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Research Article

Comparison and Mathematical Description of Ethanol Fermentation by Unconventional Substrates; Sweet Sorghum Juice, Hydrolysed from Sorghum Bagasse and Cane Molasses

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Abstract

Currently the production of biofuels has taken great strength due to the increase in fossil fuel prices as well as a reduction in the world reserves, for this reason the demand for renewable fuels has risen, as is the case of bioethanol, which is of great importance. However, in most countries, its production is restricted by the use of raw materials that are used in food, as in the case of the US and Brazil, who use corn and sugar cane for their production, however, in countries such as Mexico this is not possible due to the high demand of these raw materials as food, for this reason the use of unconventional raw materials which provide the same amounts of fermentable sugars as sugarcane, as well as the use of high performance yeasts are important cases of study for the production of them. In this research is carried out the analysis of three fermentation media using unconventional raw materials such as sweet sorghum juice, sorghum bagasse hydrolysate and sugarcane molasses to study the kinetics of the yeast *S. cerevisiae* using the models of Levenspiel and Aiba.

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Tools as Successive Quadratic Programming (SQP) and Active set re used to determinate the kinetic parameters. The approximations to Levenspiel's models presented a correlation coefficient of 0.57 to 0.974 with the use of SQP algorithms, while other algorithms such as Active-set showed values of 0.631 to 0.881 in sweet sorghum juice.

Abbreviations (if used)

μ_{max} Maximum specific growth rate (h^{-1})
q_{max} Maximum specific ethanol production rate (h^{-1}).
X Biomass or Total cell concentration (g/L)
S Substrate concentration (g/L)
P_E Ethanol concentration (g/L)
K_s Monod constant for growth (g/L)
K_{SP} Ethanol saturation constant (g/L)
K_{IS} Substrate inhibition constant (g/L)
K_P Ethanol inhibition constant for growth (g/L)
K_{P2} Ethanol inhibition constant for ethanol fermentation (g/L)
P_{xmax} Maximum ethanol concentration for growth (g/L).
P_{Emax} maximum ethanol concentration for ethanol fermentation (g/L).
$Y_{x/s}$ Biomass yield (g/g)
$Y_{p/s}$ Ethanol yield (g/g)
R^2 Correlation coefficient
$y_{(i\ obs)}$ Actual data
$y_{(i\ calc)}$ Predicted data

Introduction

Currently, the use of renewable fuels is very important due to the decrease in fossil-fuel reserves and pollution caused by the use of non-renewable sources [1,2]. One of the options that is occupying about 90% of the market of biofuels worldwide is bioethanol, which can be produced from renewable sources, as is the case of sources rich in sugars, starches or cellulose [3-5].

An important point in the subject of biofuels is the raw materials for the production of these renewable fuels, specifically for bioethanol, which is one of the most produced biofuels worldwide with a production of 120 billion liters during the year 2016 [6]. In the U.S., the first raw material used for the production of bioethanol is the corn with a production of 379,435,000 barrels [7]. In Brazil for the production of more than 24,460 million liters during 2017 [8] the main raw material is the sugarcane However, in Mexico there is a problem regarding to the use of these raw materials, since they are very important for food, their use as raw materials for the production of bioethanol represents great problems. For the specific case of corn, there is a law for the promotion and development of bioenergy that states "Grant prior permits for the production of bioenergetics from corn grain in its various forms, which will be granted only when there are surplus inventories of domestic corn production to satisfy national consumption".

For this reason, its use is unfeasible, for this and other reasons is very important the use of alternative raw materials that provide the sugars needed for the production of bioethanol.

Among the different alternatives of raw materials to produce ethanol, the sweet sorghum is reaching worldwide importance (*Sorghum bicolor* (L) Moench). It is defined as a promising bioenergetic crop, for its large production of green dough, and its tolerance to diseases, droughts and floods [9-11], as well as the use of industrial waste and by-products such as sorghum bagasse and molasses.

The kinetic parameters determine the behavior of microorganisms in different media and under specific circumstances [12]. These kinetic parameters associated with mathematical models can simulate the dynamics of microorganisms and, in many cases, with their use, it is possible to elucidate the optimum conditions of culture for the production of products of interest and value [13,14].

Usually, the mathematical optimization is used to obtain the best solutions to a specific problem or a model in an efficient way, reducing with this the high costs of experimentation and modification of the system, as well as avoiding the loss of time to carry out these changes experimentally [15]. Optimization techniques not only show results of modifications in the system but also achieve system adjustments to established functions [16]. The objective of this study is to determine the kinetic parameters of the mathematical models form fermentations carried out on three different substrates (sorghum juice, sorghum and molasses bagasse hydrolysate), based on their experimental data, in order to compare the different substrates from their affinity and productivity with the use of yeast *S. cerevisiae* ITV-01. The kinetic parameters were obtained from two different models such as the Monod model modified by Levenspiel and an exponential model proposed by Aiba through the use of MATLAB® optimization functions.

Materials and Methods

Microorganism

The strain used in this study was *S cerevisiae* ITV-01, which was isolated from sugar cane molasses by Ortiz-Zamora [17] at the Bioengineering Laboratory at the Technological Institute of Veracruz.

Inoculum Preparation and Fermentation Media

For the activation of *S cerevisiae* ITV-01, a synthetic medium consisting of 20g/L of glucose, 1g/L of yeast extract, 2g/L of ammonium sulfate, 5g/L of potassium phosphate monobasic was used and 0.4g/L of magnesium sulfate. The medium used for fermentation were sorghum juice 10-15° Brix, hydrolyzed bagasse sorghum 9-16° Brix and, sugar cane molasses 15-16° Brix; they were adjusted to 120g/L, 135g/L and 100g/L, respectively.

