY values between the control fermentation and the fermentations with 0.5 and 1 g/L of FA ($p > 0.05$). However, the addition of 1 g/L of FA resulted in a significantly higher sugar consumption compared to the addition of 1.5 g/L of FA ($p > 0.05$).

3.5.3. Phenol

The fermentation containing 0.5 g/L of phenol exhibited the highest $Y_{P/S}$ (44.46%) among all the fermentations that included phenol additions. Moreover, the $Y_{P/S}$ was determined to be 39.81% without any inhibition in fermentation with 1 g/L of phenol. The results showed that there was no statistically significant difference in the $Y_{P/S}$ values of the fermentations with 0.5 and 1 g/L of phenol added to the medium ($p > 0.05$). The usage of 5 g/L of phenol in the fermentation broth triggered the inhibition process, and the fermentation carried out with the addition of 7 g/L phenol resulted in the lowest $Y_{P/S}$, which was reported to be 1.05%. A statistically significant difference was found between the minimum $Y_{P/S}$ observed in the fermentation having 7 g/L of phenol and the highest $Y_{P/S}$ achieved from the control fermentation ($p < 0.05$). The fermentation process that included 9 g/L of phenol with a 12.07% resulted in the highest $Y_{X/S}$. However, as previously mentioned, higher phenol concentrations lead to higher sugar analysis results. Consequently, assessing the maximal microbial growth rates in this part would be more accurate. Taking into account the fermentations, including 0.5 g/L of phenol, resulted in the greatest Q_X value of 0.33 g/L/h, while the fermentation containing 5 g/L phenol caused the lowest Q_X value of 0.004 g/L/h. Comparing the Q_X values of the phenol-added fermentations and the control fermentation showed a statistically significant difference ($p < 0.05$). In prior research, acetic acid, formic acid, furfural, levulinic acid, HMF, vanillin, syringaldehyde, and phenol were chosen as inhibitors for the fermentation utilizing pure glucose and suspended *Saccharomyces cerevisiae* [32]. In that study, at quantities of 5, 10, 20, and 30 mM for furfural, HMF, and phenol, and 40, 80, 120, and 160 mM for acetic acid and formic acid, the inhibitor was added to the fermentation medium. It was observed that at concentrations higher than 80 mM, the inhibitory effects of the formic and acetic acids became apparent. Additionally, it was noted that above 20 mM concentrations of HMF, furfural and phenol showed an inhibitory effect. When previous investigations in the literature are reviewed, it is obvious that studies using suspended *Saccharomyces cerevisiae* cells were carried out on a small scale. As a result, it was determined that the

inhibitory chemicals had an inhibitory effect at lower concentrations while the cells were free. Immobilized cells instead of suspended cells were utilized in this study in order to improve the information found in the literature.

3.6. Mathematical Modeling

In this study, the effect of immobilization of *Saccharomyces cerevisiae* on ethanol production was investigated in the presence of inhibitors at different concentrations in the medium. To develop a mathematical model, kinetic parameters about biomass, sugar consumption, and ethanol production (fermentations consisting of 2.5 g/L of AA, 0.5 g/L of FA, and 0.5 g/L of phenol) were examined. All model values were presented in detail in Tables 4–6. The model with minimum errors was chosen as a representative model [8].

Table 4. Mathematical models applied to fermentation with the addition of 2.5 g/L of acetic acid.

Model/Acetic Acid	Ethanol Production					Biomass Growth					Sugar Consumption				
	RMSE (g/L)	MAE (g/L)	R2	BF	AF	RMSE (g/L)	MAE (g/L)	R2	BF	AF	RMSE (g/L)	MAE (g/L)	R2	BF	AF
MGM	1.08	0.66	0.996	0.93	1.10	0.31	0.26	0.9748	0.9	1.16	27.36	14.49	0.6327	0.69	1.49
MLM	1.70	1.61	0.9984	0.47	2.05	0.49	0.44	0.975	0.79	1.27	37.98	27.02	0.4498	0.29	3.49
MRM	1.45	1.34	0.9988	0.50	1.99	0.36	0.32	0.9745	1.3	1.34	27.25	19.64	0.681	0.35	3.02
Stannard	11.67	10.55	0.9576	0.44	4.29	1.36	1.25	0.9429	0.83	1.79	19.03	16.95	0.931	0.44	2.73
Weibull	1.27	0.90	0.9964	1.42	1.45	0.31	0.20	0.9685	1.02	1.08	3.84	2.74	0.9908	0.97	1.11
MMF	1.06	0.82	0.9966	1.53	1.57	0.16	0.13	0.9864	1	1.06	5.19	4.06	0.9825	0.76	1.36
Asymmetric	3.93	2.36	0.9639	1107.86	1118.63	1.20	0.94	0.3668	0.78	1.51	20.41	11.24	0.9023	0.37	2.7
Baranyi	2.00	1.12	0.99	2.82	2.97	1.05	0.69	0.8505	1.18	1.34	40.07	27.92	0.3424	0.29	3.47
Huang	1.07	0.74	0.9966	1.80	1.86	0.56	0.42	0.9352	0.84	1.2	40.07	27.92	0.3424	0.29	3.47
Fitzhugh	2.36	1.70	0.986	2.44	2.62	0.35	0.27	0.9615	0.96	1.13	6.73	4.25	0.9766	0.94	1.1

Table 5. Mathematical models applied to fermentation with the addition of 0.5 g/L of formic acid.

