Global NEST Journal, Vol 17, No 4, pp 816-824, 2015

HYDROLYSIS OF AGRICULTURAL WASTE TO OBTAIN REDUCING SUGARS USING CONVENTIONAL AND ULTRASOUND-ASSISTED TECHNOLOGIES

MOSCON J.M.[1]
PRIAMO W.L.[2]
SILVA J.R.F.[1]
COLLAZZO G.C.[1]
FOLETTO E.L.[1]
JAHN S.L.[1]
KUHN R.C.[1,*]
CANCELIER A.
MAZUTTI M.A.[1]

[1]*Department of Chemical Engineering*
Federal University of Santa Maria
Av. Roraima, 1000, Santa Maria, 97105-900, Brazil
[2]*Department of Food Technology, IFRS - Campus Sertão*
Sertão-RS, 88040-900, Brazil

Received: 26/12/2014
Accepted: 17/11/2015
Available online: 25/11/2015

*to whom all correspondence should be addressed:
e-mail: raquelckuhn@yahoo.com.br

ABSTRACT

The main objective of this study was to evaluate the hydrolysis of rice hulls to obtain reducing sugars by conventional and ultrasound-assisted acid hydrolysis. For this purpose, the methodology of experimental design was used to evaluating the influence of temperature, moisture content and concentration of phosphoric acid on the yield of reducing sugars. The yields at optimized conditions were of 141.1 and 162.0 $g.kg^{-1}$, for conventional and ultrasound-assisted hydrolysis, respectively. It was possible to obtain high yield using less acid and low temperature, in a manner that ultrasound can be used as a technology for process intensification.

Keywords: Acid hydrolysis, reducing sugar, ultrasound, rice hulls

1. Introduction

The fast depletion of fossil fuels has driven the world to utilize renewable-energy sources in order to reduce the total dependency on non-renewable energy sources. The growing industrialization increased the demand of fuels attempting to satisfy both the industrial and domestic requirements. Lignocellulosic agricultural residues are promising raw materials for sugar platform biorefinery on a large scale. As they are residues and wastes, they do not compete with primary food production. However, few biorefinery processes based on sugar-platform are cost-competitive in current markets because of the low efficiency and high cost of conversion processes (Diaz *et al.*, 2013).

Rice is an important agricultural culture and implies 20% of the world's nutritional energy. Rice hulls represent approximately 20% dry weight of the harvest, being an abundant lignocellulosic residue, mainly in the State of Rio Grande do Sul – Brazil, which is the major Brazilian producer of rice accounting for about 50% of all amount produced in the country. It is an abundant and low cost residue, presenting few applications nowadays (Hickert, 2010). The processing of rice generates large amounts of waste, mainly rice husk which account for more than 113 million ton generated each year in the world (Yu *et al.*, 2009). The average composition of rice residues from harvest and processing (straw and hulls) is cellulose (32–47%), hemicelluloses (19–27%) and lignin (5–24%) (Gamez *et al.*, 2006), whereas rice hulls consist on 36–40% cellulose, and 12–19% hemicelluloses (Saha *et al.*, 2005; Banerjee *et al.*, 2009; Binod *et al.*, 2010; Geddes *et al.*, 2010). They also have fats, gums, alkaloids, resins, essential oils and other cytoplasmic components (extractives), and about 12% of ashes, composed mainly by silica (80–90%), but also by K_2O, P_2O_5 (5%), CaO (4–1.2%), and small amounts of Mg, Fe, and Na. The complex chemical composition of rice hulls represents an additional barrier for the release of cellulose (Saha and Cotta, 2007; Saha and Cotta, 2008).

