Received: 20 March 2024 | Revised: 7 August 2024 | Accepted: 3 September 2024

DOI: 10.1002/cjce.25504

RESEARCH ARTICLE

THE CANADIAN JOURNAL OF CHEMICAL ENGINEERING

Optimization and modelling of bioethanol production by the fermentation of CCN-51 cocoa mucilage using the *sequential simplex* method and the *modified Gompertz* model

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Abstract

This work deals with the optimization of bioethanol production through a fermentation process of CCN-51 cocoa mucilage, based on increased concentrations of the *Saccharomyces cerevisiae* yeast. Cocoa mucilage, considered biomass waste, was selected for its high productivity and the large volumes generated in the cocoa industrial chain in Ecuador. The optimization of the fermentation process was performed using the *sequential simplex* method with two variables, and the results were experimentally confirmed by quantifying bioethanol through the microdiffusion method. The best operational conditions corresponded to a temperature of 35°C and a pH of 4. Regarding the concentration of yeast, it was found that the optimal value was 8 g/L, since lower concentrations led to low productivities, while higher concentrations resulted in inadequate functioning of the bioreactor. The best results reached a productivity of 1.35 ± 0.04 g/L · h and a maximum bioethanol concentration of 28.3 ± 0.8 g/L for a processing time of 21 h. The production of bioethanol was modelled using the *modified Gompertz* equation and simulated in MATLAB®, yielding a bioethanol production rate of 2.42 g/L · h with a correlation coefficient (R^2) of 0.95. These results contribute to the knowledge of bioethanol production using cocoa mucilage and seek to add a positive value to this residue, whose management and final disposition have both undesirable environmental and economic effects.

KEYWORDS

bioethanol production, biomass fermentation, cocoa mucilage, *modified Gompertz* model, *sequential simplex* method

1 | INTRODUCTION

Currently, one of the primary environmental challenges arises from the utilization of fossil energy sources, the combustion of which substantially contributes to water, soil, and air pollution.[1] However, these energy sources have historically played a crucial role in the economic advancement of nations and regions. In 2018, fossil fuels accounted for 81% of global energy production, with oil contributing 31%, coal 27%, and natural gas 23%.[2]

On the other hand, countries that are highly dependent on fossil fuels are energetically vulnerable due to their limited capacity to supply themselves with energy indefinitely. This is the case of Ecuador, an oil-producing country with proven reserves of 8.3 billion barrels, which would supply exclusively for a period of 20 years.[2] Therefore, there is a pressing need to search for alternative energy sources that enable a transition towards a sustainable model. In this sense, one of the most attractive and promising options is biofuels, although their massive use in the transportation sector has slowed down due to controversies about their sustainability, technological advancements, and the lack of government policies that support their development and participation in the supply of secondary energy sources.[1,3]

Among the most commonly used liquid biofuels are biodiesel and bioethanol. When blended with other fuels, they enhance the original octane rating of the pure fuel, decrease pollutant emissions, and seamlessly integrate into fuel distribution logistics systems.[4] In the transport sector, bioethanol is the most important biofuel, holding 70.5% of the total shares and with 142.6 billion litres produced in 2019.[5] The United States and Brazil are the major bioethanol producers, accounting for 84% of the total production worldwide.[5] Traditionally, this biofuel is produced from several biomasses, such as sugars, starches, and lignocellulosic residues.[6]

In this context, one of the main raw materials used for the production of bioethanol is sugarcane. For example, in Ecuador, around 79 million litres of bioethanol were produced from sugarcane in 2022.[7,8] This fact is directly linked to the strategic plan of the Ecuadorian government to use fuels with a mixture of 95% gasoline and 5% ethanol in the automotive sector.[9] However, the use of this raw material raises important concerns, such as the massive use of agricultural land and an ethical challenge derived from competition between the energy and food industries. Therefore, waste streams from the agricultural industry should be evaluated as promising materials to produce biofuels through responsible and environmentally friendly practices.[6]