Model/Formic Acid	Ethanol Production					Biomass Growth					Sugar Consumption				
	RMSE (g/L)	MAE (g/L)	R^2	BF	AF	RMSE (g/L)	MAE (g/L)	R^2	BF	AF	RMSE (g/L)	MAE (g/L)	R^2	BF	AF
MGM	1.22	0.95	0.9963	0.72	1.41	0.27	0.23	0.9662	0.90	1.15	25.56	14.06	0.6626	0.74	1.42
MLM	2.07	1.92	0.9978	0.44	2.33	0.38	0.31	0.9659	0.81	1.22	38.05	27.89	0.4715	0.31	3.20
MRM	1.74	1.58	0.9982	0.44	2.26	0.40	0.31	0.9658	1.42	1.45	27.21	20.22	0.7419	0.37	2.78
Stannard	11.45	10.26	0.9615	0.42	4.60	0.99	0.92	0.9342	0.89	1.63	19.71	17.91	0.9308	0.49	2.49
Weibull	1.48	1.01	0.9934	1.54	1.58	0.12	0.09	0.9881	1.01	1.04	2.85	2.07	0.9976	1.09	1.12
MMF	3.13	1.99	0.9966	0.89	1.29	0.09	0.07	0.9921	1.01	1.04	3.14	2.47	0.996	0.86	1.16
Asymmetric	11.62	12.35	0.3076	246.86	2115.71	0.93	0.70	0.3072	0.84	1.43	22.10	17.99	0.948	0.39	2.57
Baranyi	0.75	0.37	0.9984	1.69	1.71	0.82	0.61	0.8654	1.28	1.40	40.66	29.43	0.3229	0.30	3.24
Huang	0.36	0.24	0.9996	9.88	9.97	0.43	0.30	0.8993	0.85	1.18	40.69	29.54	0.3213	0.30	3.24
Fitzhugh	2.33	1.42	0.9845	108.82	113.29	0.28	0.22	0.9387	0.93	1.13	4.60	3.70	0.9929	1.10	1.17

Table 6. Mathematical models applied to fermentation with the addition of 0.5 g/L of phenol.

Model/Phenol	Ethanol Production					Biomass Growth					Sugar Consumption				
	RMSE (g/L)	MAE (g/L)	R^2	BF	AF	RMSE (g/L)	MAE (g/L)	R^2	BF	AF	RMSE (g/L)	MAE (g/L)	R^2	BF	AF
MGM	1.61	1.43	0.997	0.59	1.73	0.48	0.35	0.9633	0.96	1.09	28.75	15.06	0.6287	0.59	1.67
MLM	2.45	2.11	0.9961	0.44	2.29	0.49	0.44	0.9744	0.85	1.19	33.39	22.05	0.572	0.37	1.63
MRM	2.46	2.30	0.9971	0.44	2.25	0.60	0.54	0.9735	1.62	1.68	24.08	16.07	0.7282	0.47	2.21
Stannard	12.07	10.63	0.955	0.45	4.07	2.27	2.11	0.8471	0.76	2.1	16.59	14.30	0.9383	0.49	2.32
Weibull	1.33	0.96	0.9958	1.38	1.43	0.34	0.24	0.984	0.99	1.07	2.54	1.73	0.9962	0.92	1.10
MMF	1.20	0.75	0.9944	1.50	1.56	0.35	0.27	0.9822	1.03	1.09	4.07	3.09	0.9914	0.83	1.22
Asymmetric	2.30	1.26	0.9822	1.33	1.52	2.09	1.81	0.3193	0.71	1.76	26.26	21.04	0.9066	0.36	2.86
Baranyi	1.99	0.71	0.9905	1.16	1.20	1.93	1.11	0.804	1.39	1.63	35.28	22.57	0.4998	0.40	2.46
Huang	0.39	0.24	0.9996	2.13	2.15	0.54	0.34	0.967	0.93	1.11	35.27	22.56	0.5001	0.40	2.46
Fitzhugh	2.27	1.59	0.985	16.95	18.10	0.88	0.66	0.8958	0.86	1.26	5.11	3.33	0.9867	0.93	1.1

3.6.1. Model Evaluation for Acetic Acid

The experimental data of the ethanol fermentation performed in the presence of 2.5 g/L AA were predicted with ten different models. The modeling evaluation results are presented in Table 4. Based on the results, for ethanol production, the lowest RMSE value (1.06 g/L) was calculated by the MMF. MGM had the lowest MAE value of 0.67 g/L. While the Stannard model had the lowest R^2 value (0.9576), MRM had the highest R^2 value (0.9988). In addition, the BF value obtained from the MGM (0.931) is in the acceptable range for the model. The AF value obtained from the MGM (1.109) is also in the range that is compatible with the model. When all calculations were examined, the ideal model—so as to predict the observed data of ethanol production—was MGM (Figure 4). The MGM provided the lowest RMSE (0.31 g/L) and MAE values (0.26 g/L) for biomass. In the data obtained, the lowest R^2 value (0.3668) was obtained from the asymmetric model, and the highest R^2 value (0.9864) was calculated by means of the MMF model. In addition, the BF values obtained from the Weibull (1.02), MMF (1.00), and Fitzhugh (0.96) models were in the range that is compatible with the model. The AF values obtained from the MGM (1.20), Weibull (1.08), MMF (1.06), Huang (1.20), and Fitzhugh (1.13) models were also in the range that is compatible with the model. When all these values were examined, the most suitable model for biomass growth was chosen as the MMF model (Figure 4). The lowest RMSE (3.84 g/L) and MAE values (2.75 g/L) for sugar consumption were obtained from the Weibull model, and the highest RMSE value (40.08 g/L) and MAE value (27.93 g/L) were obtained from the Huang model. The Huang and Baranyi models yielded the lowest R^2 value (0.3424), while the Weibull model produced the highest R^2 value (0.9908). Additionally, the BF value obtained from the Weibull (0.97) model was in the range that is compatible with the model. The AF values obtained from the Weibull (1.11) and Fitzhugh (1.10) models were in the range that is compatible with the model. When all these values were examined, the most suitable model for sugar consumption was selected as the Weibull model (Figure 4).

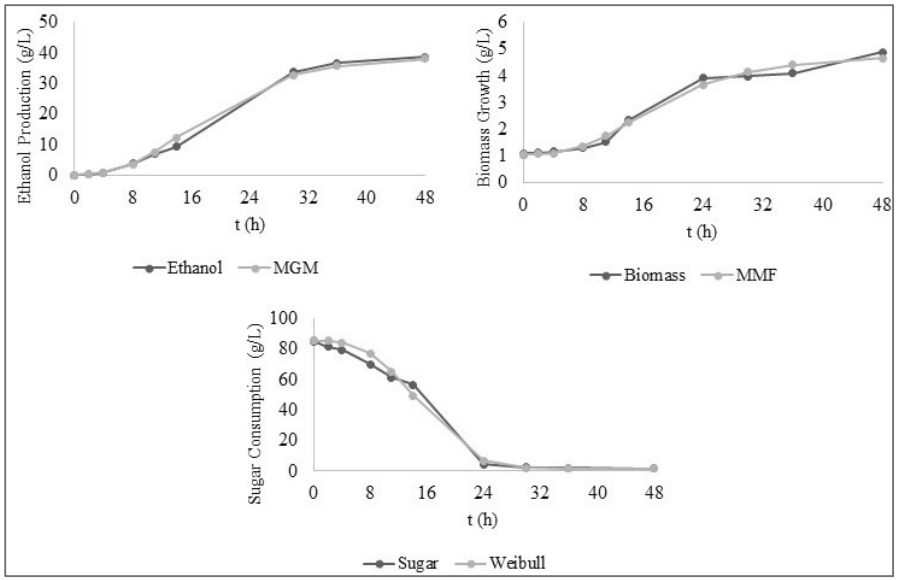


Figure 4. Graphics of models suitable for fermentation with the addition of 2.5 g/L of acetic acid.