Hydrolyzed Bagasse Sorghum

The hydrolysis of sweet sorghum bagasse was carried out in three stages [18], acid hydrolysis at H_2SO_4 concentration of 1.5% (v/v) and a liquid-solid ratio (LSR) of 5:1.

Alkaline hydrolysis using a 4.5% (v/v) hydrogen peroxide (H_2O_2) solution in a time of 45h and a liquid-solid ratio (LSR) of 16:1 and, finally an enzymatic hydrolysis with Cellic C Tec3® enzyme in a 0.05M sodium acetate buffer solution, pH 4.8, with LSR of 5:1, for 51h, at 50°C with agitation of 200r.p.m.

Fermentative Kinetics

A pre-inoculum of 12 hours of incubation of *S cerevisiae* ITV-01 is propagated to obtain a number of $6x10^6$ viable cells, which served to inoculate $3x10^6$ viable cells per mL to 300mL each of the different means of fermentation. The flasks are placed in incubation at 30°C with shaking of 250rpm. Samples are taken from the culture media every 4 hours. A cell count is performed by Thoma camera and a sample taken for further analysis by HPLC.

Biomass Measurement

The measurement of biomass is carried out by means of a curve of cell count (cc) against dry cell weight (g/L), described by the following linear correlation (equation 1):

$$dry\ cell\ weight = 3.119 \times 10^{-8} * cc + 0.1487 \qquad (1)$$

Analysis of Substrate Consumption and Metabolites Produced Using a HPLC

The substrate consumption and the production of ethanol and other metabolites of the fermentation were analyzed by HPLC. The samples taken during the kinetics were subjected to a detoxification process with 125μL of BaO 0.3M and 125μL of $ZnSO_4$ 5% W/V, to precipitate the impurities that could cause damage to the HPLC column. Subsequently, the samples were filtered and placed in vials for analysis in the HPLC. The substrates consumed (glucose and fructose) and ethanol as the metabolite of greatest interest were identified and quantified. The runs were carried out in a time of 30min with a flow rate of 0.6mL/min at 55°C and the refractive index detector at 50°C, using a mobile phase of 5mM H_2SO_4 with a Shodex column SH1011.

Kinetic Models

The modified model of Levenspiel [13] was taken to simulate the fermentative kinetics performed, this model presents the effects of inhibition by product in addition to providing information on the relationship between growth (equation 2), production of metabolites (equation 3) and substrate consumption (equation 4). In both models the parameters of Maximum specific growth rate (μ_{max}), Maximum specific ethanol production rate (q_{max}), cell concentration (X), Substrate concentration (S), Ethanol concentration (P_E), Monod constant for growth (k_s), Ethanol saturation constant (k_{SP}), Substrate inhibition constant (K_{IS}), Ethanol inhibition constant for growth (k_p), Ethanol inhibition constant for ethanol fermentation (K_{p2}), Maximum ethanol concentration for growth (P_{xmax}), maximum ethanol concentration for ethanol fermentation (P_{Emax}), biomass yield ($Y_{x/s}$) and Ethanol yield ($Y_{p/s}$), are used in their majority.

$$\frac{dx}{dt} = \mu_{\max} * x * (\frac{s}{k_s + s}) * (1 - \frac{p}{p_{x\max}}) \quad (2)$$

$$\frac{dp}{dt} = q_{\max} * x * (\frac{s}{k_{sp} + s}) * (1 - \frac{p}{p_{p\max}}) \quad (3)$$

$$\frac{ds}{dt} = -\frac{1}{Y_{x/s}}(\frac{dx}{dt}) - \frac{1}{Y_{p/s}}(\frac{dp}{dt}) \quad (4)$$

Another model used in this study is the exponential inhibition model which shows an inhibition by the product and provides information about growth (equation 5), production of metabolites (equation 6) and substrate consumption (equation 7) [19].

$$\frac{dx}{dt} = \mu_{\max} * x * (\frac{s}{k_s + s}) * e^{-\frac{p}{k_{p1}}} \quad (5)$$

$$\frac{dp}{dt} = q_{\max} * x * (\frac{s}{k_{sp} + s}) * e^{-\frac{p}{k_{p2}}} \quad (6)$$

$$\frac{ds}{dt} = -\frac{1}{Y_{x/s}}(\frac{dx}{dt}) - \frac{1}{Y_{p/s}}(\frac{dp}{dt}) \quad (7)$$

Estimation of Kinetic Parameters by Optimization

The estimation of kinetic parameters through optimization was done with the 'fmincon' function of MATLAB®, which is an optimization function that searches the minimum of a non-linear multivariable function by means of different algorithms, in our case the algorithms were used "SQP" and "Active-set" (AS) for the estimation of these parameters [20-22].

Simulation of Fermentations

With the kinetic parameters obtained through optimization, the simulations were performed by the routine ODE45 of MATLAB®, which solves numerically differential equations as the case of the kinetic models of Levespiel and Aiba, with the initial values of biomass, substrates and products.

Correlation Coefficient (R^2)

The correlation coefficient (equation 8 and 9) is used to know the fit of the model to the experimental data, when the coefficient is close to the unit, the adjustment is acceptable.

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_{i\,obs} - y_{i\,cacl})^2}{\sum_{i=1}^{n} (y_{i\,obs} - y_{av})^2} \qquad (8)$$

$$y_{av} = \frac{1}{n} * \sum_{i=1}^{n} y_{i\,obs} \qquad (9)$$

Results

Fermentative Kinetics

Figure 1 shows the fermentation kinetics performed in sorghum juice, where it is observed that the substrate is consumed after 24 hours of fermentation, obtaining 54.73g/L of ethanol. At the same time, it is observed that the biomass showed an exponential growth with a maximum of biomass until 15h of 14.73g/L. The kinetic parameters were μ_{max} = 0.309 h^{-1}, $Y_{x/s}$ = 0.116 and $Y_{p/s}$ = 0.38. These kinetic parameters indicate a high performance in the production of ethanol by *S. cerevisiae* ITV-01 and an efficiency of 74.37%.