During cocoa processing, mucilage is one of the main by-products generated, with approximately 0.39 kg of mucilage obtained per kg of cocoa beans. Thus, in 2019 alone, around 110,635 tons of this waste were generated.[10] Frequently, a part of this pulp is used to ferment the beans that will produce chocolate, but between 5% and 7% is discarded.[10] In this context, around 150 L of mucilage per ton of wet cocoa beans are discarded.[11] Therefore, it is essential to find novel routes to take advantage of this stream rather than discarding it.[12,13]

Cocoa mucilage is mainly composed of sugars, acids, and pectin.[14] Therefore, it could be used to produce bioethanol through microbial fermentations. The main operating variables that influence the fermentation process are temperature, pH, and yeast concentration.[15] Thus, the optimization of these variables is essential to maximize the amount of ethanol produced and the productivity of the process. In this sense, the *sequential simplex* method has been widely used to optimize variables in fermentation and purification processes.[16] Furthermore, due to the growing interest in industrial scale-up of fermentation processes, mathematical modelling is a vital tool for predicting and reducing process costs.[17]

From this perspective, the aim of this study was to evaluate the valorization of cocoa mucilage to produce bioethanol using *Saccharomyces cerevisiae*. In this context, the *sequential simplex* method was used to optimize the fermentation process, determining the suitable operating conditions for bioethanol production. Finally, this process was modelled using the *modified Gompertz* model in order to predict and control the process.

Therefore, the main contribution of this study is the application of the *sequential simplex* method in cocoa mucilage fermentation systems. This method acts as a decision-making tool, reducing uncertainty and offering solutions that minimize experimentation costs. Additionally, bioethanol production was modelled using the *modified Gompertz* equation to predict and control the process.

2 | MATERIALS AND METHODS

2.1 | Optimization of the fermentation process

To optimize the fermentation process, the *sequential simplex* method was used, as its successive application results in a continuous approach to the local optimum, leading to improved performance in the production process. This method is represented by a closed and convex figure made up of $(k + 1)$ points in a k-dimensional space. In the present study, fermentation temperature and yeast concentration were selected as variables to optimize due to their relevance in the fermentative processes. Therefore, when $k = 2$, the *sequential simplex* method is represented by a triangle (Figure 1).

The optimization process was carried out using Minitab® software. Table 1 shows the parameters for the construction of the initial *sequential simplex*. Each pair of temperature (X_1) and yeast concentration (X_2) values represents a point on a two-dimensional plane where the *sequential simplex* method will be displayed. Three pairs of coordinates (each pair represented by the second subscript) will constitute the *simplex* figure.

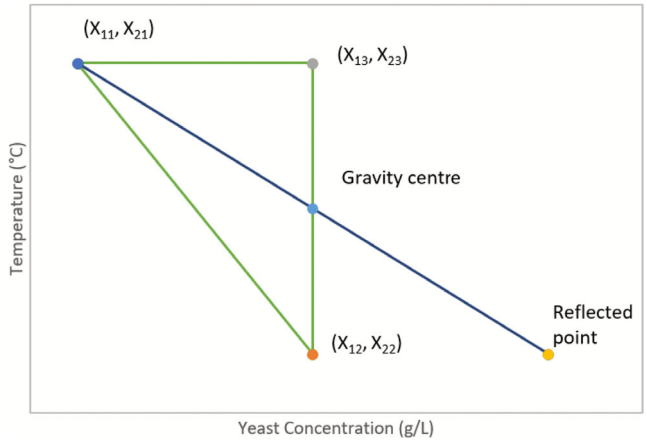

FIGURE 1 Optimization triangle of the *sequential simplex* methodology.

TABLE 1 Initial *sequential simplex.*

	Temperature (°C)	Yeast concentration (g/L)	Maximum concentration of bioethanol (g/L)
Run	X_1	X_2	Y_1
1	X_{11}	X_{21}	Y_1
2	X_{12}	X_{22}	Y_2
3	X_{13}	X_{23}	Y_3

2.2 | Cocoa mucilage

In this study, cocoa mucilage of the variety CCN-51 (Castro Naranjal Collection) was used due to its high productivity and disease tolerance properties. The cocoa was collected in Zone 6 of Ecuador (provinces of Azuay, Cañar, and Morona Santiago). Subsequently, the samples were pretreated as described by Delgado-Noboa et al.[18] Briefly, the cocoa was cut, immediately softened, and fluidized to separate the mucilaginous pulp from the rest of its components. Consequently, the samples were pasteurized (88°C, 5 min) and frozen (−18°C) until their use in the fermentation experiments.