3.6.2. Model Evaluation for Formic Acid

The limit value for ethanol production in terms of the FA was determined as 0.5 g/L and therefore, the actual data of the related fermentation were estimated by mathematical modeling (Table 1). The success of models was determined by means of the model metrics

given in Table 5. The results indicated that the minimum values of RMSE (0.36 g/L) and MAE (0.24 g/L) for ethanol production were found by the Huang model. The lowest R^2 value (0.3076) was obtained from the asymmetric model, and the maximum value of R^2 (0.9996) was calculated with the Huang model. In addition, the BF and AF values attained from the MMF model were in the range that is compatible with the model. When all these values are considered, the Huang model was the most suitable model for ethanol production, based on the lowest error value and the highest R^2 values (Figure 5). The lowest RMSE (0.09 g/L) and MAE (0.08 g/L) for biomass growth were achieved with the MMF model. In the data obtained, the lowest R^2 value (0.3072) was obtained from the asymmetric model, and the greatest R^2 value (0.9921) was attained from the MMF model. In addition, the BF values by means of Weibull (1.02) and MMF (1.02) models were in the range that is compatible with the model. The AF values obtained from the MGM (1.16), Weibull (1.05), MMF (1.05), Huang (1.19), and Fitzhugh (1.14) models were also found to be within the range compatible with the model. When considering all these values, the values predicted by the MMF model showed a high degree of agreement with the observed values of biomass growth (Figure 5). The lowest values for RMSE (2.85 g/L) and MAE (2.08 g/L) for sugar consumption were found through the Weibull model, while the highest RMSE value (40.70 g/L) and MAE value (29.54 g/L) were found using the Huang model. The Huang model yielded the lowest R^2 value (0.3213), while the Weibull model yielded the highest R^2 value (0.9976). Furthermore, the BF values from the Weibull (1.09) and Fitzhugh (1.10) models were found to be within the range that is compatible with the model. Similarly, the AF levels acquired from models of the Weibull (1.13), MMF (1.17), and Fitzhugh (1.18) were found to be within the range that is compatible with the model. When examining all these values, the best model for sugar consumption was selected as the Weibull model (Figure 5).

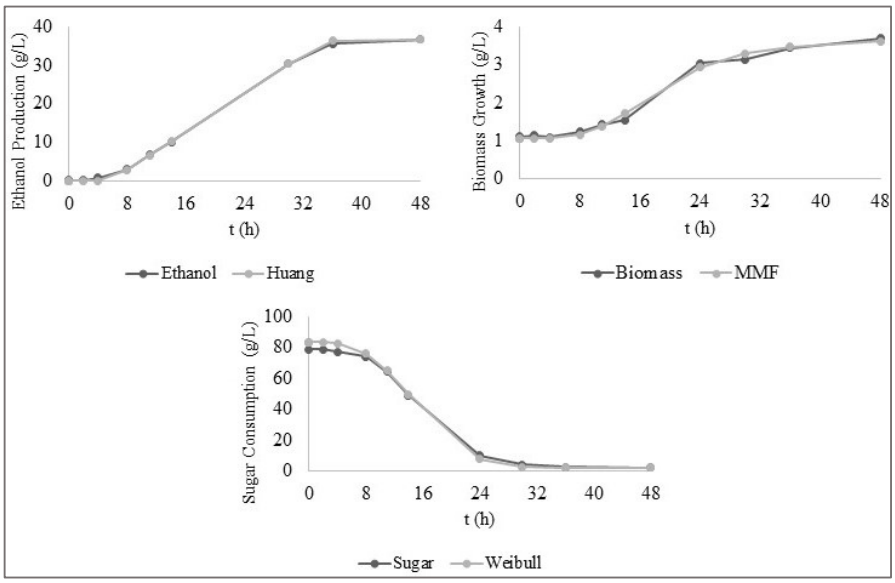


Figure 5. Graphics of models suitable for fermentation with the addition of 0.5 g/L of formic acid.

3.6.3. Model Evaluation for Phenol

Fermentations, including 0.5 g/L of phenol, were modeled with respect to the biomass, sugar consumption, and ethanol (Table 6). The lowest values of RMSE (0.39 g/L) and MAE (0.24 g/L) for ethanol production were attained through the Huang model, and the highest values were calculated using the Stannard model as 12.08 and 10.64 g/L, respectively. In the data obtained, the lowest R^2 value (0.9550) was figured out by the Stannard model, and the highest R^2 value (0.9996) was found using the Huang model. In addition, the BF value

obtained from the Baranyi (1.16) model was in the range that is compatible with the model. The AF value obtained from the Baranyi model (1.20) was also in the acceptable range for the model. When all parameters were examined, the best model for ethanol production was detected through the Huang model (Figure 6). The Weibull model was utilized for the lowest values of RMSE (0.34 g/L) and MAE (0.25 g/L) about biomass growth. In the data obtained, the lowest R^2 value (0.3193) was obtained from the asymmetric model, and the highest R^2 value (0.9840) was achieved from the Weibull model. In addition, the BF values derived from the MGM (0.97), Weibull (0.99), and MMF (1.03) models were in a range that is compatible with the model. The AF values from the MGM (1.09), MLM (1.19), Weibull (1.07), and MMF (1.10) models were also within the range consistent with the model. For sugar consumption, the lowest values of RMSE (2.55 g/L) and MAE (1.74 g/L) were taken from the Weibull model, and the greatest RMSE value (35.29 g/L) and MAE (22.57 g/L) were obtained from the Baranyi model. In the data obtained, the lowest R^2 value (0.4498) was obtained from the Baranyi model, and the maximum R^2 value (0.9962) was attained by the Weibull model. Additionally, the BF values for the Weibull (0.92) and Fitzhugh (0.94) models were within the acceptable range for the model. The AF from the Weibull (1.11) and Fitzhugh (1.10) models were also in the range that is compatible with the model. Accordingly, the most suitable model for biomass growth and sugar consumption was chosen as the Weibull model (Figure 6).