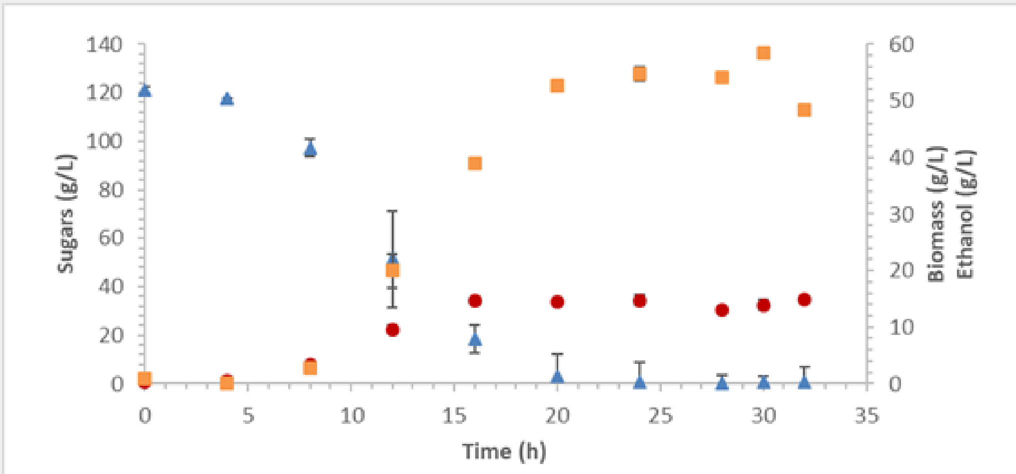

Figure 1: *Fermentation kinetics of S. cerevisiae ITV-01 in sweet sorghum juice; (▲) sugars (glucose and fructose), (●) Biomass and (■) Ethanol.*

In the fermentations made with sugarcane molasses (Figure 2), it is observed that the substrate is consumed in 26 hours, obtaining a greater production of ethanol (41.2g/L). As well as a higher biomass growth (7.915g/L) at 26 hours compared to the sorghum bagasse hydrolysate. The value of μ_{max} was $0.229h^{-1}$; the yields in biomass and product were 0.082 and 0.433 respectively. In this fermentation with cane molasses an efficiency of 84.7% was obtained in the conversion of the molasses to bioethanol after 14 hours of fermentation.

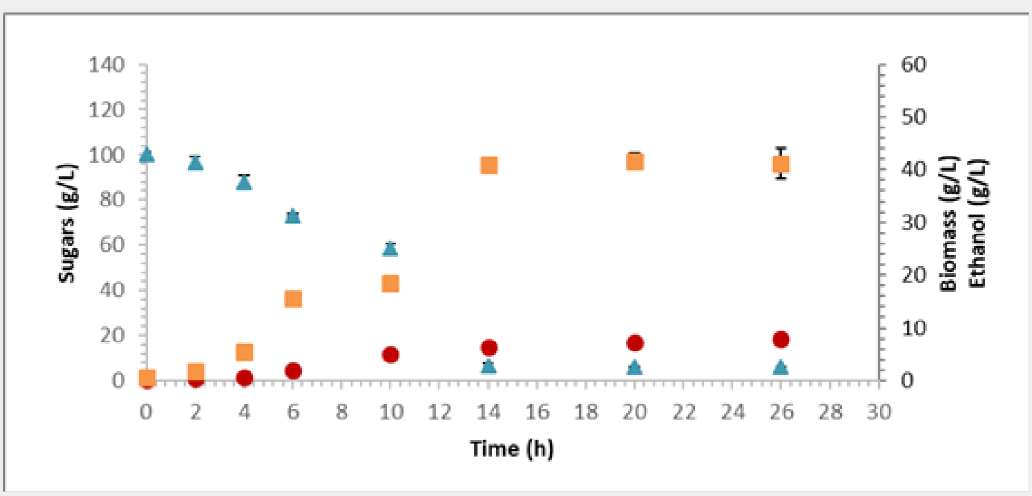

Figure 2: *Fermentation kinetics of S. cerevisiae ITV-01 in cane molasses; (▲) sugars (glucose and fructose), (●) Biomass and (■) Ethanol.*

With respect to the fermentation of *S. cerevisiae* ITV-01 with the hydrolysate of sorghum bagasse (Figure 3), the fermentation showed an efficiency of 81.5% of the bioconversion of the substrate to ethanol, obtaining 28.9g/L in 26 hours. The yeast consumed 55.6% of the total sugars obtaining a yield in product 0.417 and in biomass of 0.035. The maximum growth rate (μ_{max}) in these culture conditions was $0.136h^{-1}$. Comparing the three kinetics, we can observe that the sorghum bagasse hydrolysate is an unconventional substrate that produces lower yield in both ethanol and biomass production and the molasses is the best substrate for bioconversion to bioethanol, followed by juice of sorghum.

