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## Optimization of Ethanol Production Process from Cassava Starch by Surface Response

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**Abstract.** This work shows the study of the optimization process for producing ethanol from cassava starch based on  $2^2$  experimental designs with three central points and using statistical software. This methodology was applied to the stage of saccharification of cassava starch by acid hydrolysis as well as to the stage of fermentation using *Saccharomyces cerevisiae*. From the experimental data of acid hydrolysis, we proposed a first-order kinetic model which presented an average error of 1.87 % compared to the quadratic regression obtained. The development of a semi-continuous process showed a 89.84 % conversion of starch initially considered, yielding an ethanol concentration of 49.76 % Alc/vol.

**Keywords:** Cassava starch, acid hydrolysis, yeast fermentation, response surface, ethanol.

**Resumen.** En este trabajo muestra el estudio del proceso optimización de obtención de etanol a partir del almidón de yuca, mediante el planteamiento de un diseño  $2^2$  con tres puntos centrales y usando un software estadístico para analizar los resultados. Esta metodología fue aplicada tanto para la etapa de sacarificación del almidón de yuca mediante hidrólisis ácida como para la etapa de fermentación empleando *Saccharomyces cerevisiae*. A partir de los datos experimentales de la hidrólisis ácida, se propone un modelo cinético de primer orden el cual presentó un error promedio del 1.87 % con respecto a la regresión cuadrática obtenida. El desarrollo de un proceso semi-continuo mostró una conversión del 89.84 % del almidón inicialmente considerado, obteniéndose una concentración final de etanol del 49.76 % Alc/vol.

**Palabras clave:** Almidón yuca, hidrólisis ácida, fermentación levadura, superficie de respuesta, etanol.

### Introduction

The search for new fuels, both from biological and renewable origin, biodegradable, capable of increasing the performance of automobile engines and the need to reduce emission of gases, have contributed to use anhydrous ethanol (AE) as fuel for commercial gasoline additive worldwide [1, 2].

In recent years, Mexico has considered necessary to do structural reforms that allow further development to face the needs of the energy sector. One energy source that is little mentioned in national projects and has demonstrated its feasibility in other regions of the world is the production of ethanol [3]. Ethanol can be used in mixtures with fuels for motor vehicles. It can increase the octane index; reducing it between 10 and 15% the CO. Ethanol can be mixed with unleaded gasoline between 10 to 25% without difficulty. Ethanol could therefore replace MTBE (methyl tert butyl ether), an oxygenated product used in Mexico since 1989, although it has reduced CO<sub>2</sub> emissions it has proved to be a groundwater pollutant [4] and has a carcinogenic effect [5, 6].

Ethanol can be produced from a large number of plants, with a variation, according to the agricultural product, of the yield between the fuel consumed and generated in this process. Among the raw materials there are the fruits and vegetables such as sugar cane and beets, cereals (wheat, corn, sorghum, etc), tubers (potatoes, cassava, etc) and in general, materials from lignocellulosic or organic residues.

The growing prosperity with the use of ethanol as an alternative to fossil fuels, has created that fermentation technology

must care for several variables involved in the production of ethanol from agro-industrial waste (biomass resource, microorganisms, types of enzymes, immobilization of the microorganism, simultaneous saccharification and fermentation and improved technology) to optimize the efficiency of the process [1, 2, 7, 8, 9, 10, 11, 12].

Cassava starch has several characteristics which favor its industrial use, in general, and in particular as a raw material in ethanol production. Some characteristics of cassava starch are its high purity, neutral flavor, easy swollen, solubility, development of high viscosity and low tendency to retrograde compared with other starches such as potato, rice and corn.

Different treatment and pretreatment to improve the split of cassava starch have been studied with the aim of significantly improve the overall ethanol yield, such as: ultrasonic pretreatment [13, 14], wet oxidation (WO) pretreatment [15], combined heat treatment and acid hydrolysis [16], and alkali steeping [17], inter alia.

Acid hydrolysis is one in which starch is split by a strong acid. The economic feasibility of this procedure depends mainly on low costs in raw materials, energy and operating and low investment costs. It has been studied [18] that the hydrolysis consists of three stages: a) degradation of lignocellulosic material to fermentable sugars, b) fermentation of sugars into ethanol and c) purification of ethanol.