The hydrolysis of lignocellulosic material using a strong acid such as sulfuric or hydrochloric acids is avoided by present disadvantages such as corrosion of the equipment, production of toxic compounds such as furfural, and require repeated washings of the material, leading to a large consumption of water. Although the phosphoric acid is more expensive than sulfuric one, the neutralization of the broth led to a formation of sodium phosphate. This salt may remain in the hydrolyzed because it is used as a nutrient for microorganisms. Therefore, a filtration operation is not necessary to removing salts and decreasing the amount of nutrients needed for fermentation. Moreover, with the use of a weak acid, it requires a lower consumption of water for neutralization (Gámez *et al.*, 2006; Werle *et al.*, 2013). In order to overcoming the difficulties in hydrolysis of rice hulls, an alternative cost-effective way consists in the use of ultrasound technology, which has received great attention to the improvement of various biotechnological processes in the last decade (Subhedar and Gogate, 2013). The effect of ultrasonic irradiation is to produce cavitation in liquids to aid the progress of chemical reactions for creating bubbles and hot-spot generation. This phenomenon can be applied in the pretreatment of biomass to achieve effective processing (Bussemaker and Zhang, 2013) or to intensify the hydrolysis of lignocellulosic residues using chemical or biological catalysts (Subhedar and Gogate, 2013; Werle *et al.*, 2013).

Based on these aspects, the present article aims to evaluate the ultrasound-assisted acid hydrolysis of rice hulls using phosphoric acid to obtaining the reducing sugars. For this purpose, the methodology of experimental design was used to optimize the temperature, moisture content and concentration of phosphoric acid on the yield of reducing sugar in the presence and absence of ultrasound irradiation. All reactions were carried in the absence or near absence of free water, resulting in minimum water consumption and a low effluent production, which is defined as solid-state hydrolysis (Pandey, 2003; Moscon *et al.*, 2014).

2. Material and Methods

The rice hulls used in this study were obtained from a local processing factory (Primo Berleze, Santa Maria, Brazil). The samples, as soon as they were received, were milled, sieved and maintained at temperature of -4°C until the experiments. The reagents phosphoric acid (85 wt%, Vetec) and 3.5 dinitrosalicilic acid (Vetec) were used as received.

Experiments were carried out in an ultrasonic bath with temperature control (Unique Inc. model USC 1800A, Brazil, BR) equipped with a transducer having longitudinal vibrations. The ultrasonic unit has an operating frequency of 40 kHz and a maximum-rated electrical power output of 132 W. The ultrasonic transducer (surface area of 282.2 cm^2) is fitted at the bottom of the bath horizontally along the length of bath. All reactions were carried out using 10 g of dry rice hulls. Before the reactions, the moisture content and acid concentration were adjusted at specified value taking as basis the mass of solids. Afterwards, the beaker containing rice hulls were maintained at the center of the ultrasound bath at specified temperature by 240 min and, at the end, the whole content of the beaker was sampled for sugar extraction. The same experiment was carried out in the presence and absence of ultrasound irradiation for sake of comparison. For the experiments without ultrasound, the equipment was used as thermostatic bath. In this work, after preliminary tests, it were evaluated the effects of temperature (30-65 °C), moisture content (40-80 wt%) and concentration of phosphoric acid (7.5-30 wt%) on the amount of reducing sugar released by mean a central composite rotational design (CCRD) for three independent variables. All the results were analyzed using the software Statistica® 8.0 (Statsoft Inc., Tulsa, OK, USA), considering a significance level of 90%.

After the hydrolysis, the reducing sugars were extracted from the solid material using distilled water at solid to liquid ratio of 0.1 (0.1 grams of solid for each gram of water). The extractions were carried out at 30°C under orbital shaker agitation of 150 rpm during 30 minutes. Afterwards, the solution was filtered by vacuum filtration (Whatman qualitative filter paper, grade 1) and the supernatant was used to determine the amount of sugars by the 3.5-dinitrosalicylic acid method (DNS) (Miller, 1959). The amount of reducing sugar released was assessed by taken an aliquot free of solids. All results were expressed as gram of reducing sugar per kg of dry rice hulls.

