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# ACID HYDROLYSIS OF WATER HYACINTH TO OBTAIN FERMENTABLE SUGARS

## *HIDRÓLISIS ÁCIDA DEL JACINTO DE AGUA PARA OBTENER AZÚCARES FERMENTABLES*

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## ABSTRACT

In order to take advantage of the aquatic weed water hyacinth (*Eichhornia crassipes*) as lignocellulosic feedstock for the production of reducing sugars, a bromatological characterization of the leaves and stems of the plant was performed. Then, the effects of the sulfuric acid concentration (1 - 3% v/v), the concentration of solids (10 to 12.5% w/v) and the reaction time (15 - 25 min) at 121°C/15 psi in the production reducing sugars were studied by employing a central composite design. It was found that the acid concentration was the most significant variable. The optimal conditions were solids 11.25% p/v, $H_2SO_4$ 2% v/v and 20 minute reaction time. These conditions yielded a concentration of reducing sugars of 33.3 g/L.

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## RESUMEN

Con el propósito de aprovechar la maleza acuática Jacinto de Agua *(Eichhornia crassipes)* como materia prima lignocelulósica para la obtención de azúcares reductores, se realizó en primera instancia una caracterización bromatológica de las hojas y tallos de la planta. Luego se estudió el efecto de la concentración de ácido sulfúrico (1 - 3% v/v), la concentración de sólidos (10 - 12.5% p/v) y el tiempo de reacción (15 - 25 min.) a 121°C/15 psi, en la producción de azúcares reductores, empleando para ello un diseño central compuesto. Se encontró que la concentración de ácido fue la variable más significativa. Las condiciones óptimas fueron 11.25% p/v de sólidos, 2% v/v $H_2SO_4$ y 20 minutos de tiempo de reacción. A estas condiciones se obtuvo una concentración de azúcares reductores de 33.3 g/L.

**Palabras clave:** *Eichhornia crassipes, Lignocelulosa, Biocombustible, Azúcares reductores, Pretratamiento con ácido sulfúrico.*

## RESUMO

Com o propósito de aproveitar a erva aquática Aguapé (*Eichhornia crassipes*) como matéria-prima lignocelulósica para a obtenção de açúcares redutores, foi realizada em primeira instância uma caracterização bromatológica das folhas e talos da planta. Posteriormente, foi estudado o efeito da concentração de ácido sulfúrico (1 - 3% v/v), a concentração de sólidos (10 - 12.5% p/v) e o tempo de reação (15 - 25 min.) a 121°C/15psi, na produção de açúcares redutores, utilizando para tal fim um desenho central composto. Encontrou-se que a concentração de ácido foi a variável mais significativa. As condições ótimas foram 11.25% p/v de sólidos, 2% v/v $H_2SO_4$ e 20 minutos de tempo de reação. Nestas condições foi obtida uma concentração de açúcares redutores de 33.3 g/L.

**Palavras-chave:** *Eichhornia crassipes, Lignocelulose, Biocombustível, Açúcares redutores, Pré-tratamento com ácido sulfúrico*

## 1. INTRODUCTION

Water hyacinth (*Eichhornia crassipes*), also known as water Buchón, Taruya, Camalote, Mop, Water Violet, is an aquatic plant native to the rivers of the Amazon Basin in South America (Gunnarsson & Petersen, 2007). *Eichhornia crassipes* is a floating aquatic weed, pop-rooted with rhizomes or stolons. The plant is variable in size and may grow in poor nutrient concentration, up to 0.05 and 0.01 ppm of nitrogen and phosphate (Moran, 2006; Cronk & Fennessy, 2001; Reddy & D'Angelo, 1990). Water hyacinth reproduces sexually by seed propagation or vegetative regeneration. Sexual reproduction takes place by the development of tubers on the basis of the rosette; they can grow up to 30 cm in length before developing a rosette daughter; the intensity of spreading by these means can result in a doubling of the infested area from 6 to 15 days. Studies suggest that water hyacinth increases biomass up to 12% per day and biomass accumulation rate can range from 7.7 to 60 g/m²/day (Ramírez, 2005; Reddy & D'Angelo, 1990).