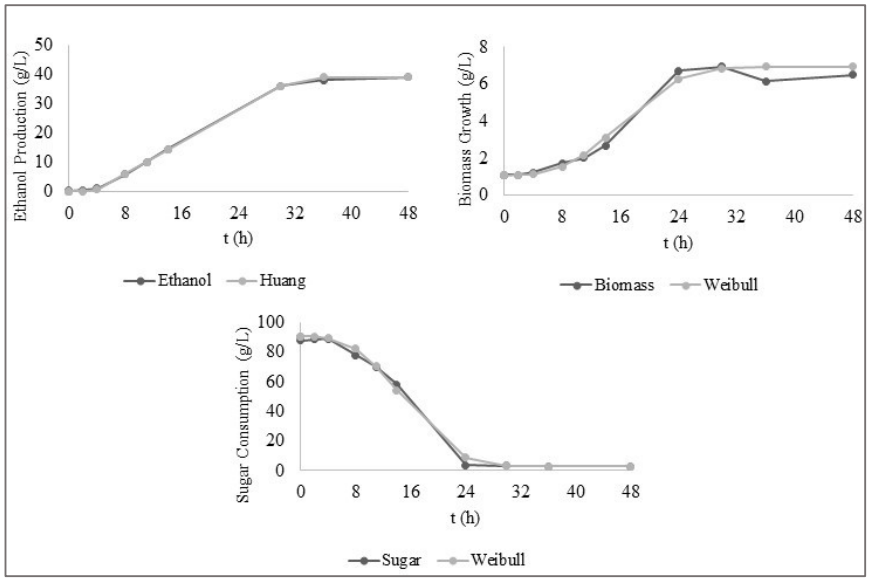


Figure 6. Graphics of models suitable for fermentation with the addition of 0.5 g/L of phenol.

3.7. Sensitivity Analysis for Inhibitors

The 5% response analysis illustrates how model parameters impact forethoughts. When upsurging or cutting down biomass, ethanol, or sugar values by 5%, the variations acquired in forethoughts underscore the model's adaptability to parameter modification. These alterations can ensure costly realizations of the robustness and stability of the fermentation model. To summarize, Table 7a–c encapsulates a comprehensive view of the fermentation dynamics, encompassing experimental values, forethoughts, and sensitivity analyses. This table serves as a costly resource for cognizance of the entangled ties between fermentation times, cell development, ethanol creation, and sugar utilization and to examine the model's effectiveness under parameter alterations. Table 8 shows the ID, ED, and IAD that were determined based on model parameters by upsurging or cutting down by 5%.

Table 7. (**a**) Exploratory and estimated ascetic acid inhibitor fermentation measurements together with biomass growth, ethanol production, and sugar consumption data gained by upsurging and shrinking the model parameters by 5% for response analysis. (**b**) Exploratory and estimated formic acid inhibitor fermentation measurements, together with biomass growth, ethanol production, and sugar consumption data gained by upsurging and shrinking the model parameters by 5% for response analysis. (**c**) Exploratory and estimated phenol inhibitor fermentation measurements, together with biomass growth, ethanol production, and sugar consumption data gained by upsurging and shrinking the model parameters by 5% for response analysis.

(**a**)

	Time	Biomass	Product	Sugar	Biomass_Pred	Biomass_5% Increase	Biomass_5% Decrease	Product_Pred	Product_5% Increase	Product_5% Decrease	Sugar_Pred	Sugar_5% Increase	Sugar_5% Decrease
0	0	1.07	0.04	84.95	0.84	0.92	0.78	0.00	0.00	0.00	83.71	88.75	78.59
1	0	1.10	0.09	85.22	0.84	0.92	0.78	0.00	0.00	0.00	83.71	88.75	78.59
2	2	1.13	0.26	81.36	1.03	1.11	0.95	0.00	0.00	0.00	81.98	87.24	76.63
3	4	1.16	0.92	79.20	1.23	1.32	1.13	0.04	0.02	0.07	79.66	85.20	73.99
4	8	1.31	3.85	70.03	1.65	1.77	1.54	1.34	1.06	1.66	72.45	78.80	65.92
5	11	1.53	6.96	61.09	1.99	2.13	1.86	5.39	4.78	5.98	63.96	71.13	56.66
6	14	2.35	9.34	56.59	2.33	2.48	2.18	12.19	11.59	12.68	52.24	60.22	44.29
7	24	3.93	33.84	4.27	3.38	3.58	3.19	32.01	33.05	30.88	6.19	10.42	3.31
8	30	4.00	36.64	2.35	3.91	4.12	3.69	36.43	38.03	34.79	0.08	0.29	0.02
9	36	4.10	38.14	2.03	4.33	4.57	4.10	38.09	39.92	36.26	0.00	0.00	0.00
10	48	4.89	37.73	1.76	4.93	5.18	4.67	38.89	40.83	36.96	0.00	0.00	0.00

	time	MFT_biomass pred	MFT_biomass increase	MFT_biomass decrease	MFT_product pred	MFT_product increase	MFT_product decrease	MFT_sugar pred	MFT_sugar increase	MFT_sugar decrease
0	0	0.84	0.92	0.78	0.00	0.00	0.00	83.71	88.75	78.59
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	1.87	2.03	1.72	0.00	0.00	0.00	165.69	175.99	155.22
3	4	2.25	2.43	2.08	0.04	0.03	0.07	161.64	172.44	150.62
4	8	5.76	6.18	5.34	2.77	2.17	3.46	304.20	328.00	279.83
5	11	5.47	5.85	5.10	10.10	8.76	11.46	204.61	224.90	183.88
6	14	6.48	6.91	6.06	26.37	24.56	27.99	174.30	197.02	151.43
7	24	28.57	30.31	26.86	221.00	223.19	217.84	292.14	353.22	238.03
8	30	21.87	23.11	20.64	205.32	213.22	197.03	18.81	32.14	9.99
9	36	24.72	26.07	23.37	223.57	233.84	213.15	0.23	0.86	0.05
10	48	55.55	58.48	52.62	461.92	484.47	439.28	0.00	0.00	0.00

Table 7. *Cont.*

(**b**)