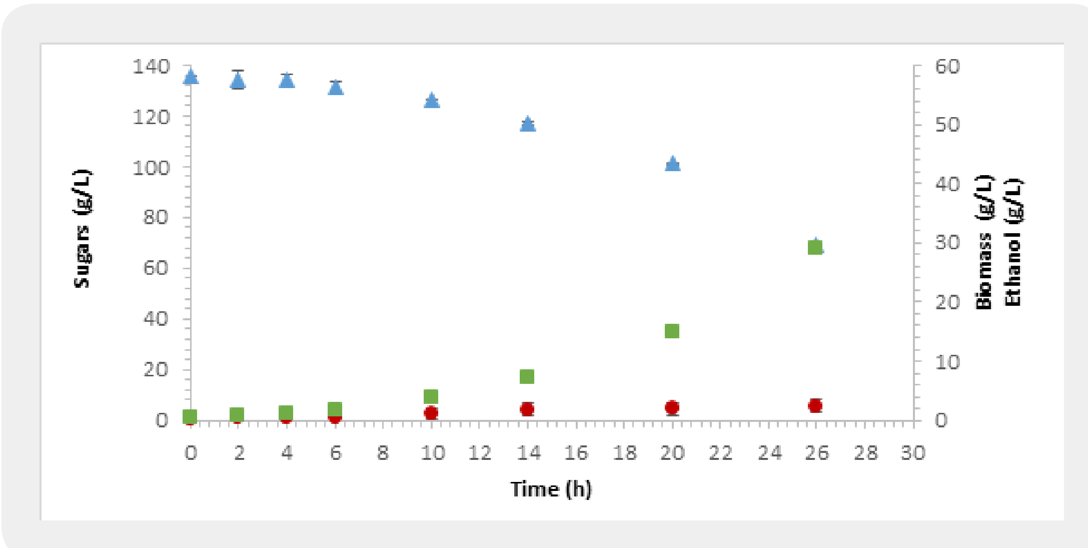

Figure 3: *Fermentation kinetics of S. cerevisiae ITV-01 with sorghum bagasse hydrolysate; (▲) sugars (glucose and fructose), (●) Biomass and (■) Ethanol.*

Figure 4 shows a comparison of the kinetic parameters of the fermentations for the production of ethanol by *S. cerevisiae* ITV-01, with the different substrates. In this figure, it is observed that the yields of ethanol produced with the hydrolysate of sorghum bagasse, molasses and sorghum juice are similar statistically ($p = 0.465$). This is due to the hydrolysate to which the substrate was not consumed in its entirety by the presence of inhibitors such as 5-hydroxymethylfurfural produced due to the acid-alkaline hydrolysis process used for the extraction of fermentable sugars. These compounds can reach levels to inhibit yeast growth and consequently ethanol yield [23,24], while with cane molasses higher yields are obtained due to the osmotolerance of yeast *S. cerevisiae* ITV-01.

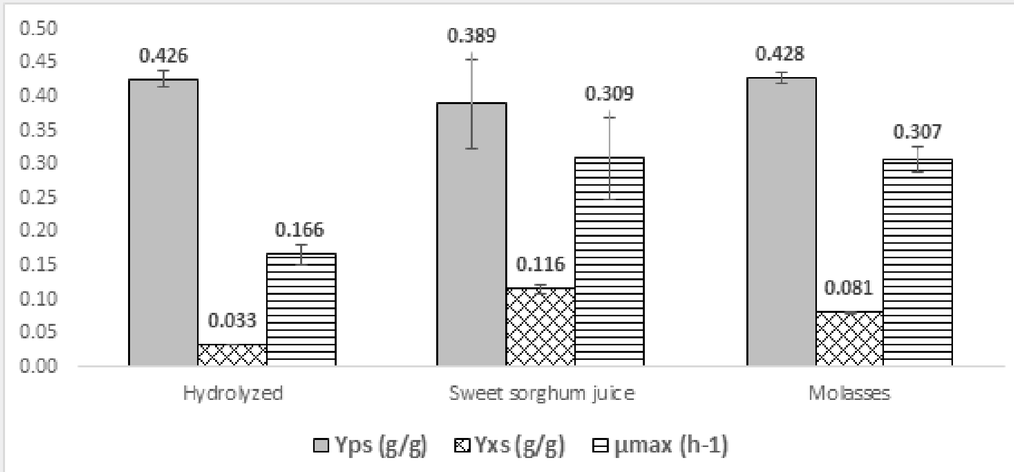

***Figure 4:** Comparison of the kinetic parameters of the different fermentations with S. cerevisiae ITV-01.*

Determining the Kinetic Parameters

Approaches were made to the Levenspiel and Aiba models by using the 'fmincon' function of MATLAB®, comparing the adjustment made for the algorithm "SQP" as for "Active-set", for each of the experiments, substrates or sugars (S) (Figure 5), product or ethanol (P) (Figure 6) and biomass (x) (Figure 7).

