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Hydrolysis of rice straw and rice husk for production of second generation (2G) ethanol

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ABSTRACT : *This paper presents the evaluation of acid hydrolysis of rice straw and rice husk for the production of 2G ethanol, at different temperatures, after pretreatment of hydration in phosphoric acid solution, 5, 10 and 15%, exposure to ultrasound in 5, 10 and 15minutes, following experimental planning 23. It was used Sacaromyces cerevisiae yeast, under agitation at 130 rpm, at 36ºC, for 48 hours. The statistical analysis of the results evaluated the influence of the effect of the variables, hydrolysis temperature, time of exposure to ultrasound and acid concentration. The results indicated that the concentration of 15% phosphoric acid followed by the temperature of 70 ºC were significant. The analysis of the estimated effects and the response surfaces confirmed that the temperature, concentration, and the interaction between temperature and concentration were statistically significant in the hydrolysis of the material and resulted in a model with good fit between the predicted values and the values obtained experimentally. It indicates that it can be used in the prediction of °BRIX values, within the studied limits. The statistical analysis of the ethanol results indicated that the temperature was statistically significant, while acid concentration and ultrasound exposure time were not significant in the conditions studied.*

KEYWORDS *-Rice husk and straw, Hydrolysis, Fermentation, Ethanol 2G.*

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I. INTRODUCTION

Agriculture occupies a large and important space in the Brazilian economy, resulting from many factors, among which, the similarity of a global trend in the increase of the population and the consequent increase in the demand for food and energy.

Considering food production, Brazil currently produces about 12.7 million tons of rice, being among the ten largest producers in the world [1].

From this volume of production, it is estimated that about 2.74 million tons of rice husk are produced per year throughout the national territory [2], in addition to the straw left over in the fields after harvest, adding up to a significant amount in waste.

These residues are mostly used as a food supplement for animals or as a material for composting. However, there is the possibility of processing and transforming them into ethanol, adding value to the waste and providing an alternative for biofuel production.

Biofuels fall into the category of environmentally friendly products with renewable energy sources, making them an attraction for world economies. With regard to the production of biofuels, it is evaluated the limitation of available natural resources and space for cultivation, as well as the competition of arable area with food production.

Among biofuels, ethanol has great representativeness not only restricted as fuel, but as an important raw material in the chemical and pharmaceutical industry for the manufacture of products for personal use and surfaces in the fight against viruses, fungi and bacteria. This demand reinforces the importance of studying potentially usable raw materials for their production, minimizing competition with food sources.

In this perspective, there is a growing demand for studies evaluating the use of agro-industrial residues for ethanol production, called second generation ethanol (2G ethanol), with different methods presented in the specialized literature that support the conversion of a biomass of interest into ethanol. This route of ethanol production from lignocellulosic raw material, from crop residues and agroindustrial processing, requires pre-treatment processes (chemical or physical), followed by cellulosic hydrolysis (acid, basic or enzymatic), to enable fermentation of interest.

The acid hydrolysis of biomass from lignocellulosic source for ethanol production is performed with acids: nitric (HNO_3), hydrochloric (HCl), sulfuric (H_2 SO_4) and phosphoric (H H_3PO_4), usually in diluted solutions [1,4].

The acids act in the solubilization of lignin and hemicellulose of biomass and hydrolyze polysaccharides in monosaccharides, facilitating a hydrolysis by enzymes in a subsequent process [4,5].

Hemicellulose degrades under milder operating conditions, requiring more severe conditions for cellulose hydrolysis, which can cause the degradation of sugars already obtained from hemicellulose as well as its degradation, forming fermentation inhibitors [6].

The main difference between the acids used is in the concentration, more diluted or more concentrated, so that in the operationalization with concentrated solutions the temperatures are reduced, and solutions diluted at higher temperatures, mainly aiming at a reduction in process costs, without many changes in the method [4].

The use of concentrated acids presents disadvantages such as toxicity, corrosion of equipment, production of compounds such as phenolic acids, furfural and undesirable aldehydes for the processes of enzymatic hydrolysis and fermentation [4,5].