The aim of this study was to optimize the acid hydrolysis of cassava starch, determining the kinetic model depending on the concentration of starch and the reaction time and their

subsequent fermentation of alcohol with *Saccharomyces cerevisiae*, based on  $2^2$  experimental designs with three central points and using statistical software to define the experimental area through a response surface.

## Results and Discussion

### Kinetic model of acid hydrolysis

Figure 1 shows the experimental results of starch degradation with respect to time. It is noted that the starch concentration decreases following the linear behavior, as demonstrated by the linear correlation obtained with adjustment coefficient of 0.9911 ( $R^2$ ).

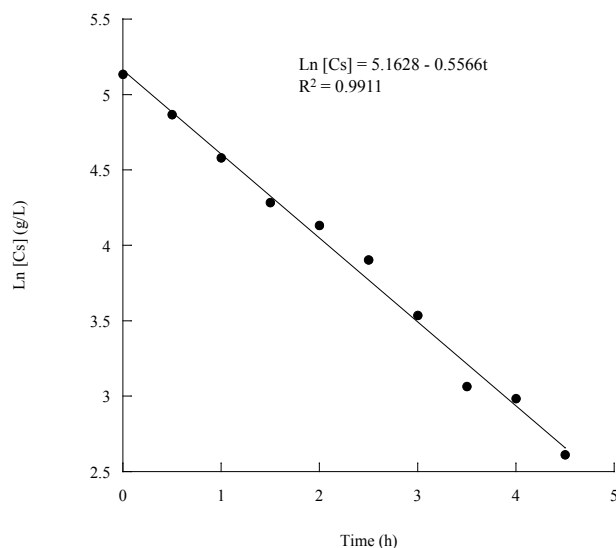
From the experimental data of starch concentration and using the equation 9, it was found an average value of  $k$  being obtained  $k_{\text{average}} = 0.542169 \text{ h}^{-1}$ , substituting this value and considering  $[Cs_i] = 170 \text{ g/L}$ , the resulting kinetic model was:

$$[Cs] = 170 e^{-0.542169t} \quad (1)$$

Performing the analysis at concentrations of 150 and 190 g/L, the average value of  $k$ 's were 0.523491 and 0.541425 respectively, whose standard deviation between the three values of  $k$  was 0.010575. The proposed model in equation 1 was employed in this study.

A second order regression was carried out using the value of starch concentration with respect to time in order to compare with the kinetic model and with the experimental data, obtaining:

$$[Cs] = 169.673 - 67.533 * t + 7.774 * t^2 \quad (2)$$



**Fig. 1.** Behavior of starch degradation caused by the effect of acid hydrolysis with  $H_2SO_4$ .

Table 1 shows the comparison between the kinetic model (equation 1) and the quadratic regression model (equation 2) on the average error and the adjustment coefficient ( $R^2$ ).

The standard deviation between the model and experimental data of cassava starch degradation was 4.12 % and the experimental data and the quadratic regression was 5.22 % (Figure 2). The most significant variation was founded as  $t = 0$  due to the independent term of the quadratic model with respect to the kinetic model and experimental data.

From the data obtained and considering an initial concentration of starch from 170 g/L was quantified the value of the reaction rate ( $r$ ) for different intervals of reaction (Table 2) being obtained as  $r_{\text{avg}} = 76.0318 \text{ g/h L}$  for this process.

Using the model established in equation 1, a simulation of the process was done, considering the concentrations used in the experimental design 150, 170 and 190 g/L. Figure 3 shows the dynamic behavior of the cassava starch degradation, according to the points in the experimental design.

It can be observed that starch degradation was significantly reduced after 4 h, being less than 2 % in the fifth hour. This allowed to establish that the processing time used in testing the experimental design is 4.5 h, due to the conversion was significantly reduced, impacting directly in a reduction of energy consumption.