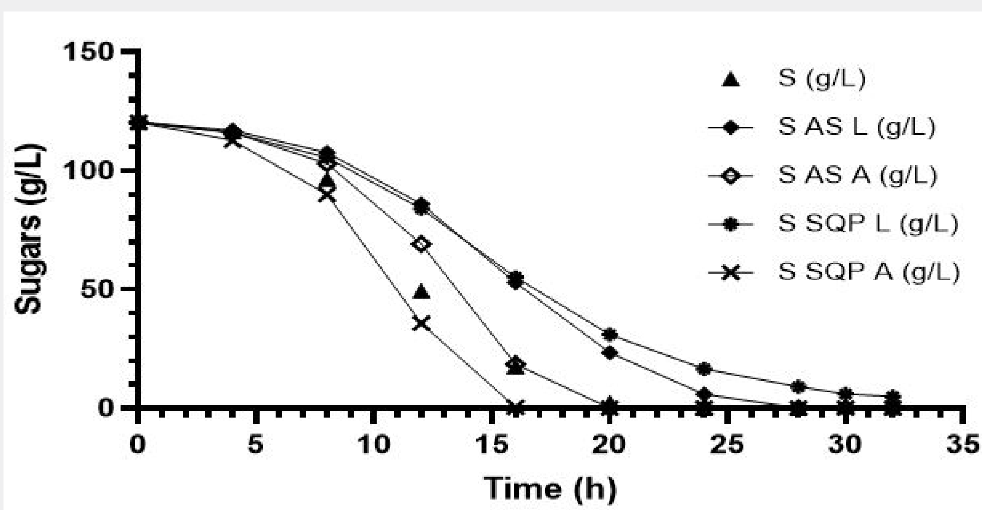

***Figure 5:** Comparison of fit of substrate consumption between models in sweet sorghum juice.*

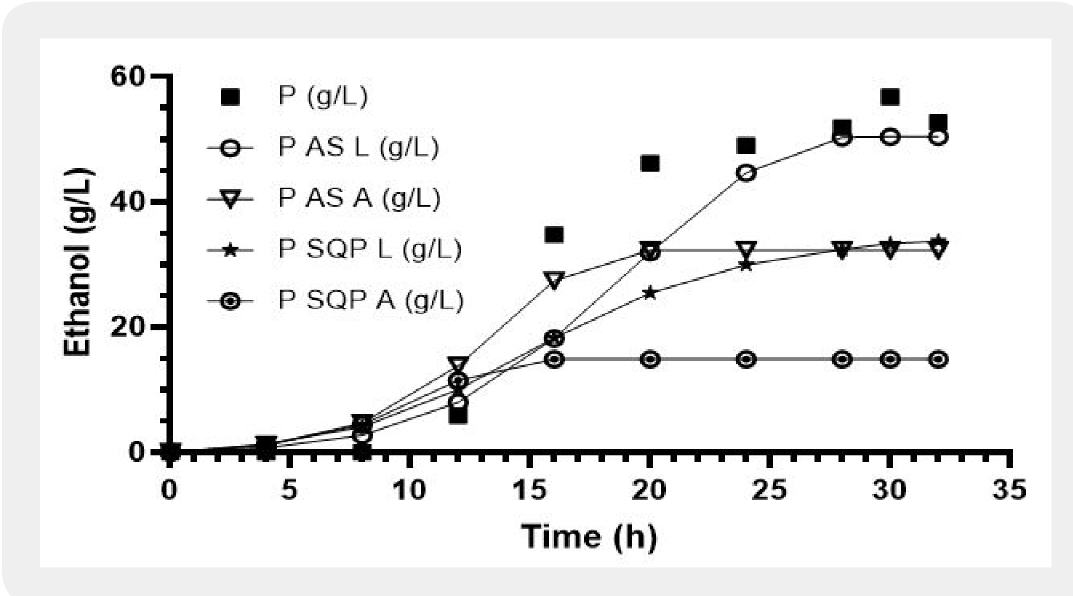

Figure 6: *Comparison of fit of product formation between models in sweet sorghum juice.*

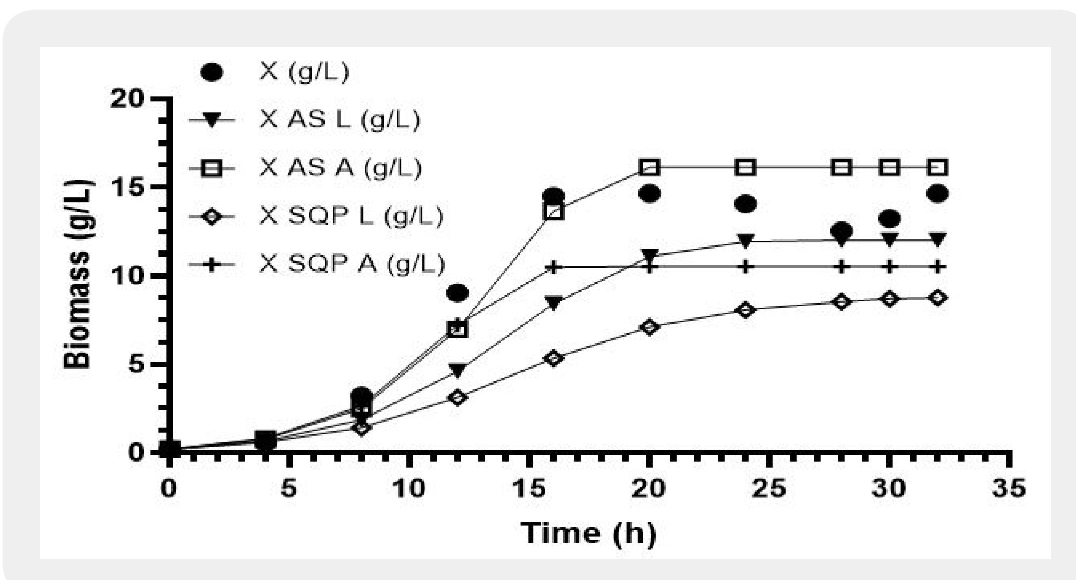

Figure 7: *Comparison of fit of biomass formation between models in sweet sorghum juice.*

You can observe the change in the fit by means of the application of different algorithms (Figure 8)