2.3 | Fermentation

The fermentation experiments were carried out in a 2 L batch bioreactor (*Biotron GX Single* Vessel) at 35°C, pH 4, and stirring at 250 rpm, which ensures a perfectly mixed reactor and contributes to managing substrate viscosity. The fermentation volume was 80% of the total capacity of the bioreactor. For all fermentation experiments, cocoa mucilage was used without the addition of any nutrients, as this residue has a composition rich in sugars, acids, and other micronutrients that support the microbial fermentation process.[19] The initial concentration of soluble solids in the fermentation liquor was 17° Brix, corresponding to a total carbohydrate concentration of 231 ± 3 g/L. During the fermentation experiments, samples were collected periodically for subsequent analysis.

In this study, *S. cerevisiae* (commercial bakery yeast) was used because it is a model strain for ethanol production and has a wide range of applications in both research and industrial settings. *S. cerevisiae* is frequently used as a fermenting agent due to its resilience in environments with high ethanol and sugar concentrations, as well as its tolerance to low pH levels. Additionally, this yeast was selected for its robustness, high performance, and bioethanol productivity. Consequently, *S. cerevisiae* is widely used in biofuel biosynthesis.[20–22] In this context, *S. cerevisiae* yeast from the Levapan brand was used as a bioethanol-producing microorganism. This yeast was activated in the cocoa mucilage itself to establish the lag phase.

2.4 | Analytical methods

The bioethanol concentration was determined using the microdiffusion method.[23] To this end, a closed chamber known as a Conway cell was used, which allows for the determination of substances susceptible to volatilization.[24] This chamber consists of two compartments in which chemical equilibrium is reached after a certain period. Ethanol is placed in the first compartment, which, due to its high vapour pressure and test temperature, volatilizes, heading towards the second compartment. Once it reaches the second compartment, the oxidation of ethanol to acetic acid occurs due to the presence of a mixture of potassium dichromate in sulphuric acid. Subsequently, the unreacted potassium dichromate is quantified by the release of iodine in the presence of potassium iodide. The released iodine is titrated with sodium thiosulphate, using starch as an indicator.

For the quantification of sugars, the phenol–sulphuric acid test was used. This method allows for the determination and quantification of various sugars, including polysaccharides, oligosaccharides, monosaccharides, and their derivatives. In this test, the colour intensity is directly related to the concentration of total carbohydrates. The assay was performed in triplicate by measuring the absorbance at a wavelength of 490 nm using a Thermo Scientific UV–Visible Genesys 180 spectrophotometer.[25] The calibration curves were previously established using D-(+)-glucose as a standard.

Biomass quantification was performed by lyophilization followed by gravimetric determination. Specifically, the samples from the fermentation experiments were centrifuged at 4000 rpm for 15 min to discard the

supernatant, and the pellet containing the biomass was stored in liquid nitrogen at −190°C to avoid degradation reactions. Once the experimental process was completed, the samples were lyophilized in an Armfield FT 33 lyophilizer for 48 h, with the first 24 h spent freezing and the last 24 h spent drying. Finally, cell weights were determined gravimetrically.[26]

2.5 | Productivity of the fermentative process

The productivity of the fermentation process was defined as the ratio of the maximum value of bioethanol concentration to its corresponding fermentation time, according to Equation (1).[27]

$$\text{Productivity} = \frac{\text{Maximum bioethanol concentration}}{\text{Time to achieve maximum bioethanol concentration}} = \frac{\left[\frac{g}{L}\right]}{[h]} \quad (1)$$

2.6 | Modelling of the bioethanol production

To model bioethanol production, the *modified Gompertz* equation was used (Equation (2)). Thus, to obtain the maximum bioethanol production rate (r_{pm}) and the correlation coefficient between the model results and the experimentally obtained results, the Levenberg–Marquardt nonlinear least squares method routine programmed in MATLAB® was used.

$$P = P_{max} * exp\left\{-\exp\left[\left(\frac{r_{pm} * \exp(1)}{P_{max}}\right) * (t_L - t) + 1\right]\right\} \quad (2)$$

where P is the bioethanol concentration at time t (g/L); P_{max} is the maximum concentration of bioethanol (g/L); r_{pm} is the maximum bioethanol production rate (g/L · h); t_L is the lag phase (h); and t is the fermentation time (h).