Water hyacinth is considered an important raw material for agricultural fertilizers, animal feed, biogas, paper, cardboard and building materials. It is also useful in the absorption of some heavy metals in water and, currently, there is scientific and technological interest in obtaining ethanol from it. (Ganguly, Chatterjee & Dey, 2012; Kumar, Singh & Ghosh, 2009a; Mishima *et al.*, 2008).

The use of this biomass to obtain ethanol is an alternative to help relieving problems associated with this plant that because of its rapid growth and spread in the absence of controlling species has nowadays created an imbalance situation of one of the major wetlands in the country: Zapatosa Swamp. Zapatosa Swamp has an area of 36000 hectares in the dry season and 50000 hectares in winter time, where 30% is occupied by weeds, equivalent to 10800 and 15000 hectares respectively. This situation dramatically affects the urban area of Chimichagua and Saloa villages which belong to the Department of Cesar (Rangel *et al.*, 2007). This aquatic weed invades in a short time the waterway, obstructing the generation of power, irrigation, navigation and fishing. It also restricts the passage of light to the underwater environment and depletes oxygen levels (Ganguly *et al.*, 2012; Ma *et al.*, 2010; Kumar *et al.*, 2009a).

There are chemical characterization reports that indicate that water hyacinth contains 18 - 35% cellulose, 18 - 49% hemicellulose and 3.5 - 9% of lignin composition, able enough to obtain fermentable sugars through different physical pretreatments, physicochemical, chemical and biological processes (Kumar, Barret, Delwiche & Stroeve, 2009 b; Mishima *et al.*, 2008; Gunnar-sson & Petersen, 2007; Nigam, 2002). Some research works show that acid pretreatment has the property of hydrolyzing hemicellulose of the xylose material and other degradative compounds of the hydrolysis of this polymer. It remains thereby an insoluble form called cellulignin, a fraction characterized by its high content of cellulose and lignin, and low content of hemicellulose (Peñuela, Da Silva, Bezerra De Sousa & Pereira, 2007).

The pretreatment with dilute sulfuric acid to 2% v/v of water hyacinth has been effective in the removal of sugars in the hemicellulose fraction, especially pentoses, after seven hours of reflux and stirring at 250 rpm, yielding 18.8 g of Reducing Sugars (RS)/100 g dry biomass of which pentoses totaled 13.3 g RS/100 g RS of dry biomass (Kumar *et al.*, 2009b). Comparative studies have also been made of dilute acid hydrolysis with new pretreatment methods such as ionic liquids, raw glycerine, and pure glycerine on water hyacinth with the purpose of transforming the hydrolyzed cellulose into ethanol.

The performance of total RS in the pretreatment with both pure glycerine and raw glycerine, as compared to dilute acid hydrolysis, did not differ significantly, whereas pretreatment with ionic liquids was less effective (Guragain *et al.*, 2011).

During the pretreatment of the lignocellulosic material with sulfuric acid (0.2 - 2%) not only can sugar be obtained from hydrolysis and solubilization of cellulose and hemicelluloses. Also, due to the high temperatures (121 - 220°C) they degrade, especially sugars from hemicellulose, two compounds derived from furan are originated: furfural, formed from the degradation of pentoses (xylose and arabinose) and 5-hydroxymethylfurfural (HMF), formed as a result of degradation of hexoses (glucose, mannose and galactose) (Balat, 2011; Oliva, 2003). In order to mitigate this undesirable effect, some detoxication methods have been employed

like heating at high temperature and alkalinazing with *Ca(OH)₂* (Isarankura *et al.*, 2007). However, one of the biggest challenges to increase the performance of the transformation of lignocellulosic biomass into ethanol is the fermentation of all sugars present in biomass ($C_5$ and $C_6$). This has been worked intensively to obtain genetically modified microorganisms that can transform xylose and arabinose into ethanol. Recently, DNA technology has been used to provide some micro-organisms with the feature of fermenting five-carbon sugars with high efficiency. An example is the bacterial strain of the species *Escherichia coli*, developed by the University of Florida (USA) which can ferment sugars of six and five carbons at the same time (Patrouilleau, Lacoste, Yapura & Casanovas, 2006).