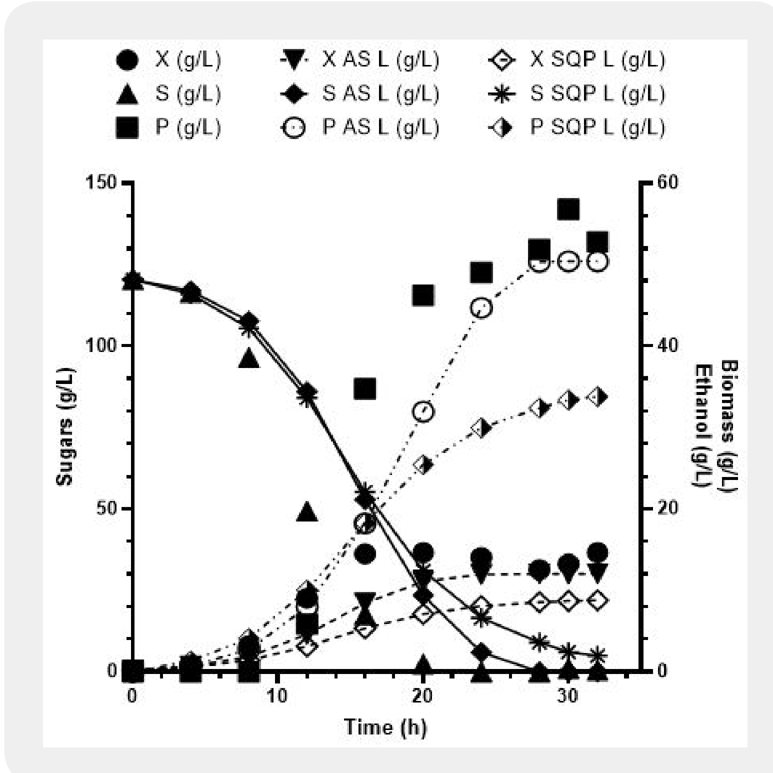

Figure 8: *Comparison of fit between algorithms sweet sorghum juice.*

In the case of sorghum juice, a good approximation was obtained by means of the Levenspiel model using an SQP algorithm that shows the highest correlation coefficients, however, the product is not well described, with coefficient of 0.57, followed by this the SQP-legacy which is a variant of SQP shows a nice correlation coefficient for the Aiba model (Table 1).

Table 1: *Correlation coefficients for each model and algorithm with sweet sorghum juice.*

R^2				
Algorithm	**Model**	**X (g/L)**	**S (g/L)**	**P (g/L)**
Active-set	Levenspiel	0.631	0.809	0.881
SQP	Levenspiel	0.845	0.974	0.570
Active-set	Aiba	-0.255	0.765	0.487
SQP	Aiba	0.660	0.968	-0.534
Interior-point	Levenspiel	0.007	0.315	-3.710
SQP-legacy	Levenspiel	-0.225	0.794	0.494
Interior-point	Aiba	0.148	0.753	0.126
SQP-legacy	Aiba	0.844	0.973	0.258

To know the value of the kinetic parameters it is necessary that the models conform to the experimental data and describe in this way the fermentative system, for this reason the fittest models were taken to know the kinetic parameters they describe (Table 2).

Table 2: *Calculated kinetic parameters for sweet sorghum juice.*

Parameters	In this study	Levenspiel active-set	Levenspiel sqp	Aiba sqp-legacy
μ_{max} (h^{-1})	0.309	0.951	0.400	0.392
Q_{max} (h^{-1})	1.308	0.449	1.130	1.000
Y_{xs} (g/g)	0.116	0.164	0.184	0.200
Y_{ps} (g/g)	0.380	1.041	0.490	0.490
P_{mx} (g/L)		65.029	40.000	40.000
P_{mp} (g/L)		131.578	42.025	40.000
k_s (g/L)		284.319	64.909	28.465
k_p (g/L)		5.000	5.000	13.818
k_{sp} (g/L)		1.739	36.039	49.397
k_{p2} (g/L)		10.000	10.000	80.000

In the case of molasses tests were carried out between the different models, concluding that the SQP algorithm and the Levenspiel and Aiba models had a very similar adjustment, in Biomass (x) (Figure 9), sugars (S) consumption (Figure 10) and ethanol (P) production (Figure 11).

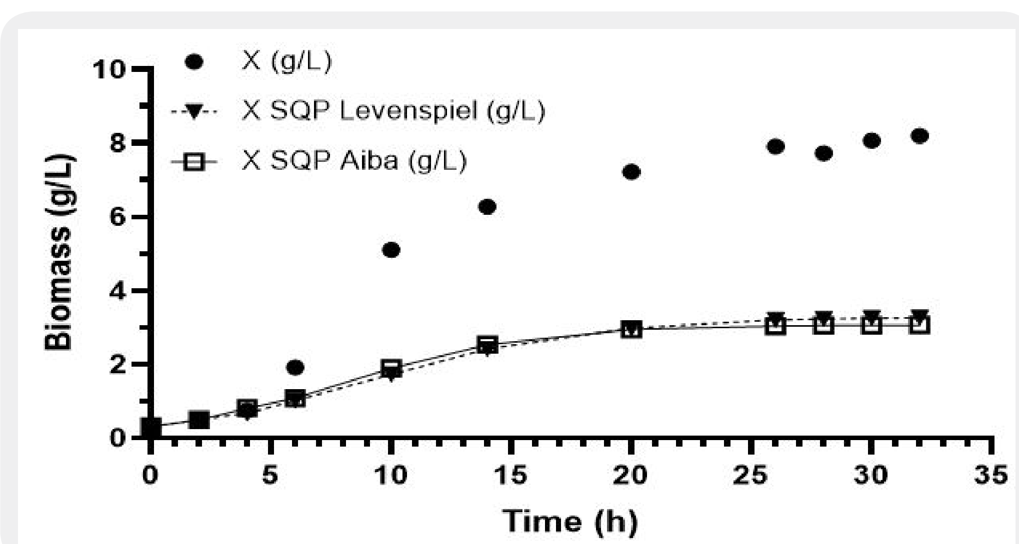

Figure 9: *Comparison of fit of biomass formation between algorithm in molasses.*