3 | RESULTS AND DISCUSSION

3.1 | Optimization through the *sequential simplex* method

In order to optimize the fermentation process through the *sequential simplex* method, the R_1, R_2, and R_3 values (see Table 2) were selected from previous experiments conducted by our research group.

During the optimization process, it is essential to consider the operating limits of the variables to be studied. In this context, the temperature was established to be below 40°C. This decision was driven by the potential inhibitory effects that elevated temperatures can exert on microorganisms, which may lead to decreases in the performance of the bioprocess.[28] Thus, this value was in line with what was described by Lip et al.[29] in their study on the continuous growth of industrial *S. cerevisiae* strains at sub- and supra-optimal temperatures. In fact, the authors suggest the need to control this parameter, considering that both the production of new cells and ethanol are exothermic reactions. On the other hand, the variation in the yeast concentration was established between 3 and 20 g/L. These limits agree with those reported by Vázquez,[30] who found that an increase above these limits does not lead to significant improvements in bioethanol production.

The results of the theoretical points (reflected points) obtained using the *sequential simplex* method are presented in Table 3. Thus, R_i ($_i$ >3) represents the new point obtained with the temperature and yeast concentration data (X_{1i}; X_{2i}) and i the successive iteration along the optimization process. The response variable Y_1 represents the concentration of bioethanol, which is the variable expected to be maximized.

As can be deduced from Table 3, the application of the *sequential simplex* method made it possible to obtain the theoretical values for the maximum concentration of bioethanol under certain operating conditions. In this context, there was an increasing trend in the concentration of bioethanol with the increase in both temperature and yeast concentration. Thus, the maximum concentration of bioethanol was 39.61 g/L when using a temperature of 37.1°C and a yeast concentration of 20 g/L. This optimization method has been used in different studies to produce bioethanol from biomass waste. For instance, Singh et al.[31] used this method to evaluate the performance of *Sorghum durra* as a raw material to produce bioethanol,

TABLE 2 Initial values of the parameters selected for the *sequential simplex* method.

Run	Temperature (°C)	Yeast concentration (g/L)	Maximum concentration of bioethanol (g/L)
R_i	X_1	X_2	Y_1
1	35.0	3	25.41
2	35.1	5	26.80
3	35.5	6	27.70

with 40°C being the optimal temperature value for enzymatic saccharification in bioethanol production. Likewise, Cardoso et al.[32] applied the *sequential simplex* method to optimize the production of ethanol from palm fruits. In this sense, a yeast concentration of 5.7 g/L allowed the production of 66.8% alcohol under optimal conditions, with an experimental determination coefficient (R^2) of 0.97. On the other hand, Caqueret et al.[33] utilized the *sequential simplex* method to optimize the operating conditions in the purification of bioethanol from sugar beets by distillation. In their work, both the temperature and ethanol/vinasse (w/w) ratio of the precipitation were optimized, obtaining values of 21°C and 4.6, respectively, which allowed the separation of 52.4% of the dry matter of vinasse. Finally, Aboytes-Ojeda et al.[34] developed a hybrid method to minimize the cost of the biomass supply chain for biofuel production, validating the proposal with a real-world scenario.

In general, the *sequential simplex* method is appropriate when little noise is detected especially in cases with few factors, as in this study. Hence, it is unsurprising that this method is employed in laboratory-scale equipment to optimize configurations, particularly when the number of factors remains limited.[35] Nevertheless, when it comes to real applications at an experimental level, it is essential to consider the operational limits of the equipment.

TABLE 3 Evolution of values for the variables involved in the *sequential simplex* method.