The most used acid in this type of hydrolysis is sulfuric acid [4,7]. and when at a concentration of 0.05–5% it can reduce the formation of undesirable compounds [8,9]. This diluted acid avoids the use of enzymes to hydrolyze hemicellulose with reduction in production cost [4]. It was used with good yield results under different experimental conditions in rice straw residues [10], canola residues [11] and grass [12]. However, it presents high dangerousness of operation being highly corrosive to equipment.

Although the processes that use acid still represent a high cost with the use of energy and water, they indicate improved carbohydrate yield; thus, phosphoric acid is non-toxic, low cost compared to other acids [3,7]. It has advantages in the process when it is neutralized with sodium hydroxide forming sodium phosphate, a nutrient for microorganisms in the fermentation medium, and can be a beneficial, efficient and economical alternative [13].

The use of ultrasound can activate some types of mechanisms, such as mechanical rupture, agitation, localized heating, diffusion[14] improves mass transfer in heterogeneous systems by thermal effects, or through cavitation [15]. The fluctuations in the pressure of the ultrasound sound field cause the formation, growth and collapse of microbubbles within the liquid generating high turbulence releasing heat as a form of energy, significantly raising pressure and temperature [16].

Acid hydrolysis assisted by ultrasound was considered more effective in 44% when compared to the common one [17], and has the advantage of being a clean, non-toxic and easy-to-use process and, when used in biomass, it improves the extraction of lignin, hemicellulose and cellulose, thus increasing the efficiency of hydrolysis [18]. However, studies of assisted enzymatic hydrolysis of ultrasound showed unfavorable results, with the formation of inhibitory products by irradiation [19]. Although there are controversies in the use of ultrasound, there was effectiveness in the pre-treatment of rice residues and subsequent enzymatic treatment, improving yield of reducing sugars due to changes in the crystalline structure of cellulose [20].

In general, the production of 2G ethanol in Brazil presents great challenges to solve technical problems in the production process, among which stands out the pre-treatment stage of the sample, with high cost of reagents, enzymes and equipment for research and or production. This research aims in the foreground to seek low-cost alternatives for its realization so that it can be replicated in later studies.

Following the different arguments presented, this article presents the research that aimed to evaluate different conditions of acid hydrolysis of straw and rice husk for 2G ethanol production through experimental planning.

II. MATHERIAL AND METODS

The hydrolysis of rice husk and rice straw was performed according to adaptation of methodology [17], following experimental planning 2^3, in eleven random experiments, the pretreatment sequence of rice husk and rice straw aliquots, hydrolysis followed by neutralization, evaluation of ° Brix, alcoholic fermentation and ethanol content evaluation.

The pretreatment of biomass (rice husk and rice straw) was performed by adding 5g portions of each fraction in a round-bottomed balloon of 250 mL, totaling 10 g, on which 100 mL of phosphoric acid solution (H_3PO_4) 5%, 10% and 15% were added, and remained in hydration for a period of 24 hours.

After the hydration period, the biomass was heated in a heating mantle at temperatures of 30, 50 and 70 ºC, according to experimental planning, for one hour. A condenser was mounted so that the vapors generated during the process were condensed back into the balloon.

After heating procedure in a heating mantle, the round bottom balloon containing the biomass was taken for exposure to ultrasound at a continuous frequency of 45Hz, at times of 10, 20 and 30 minutes, according to experimental planning.

After ultrasound treatment, the sample was filtered with the aid of funnel, gauze and cotton filter, due to the high viscosity of the medium and high concentration of solids. After the first filtration, biomass washes were carried out with 150 mL of ultrapure water, aiming to extract as much of the fermentable sugars formed during acid hydrolysis. The collected solutions were neutralized at pH 7.0 [21] with sodium hydroxide solution 1 $moL.L^{-1}$and filtered again.

The concentration of soluble solids in hydrolyzed neutralized at pH 7.0, obtained under the different study conditions and of the respective fermented ones was analyzed by refractometry, in a portable refractometer TE697-SZ. In addition to the samples, white acid solutions were prepared at concentrations of 5%, 10% and 15%, neutralized with sodium hydroxide solution at pH 7.0, in triplicate to discount the values of the samples. The result was expressed in °Brix in which each unit of this measure corresponds to one gram of sugar per hundred milliliters of solution.