3. Results and Discussion

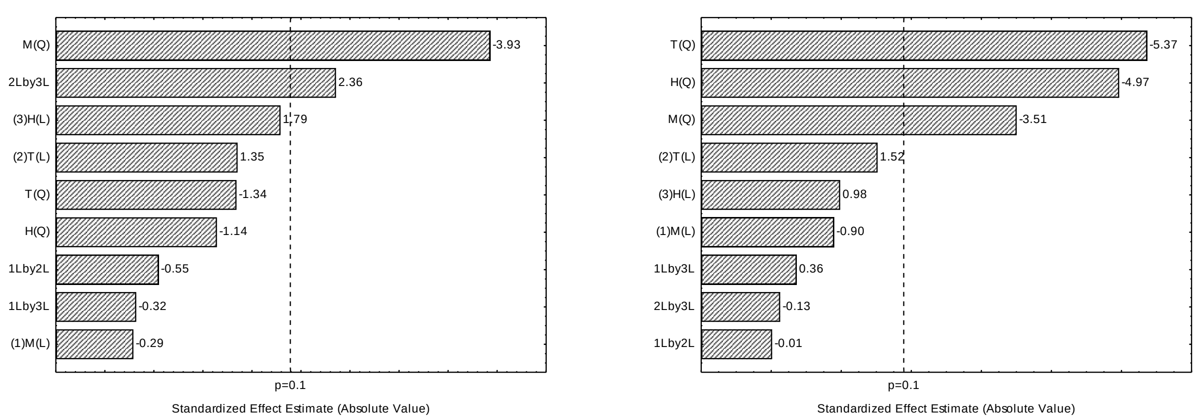
Table 1 presents the reducing sugars yield and hydrolysis efficiencies obtained in the seventeen runs of the CCRD for the conventional and ultrasound-assisted acid hydrolysis. The reducing sugars yield for conventional hydrolysis ranged from 6.4 $g.kg^{-1}$ (run 10) to 143.5 $g.kg^{-1}$ (run 7), whereas for ultrasound-assisted hydrolysis from 22.5 $g.kg^{-1}$ (run 1) to 159.6 $g.kg^{-1}$ (run 17). The mean yields of reducing sugars (considering the 17 runs) were 60.9 and 79.5 $g.kg^{-1}$, in the conventional and ultrasound-assisted hydrolysis, respectively. This result showed that the uses of ultrasound can be considered a tool to promoting the process intensification. In a general way, the yields of reducing sugars in the ultrasound-assisted hydrolysis were always greater than for conventional one.

Table 1. Matrix of the CCRD expressing the yield of reducing sugars for reactions carried out using acid hydrolysis and US assisted-acid hydrolysis after 4 h of reaction

Run	Moisture Content (wt%)	Temperature (°C)	Acid concentration (wt%)	Conventional Yield ($g\ kg^{-1}$)	Ultrasound Yield ($g\ kg^{-1}$)
1	48 (-1)	37.0 (-1)	12.1 (-1)	12.9	22.5
2	72 (1)	37.0 (-1)	12.1 (-1)	18.3	30.4
3	48 (-1)	58.0 (1)	12.1 (-1)	12.4	65.5
4	72 (1)	58.0 (1)	12.1 (-1)	9.1	52.5
5	48 (-1)	37.0 (-1)	25.4 (1)	14.0	33.6
6	72 (1)	37.0 (-1)	25.4 (1)	21.6	36.0
7	48 (-1)	58.0 (1)	25.4 (1)	143.5	51.0
8	72 (1)	58.0 (1)	25.4 (1)	107.4	73.2
9	40 (-1.68)	47.5 (0)	18.8 (0)	12.3	139.1
10	80 (1.68)	47.5 (0)	18.8 (0)	6.4	70.5
11	60 (0)	30.0 (-1.68)	18.8 (0)	93.8	46.9
12	60 (0)	65.0 (1.68)	18.8 (0)	71.9	72.1
13	60 (0)	47.5 (0)	7.5 (-1.68)	91.6	44.8
14	60 (0)	47.5 (0)	30.0 (1.68)	85.4	93.7
15	60 (0)	47.5 (0)	18.8 (0)	105.8	165.8
16	60 (0)	47.5 (0)	18.8 (0)	99.8	155.1
17	60 (0)	47.5 (0)	18.8 (0)	129.8	159.6