Reflected point	Temperature (°C)	Yeast concentration (g/L)	Maximum concentration of bioethanol (g/L)
R_i[a]	X_1	X_2	Y_1
4	35.6	8	29.49
5	36.0	10	31.23
6	36.1	12	32.99
7	36.5	14	34.65
8	36.6	16	36.36
9	37.0	18	37.94
10	37.1	20	39.61

[a]R_i is the new point generated by the sequential simplex method with the variable required in a new optimization experiment.

3.2 | Fermentative process

The productivity of the fermentative process was calculated using the maximum value of bioethanol concentration and the time required to reach it (t_{max}), according to Equation (1). Both parameters were obtained experimentally. Table 4 shows the values of these parameters for experiments carried out at 35°C and a pH of 4.

As can be seen in Table 4, the experiments with a yeast concentration of 8 g/L reached a maximum bioethanol concentration of 28.3 ± 0.8 g/L. This value was 9% lower than the maximum bioethanol concentration achieved (31.2 ± 1.4 g/L) when a yeast concentration of 14 g/L was used. However, the maximum concentration of bioethanol was reached in 21 h with a yeast concentration of 8 g/L, compared to the 25 h required for the yeast concentration of 14 g/L. This fact led to a maximum productivity value of 1.35 ± 0.04 g/L · h when the yeast concentration was 8 g/L. This maximum value is fundamental from the point of view of the biotechnological application of the process. Thus, lower yeast concentrations trigger lower productivity in the process, likely stemming from an initially low cell concentration that decelerates the bioprocess and diminishes bioethanol yield. Conversely, cell concentrations exceeding 8 g/L also exhibited decreased productivities, possibly due to the increased demand for essential micronutrients.

The resulting response surface (Figure 2) illustrates the effect of yeast concentration on both maximum bioethanol concentration and process productivity. This graph, therefore, outlines the optimization of the

TABLE 4 Experimental results of the effect of yeast concentration on the maximum bioethanol concentration, time to maximum bioethanol concentration, and productivity of bioethanol in fermentative processes of CCN-51 cocoa mucilage.

Yeast concentration (g/L)	Maximum bioethanol concentration (g/L)	Time for maximum bioethanol concentration (h)	Productivity (g/L · h)
3	24.9 ± 0.1	36	0.69 ± 0.01
5	25.4 ± 0.7	24	1.06 ± 0.03
6	26.4 ± 0.3	23	1.14 ± 0.01
8	**28.3 ± 0.8**	**21**	**1.35 ± 0.04**
10	25.9 ± 0.1	21	1.23 ± 0.01
12	28.4 ± 0.3	24	1.18 ± 0.01
14	31.2 ± 1.4	25	1.24 ± 0.06

variables in the cocoa mucilage fermentation process. In this context, Shafaei et al.[36] used a response surface to demonstrate the effects of independent variables on bioethanol concentration and yield response. Another study conducted by Maturano et al.[37] examined how temperature, yeast concentration, and time factors affect ethanol concentration. Therefore, identifying the optimal combination of these two parameters can improve bioethanol productivity.[38]

The values achieved in the present study were comparable to those previously obtained by other researchers. For instance, Chang et al.[39] obtained a bioethanol concentration of 48.7 g/L after 30 h of fermentation with an ethanol productivity of 1.62 g/L · h when they fermented glucose solutions. The slight differences compared to our results could probably stem from the type of yeast strain used[40] and due to the existence of a risk of inhibition when thick liquor from residual biomass is used.[41] On the other hand, Phukoetphim et al.[42] in their study of ethanol production from sweet sorghum juice, reached a maximum bioethanol concentration of 54 g/L (36 h) and a productivity of 1.8 g/L · h. These observed differences are probably linked to the initial substrate concentrations, since in the research by Phukoetphim et al.,[42] the initial substrate presented a concentration of 160 g/L of total sugar. In this regard, it is interesting to highlight that excessive sugar concentrations generate stress due to osmotic pressure, which results in growth restriction and decreased viability of the yeast. Additionally, this could increase the production of byproducts, including glycerol.[43]