Alcoholic fermentation was performed following procedures based on modifications of the methodologies [22,23,24,25], preceded by the hydration phase of Ca-11 yeast, for which 0.25 grams of yeast were at 30mL of water for 30 minutes. After this time, the hydrated yeasts were added to the 500mL kitazatos containing the hydrolyzed prepared according to experimental design, closed with stoppers, and at the side outlet a silicone hose was fitted, immersed in water so that there was no oxygen inlet and allowed carbon dioxide output, conducted in rotary Shaker under operating conditions at 36ºC and 130rpm, over a period of 48 hours.

The evaluation of ethanol concentration in fermented rice husk and rice strawwas performed by the modified method [26], under experimental conditions evaluated and optimized in several previous experiments in Gas Chromatography 6100GC, Young Lin brand, with flame ionization detector (FID), equipped with headspace with automatic injector, capillary column ZB-WAX, 30 meters, 0.25 mm and 0.25 micrometers available in the Laboratory of Instrumental Methods at Federal Technological University of Paraná, Campus Ponta Grossa.

III. RESULTS AND DISCUSSION

The results of the concentration of soluble solids in the hydrolyzed rice husk and rice straw in °Brix, corresponding to each planning experiment are presented in Table I, where minimum values of 2.2 are observed for sample 7 to maximum 4.9 for sample.

From the analysis of variance of the values of °BRIX of the hydrolyzed it was observed that the concentration of phosphoric acid was significant in the hydrolysis of rice husk and rice straw, followed by temperature. Statistical significance was confirmed with reliability of 95%, with statistically significant factors those with p-value lower than 0.05. In addition, from the f-test, it is possible to affirm the significance of the factors if the calculated value is greater than the tabulated value.

Table I. Concentration of soluble solids in hydrolyzed and ethanol in the fermented rice husk and rice straw

Experiment (Sample)	Hydrolyzed °BRIX	Ethanol ($g.kg^{-1}$ of biomass)
1	2,3	4,52
2	3,1	0,82
3	3,3	1,23
4	2,7	1,87
5	3,2	1,30
6	4,3	10,01
7	2,2	0,41
8	3,6	1,41
9	4,9	9,73
10	2,4	1,57
11	2,6	1,39

The analysis of the effects of variable phosphoric acid concentration, temperature and ultrasound exposure time in the °BRIX of the hydrolyzed (Table 4) corroborates the significance of the effect of phosphoric acid concentration on rice husk and rice straw hydrolysis with a value of 7.50, followed by the significant effect of temperature with a value of 5.04, and the non-significant effect of ultrasound with a value of 1.50.

From the results obtained in the ANOVA analysis and in the analysis of the estimated effects, it is observed that the hydrolysis temperature, the concentration and the interaction between temperature and concentration were statistically significant in relation to the increase of °BRIX in the hydrolysis of the lignocellulosic material of rice husk and rice straw.

Considering the significant factors, we obtained the equation that represents the research model as:

Y= 3,1454 +0,4625T + 0,6875C + 0,3635TC, Equation1

Where:Y is the value of the BRIX °; T is the temperature in coded value and C is the concentration of phosphoric acid in coded value.

From equation 2 it was possible to plot a graph of values predicted by the model versus values obtained experimentally for the °Brix of the hydrolyzed (Figure 1).

The predicted values and the values obtained for °Brix experimentally in the hydrolyzed were close, which indicates a good fit of the model, which can be used to predict values of the ° BRIX from the values of temperature and acid concentration, provided that they are between the maximum and minimum limits delimited by the model. In this scenario, it can be assumed that the linear adjustment is appropriate to represent the behavior of brix degrees. The highest value obtained for °BRIX through the equation is 4.69 for temperature and acid concentration at the highest encoded value (+1), respectively at 70 °C and 30%.

The highest values of °BRIX in hydrolyzed were obtained when the highest concentrations of phosphoric acid were used in the process. This action of phosphoric acid on lignocellulose hydrolysis has been observed in previous studies at high concentration, indicating that a greater amount of acid considerably influences the hydrolysis of lignocellulosic material [27].