Another example is the introduction of operons that encode enzymes for xylose assimilation and pentose phosphate pathway in *Zymomona mobilis*, (Zhang *et al.*, 1995), recombinant strains of *E. coli* with genes from *Z. mobilis* for more efficient conversion of py-ruvate to ethanol (Dien, Nichols, O'Bryan & Bothast, 2000), *E. coli* strains of recombinant material for the production of endoglucanase (Wood, Beall & Ingram, 1997) and, finally, plasmids with genes of xylose reduc-tase and xylitol dehydrogenase of *P. stipitis* in *Saccharomyces spp.* for efficient co-fermentation of glucose and xylose (Ho, Chen & Brainard, 1998). Based on the significant contents of hemicellulose/cellulose and low lignin contents in water hyacinth, and the possibility that through acid hydrolysis a significant amount of RS can be obtained, this work evaluates the effect of the concentration of solids, acid concentration and reaction time on reducing sugar production, as a pre-processing stage of the acid hydrolyzate transformation to ethanol water hyacinth.

## 2. METHODOLOGY

### Substrate Preparation

The water hyacinth was collected from the Zapatosa Swamp from Chimichagua Township, located in the Department of Cesar. The aquatic plant collected was washed with distilled water to remove dirt. Leaves and stems were processed separately, milled in fresh, dried at 105°C/6 hours and subjected to a mechanical spraying, taking them to a size of 1.18 mm, in order to increase the contact surface between the sample and the sulfuric acid solution. Biomass was prepared by mixing equal parts of ground leaves and stems. A smaller size increases the contact between the solid and the acid but leads to additional energy costs on the comminution of the same. Therefore, the size obtained not only establishes favo-rable processing conditions but also additional savings (Gutiérrez & Arias, 2009; Misson, Haron, Ahmad & Saidina, 2009).

### Acid Hydrolysis

Hydrolysis experiments were carried out in 200 ml Erlenmeyer flasks to the solids concentration, acid con-centration and reaction time according to experimental design. The heating was performed in autoclave at 121°C/15 psi. Two mL of samples were taken from the supernatant and centrifuged at 5000 rpm for analysis of RS.

### Statistical Approach

An experimental method was designed to optimize the acid hydrolysis using a Central Composite Design and response surface methodology. The effects of sol-ids concentration were evaluated, as well as the acid concentration, and reaction time on the release of RS. Table 1 shows the experimental matrix.

**Table 1.** Description of the levels of the variables used in the central composite design.

| Variable | Low (-1) | High Level (+1) | Axial Point | | Central Point (0) |
| --- | --- | --- | --- | --- | --- |
| | | | Low -1.68 | High +1.68 | |
| Solids (%w/v) | 10 | 12.5 | 9.15 | 13.35 | 11.25 |
| Acid concentration (%v/v) | 1 | 3 | 0.32 | 3.68 | 2 |
| Time (min.) | 15 | 25 | 11.59 | 28.40 | 20 |

Regression analysis and analysis of variance (ANOVA) were performed using the software Statgraphics Centurion™ XV (StatPoint Inc., USA). Using response surface methodology, the concentration of RS released was analyzed with the following second-order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_i {}_< \sum_j b_{ij} x_i x_j \qquad (1)$$

Where $Y$ is the response variable, $x_i$ and $x_j$ are the independent variables in encoded units, and $\beta_0$, $\beta_i$, $\beta_{ii}$, $b_{ij}$ settings are the coefficients of the model to the independent terms, linear, quadratic and interactive, respectively.

### Analytical Methods

The physicochemical characterization of water hyacinth was performed using the following methods: moisture, gravimetric method 930.15/90 of the AOAC, ashes method 942.05/90 of AOAC, raw fiber, AOAC 962.09/90; proteins, Kjeldahl method, according to 955.04/90 of AOAC; fat, 920.39/90 technique of AOAC, pH, technique 10.041/84 of AOAC. The content of cellulose, hemicellulose and lignin were determined by the detergent extraction method (Robertson & Van Soest, 1981).