Considering only the runs 15-17 (central points of the CCRD), the increasing in the yield for the ultrasound-assisted hydrolysis was 55% higher than conventional one, which is statistically significant by Tukey test (p<0.1). The highest yield obtained in the presence of ultrasound can be associated with the partial decomposition of the lignin network structure due to cavitation effects (Bussemaker and Zhang, 2013). The ultrasonic irradiation can break the bind of lignin to some extent, and releases more cellulose and hemicellulose on the surface of rice hulls (Shi *et al.*, 2013). Zhang *et al.* (2007) used phosphoric acid at 50°C to dissolve hemicellulose and lignin, reducing cellulose crystallinity and increasing its digestibility. The same authors concluded that the use of concentrated phosphoric acid (> 81%) was an ideal solvent, because the dissolution of cellulose by phosphoric acid occurs at low temperatures, and phosphoric acid may dissolve the cellulose in the presence of water. Furthermore, the regenerated cellulose remains in the amorphous form, suitable for the hydrolysis, and the residual phosphorous acid has no inhibitory effects on the sequential hydrolysis and fermentation.

The experimental results presented in the Table 1 were used to determining the effects of the studied variables on yield of reducing sugars. The effects were expressed in the form of Pareto chart, which is presented in the Fig. 1. Pareto chart is graphic manner, much used in statistical analysis, to provide information on main and cross interaction effects (variables), whether they are positive or negative and also the magnitude of the effect (Haaland, 1989). For conventional hydrolysis (Fig. 1a), the quadratic term for moisture content as well as the interaction term for temperature and acid concentration were statistically significant (p<0.1), whereas other terms as linear and interaction were not significant in the studied range (p<0.1). The negative sign of the quadratic terms indicate the presence of a maximum point for moisture content in the evaluated range, whereas the increase of temperature and acid concentration can result in the increase of the yield, since the interaction effect of this term was positive. For the ultrasound assisted hydrolysis (Fig. 1b), it was observed that only the quadratic terms of the independent variables were statistically significant (p<0.1) in the evaluated range. The negative signs of the quadratic terms indicate the presence of a maximum point for all variables in the evaluated range.



(a) (b)

Figure 1. Pareto Chart showing the effects of linear, quadratic and interaction terms of independent variables on the yielding of reducing sugars obtained by conventional (a) and ultrasound-assisted acid hydrolysis (b) (where T is temperature, M is moisture content, H is acid concentration, 1Lby2L is the interaction between temperature and moisture content, 2Lby3L is the interaction between moisture content and acid concentration and 1Lby3L is the interaction between temperature and acid concentration, L is linear and Q is quadratic terms, respectively)

In order to obtain maximum yield of reducing sugars, two empirical models were generated from the data for optimization purposes. Eq. 1 presents the significant terms ($p<0.1$) concerning to conventional hydrolysis, and Eq. 2 referring to ultrasound-assisted hydrolysis.

$$FS=112.78-39.55\ M^2+28.13\ TH \quad (1)$$

$$FS_{US}=161.97-25.65\ M^2-41.71\ T^2-38.26\ H^2 \quad (2)$$

where: *FS* and FS_{US} are the yielding of reducing sugar ($g.kg^{-1}$) obtained by conventional and ultrasound-assisted acid hydrolysis, respectively; *T*, *M* and *H* are the coded temperature, moisture content and acid concentration, respectively.