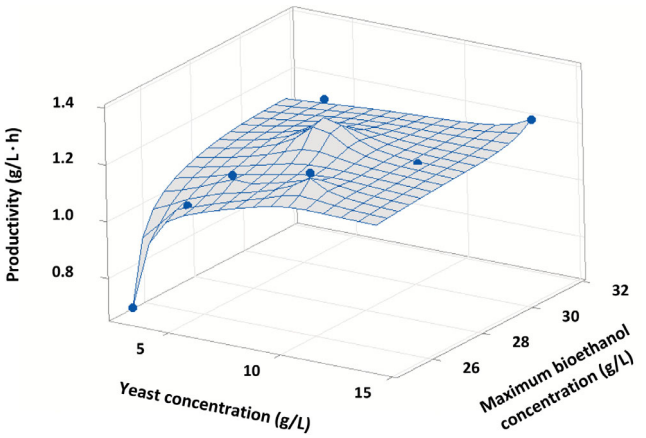

FIGURE 2 Effect of yeast concentration on bioethanol production in fermentative processes of CCN-51 cocoa mucilage.

Figure 3A shows substrate consumption, biomass concentration, and bioethanol concentration for the previously optimized conditions (yeast concentration of 8 g/L). Thus, a maximum level of both bioethanol and biomass concentration was observed at a similar time point (21 h). From this moment onward, despite the substrate concentration continuing to decrease, the bioethanol concentration decreased slightly until the end of the experiment. This phenomenon is likely associated with an inhibition of yeast cell viability or a decrease in the specific fermentation rate. In fact, it was observed that, after 21 h of fermentation, cellular growth slowed down, likely due to product inhibition. In this context, Joannis-Cassan et al.[43] suggested that inhibition depends on various factors, including parameters such as temperature, substrate concentration, and product concentration. Additionally, it may also be attributed to batch fermentation, as indicated by Fan et al.,[44] who suggested that batch fermentations do not allow for extending the lifespan of the culture, reaching maximum viable cell concentration, or achieving a higher fermentation product concentration.

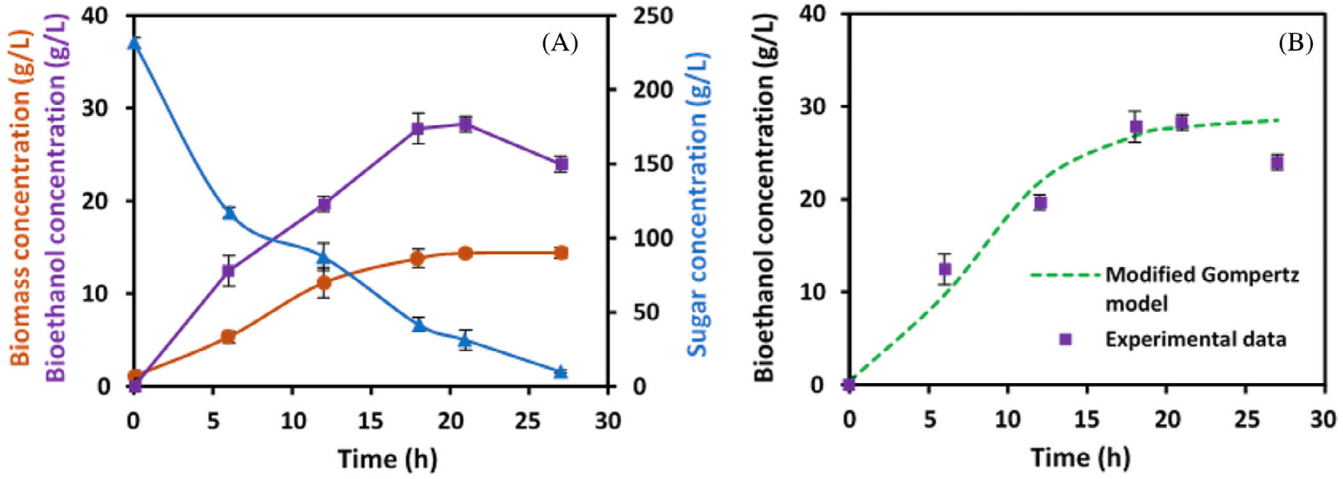

FIGURE 3 (A) Evolution of the CCN-51 cocoa mucilage fermentation process under optimal conditions and (B) comparison between experimental concentrations of bioethanol and predicted values by the *modified Gompertz* model.