The main effect of temperature was also statistically significant regarding the increase of soluble solids in hydrolyzed as observed in other studies where the increase in temperature increased the amount of glucose in the medium [28].

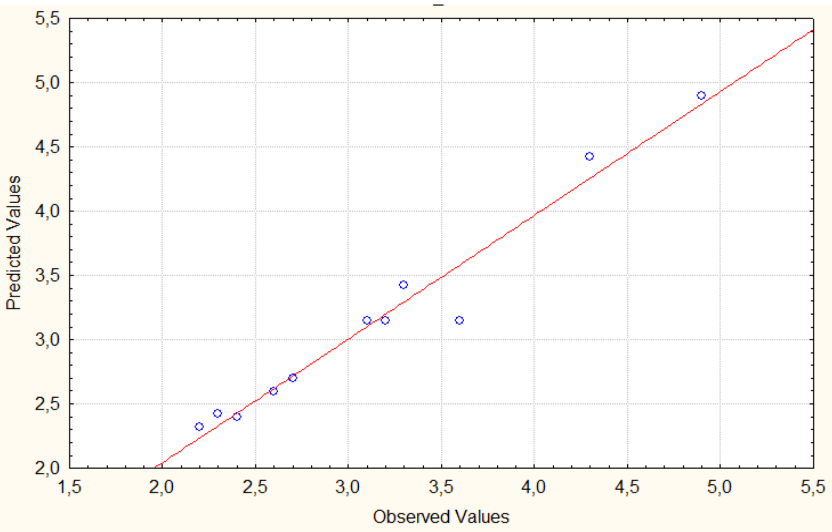

Fig. 1: Values predicted by the model versus experimentally obtained values for the °BRIX of hydrolyzed

Response surfaces were plotted to evaluate the combined effect of the variables studied and the statistical significance of phosphoric acid concentration, ultrasound exposure time and hydrolysis temperature in the values of °Brix of the hydrolyzed.

The response surface of the combined effect of temperature versus concentration, shown in Figure 2 (a), indicates that the ° Brix content of hydrolyzed increases with phosphoric acid concentration from 5 to 15%, and with the temperature increase from 30°C to 75°C. There is also an increase in the values of °Brix of the solution.

The response surface of the combined effect of temperature versus ultrasound shown in Figure 2 (b) indicates a significant influence of temperature on the temperature of the solution °Brix while ultrasound does not demonstrate this same effect.

The response surface of the combined effect of the concentration versus ultrasound shown in Figure 2 (c) indicates a considerable influence of the acid concentration during hydrolysis in the °BRIX of the solution, while ultrasound indicates an increase in BRIX without the same significance.

The results obtained in this research did not indicate significant action of ultrasound in the hydrolysis of rice husk and rice straw and are in agreement with results obtained by Perrone (2015) considered unfavorable as pretreatment in enzymatic hydrolysis. However, the results obtained are in disagreement with other studies that obtained an increase in the yield of reducing sugars [17, 18, 20].