### Determination of Reducing Sugars

Reducing sugars were determined by the method of 3.5 - dinitrosalicylic acid (DNS) (Miller, 1959). The performance of diluted acid hydrolysis was calculated as follows:

$$Hydrolysis\ yield\ (\%) = \frac{Sugars\ reducing\ (g) \times 0.9 \times 100}{Polysaccharides\ (cellulose + hemicellulose,\ g)} \qquad (2)$$

The 0.9 value corrects the contribution of a mole mass of water for each hydrolyzate glycosidic bond.

### Severity Factor

To estimate the degree of severity of the acid hydrolysis process, an autoclave parameter developed by Silverstein *et al.* (2007) was used.

$$M_0 = C \times B^{0.849} \times exp\left(\frac{T_r - 100}{14.75}\right) \qquad (3)$$

Where $M_0$ is the severity parameter, $C$ is the residence time, $B$ is the concentration of acid, $T_r$ is the reaction temperature in °C, 100 is the reference temperature in °C, and 14.75 is the conventional activation energy, assuming that the overall reaction is hydrolytic and first order.

### Validation

The optimal conditions determined from the design analysis were validated experimentally. For this purpose, the evaluation of three replicates to these conditions was carried out.

## 3. RESULTS AND DISCUSSION

### Characterization of Water Hyacinth

The results of the physicochemical characterization and lignocellulosic water hyacinth in a 1:1 ratio of leaves and stems are shown in Tables 2 and 3.

In analyzing the results obtained in the characterization of water hyacinth (*Eichhornia crassipes*) reported

**Table 2.** Physicochemical characterization of water Hyacinth (*Eichhornia crassipes*).

| Parameter (%) | Abdelhamid & Gabr, 1991 | Poddar, Mandal & Banerjee, 1991 | Nigam, 2002 | Kumar et al., 2009a | This Study |
|---|---|---|---|---|---|
| Moisture | - | - | 92.8 - 95 | 92.6 - 95 | 93.10±1.01 |
| Ash | - | - | - | - | 1.614±0.068 |
| Crude protein | 20 | 16.25 | 13.30±0.02 | 12.60 ± 0.018 | 12.716±0.494 |
| Crude fiber | 18.9 | 16.34 | - | - | 19.188±0.558 |
| Fat | 3.47 | 1.61 | - | - | 1.419±0.085 |

**Table 3.** Characterization lignocellulosic of water Hyacinth  (*Eichhornia crassipes*).

| Component* | Abraham & Kurup, 1996 | Nigam, 2002 | Gunnarsson & Petersen, 2007 | Kumar et al., 2009a | Sornvoraweat & Kongkiattikajorn, 2010 | This Study |
|---|---|---|---|---|---|---|
| Cellulose | 35.0 | 18.2 | 19.5 | 18.4 | 19.0 | 31.67 |
| Hemicellulose | 18.3 | 48.70 | 33.4 | 49.2 | 32.7 | 27.33 |
| Lignin | - | 3.5 | 9.3 | 3.6 | 4.4 | 3.93 |

*The component values are expressed in g per 100 g dry matter.

in Table 2, and making a parallel with other works, it is concluded that the moisture content calculated in this research (93.10%) is in the range reported by other authors. As for the results of the percentages of ash and fats obtained (1.614 and 1.419%), the percentage of fiber (19.188%) is found in the range reported by Abdelhamid and Gabr (1991) and Poddar *et al.* (1991). The differences found may be because the nutritional conditions of habitats affect the metabolic processes of the plant, resulting in different chemical characterizations. The evident raw protein content (12.716%) of water hyacinth makes it attractive as a source of nitrogen in bioconversion processes (Sornvoraweat & Kongkiattikajorn, 2010).