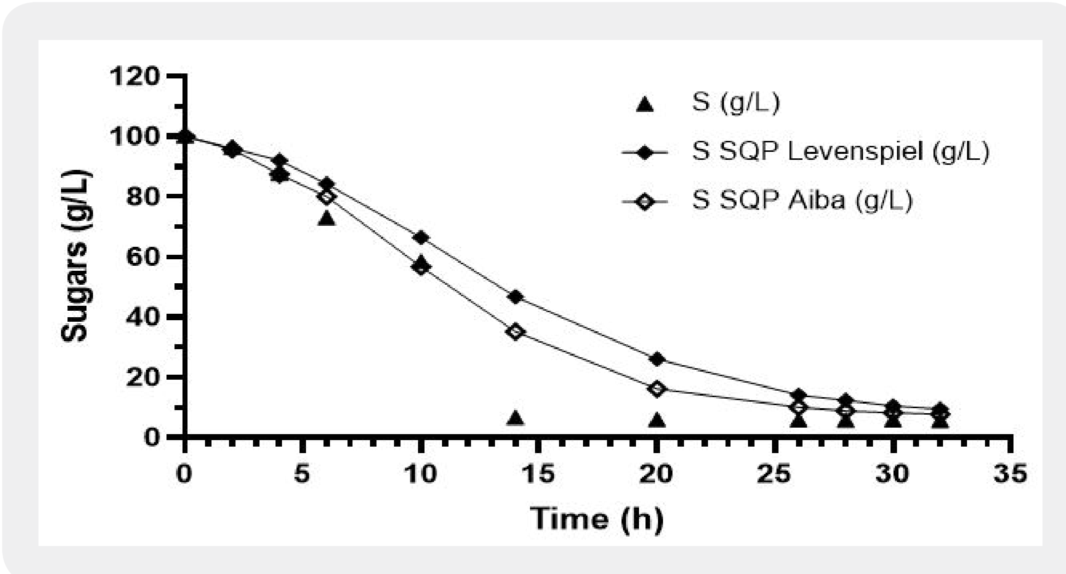

Figure 10: *Comparison of fit of sugars consumption between algorithm in molasses.*

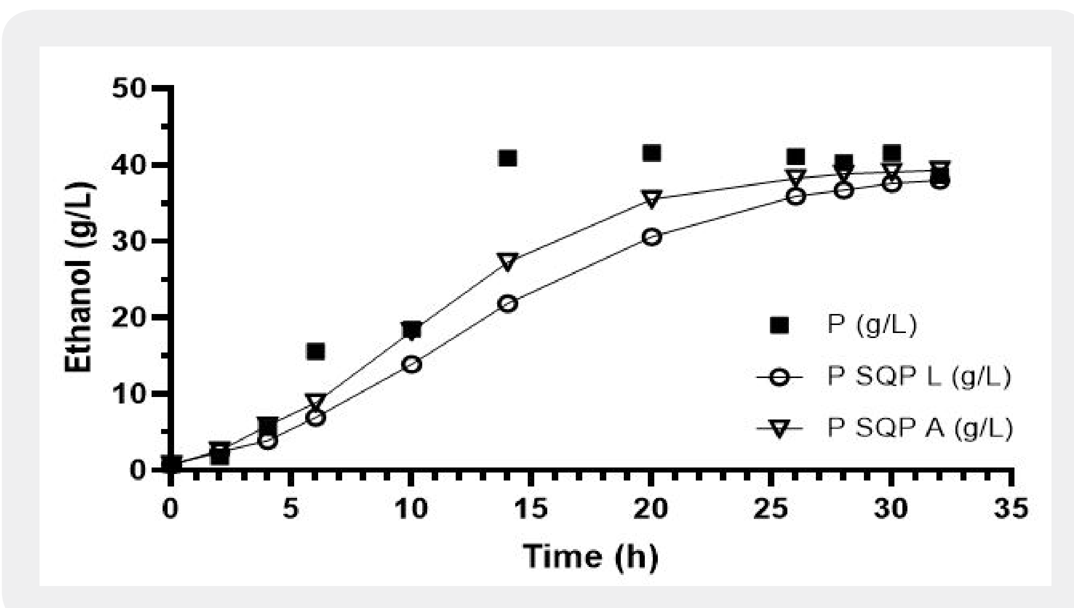

Figure 11: *Comparison of fit of ethanol formation between algorithm in molasses*

With this information, the simulation of fermentative kinetics was performed to obtain the value of the kinetic parameters (Table 3) and to determine the adjustment of the models to the experimental data by means of the correlation coefficient (Table 4).

Table 3: *Calculated kinetic parameters for molasse.*

Parameters	In this study	Levenspiel	Aiba
		sqp	sqp
μ_{max} (h^{-1})	0.307	0.400	0.368
Q_{max} (h^{-1})	0.310	1.773	2.454
Y_{xs} (g/g)	0.081	0.200	0.200
Y_{ps} (g/g)	0.427	0.490	0.490
P_{mx} (g/L)		40.000	40.000
P_{mp} (g/L)		40.000	40.000
k_s (g/L)		92.701	52.333
k_p (g/L)		5.000	80.000
k_{sp} (g/L)		1.000	1.000
k_{p2} (g/L)		10.000	10.000

Table 4: *Correlation coefficients for each model and algorithm with molasses.*

R^2				
Algorithm	**Model**	**B**	**S**	**P**
SQP	Levenspiel	-0.253	0.951	0.911
SQP	Aide	-0.425	0.921	0.874

For the sorghum bagasse hydrolysate tests were carried out between the different models, concluding that the SQP algorithm and the Levenspiel and Aiba models had a very similar adjustment, in Biomass (x) (Figure 12), sugars (S) consumption (Figure 13) and ethanol (P) production (Figure 14).

In the same way, the kinetics of the sorghum bagasse hydrolysate were analyzed, and its kinetic parameters were determined through optimization (Table 5)

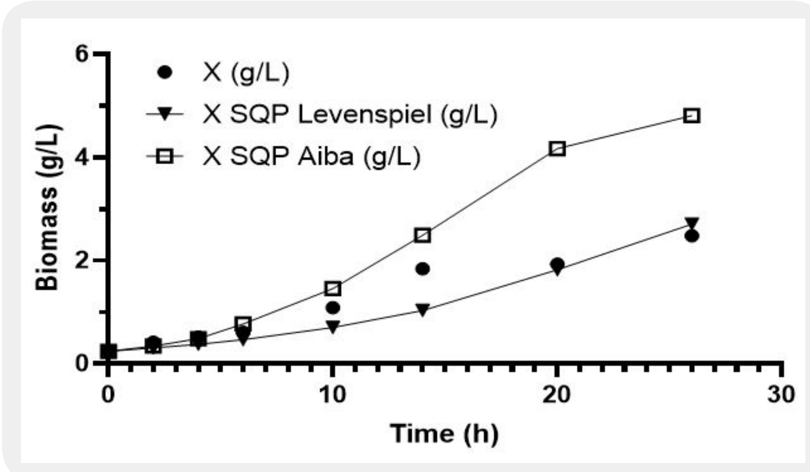

Figure 12: *Comparison of fit of biomass formation between algorithm in sorghum bagasse hydrolysate.*