### Optimization of acid hydrolysis process

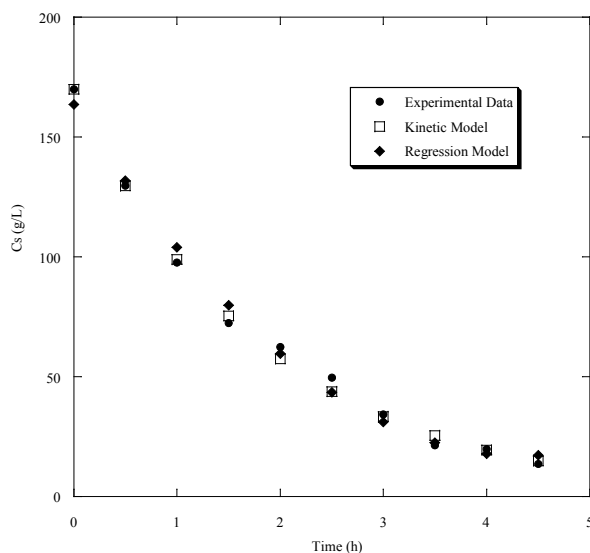
The results of experimental design were analyzed using the statistical software NCSS-2004 [19]. For the purpose of defining an experimental space it was determined the optimization trend by adjusting the second order of the results (equation 3), obtaining an adjustment of 99.09 %.

**Table 1.** Evaluation of average error and adjustment coefficient for different models.

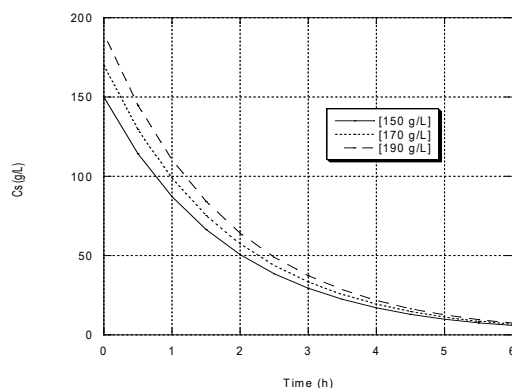
Model	Average Error	R2
Quadratic Regression	5.15 %	0.9955
Kinetic Model	3.28 %	0.9982

**Table 2.** Rate of reaction for acid hydrolysis function within a specific timeframe.

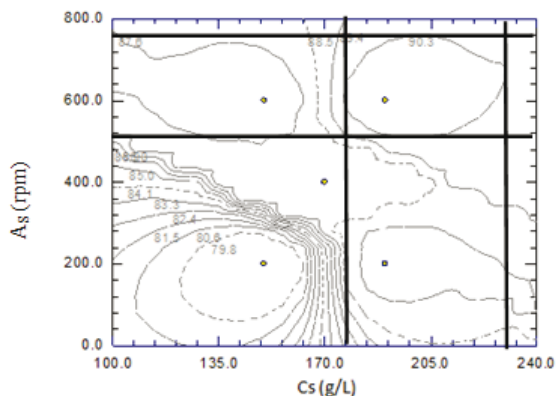
Time (h)	Rate of reaction g/h L
0.0	92.1687
0.5	70.3544
1.0	52.9345
1.5	39.2934
2.0	33.8388
2.5	26.9206
3.0	18.6223
3.5	11.6179
4.0	10.7329
4.5	7.3912



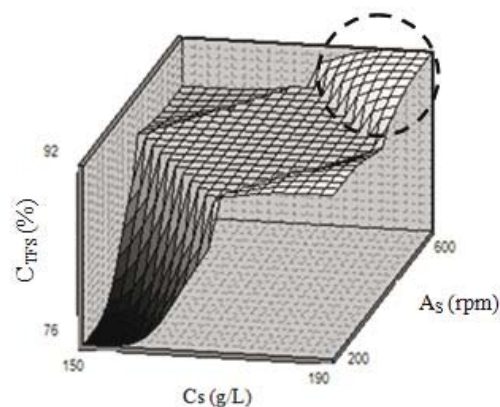
**Fig. 2.** Behavioral comparison of experimental data against kinetic (equation 1) and the regression models (shown on equation 2).



