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Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production

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ABSTRACT

The structural complexity of the lignocellulosic materials hinders enzymatic hydrolysis for what their conversion to bioethanol requires a pretreatment step. The aim of this work was to optimize the pretreatment of rice hulls with diluted acid. A Central Composite Design (CCD) was used to obtain regression equations in function of the variables: acid concentration and heating time. Optimal conditions were obtained by the Desirability function. As a strategy to obtain the best solid material for the production of ethanol by fermentation minimizing sugars degradation, the optimization of the pretreatments was performed following three scenarios. The optimum was established as the conditions that maximize glucans in the solid and xylose in the liquid (0.3% (w/v) of sulfuric acid and a 33 min). The pretreated rice hull in those conditions was treated enzymatically. The performance of the enzymatic hydrolysis was about 50% (25% of total sugars present) over a period of 48 h of reaction, and the efficiency of conversion of dissolved sugars to bioethanol was of 84%.

1. Introduction

Bioenergy – energy produced from biomass – offers the opportunity to reduce not only the carbon dioxide emissions but also the dependence of energy imports, and as well as to diversify the energy matrix, reducing the oil dependence (United Nations Organization for Food and Agriculture, 2008). Second generation bioethanol is based on raw materials rich in complex carbohydrates like cellulose. This becomes an interesting alternative to reduce competition with the food industry and to generate an added value to the agro-industrial residues (Ahring et al., 2007).

The lignocellulosic materials are formed by three structural polymers: cellulose, hemicelluloses and lignin and small quantities of other compounds (Fengel and Wegener, 1984). Among these components, carbohydrates (cellulose and hemicelluloses) can be saccharified and eventually fermented to obtain bioethanol. Although there is some background on the fermentation of mixtures of hexoses and pentoses, the processes are complex and they are not yet used industrially (Dien et al., 1998; Kuhad et al., 2011; Lindsay et al., 1995).

The availability of surface area for the enzymatic attack of cellulose fibers, the lignin content, and the crystallinity and degree of polymerization of cellulose, are the most important factors that limit their enzymatic hydrolysis (Ahring et al., 2007; Hendriks and Zeeman, 2009; Kumar et al., 2009; Mosier et al., 2005; Sun, 2010; Taherzadeh and Karimi, 2008; Zheng et al., 2009). Therefore, bioethanol production from lignocellulosic materials demands a pretreatment, that can be carried out by different techniques, using alkalis, acids, heat, pressure, solvents, etc. (Ahring et al., 2007; Fengel and Wegener, 1984; Hendriks and Zeeman, 2009; Kumar et al., 2009; Mosier et al., 2005; Sun, 2010; Taherzadeh and Karimi, 2008; Zheng et al., 2009).

Rice implies 20% of the world's nutritional energy. Rice hulls represent approximately 20% dry weight of the harvest, being an abundant lignocellulosic residue in the North East of Argentina (NEA). Rice hulls cover and protect the grain during its growth, and essentially consist of four layers, among which there are an outer epidermis coated with a thick cuticle layer of highly silicified cells, and the sclerenchyma, with thick lignified and silicified walls (Sun, 2010). The average composition of rice residues from harvest and

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processing (straw and hulls) is: cellulose (32–47%), hemicelluloses (19–27%) and lignin (5–24%), (Binod et al., 2010), whereas rice hulls consist on 36–40% cellulose, and 12–19% hemicelluloses (Banerjee et al., 2009; Saha et al., 2005; Saha and Cotta, 2007, 2008). 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 (Balconi Bevilaqua, 2010; Diel Rambo, 2009). The complex chemical composition of rice hulls represents an additional barrier for the release of cellulose.

The chemical pretreatment with diluted sulfuric acid solutions combined with enzymatic hydrolysis is considered a promising method for the production of lignocellulosic bioethanol (Ahring et al., 2007; Kumar et al., 2009). This kind of pretreatment promotes hydrolysis of hemicelluloses and part of the amorphous cellulose that results in high recovery of hemicelluloses as monomers in the liquid fraction and high cellulose content in the solid fraction (Hendriks and Zeeman, 2009; Linde et al., 2008; Sassner et al., 2008; Wyman et al., 2005; Yang and Wyman, 2008).