	Time	Biomass	Product	Sugar	Biomass_Pred	Biomass_5% Increase	Biomass_5% Decrease	Product_Pred	Product_5% Increase	Product_5% Decrease	Sugar_Pred	Sugar_5% Increase	Sugar_5% Decrease
0	0	1.06	0.00	83.64	1.14	1.19	1.08	0.27	0.29	0.26	79.59	83.57	75.61
1	0	1.12	0.08	78.62	1.14	1.19	1.08	0.27	0.29	0.26	79.59	83.57	75.61
2	2	1.15	0.19	78.87	1.14	1.19	1.08	0.27	0.29	0.26	79.59	83.57	75.61
3	4	1.10	0.75	77.24	1.14	1.19	1.08	0.27	0.29	0.26	79.59	83.57	75.61
4	8	1.25	2.97	74.08	1.14	1.19	1.08	1.56	1.04	2.07	74.71	79.87	69.67
5	11	1.43	6.70	64.40	1.32	1.32	1.31	6.80	6.47	7.07	62.54	67.11	58.10
6	14	1.54	10.07	48.93	1.73	1.76	1.70	12.07	12.00	12.08	50.36	54.32	46.52
7	24	3.05	30.42	9.94	2.86	2.98	2.73	29.64	30.44	28.77	9.75	11.68	7.95
8	30	3.14	35.66	3.81	3.27	3.43	3.10	36.13	37.93	34.33	2.65	2.78	2.52
9	36	3.44	36.09	2.34	3.49	3.67	3.31	36.15	37.96	34.34	2.65	2.78	2.52
10	48	3.70	36.67	1.80	3.64	3.82	3.45	36.15	37.96	34.34	2.65	2.78	2.52

	time	MFT_biomass pred	MFT_biomass increase	MFT_biomass decrease	MFT_product pred	MFT_product increase	MFT_product decrease	MFT_sugar pred	MFT_sugar increase	MFT_sugar decrease
0	0	1.14	1.19	1.08	0.27	0.29	0.26	79.59	83.57	75.61
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	2.27	2.39	2.16	0.55	0.58	0.52	159.18	167.14	151.22
3	4	2.27	2.39	2.16	0.55	0.58	0.52	159.18	167.14	151.22
4	8	4.55	4.77	4.32	3.67	2.65	4.66	308.60	326.88	290.56
5	11	3.68	3.76	3.59	12.54	11.27	13.71	205.87	220.47	191.65
6	14	4.57	4.61	4.52	28.32	27.71	28.73	169.34	182.14	156.93
7	24	22.93	23.70	22.12	208.56	212.23	204.22	300.53	329.99	272.35
8	30	18.37	19.25	17.48	197.31	205.11	189.29	37.20	43.38	31.39
9	36	20.27	21.32	19.23	216.84	227.66	206.02	15.90	16.69	15.10
10	48	42.76	44.98	40.55	433.79	455.48	412.10	31.79	33.38	30.20

Table 7. *Cont.*

(**c**)

	Time	Biomass	Product	Sugar	Biomass_Pred	Biomass_5% Increase	Biomass_5% Decrease	Product_Pred	Product_5% Increase	Product_5% Decrease	Sugar_Pred	Sugar_5% Increase	Sugar_5% Decrease
0	0	1.07	0.02	90.62	1.24	1.30	1.17	0.38	0.40	0.36	89.04	93.49	84.59
1	0	1.08	0.05	88.10	1.24	1.30	1.17	0.38	0.40	0.36	89.04	93.49	84.59
2	2	1.10	0.43	88.50	1.24	1.30	1.18	0.38	0.40	0.36	89.04	93.49	84.59
3	4	1.21	1.01	88.95	1.24	1.30	1.18	0.38	0.40	0.36	89.04	93.49	84.59
4	8	1.72	5.83	78.12	1.24	1.30	1.18	4.46	4.09	4.78	82.55	88.34	76.92
5	11	2.01	9.92	70.03	1.70	1.55	1.83	10.26	10.17	10.29	68.15	73.24	63.23
6	14	2.68	14.31	58.36	3.09	3.00	3.14	16.05	16.26	15.79	53.74	58.11	49.54
7	24	6.71	36.09	3.57	6.35	6.64	6.06	35.33	36.53	34.06	5.77	7.69	4.18
8	30	6.94	38.02	3.33	6.60	6.93	6.27	38.40	40.32	36.48	2.89	3.04	2.75
9	36	6.15	38.05	2.74	6.61	6.94	6.28	38.40	40.32	36.48	2.89	3.04	2.75
10	48	6.48	39.09	2.73	6.61	6.94	6.28	38.40	40.32	36.48	2.89	3.04	2.75

	time	MFT_biomass pred	MFT_biomass increase	MFT_biomass decrease	MFT_product pred	MFT_product increase	MFT_product decrease	MFT_sugar pred	MFT_sugar increase	MFT_sugar decrease
0	0	1.24	1.30	1.17	0.38	0.40	0.36	89.04	93.49	84.59
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	2.47	2.60	2.35	0.75	0.79	0.71	178.08	186.98	169.17
3	4	2.47	2.60	2.35	0.75	0.79	0.71	178.08	186.98	169.17
4	8	4.95	5.20	4.70	9.68	8.97	10.27	343.19	363.67	323.00
5	11	4.41	4.28	4.51	22.08	21.39	22.60	226.06	242.37	210.21
6	14	7.18	6.84	7.47	39.47	39.65	39.12	182.84	197.02	169.15
7	24	47.19	48.21	46.00	256.92	263.95	249.27	297.58	328.98	268.60
8	30	38.84	40.69	36.97	221.19	230.54	211.62	26.00	32.18	20.79
9	36	39.63	41.61	37.64	230.39	241.91	218.87	17.36	18.23	16.49
10	48	79.36	83.33	75.39	460.79	483.83	437.75	34.71	36.46	32.99

Table 8. ED, ID, and IAD values calculated for economic analysis.