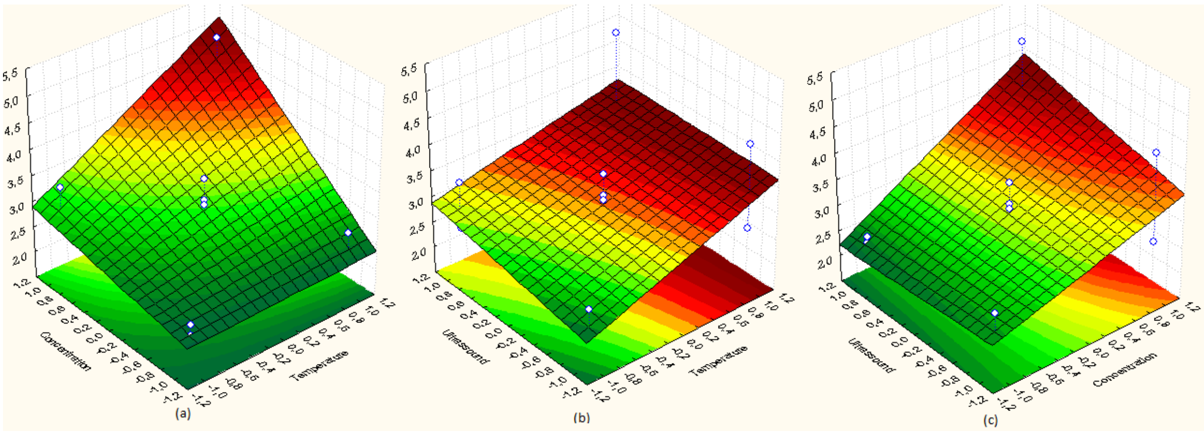

Fig. 2:Response surfaces of the combined effect (a) of temperature versus concentration (b) of temperature versus ultrasound (b) of concentration versus ultrasound in hydrolysis in °BRIX

The results of the 2G ethanol content in the fermented rice husk and rice straw corresponding to each planning experiment are presented in Table 1. It presented minimum values of 0.41 g. kg^{-1} of biomass for sample 7 and maximum of 10 $g.kg^{-1}$ of biomass for sample 6.

The ANOVA of the ethanol production values indicated that temperature is the only significant variable for ethanol production, unlike °BRIX where the concentration and the combined effects between temperature and concentration were significant.

The analysis of the effects of the variable concentration of phosphoric acid, temperature and ultrasound time on ethanol production in fermented individuals confirms the significance of the effect of temperature on ethanol production in fermented rice husk and rice straw with a value of 3.91, and the non-significant effects of the concentration with a value of 2.54, and ultrasound with a value of -0.35.

These results obtained in the production of ethanol in relation to the use of phosphoric acid (H_3PO_4) in hydrolysis showed the same tendency to recommend the use of diluted solutions according to previous studies [3, 4].

While the results obtained in ethanol production in relation to the use of ultrasound in hydrolysis may have occurred for the same reasons presented in previous research that considered unfavorable ultrasound in the pretreatment of enzymatic hydrolysis, due to the formation of inhibitory products by irradiation [19].

IV. CONCLUSION

The results obtained allowed to evaluate the effect of different conditions of acid hydrolysis of rice straw and rice husk for 2G ethanol production through experimental planning 2^3.

The hydrolysis performed under temperature conditions of 70°C, 30 minutes of ultrasound and 15% of phosphoric acid showed that the highest value of ° BRIX obtained was 4.9. Statistical data analysis indicated that a concentration of 15% of phosphoric acid followed by the temperature of 70 °C were significant and the analysis of the estimated effects indicated that the hydrolysis temperature, the concentration and the interaction between temperature and concentration were statistically significant in the hydrolysis of the material used.

The statistical data analysis in °Brix and the construction of the response surfaces confirmed that the statistically significant variables for hydrolysis were the temperature and concentration of phosphoric acid in the medium, as well as the combined effect between them. These data resulted in a model with good adjustment between the predicted values and the values obtained experimentally and indicates that it can be used in the prediction of values of °BRIX, as long as they are within the limits studied.

Ethanol production obtained under the conditions established in the experimental planning was 0.40 $g.kg^{-1}$of biomass under conditions of 30°C, 10 minutes of ultrasound and 5% of phosphoric acid in the medium and 9.73 $g.kg^{-1}$of biomass, under conditions of 70°C, 30 minutes of ultrasound and 15% phosphoric acid. The statistical analysis of the data indicated that the temperature was statistically significant, while acid concentration and ultrasound exposure time were not significant in the conditions studied.

Ethanol production obtained from fermentation was 0.40 $g.kg^{-1}$of biomass under conditions of 30°C, 10 minutes of ultrasound and 5% of phosphoric acid in the medium, and 9.73 $g.kg^{-1}$of biomass under conditions of 70°C, 30 minutes of ultrasound and 15% phosphoric acid. The statistical data analysis indicated that the temperature was statistically significant, while acid concentration and ultrasound exposure time were not significant in the conditions studied.

The results of the comparison model between the ethanol values obtained experimentally and the values estimated by the model showed a significant difference, whose lack of adjustment does not indicate its use in predicting ethanol yield values within the limits of the variables studied.

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