Therefore, an alternative to mitigate these consequences could be the use of a fed-batch fermentation strategy.

3.3 | Kinetic modelling

Kinetic modelling is considered one of the crucial steps in the assessment of the fermentation processes for subsequent large-scale applications. This modelling evaluates the effect of variations in operating conditions, leading to improvements in bioprocess performance, productivity, and the reduction of undesirable byproducts, ultimately reducing costs and improving product quality.[45] In this sense, the experimental results for bioethanol production from CCN-51 cocoa mucilage during batch fermentation were fitted to the *modified Gompertz* kinetic model. Thus, Figure 3B shows the comparison between the experimental data and the results of the parametric adjustment to the *modified Gompertz* model.

The results showed that an optimal yeast concentration of 8 g/L enabled the achievement of a bioethanol concentration slightly higher than that observed in our previous study, as well as a significantly higher process productivity.[18] A short lag phase of 2 h was observed for bioethanol production, which coincided with the lag phase of yeast cell growth, suggesting that 2 h was required for yeast cells to adapt to the culture medium and initiate ethanol biosynthesis. These findings were consistent with other experimental systems previously reported by Phukoetphim et al.,[42] who noticed a 2 h lag phase in ethanol production when fermenting sweet sorghum juice with an initial sugar concentration of 160 g/L. On the other hand, a maximum bioethanol concentration of 28.3 ± 0.8 g/L was observed after 21 h of fermentation (Figure 3B), which was associated with the efficient metabolization of the substrate by yeast cells. These values were similar to those reported by Chohan et al.[46] when they studied the valorization of potato skin waste to produce bioethanol through simultaneous saccharification and fermentation. Specifically, in their research, a maximum bioethanol concentration of 23.5 g/L was reached after 16 h of fermentation. Interestingly Chohan et al.[46] reported an immediate decrease in the concentration of bioethanol, probably due to the depletion of the substrate and the oxidation of ethanol for the formation of organic acids.

The experimental data fit the *modified Gompertz* model (Figure 3B). The value of the correlation coefficient between the experimental results and the kinetic modelling results was $R^2 = 0.95$, indicating a high correlation. This fact suggests that the *modified Gompertz* equation is capable of adequately describing the production of bioethanol from CCN-51 cocoa mucilage. In this context, the *modified Gompertz* model was an ideal tool to support and estimate the kinetic behaviour of the fermentation process in terms of the maximum concentration of bioethanol, its maximum production rate, and the reaction time, demonstrating its effectiveness in optimizing the bioenergy conversion process.

Table 5 shows the main kinetic parameters obtained in our research, which are compared with those reported by other researchers in previous works. Thus, the maximum concentration of bioethanol (P_{max}) was 28.51 g/L, which aligns with findings from previous studies on the kinetic modelling of bioethanol production from potato peel waste (15.48 g/L)[46] and sorghum leaves (17.15 g/L).[47] However, when the production of bioethanol from sweet sorghum juice was studied, a P_{max} of 88.48 g/L was obtained, which is significantly higher than that obtained in our study. This is probably because sweet sorghum juice was used, which had a sugar concentration of 240 g/L. Furthermore, in their study, the yeast

TABLE 5 Comparison of the kinetic parameters of bioethanol production using the *modified Gompertz* model in relation to previous research.

Substrate	P_{max} (g/L)	r_{pm} (g/L · h)	t_L (h)	Reference
Mead	8.50	0.27	1.98	García et al.[25]
Sugar beet raw juice	73.31	4.39	1.04	Dodić et al.[41]
Sweet sorghum juice	88.48	1.82	2.98	Phukoetphim et al.[42]
Corn cobs	42.24	2.39	1.98	Sukai and Kana[45]
Potato peel waste	15.48	1.51	4.66	Chohan et al.[46]
Sorghum leaves	17.15	0.52	6.31	Rorke and Gueguim[47]
Manihot glaziovii starch	87.47	1.84	2.94	Sebayang et al.[48]
Oil palm frond juice	3.79	0.08	0.77	Srimachai et al.[49]
Cocoa mucilage waste	68.50	2.03	18.56	Ayala et al.[50]
CCN-51 cocoa mucilage	28.51	2.42	2.00	This study

Abbreviations: P_{max}, maximum concentration of bioethanol (g/L); r_{pm}, maximum bioethanol production rate (g/L · h); t_L, lag phase (h).