Scenario/Acetic Acid	Sensitivity Metrics	Type	Biomass Growth	Ethanol Production	Sugar Consumption
0	ED	5% increase	5.969	7.050	4.137
1	ED	5% decrease	−4.747	−3.101	−7.778
2	ID	5% increase	69.450	−52.478	−52.478
3	ID	5% decrease	−65.602	104.489	104.489
4	IAD	5% increase	69.450	52.478	52.478
5	IAD	5% decrease	65.602	104.489	104.489
Scenario/Formic acid	Sensitivity metrics	Type	Biomass growth	Ethanol production	Sugar consumption
0	ED	5% increase	3.405	3.511	−0.081
1	ED	5% decrease	−6.664	−6.347	−9.595
2	ID	5% increase	41.363	−6.055	−6.055
3	ID	5% decrease	−42.226	7.005	7.005
4	IAD	5% increase	41.363	6.055	6.055
5	IAD	5% decrease	42.226	7.005	7.005
Scenario/Phenol	Sensitivity metrics	Type	Biomass growth	Ethanol production	Sugar consumption
0	ED	5% increase	0.105	3.137	3.172
1	ED	5% decrease	−9.437	−6.685	−6.654
2	ID	5% increase	29.656	22.890	22.890
3	ID	5% decrease	−31.466	−25.671	−25.671
4	IAD	5% increase	29.656	22.890	22.890
5	IAD	5% decrease	31.466	25.671	25.671

The findings demonstrated a 5% increase in biomass, ethanol, and sugar consumption for AA, indicating a positive effect of the ED on ethanol, biomass, and sugar at the end of fermentation. However, a decline in the ID and ED due to a 5% decrease in the model parameters revealed an inadequate contribution to biomass growth, ethanol production, and sugar consumption (Table 8). Nearly identical to those obtained from the model parameters upturned (5%) were the IAD values obtained with a 5% decrease.

The outcomes, having a positive ED, demonstrated, for FA, a 5% increase that carries out a beneficial influence on biomass and ethanol. Yet, when the values of the model factors were upped by 5%, only the ED value was not positive for sugar consumption in the fermentation broth. This is on the grounds of a relatively advanced value rather than the exploratory sugar data, as the fermentation concludes. In contrast, factors that resulted negatively in the ED and ID (because of a 5% rise) of ethanol and sugar in the fermentation broth demonstrated an undesirable influence on sugar, biomass, and ethanol (Table 8). Furthermore, the IAD values with a 5% decrease could be referred to as very close to those procured by a 5% increase for every parameter of the model (Table 8).

The findings indicated that a 5% rise in biomass, ethanol, and sugar was associated with a positive effect of the ED on these parameters at the end of fermentation. In spite of that, a 5% decrease in the model coefficients led to a reduction in the ED and ID for biomass, ethanol, and sugar in the fermenting solution, indicating an undesirable influence on biomass, ethanol, and sugar (Table 8). Furthermore, the IAD values with a 5% decrease in Table 8 were vitally about those obtained by a 5% increase in each model parameter.

3.8. Economic Analysis for Ethanol Production

A brief financial practicability of fermentative creation in a medium was performed by computing the financial performance and financial efficiency using data from experimental and anticipated ethanol formation. Sources of carbon, nitrogen, and minerals were basic cost inputs. Consequently, these cost parameters were evaluated within the context of financial viability. The costs of the components used in the medium and of the used reference ethanol were ascertained from Sigma-Aldrich (St. Louis, MO, USA) and are indicated in Table 9.

Table 9. Overheads of nutrients exploited in the fermentation broth.

Components	Cost_per_Gram_in_Euro	Concentration_Gram_per_Liter
Acetic acid	0.0336	2.5
Formic acid	0.0233	0.5
Phenol	0.147	0.5
Glucose	0.0604	50
Yeast extract	0.22304	6
Ammonium sulphate	0.0934	4
Magnesium sulfate heptahydrate	0.0556	1.5
Calcium chloride dihydrate	0.0596	1
Potassium dihydrogen phosphate	0.0562	0.3

The tentative and forecasted economic yield and efficiency values have significant importance in the election of medium formulation for budget-friendly ethanol fabrication. As demonstrated by the results for AA, the medium demonstrated economic returns of EUR 0.246 and 0.251 of ethanol/EUR of the nutrients for both tentative and forecasted financial outcomes at 36 and 48 h of fermentation, respectively. The medium exhibited an economic return of EUR 0.009 and 0.009 of ethanol/EUR of nutrients.h at 24 h of fermentation, respectively. When Figure 7a is analyzed, the economic return curve acquired for the medium was equivalent to its tentative and forecasted ethanol fabrication curve. Consequently, the optimal economic return value of EUR 0.246 of ethanol/EUR of nutrients.h was obtained from the ethanol at 36 h of fermentation. Following Figure 7a, as the tentative and forecasted financial outcome for the medium throughout the time of the time-trend of the process was below EUR 1 of ethanol/EUR of nutrients, the medium exploited is not savings-conscious enough to generate ethanol in the medium.

According to the findings for FA, the fermentation medium demonstrated an economic output of EUR 0.24 and 0.236 of ethanol/EUR of nutrients for both experiential and presaged at 48 and 48 h of fermentation and economic productivities of EUR 0.008 and 0.008 of ethanol/EUR of nutrients.h for the experiential and presaged at 24 and 24 h of fermentation, respectively. When Figure 7b is analyzed, the economic output curve achieved for the brewing medium was equivalent to its experiential and presaged ethanol formation curves. Consequently, a peak economic output value of EUR 0.24 of ethanol/EUR of nutrients was achieved from the ethanol level at 48 h of fermentation. As per Figure 7b, on account of the experiential and presaged economic yields for the brewing broth through the temporal profile of fermentation being below EUR 1 of ethanol/EUR of nutrients, the brewing medium practiced is not efficient in creating ethanol in the brewing medium.

As indicated by the findings for P, the fermentation medium illustrated economic returns of EUR 0.252 and 0.248 of ethanol/EUR of nutrients for both investigational and presaged at 48 and 48 h of fermentation and economic productivities of EUR 0.01 and 0.01 of ethanol/EUR of nutrients.h for the investigational and presaged at 24 and 24 h of fermentation, respectively. When Figure 7c is reviewed, the economic return curve procured for the bioreactor solution corresponds to its investigational and presaged ethanol formation curve. Hence, the uppermost economic return value of EUR 0.252 of ethanol/EUR of nutrients was procured from the ethanol amount at 48 h of fermentation. In light of Figure 7c, as the investigational and presaged values of financial return from the bioreactor solution throughout the time of the time-behavior of fermentation were short of EUR 1 of ethanol/EUR of nutrients, the bioreactor solution exercised is not economical to create ethanol in the bioreactor solution.