Table 3 shows that the cellulose and hemicellulose content of water hyacinth determined in this study has a composition similar to that reported by Abraham and Kurup (1996). In this study we can appreciate a slight difference of 4.34% between holocellulose fractions (cellulose + hemicellulose). Much of the research shows that the water hyacinth hemicellulose substantially predominates. However, in this study the cellulose fraction is predominant. The differences in the composition of water hyacinth lignocellulosic may lie in the use of different methods for the quantification of structural carbohydrates, because the methods of acid and neutral detergent fiber followed by Robertson and Van Soest (1981) were designed to characterize pasture and forage. The low content of lignin present in the water hyacinth allow better utilization of cellulose and hemicellulose in the hydrolysis process.

### *Acid Hydrolysis*

Table 4 shows the experimental results under the various conditions according to the experimental de-

sign. The data shown in RS obtained vary from 4.9 to 34.3 g/L.

**Table 4.** Experimental conditions and results of reducing sugars.

| A: Solids (% w/v) | B: Acid Concentration (% v/v) | C: Time (min) | Y: Reducing Sugars (g/L) |
|---|---|---|---|
| 10.00 | 3.00 | 15.00 | 20.44 |
| 13.35 | 2.00 | 20.00 | 30.12 |
| 11.25 | 2.00 | 28.40 | 4.90 |
| 12.50 | 1.00 | 25.00 | 17.35 |
| 9.15 | 2.00 | 20.00 | 17.30 |
| 12.50 | 1.00 | 15.00 | 9.48 |
| 10.00 | 1.00 | 25.00 | 11.86 |
| 10.00 | 1.00 | 15.00 | 9.99 |
| 12.50 | 3.00 | 25.00 | 31.70 |
| 11.25 | 3.68 | 20.00 | 23.38 |
| 11.25 | 2.00 | 11.59 | 19.30 |
| 10.00 | 3.00 | 25.00 | 22.28 |
| 11.25 | 0.32 | 20.00 | 2.41 |
| 12.50 | 3.00 | 15.00 | 23.75 |
| 11.25 | 2.00 | 20.00 | 33.45 |
| 11.25 | 2.00 | 20.00 | 32.37 |
| 11.25 | 2.00 | 20.00 | 34.33 |
| 11.25 | 2.00 | 20.00 | 33.06 |

ANOVA (see Table 5) shows that the acid concentration and the quadratic terms of acid concentration and time have a P-value less than 0.05, indicating that they have a significant effect on the production of RS.

**Table 5.** ANOVA for reducing sugars.

| Source* | Sum of Squares | D.F. | M.S | Ratio F | P-value |
|---------|------|-----|-----|------|------|
| A | 112.923 | 1 | 112.923 | 5.27 | 0.0509 |
| B | 526.02 | 1 | 526.02 | 24.53 | 0.0011 |
| AA | 92.7006 | 1 | 92.7006 | 4.32 | 0.0712 |
| BB | 539.507 | 1 | 539.507 | 25.16 | 0.0010 |
| CC | 586.946 | 1 | 586.946 | 27.37 | 0.0008 |
| Lack of Fit | 47.7628 | 4 | 11.9407 | 0.56 | 0.7005 |
| Pure error | 171.551 | 8 | 21.4439 | | |
| Total (corr.) | 1792.02 | 17 | | | |

*A=Solids (% w/v), B=Acid concentration (%v/v), C=Time (min).
M. S: Mean Square; D. F: Degree freedom.

Figure 1 shows that the acid concentration *(B)* is one factor that has a significant positive effect on the production of RS.
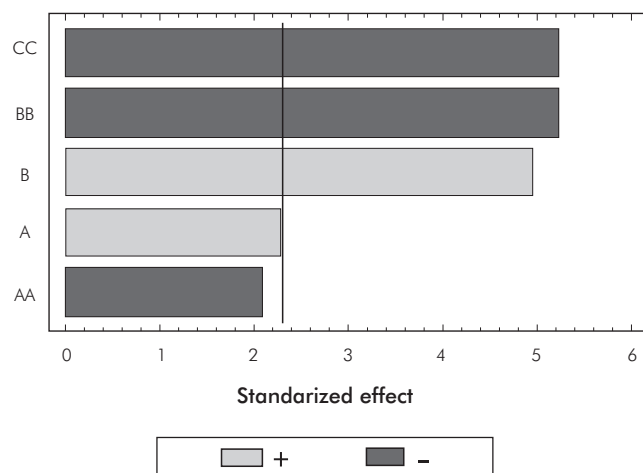


**Figure 1.** Standardized Pareto chart for reducing sugars. A=Solids (% w/v), B=Acid concentration (%v/v), C=Time (min).