**Fig. 3.** Kinetic behavior of starch degradation at different initial concentrations.



(a)



(b)

**Fig. 4.** Behavior of acid hydrolysis process using the 22 experimental designs with 3 central points a) graphical contours showing the highest yield region, b) Surface Response showing a saddle point in the center and maximum conversion at the dotted region.

$$C_{TFS} = 10.879 + 0.426C_{S_i} + 0.121A_s - 2.02E - 0.4C_{S_i}^2 - 3.78E - 0.5A_s^2 - 4.66E - 0.4C_{S_i}A_s \quad (3)$$

The methodology of the response surface (RSM) was used in order to determine the optimal working region. The contour graph (Figure 4a), shows that the highest yield was obtained in the region from 177 to 233 g/L, and from 510 to 760 rpm. Figure 4b clearly shows a saddle point whereas the central experimentation conditions, while the conditions of higher concentration and agitation present the maximum conversion of starch to glucose.

TFS optimal production from cassava starch through acid was obtained for a starch concentration of 190 g/L of starch and an agitation of 600 rpm with sulfuric acid (90.5 % conversion). The results obtained in this work were higher than those reported in [20] who employ 1.5 h more in the process, reaching a conversion of 4.3 % lower.

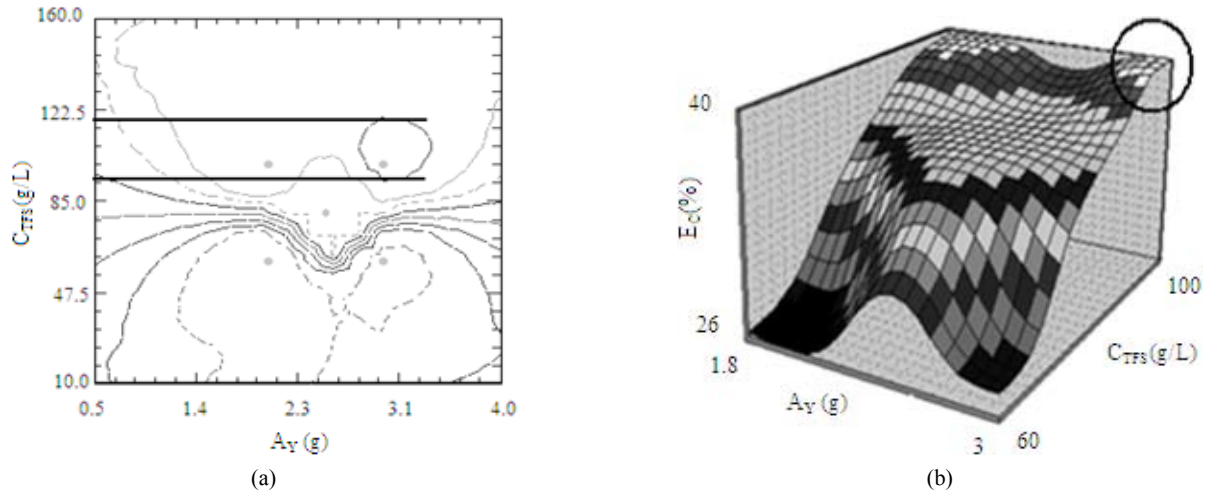
### Optimization of the fermentation process

Statistical software NCSS-2004 was employed to analyze the results, considering the ethanol content ( $E_C$ ) in % Alc/vol as the response variable. The trend of optimization was identified by initially adjusting (equation 4) and secondly (equation 5), obtaining an adjustment of 82.56 % and 91.43 % respectively, which guarantees the reliability of the correlations.

$$E_C = 11.3514 + 0.6599A_y + 0.2685C_{TFS} \quad (4)$$

$$E_C = -4.7299 - 62.7889A_y + 2.6915C_{TFS} + 13.0177A_y^2 - 0.205A_yC_{TFS} - 0.0418C_{TFS}^2 \quad (5)$$

A study was conducted in the optimal region from the contour plot (Figure 5). It was possible to observe an optimal region by considering a mass of yeast *Saccharomyces cerevisiae* between 2.78 and 3.34 g and a glucose concentration between 94.5 and 122.5 g/L. Figure 5b shows that the %



**Fig. 5.** Fermentation process with *Saccharomyces cerevisiae*, a) Contour plot showing the highest yield region, b) Response surface showing a maximum region to conversion ethanol.

alcohol increased proportionally as with the concentration of glucose in the range previously analyzed. However an excessive concentration of glucose can stop or prevent fermentation because an osmosis process would begin [21]. The optimal values of ethanol concentration were obtained with  $C_{TFS}$  of 100 g/L and an  $A_Y$  of 3 g producing ethanol with 38.83 % Alc/vol, 57 % higher than those obtained using sorghum and potato [22, 23, 24].