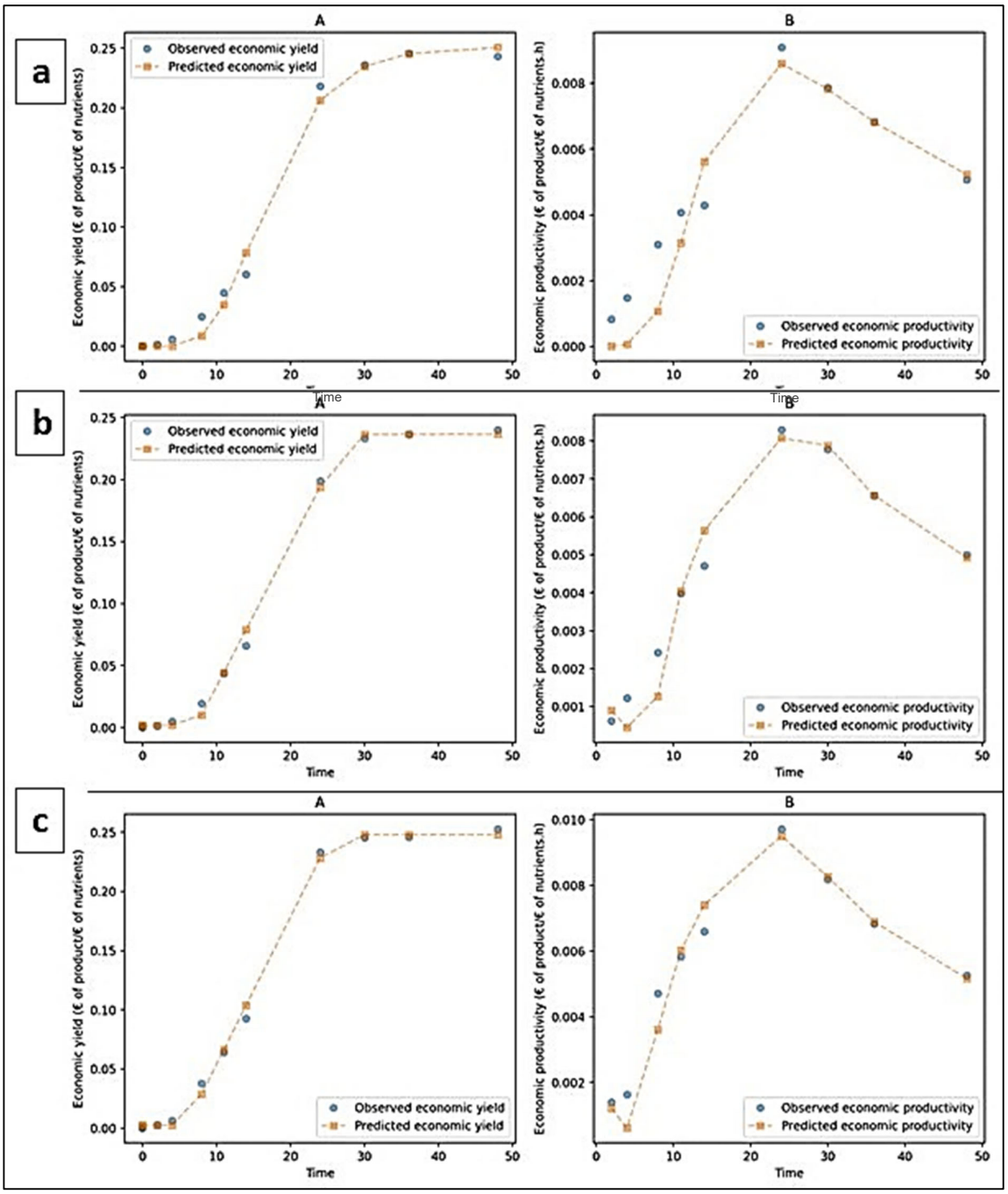


Figure 7. Dynamic profiles of financial performance and productive output of ethanol production in the fermentation substrate. (A) Financial performance and (B) productive output from the fermentation substrate ((**a**) for acetic acid, (**b**) for formic acid, (**c**) for phenol).

4. Conclusions

In this study, the impact of three inhibitor components, including the AA, FA, and phenol concentrations, on the tolerance of immobilized *S. cerevisiae* during ethanol production in a glucose-based medium was investigated. Subsequently, ten different mathematical models were applied to forecast the observed data of the fermentations using the rates of the best ethanol production, sugar consumption, and biomass. Based on the results, the inhibition was observed at AA concentrations of 7.5 g/L and above. Ethanol production using fermentation broth, including 1.5 g/L of FA, was prevented with the initiation of inhibition in the medium. As for the phenol, the inhibition started in the medium (added

5 g/L of phenol), and the same inhibition occurred in fermentations with the addition of 7 to 9 g/L of P. Regarding modeling, for ethanol production, the MGM model was chosen for fermentation with AA (2.5 g/L) (R^2 = 0.996), and the Huang model was detected as the suitable model for the fermentation with the addition of FA (0.5 g/L) (R^2 = 0.9996) and phenol (0.5 g/L) (R^2 = 0.9996). When this study was compared with the literature, it was seen that the immobilization process expanded the tolerance range and protected the cells. At the same time, it opens a positive door for studies in terms of time, cost, and energy with the determined tolerance ranges.