Crossed factors (*AA, CC* and *BB*) have no physical meaning and are used to improve the mathematical fit of statistical model based on experimental data. The solid concentration, although it did not exert a significant effect on the conditions studied, makes a great contribution in obtaining fermentable sugars, a fact reflected in the p-value.

The statistical analysis yielded a mathematical model to predict outcomes within the range of each variable. The regression equation, with variables in encoded units and with the terms that have a significant and measurable effect on the release of RS, is as follows:

$$R.S = 33.1473 + 2.87552 \times A + 6.20621 \times B - 2.70714 \times A^2 - 6.53084 \times B^2 - 6.81191 \times C^2 \quad (4)$$

Where RS is the concentration of RS (g/L), *A* is the concentration of solids (% w/v), *B* is the concentration of sulfuric acid (% v/v) and *C* is the reaction time. The regression coefficient value for this model ($R^2 = 0.88$) indicates that the quadratic model fitted explains 88% of the variability in the production of RS. The effects of acid concentration and reaction time, keeping the concentration of solids constant, are shown in Figure 2.
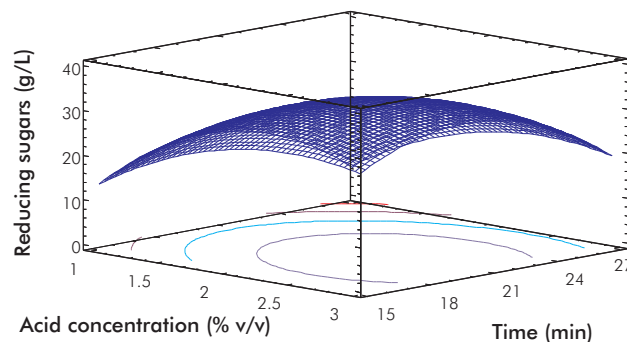


**Figure 2.** Response surface reducing sugars as a function of the acid concentration and reaction time to a solid concentration of 11.25% w/v.

It can be observed that in the central region there is a maximum. As the acid concentration increases, there is a greater amount of sugars released, but there comes a point at which it begins to decrease. This may be explained by the greater presence of acid catalyst that produces transformations of hexose and pentose to 5HMF and furfural respectively, and the formation of organic acids such as lactic acid, propionic acid, levulinic acid and formic acid, among others. Figure 3 shows a slight increase in the concentration of RS to increase the solid concentration in both high and low concentrations of acid. However, in high acid concentrations more sugars are released than in low acid concentrations and, as Figure 2 shows, there is a maximum in the experimental region. This behavior is consistent with the reported

by Ganguly *et al.* (2012), who claims that at high acid concentrations a greater hydrolytic effect is obtained.
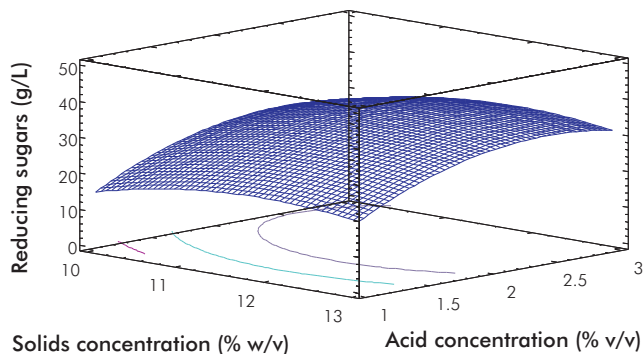


**Figure 3.** Response surface reducing sugars as a function of the solids concentration and acid concentration of 20 minutes reaction time.