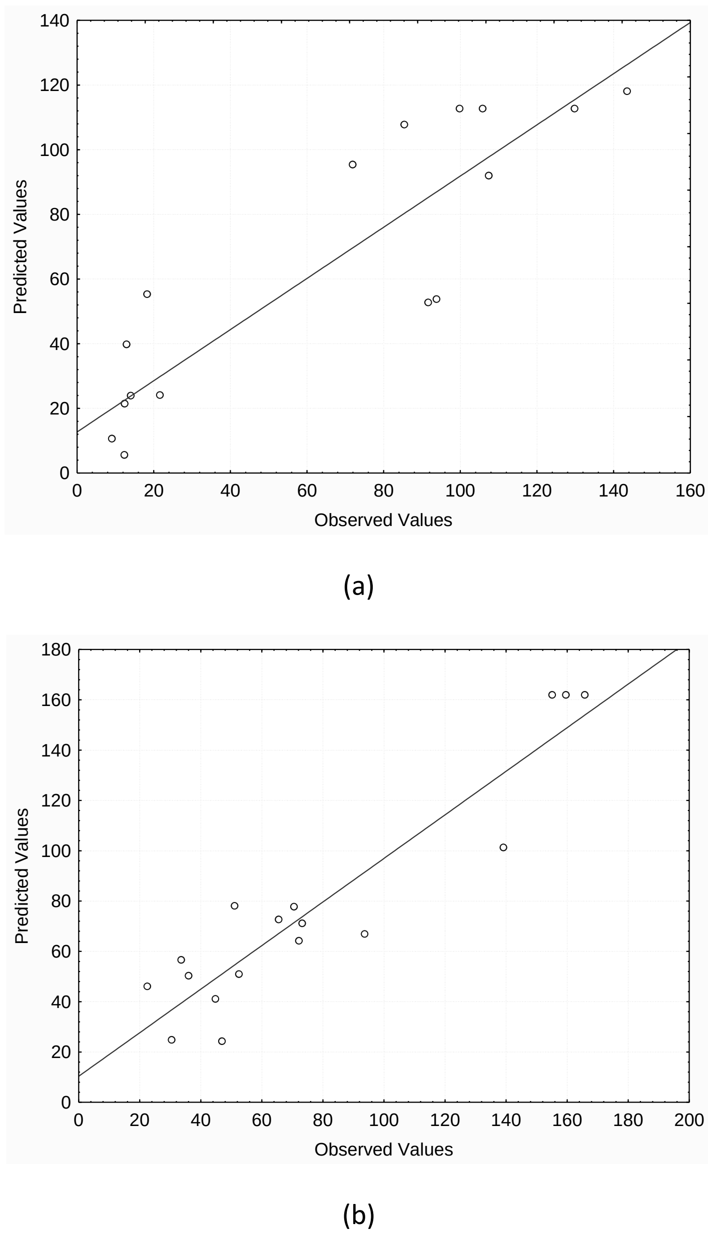
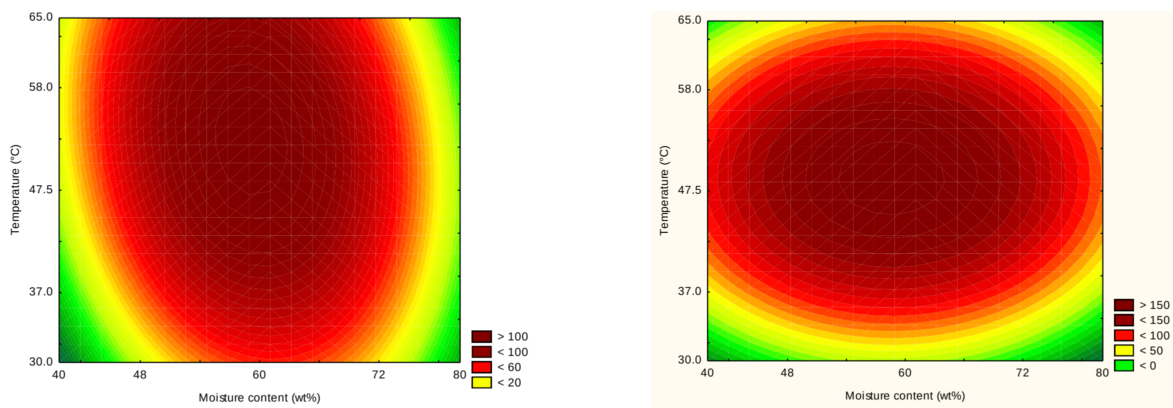


Figure 2. Predicted vs. observed values by conventional (a) and ultrasound-assisted acid hydrolysis (b)

These models were validated by analysis of variance (ANOVA). The calculated F-test for Eq. 1 and 2 were about 3.2 and 6.5 times greater than the tabulated ones for significance at p=0.1, and the determination coefficients (R^2) were 0.7915 and 0.8707, respectively. A practical rule for evaluating the determination coefficient is that it should be at least 0.75 or greater (Haaland, 1989).