To study the influence of concentration of the total fermentable sugar ( $C_{TFS}$ ) and the amount of yeast ( $A_Y$ ) on ethanol content ( $E_C$ ) we used the NCSS-2004 software, a second-order Taylor-series model was used. Equation 6, shows the mathematical model obtained,  $A_Y$  was a variable no significant, with an adjustment of 91.21 %. This established that the optimal value of  $A_Y$  (2.5 g) was sufficient to achieve an optimal investment of glucose into ethanol.

$$E_C = -28.27 + 1.3385C_{TFS} - 0.0066875C_{TFS}^2 \quad (6)$$

#### Optimized semi-continuous process for production of ethanol

From the optimal conditions obtained (both hydrolysis and fermentation), acid hydrolysis was realized obtaining a final conversion of 89.84 % the TFS initially considered, which compared with the value predicted by the kinetic model of equation 4 which shows a deviation of 14 % from the experimental value (Figure 6), which validates the proposed kinetic model fit. Subsequently it was carried out the fermentation yielding ethanol concentration of 49.76 % which equates to an increase above 60 % higher than in other investigations [2, 9, 25, 26] which considered different conditions (bacterium, type of enzymes, yeast species, process design), demonstrating the optimization of the process.

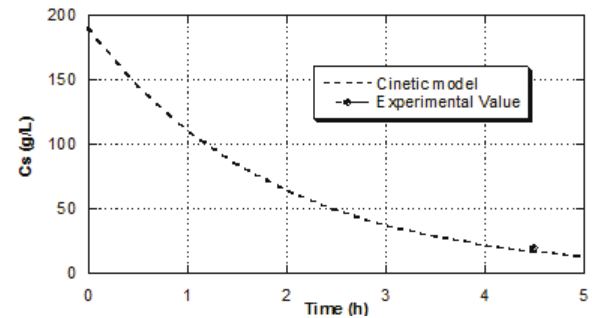
## Conclusions

Kinetic model of acid hydrolysis of cassava starch proposed together with the experimental design approach allowed determining the optimal conditions at hydrolysis and fermentation steps, from second-order polynomial, contours plot and response surface fits. Using the optimal conditions obtained (under hydrolysis and fermentation), in semi-continuous process, substantially increased the final concentration of ethanol, reducing the total process time.

## Experimental

### Kinetic model of the acid hydrolysis

A starch solution with a concentration of 170 g/L at pH of 0.8 was prepared, using sulfuric acid 20 % (w/w). The solution was brought to the boil at a temperature of 98 °C with reflux at atmospheric pressure and a agitation speed of 400 rpm to perform the conversion of cassava starch to total fermentable



**Fig. 6.** Representation of kinetic model for the degradation of starch at different initial concentrations.

sugar (TFS). Once filtered the hydrolysate was neutralized with NaOH, centrifuging the solution to remove the salts generated in the neutralization process.

It employed a HPLC chromatograph Dionex ICS-3000 for determination of TFS. They were injected 2.5 mL of this one using a mobile phase of 200 mM NaOH with a flow of 0.25 mL/min at room temperature with a CarboPac™ PA1 column (2 x 200 mm) using an ED40 electrochemical detector with the AOAC method 996.4 [27]. External standard was used to validate the data.

The model and kinetic data were important in the design, development and operation of carbohydrate conversion processes [13]. The proposed model was based on an irreversible first order homogeneous reaction for hydrolysis with  $H_2SO_4$ , where the average constant was a function of the concentration of starch ( $C_S$ ) and the time (t). The saccharification reaction is obtained by equation 7.