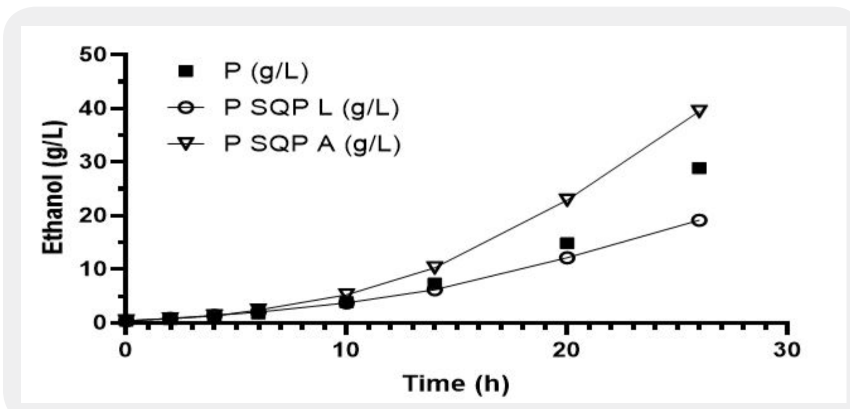

Figure 13: *Comparison of fit of sugars consumption between algorithm in sorghum bagasse hydrolysate.*

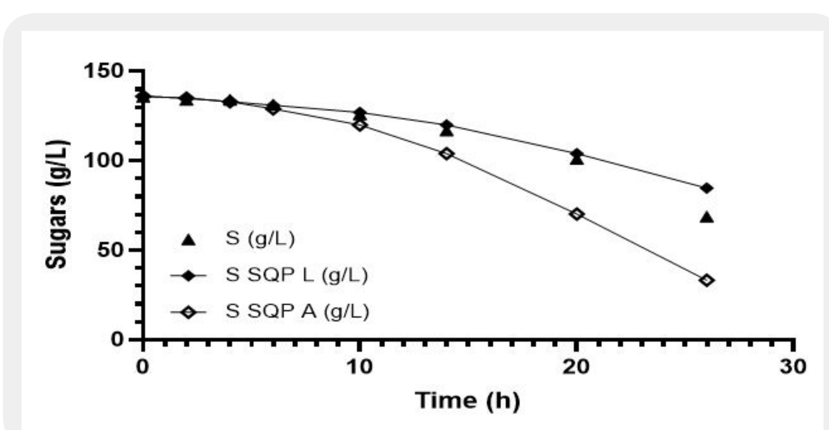

Figure 14: *Comparison of fit of ethanol formation between algorithm in sorghum bagasse hydrolysate.*

Table 5: *Calculated kinetic parameters for hydrolyzed*

Parameters	In this study	Levenspiel	exponencial
		sqp	sqp
μ_{max} (h^{-1})	0.166	0.137	0.400
Q_{max} (h^{-1})	0.310	0.833	0.686
Y_{xs} (g/g)	0.033	0.193	0.200
Y_{ps} (g/g)	0.425	0.490	0.490
P_{mx} (g/L)		46.335	40.000
P_{mp} (g/L)		48.501	300.000
k_s (g/L)		30.134	152.949
k_p (g/L)		5.000	9.915
k_{sp} (g/L)		3.317	1.000
k_{p2} (g/L)		10.000	10.000

Discussions

The production of ethanol by unconventional raw materials like sweet sorghum juice and industrial waste such as sorghum bagasse or by-sub products as sugarcane molasses, may be a better option for bioethanol production in Mexico [25,26].

The ethanol yields of sorghum hydrolysate, molasses and sorghum juice are very similar ($p = 0.46$), however, there is a significant difference between the yield in biomass for each of the media ($p = 0.0005$). The strain *S. cerevisiae* ITV-01 has great advantages over other commercial yeasts, since it is osmotolerant at high concentrations of sugar, which can resist up to 260g/L of sugar in the culture medium, without affecting the production of ethanol [17,26]. In this research the concentration of sugars ranging between 100 and 130g/L of sugars was studied for this reason models of inhibition were not handled by substrate because as mentioned this yeast is able to resist up to 260g/L of sugars.

The ethanol yields obtained in this study are similar to those presented by [26] who preserved a yield of 0.41g/g with an initial concentration of 150g/L of glucose while in our study we have a concentration of 120g/L of sugars (glucose and fructose) for a yield of 0.38g/g in sorghum juice.

The model that showed the best results for the representation of the experimental data was the Levenspiel's model in conjunction with the SQP algorithm, however there is a lack of adjustment that may be due to the inhibition by product not taken into account [27,28].

Obtaining of the kinetic parameters by means of mathematical optimization presents great advantages reducing the time of experimentation as well as reducing the costs, nevertheless, it is of great importance to take into account the great amount of factors that can affect in these determinations, as it is the case of inhibitions by substrate, by product or by other organisms present in the environment, all this can be similarly adjusted by a model, however the complexity of these models will grow as more factors are added [14,29].

Conclusions

As a conclusion we can see that the behavior of the yeast *S. cerevisiae* can be modeled by different models which adapt to the means where it is developed [13,30,31], however, a study is necessary on the factors that can affect its growth or the production of certain metabolites to simulate their behavior in these, nevertheless it is possible to opt for these parameters by mathematical means such as those used here [32,33].

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Conflicts of Interests

There is no conflict of interest between the authors. We all agree that the results of this work be published.

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