Upon estimating the optimal value, it was found that with a solid concentration of 12%, acid concentration 2.5% v/v and 20 minutes reaction, a 35.7 g/L of reducing sugar was obtained. By validating these conditions, and triplicating them, a reducing sugar concentration of 33.2±0.2 g/L was obtained, a value very close to that estimated by the model: 33.3±1.3 g/L. After performing a test to compare means, a P-value greater than 0.05 was obtained, indicating that there are no significant differences between them. A similar result is obtained under the conditions of the central design point, with a concentration of acid and a lower solids concentration, which is economically advantageous. Taking the economic factor as criterion, and without characterizing the inhibitory products generated in the pretreatment that conditions the selection depending on the destination of the pretreatment product, optimal conditions were chosen as 11.25% w/v solids, 2% $H_2SO_4$, and a reaction time of 20 minutes at 121°C/15 psi. Under these conditions a conversion of cellulose and hemicellulose of 45% in RS is achieved; that is, 29.33 g of RS/100 g of dry biomass, which exceeds the value obtained by

Harun, Dayang, Zainal and Yunus (2011), Kumar *et al.* (2009b) and Nigam (2002a), as shown in Table 6.

This shows the greatest hydrolytic effect of the conditions found here. This effect can be attributed to the cleavage of hemicellulose in the sugars contained in it, such as xylose, and sugars such as mannose, arabinose also present in hemicellulose, and even glucose in minor proportions, given the hydrolytic characteristics of acid agent over structural carbohydrates of lignocellulosic biomass. This suggests that the solid fraction is rich in cellulose so it can further be enzymatically zaccharized into glucose. The liquid fraction can be fermented directly by a microorganism to ferment pentoses with prior detoxification, such as *Pichia stipitis* (Sánchez, Gutiérrez, Muñoz & Rivera, 2010).

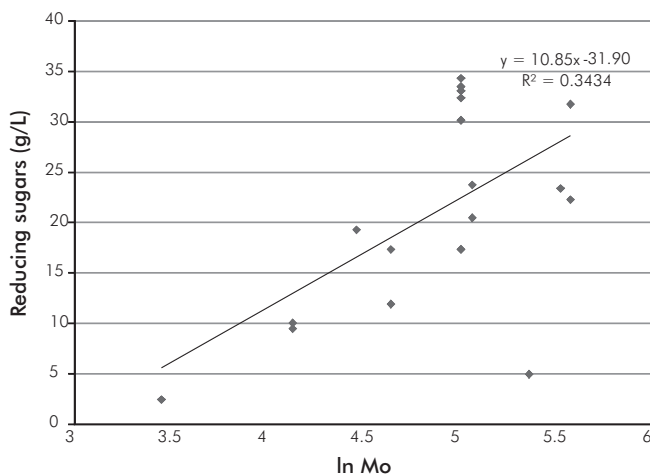**Figure 4.** Reducing sugars vs ln (severity parameter).



Figure 4 shows the poor relationship between the severity parameter and RS, indicating the inability of the severity parameter to predict the dependence of the response variable to the independent variables, in the absence of other variables such as the percentage of solids.

**Table 6.** Reducing sugars obtained in acid pretreatment, g/100 g of dry biomass.

| References | Nigam, 2002 $H_2SO_4$ 1% v/v, refluxby 7 h, 250 rpm | Kumar *et al.*, 2009b $H_2SO_4$ 2% v/v, refluxby 7 h, Env. Temp., 250 rpm | Harun *et al.*, 2011 $H_2SO_4$ 5% v/v, 121°C, 60 min | This study |
|---|---|---|---|---|
| Reducing sugars | 21 | 18.8 | 12 | 29.33 |

## 4. CONCLUSION

• The optimal conditions to obtain a significant yield of RS are in a ratio of 11.25% w/v of solids with a concentration of 2% v/v of sulfuric acid over 20 minutes at 121°C/15 psi. The variable that has a significant and positive effect on the studied conditions of acid hydrolysis of the water hyacinth is the acid concentration, the effect of which was favored by the high pressure at which the substrate was submitted. The central composite design optimizes sugar performance based on dry biomass, with 29.33 g of RS/100 g of dry biomass.

## ACKNOWLEDGEMENTS

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