Getting the rate of equation for species A from the equation 7 is:

$$-\frac{d[C_S]}{dt} = -r_{C_S} = k_1[C_S] \quad (8)$$

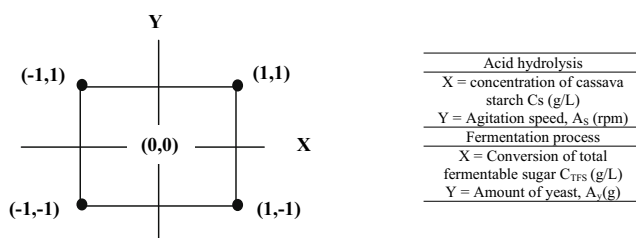
Getting the integrated solution of the equation 8 it was obtained an exponential drop of starch and its corresponding exponential increase in TFS

$$[C_S] = [C_{S_0}] e^{-k_1 t} \quad (9)$$

### Design of the Experiment

Acid hydrolysis and fermentation process were developed using a  $2^2$  experimental designs with three central points. This statistical technique allowed evaluating the influence among the most important factors as well as the significant interaction, using a small number of trials.

Seven tests with three central points were made, Figure 7, shows the design of the experiment. Each experiment was made randomly so that errors are independently distributed. Specific values to acid hydrolysis and fermentation process are shown on Table 4.



**Fig. 7.** Factorial Design  $2^2$  with 3 central points used both in acid hydrolysis and fermentation process.

**Table 4.** Factorial design data used in acid hydrolysis and fermentation process.

X	Y	Acid hydrolysis		Fermentation process	
		$C_S$ (g/L)	$A_S$ (rpm)	$C_{TFS}$ (g/L)	$A_y$ (g)
0	0	170	400	80	2.5
0	0	170	400	80	2.5
0	0	170	400	80	2.5
-1	-1	150	200	60	2.0
1	-1	190	200	100	2.0
-1	1	150	600	60	3.0
1	1	190	600	100	3.0

### Optimization of the acid hydrolysis process

To achieve the hydrolysis process it was employed a  $2^2$  experimental design with three central points for considering two independent variables: 1) concentration of cassava starch ( $C_S$ ) and 2) agitation speed ( $A_S$ ), being the response variable the percent conversion of starch to total fermentable sugar ( $C_{TFS}$ ).

The starch was hydrolyzed for 4.5 h, according to kinetic analysis of the process, to 98 °C to a pH 0.8 using  $H_2SO_4$  to 30 % (w/w). Three levels of concentrations of starch (150, 170 and 190 (g/L)) and three agitation speeds (200, 400 and 600 rpm) were considered. The TFS was quantified using 996.4 AOAC method's.

The experiments were randomly conducted, the order is shown under Table 5.

### Optimization of fermentation process

Completed the saccharification process, the syrup was filtered, adjusting to pH 5 with NaOH 5N and considering the concentration of TFS at three different levels (100, 80, 60 g/L). The fermentation media was enriched with nitrogen ( $(NH_4)_2SO_4$ ), phosphate ( $KH_2PO_4$ ) and magnesium ( $MgSO_4$ ) at concentrations of 0.96 g/L, 0.02 g/L and 0.5 g/L, respectively. A total volume of 800 mL was considered. Subsequently, the medium was sterilized by autoclave (121 °C and 1.2 kg/cm<sup>2</sup> pressure) during 15 min. 200 mL from the fermentation media were taken adding the mass of yeast *Saccharomyces cerevisiae* to employ (2, 2.5 and 3 g) activated by aeration for 15 min at a

**Table 5.** Experimentation sequence for acid hydrolysis process.

Experiment	$C_S$ (g/L)	$A_S$ (rpm)
1	170	400
2	150	200
3	170	400
4	190	600
5	170	400
6	150	600
7	190	200

**Table 6.** Experimentation sequence for fermentation process.

Experiment	CTFS(g/L)	Ay(g)
1	80	2.5
2	80	2.5
3	100	2.0
4	80	2.5
5	100	3.0
6	60	2.0
7	60	3.0

temperature of  $30\text{ }^{\circ}\text{C} \pm 0.1\text{ }^{\circ}\text{C}$ , incorporating the remaining volume of the fermentation media. The concentration of sugars was determined using the Fehling method. Finally, the grape obtained was distilled using a Vigreux column. The ethanol content was determined using the densitometer DMA 35N manufactured by Anton Par.

The experiments were randomly conducted, the order is shown on Table 6.

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