The lowest value for determination coefficient obtained for conventional hydrolysis can be attributed to the low uniformity of solid-state media. How the media was not homogenized during the reactions, the experimental variance among the experiments is greater than for ultrasound-assisted reaction, where the ultrasonic irradiation promotes a better media homogenization since it improves the mass transfer. This could be implies in a worst fitting of experimental data. However, the Figure 2 shows the predicted versus experimental values for conventional (a) and ultrasound-assisted acid hydrolysis (b) for demonstrated the adequacy of the model. However, both models were validated by ANOVA and can be used for optimization purposes. The applicability of these models is explored in Fig. 3 to 5.

Fig. 3 presents the influence of the moisture content and temperature on the yield of reducing sugars for conventional (Fig. 3a) and ultrasound-assisted hydrolysis (Fig. 3b). For conventional hydrolysis, the maximum yield of reducing sugar predicted is around 100 $g.kg^{-1}$ for 60 wt% of moisture content and temperature ranging from 45 to 62 °C. For ultrasound-assisted hydrolysis, the maximum yield of reducing sugar predicted is around 175 $g.kg^{-1}$, almost two times greater than conventional one. The highest yield can be obtained for a temperature ranging from 44 to 48°C and moisture content from 54 to 64 wt%.

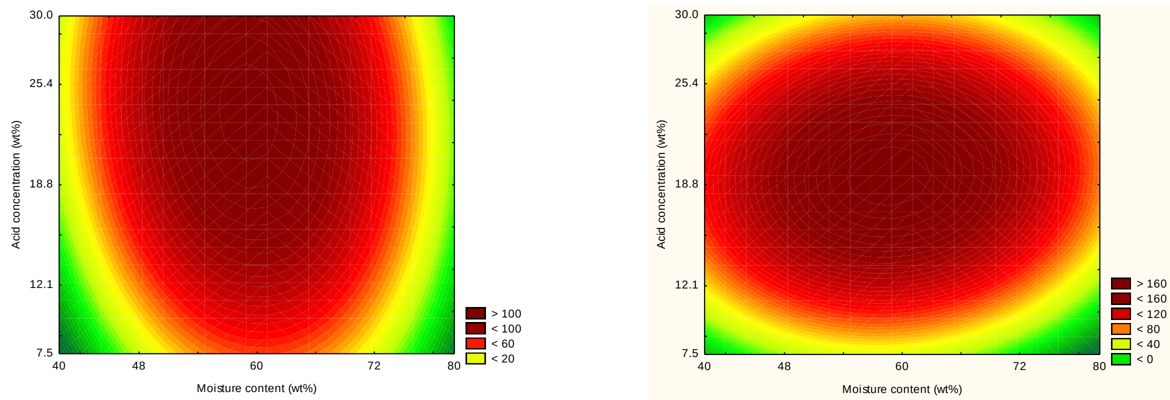


(a) (b)

Figure 3. Contour plots showing the influence of temperature and moisture content on the yielding of reducing sugars obtained by conventional (a) and ultrasound-assisted acid hydrolysis (b). Acid concentration was maintained at the central point of the CCRD (18.8 wt%)

Fig. 4 presents the influence of the acid concentration and moisture content on the yield of reducing sugars for conventional (Fig. 4a) and ultrasound-assisted hydrolysis (Fig. 4b). The highest yield was obtained for moisture content around 60 wt% for both systems. However, the optimized region for acid concentration ranged from 18 to 28 wt% for conventional hydrolysis and from 16 to 20 wt% for ultrasound-assisted hydrolysis.