S. cerevisiae NP-01 was used. On the other hand, it is important to highlight that although sweet sorghum juice, sugar beet raw juice, and *Manihot glaziovii* starch allowed for high P_{max} values (>70 g/L), these substrates threaten food security since they generate competition between the food and energy industries. This fact further enhances the feasibility of cocoa mucilage as a sustainable substrate for the bioethanol industry.

Regarding the value of process productivity (r_{pm}), a value of 2.42 g/L · h was obtained when CCN-51 cocoa mucilage was used as a substrate for *S. cerevisiae*. Although Dodić et al.[41] obtained an r_{pm} value of 4.39 g/L · h when they used sugar beet raw juice, values of 2.39 and 1.84 g/L · h were observed using corn cobs[45] and *Manihot glaziovii* starch,[48] respectively, which were similar to the values obtained in this study. The lag time (t_L) observed in this work was 2 h, comparable to values reported in studies involving corn cobs,[45] sweet sorghum juice,[42] and *Manihot glaziovii* starch.[48] Remarkably, the t_L observed in this study was even lower than those documented for potato peel waste[46] and sorghum leaves,[47] underscoring the swift adaptation of *S. cerevisiae* to the cultivation conditions, which benefits the application of the biotechnological process.

4 | CONCLUSIONS

The present study evaluated the production of bioethanol from CCN-51 cocoa mucilage through fermentation with *S. cerevisiae*. Accordingly, the operating variables were optimized using the *sequential simplex* method. It was found that a temperature of 35°C and a yeast concentration of 8 g/L allowed for obtaining a bioethanol concentration of 28.3 ± 0.8 g/L in a fermentation time of 21 h. Furthermore, under these conditions, a maximum process productivity of 1.35 ± 0.04 g/L · h was reached, which was significantly higher than that observed in experiments with yeast concentrations either higher or lower than 8 g/L. Subsequently, bioethanol production was modelled in MATLAB® using the *modified Gompertz* kinetic model, resulting in an r_{pm} of 2.42 g/L · h with a correlation coefficient of 0.95.

These results demonstrate that CCN-51 cocoa mucilage is a highly promising substrate for bioethanol production, without the need to supplement any micronutrients. In this sense, the present study developed a route for the sustainable use of cocoa biomass residues, successfully applying the *modified Gompertz* model for fermentation with *S. cerevisiae*, enabling the prediction and control of the fermentative system. Therefore, an encouraging strategy is proposed to generate value from this waste, addressing the serious environmental and health effects associated with its use and final disposal.

AUTHOR CONTRIBUTIONS

Jorge Delgado: Conceptualization; funding acquisition; validation; supervision; resources; formal analysis; visualization; project administration. **Andrea Serpa:** Investigation; writing – original draft; methodology; data curation. **Juan F. Moreno:** Writing – review and editing; methodology; data curation; conceptualization; validation; formal analysis. **Tamara Bernal:** Conceptualization; methodology; validation; writing – review and editing; formal analysis; data curation; software. **Fausto Posso:** Conceptualization; visualization; validation; writing – review and editing; formal analysis. **Oscar Tenesaca:** Conceptualization; writing – original draft; methodology; investigation; software; data curation; resources.

ACKNOWLEDGEMENTS

The authors thank the University of Cuenca (Ecuador) and its Faculty of Chemical Sciences for their support of equipment and materials.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/cjce.25504.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: J. Delgado, A. Serpa, J. F. Moreno, T. Bernal, F. Posso, O. Tenesaca, *Can. J. Chem. Eng.* **2024**, 1. https://doi.org/10.1002/cjce.25504