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References

1. Turhan, I.; Bialka, K.L.; Demirci, A.; Karhan, M. Ethanol production from carob extract by using *Saccharomyces cerevisiae*. *Bioresour. Technol.* **2010**, *101*, 5290–5296. [CrossRef] [PubMed]
2. Filho, J.S.O.; Santos, O.A.Q.; Rossi, C.Q.R.; Diniz, Y.V.F.G.; Fagundes, H.S.; Pinto, L.A.S.R.; Pereira, W.; Pereira, M.G. Assessing the effects of harvesting with and without burning and vinasse application in sugarcane crops: Evaluation of soil fertility and phosphorus pools in different ethanol production systems. *Agric. Ecosyst. Environ.* **2021**, *307*, 107233. [CrossRef]
3. Cardona, C.A.; Sanchez, O.J. Fuel ethanol production: Process design trends and integration opportunities. *Bioresour. Technol.* **2007**, *98*, 2415–2457. [CrossRef]
4. Fatehi, P. Recent advancements in various steps of ethanol, buta-nol, and isobutanol productions from woody materials. *Biotechnol. Prog.* **2013**, *29*, 297–310. [CrossRef] [PubMed]
5. Chatzimaliakas, P.F.; Koutsaftis-Fragkos, E.; Mai, S.; Malamis, D.; Barampouti, E.M. From Microalgae to Biofuels: Investigating Valorization Pathways Towards Biorefinery Integration. *Processes* **2024**, *12*, 2936. [CrossRef]
6. Adigüzel, A.O. Biyoetanolün genel özellikleri ve üretimi için gerekli hammadde kaynakları. *Bitlis Eren Üniversitesi Fen Bilim. Derg.* **2013**, *2*, 204–220.
7. Koga, N. An energy balance under a conventional crop rotation system in northern Japan: Perspectives on fuel ethanol production from sugar beet. *Agric. Ecosyst. Environ.* **2008**, *125*, 101–110. [CrossRef]
8. Erkan, S.B.; Coban, H.B.; Turhan, I. Evaluation of the inhibitory effect of 5-hydroxymethylfurfural (HMF) on ethanol fermentation by using immobilized *Saccharomyces cerevisiae* in stirred-tank bioreactor and mathematical modeling. *Fuel* **2022**, *317*, 123499. [CrossRef]
9. Menon, V.; Rao, M. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. *Prog. Energy Combust. Sci.* **2012**, *38*, 522–550.
10. Zhou, J.; Chen, L.; Zhang, R.; Zhao, W. Study on Oxidation Activity of Hydrogenated Biodiesel–Ethanol–Diesel Blends. *Processes* **2024**, *12*, 462. [CrossRef]
11. Jonsson, L.J.; Martin, C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* **2016**, *199*, 103–112. [CrossRef] [PubMed]
12. Almeida, J.R.M.; Modig, T.; Petersson, A.; Hahn-Hagerdal, B.; Liden, G.; Gorwa-Grauslund, M.F. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. *J. Chem. Technol. Biotechnol.* **2007**, *82*, 340–349. [CrossRef]
13. Klinke, H.B.; Thomsen, A.B.; Ahring, B.K. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl. Microbiol. Biotechnol.* **2004**, *66*, 10–26. [CrossRef] [PubMed]

14. Shinto, H.; Tashiro, Y.; Yamashita, M.; Kobayashi, G.; Sekiguchi, T.; Hanai, T.; Kuriya, Y.; Okamoto, M.; Sonomoto, K. Kinetic modeling and sensitivity analysis of acetone–butanol–ethanol production. *J. Biotechnol.* **2007**, *131*, 45–56. [CrossRef] [PubMed]
15. Germec, M.; Turhan, I. Kinetic modeling and sensitivity analysis of inulinase production in large-scale stirred tank bioreactor with sugar beet molasses-based medium. *Biochem. Eng. J.* **2021**, 176. [CrossRef]
16. Türker, M. (Ed.) Matematiksel modellemenin temel ilkeleri. In *Biyoreaksiyon Mühendisliği: Biyolojik Proseslerin Kinetiği ve Modellenmesi*; Su Vakfı Yayınları: İstanbul, Turkey, 2005; pp. 65–81.
17. Sablani, S.S.; Datta, A.K.; Rahman, M.S.; Mujumdar, A.S. *Handbook of Food and Bioprocess Modeling Techniques*; CRC Press: Boca Raton, FL, USA, 2006.
18. Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; van Riet, K. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875–1881. [CrossRef] [PubMed]
19. Stannard, C.J.; Williams, A.P.; Gibbs, P.A. Temperature/growth relationships for psychrotrophic food-spoilage bacteria. *Food Microbiol.* **1985**, *2*, 115–122. [CrossRef]
20. Weibull, W. A statistical distribution function of wide applicability. *J. Appl. Mech.* **1951**, *18*, 287–293. [CrossRef]
21. Morgan, P.H.; Mercer, L.P.; Flodin, N.W. General model for nutritional responses of higher organisms. *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 4327–4331. [CrossRef]
22. Dantigny, P.; Nanguy, S.P.; Judet-Correia, D.; Bensoussan, M. A new model for germination of fungi. *Int. J. Food Microbiol.* **2011**, *146*, 176–181. [CrossRef]
23. Baranyi, J.; Roberts, T.A. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* **1994**, *23*, 277–294. [CrossRef] [PubMed]
24. Huang, L.H. Optimization of a new mathematical model for bacterial growth. *Food Control* **2013**, *32*, 283–288. [CrossRef]
25. Fitzhugh, H.A., Jr. Analysis of growth curves and strategies for altering their shape. *J. Anim. Sci.* **1976**, *42*, 1036–1051. [CrossRef] [PubMed]
26. Lee, K.H.; Choi, I.S.; Kim, Y.G.; Yang, D.J.; Bae, H.J. Enhanced production of bioethanol and ultrastructural characteristics of reused *Saccharomyces cerevisiae* immobilized calcium alginate beads. *Bioresour. Technol.* **2011**, *102*, 8191–8198. [CrossRef] [PubMed]
27. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
28. Germec, M.; Ozcan, A.; Yilmazer, C.; Tas, N.; Onuk, Z.; Demirel, F. Ethanol fermentation from microwave-assisted acid pretreated raw materials by *Scheffersomyces stipitis*. *Agrolife Sci. J.* **2017**, *6*, 112–118.
29. Chai, T.; Draxler, R.R. Root mean square error (RMSE) or mean absolute error (MAE) arguments against avoiding RMSE in the literature. *Geosci. Model Dev.* **2014**, *7*, 1247–1250. [CrossRef]
30. Ross, T.; Dalgaard, P.; Tienungoon, S. Predictive modelling of the growth and survival of Listeria in fishery products. *Int. J. Food Microbiol.* **2000**, *62*, 231–245. [CrossRef] [PubMed]
31. de Souza Abud, A.K.; da Silva, A.D.S.; de Oliveira Junior, A.M. Effect of inhibitors on ethanol production by Pichia stipitis in a complex culture media. *Acta Sci. Technol.* **2017**, *39*, 223. [CrossRef]
32. Li, Y.C.; Gou, Z.X.; Zhang, Y.; Xia, Z.Y.; Tang, Y.Q.; Kida, K. Inhibitor tolerance of a recombinant flocculating industrial *Saccharomyces cerevisiae* strain during glucose and xylose co-fermentation. *Braz. J. Microbiol.* **2017**, *48*, 791–800. [CrossRef] [PubMed]

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