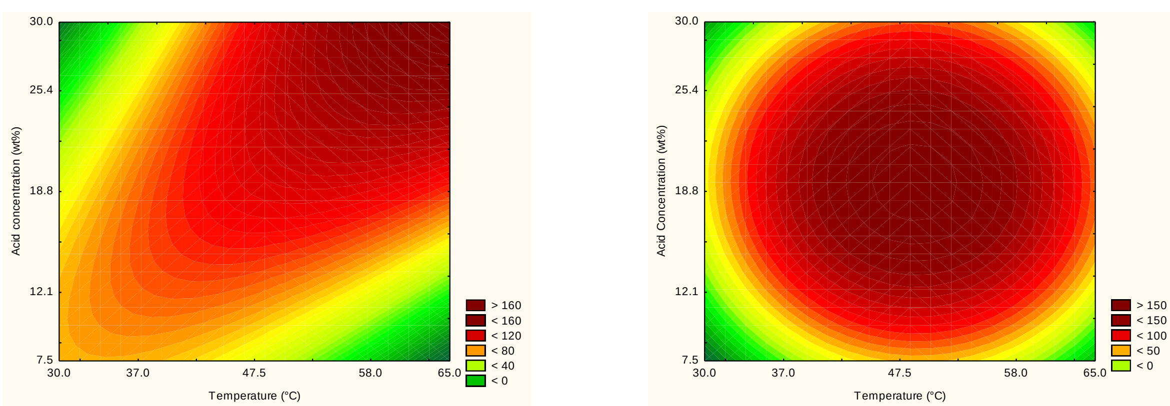
Fig. 5 presents the influence of the acid concentration and temperature on the yield of reducing sugars for conventional (Fig. 5a) and ultrasound-assisted hydrolysis (Fig. 5b). For conventional hydrolysis, the highest yield predicted was around 140 $g.kg^{-1}$ for acid concentration ranging from 24 to 30 wt% and temperature from 58 to 65 °C. By the other hand, the optimized yield for ultrasound-assisted hydrolysis presented a centered region for both independent variables. The results presented in Fig. 5 demonstrated that the ultrasound can be used as an alternative to process intensification, since it is possible to use fewer acid and lower temperature for the obtaining the higher yields of sugars compared to the conventional hydrolysis.



(a) (b)

Figure 4 Contour plots showing the influence of acid concentration and moisture content on the yielding of reducing sugars obtained by conventional (a) and ultrasound-assisted acid hydrolysis (b). Temperature was maintained at the central point of the CCRD (47.5°C)

The ultrasonic treatment changes the structure of cellulose and enhances the accessibility of the acid to it. Ultrasonic treatment can also assist the mass transfer of the dissolved components from lignocellulosic biomass and enhance the release of polysaccharides (Guo *et al.*, 2012).



(a) (b)

Figure 5. Contour plots showing the influence of acid concentration and temperature content on the yielding of reducing sugars obtained by conventional (a) and ultrasound-assisted acid hydrolysis (b). Moisture content was maintained at the central point of the CCRD (60 wt%).

4. Conclusions

This paper presented experimental data for hydrolysis of rice hulls to obtaining reduced sugars by conventional and ultrasound-assisted acid hydrolysis. The optimized condition obtained from analysis of contour plots for conventional hydrolysis was moisture content 60 wt%, acid concentration 25.4 wt% and 65 °C, being obtained a yield about 141.1 $g.kg^{-1}$. For ultrasound-assisted hydrolysis, the optimized condition was moisture content 60 wt%, acid concentration 15 wt% and 45 °C, resulting in a yield of 162.0 $g.kg^{-1}$. The yield of reducing sugar obtained by ultrasound-assisted hydrolysis at optimized condition was around 15% higher than for the conventional hydrolysis. Besides the increase in the yield obtained, the optimized acid concentration and temperature were lower than for conventional one. In practice, it is possible to obtain high yield using less acid and low temperature, in a manner that ultrasound can be used as a device for process intensification.

Acknowledgements

The authors thank CNPq and CAPES for the financial support of this work and scholarships.

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