Due to the high temperatures and the acid conditions of the pretreatment, the sugars released by hydrolysis are degraded generating two compounds derived from furan: furfural (degradation of pentoses: xylose and arabinose) and 5-hydroxymethilfurfural or HMF (degradation of hexoses: glucose, mannose and galactose). Furfural can also degrade into formic acid or polymerize, while HMF generates equimolecular quantities of formic and levulinic acids. In addition to these two aliphatic acids, acetic acid is generated from the hydrolysis of the hemicelluloses acetyl groups (Ahring et al., 2007; Fengel and Wegener, 1984; Megawati et al., 2010; Oliva Domínguez, 2003; Talebnia et al., 2010). These degradation compounds reduce the total yield of monomeric sugars, and they are also inhibitors of the fermentation process (Olsson and Hahn-Häigerdal, 1996; Palmqvist and Hahn-Hägerdal, 2000). Very little literature is available on the pretreatment of rice hulls. Saha et al. (2005) studied the combination of pretreatment with diluted acid and enzymes in a range of conditions, concluding that the process must be optimized.

Once pretreated, the solid is saccharified by the enzymes, and the resulting glucose is then fermented to bioethanol. Regardless of the purification of the solid fraction to produce bioethanol, it is important to maintain the fraction of dissolved sugars (mostly xylose, product of the hydrolysis of hemicelluloses), leading to their use for the manufacture of byproducts. The pretreatment liquid containing dissolved sugars can also be fermented directly by the appropriate microorganisms, but the degradation compounds are inhibitors of the fermentation process.

In this work, the optimization of the pretreatments was performed by means of the combination of the equations, using the Desirability function, following three scenarios: (a) the maximization of xylose concentration and the minimization of furfural, both in the liquid; (b) the maximization of glucans and the minimization of xylans, both in the solid; (c) the maximization of glucans in the solid and of xylans in the liquid. A Central Composite Design (CCD) was used for this purpose, obtaining the regression equations based on the two independent variables: acid concentration of the solution and time. To validate the experiments, the solid fraction pretreated in the optimum condition was hydrolyzed with enzymes, and the hydrolysate was converted to bioethanol.

2. Materials and methods

2.1. Materials

The rice hulls, from plantations of Chaco, Argentina, were supplied by a local rice company. The raw materials were milled (Ariete burr mill coffee grinder, 100 W power, 280 g capacity) to a size less than 10 mm × 1 mm. The milled materials were stored at room temperature until further use.

The commercial enzymes, endoglucanases (NS 50013 Product) and cellobiases (NS 50010 Product) were provided by Novozymes S.A. The *Saccharomyces cerevisiae* inoculums used was commercial grade (Baker's yeast).

2.2. Characterization trials

2.2.1. Characterization of solids

The characterization of the raw material was accomplished using the National Renewable Energy Laboratory, Laboratory Analytical Procedure, Technical Reports (NREL/TP). It included: total solids and humidity, extractable substances in water and ethyl alcohol (NREL/TP 510-42621), structural carbohydrates: glucans, xylans and arabinans, acetyl groups (NREL/TP 510-42619), insoluble acid lignin, soluble acid lignin (NREL/TP 510-42618), and ashes (NREL/TP 510-42622). The same analyses, except for the determination of extractable substances in water and alcohol, were carried out on the resulting solids from all stages.

2.2.2. Characterization of liquids

The liquids from all stages were characterized by the determination of sugars content (glucose, xylose, and arabinose) and degrading products: furfural, 5-hydroxymethilfurfural and organic acids (formic and acetic). The degraded hexoses were calculated as the product of the HMF concentration by the stoichiometric factor 1.4286, and the degraded pentoses as the product of furfural concentration by the stoichiometric factor 1.5625 (*Mr glucose*/*Mr HMF and Mr xylose*/*Mr furfural* respectively being *Mr* the molar mass of the compound).

The quantification of sugars, organic acids and degradation products was carried out by HPLC liquid chromatography (Waters HPLC System), using an Aminex-HPX87H column (BIO-RAD) with the following chromatographic conditions: H_2SO_4 4 mM as eluent, 0.6 ml/min, 35 °C and refractive index and diode array detectors.

The structural carbohydrates of the raw material and the pretreated solid (glucans, xylans and arabinans) were calculated as the product of the sugar concentration of six and five carbons in the hydrolysates, by the stoichiometric factors 0.90 and 0.88, respectively (NREL/TP 510-42619). The acetyl groups were determined as the product of the acetic acid concentration by the stoichiometric factor 0.717 (NREL/TP 510-42619).

2.2.3. Quantification of bioethanol

The ethanol resulting from the fermentation trials was analyzed by Gas Chromatography (GC) using a Shimadzu GC 14B system equipped with a Mega Bore DB-WAX (0.53 mm ID × 1.5 μm film thickness) column and Flame Ionization Detector (FID). Chromatographic conditions were: air at 60 kPa as carrier gas, oven temperature 30 °C, temperature of the injector 180 °C and temperature of the detector 250 °C. The ethanol standards were prepared using commercial grade ethanol and methyl butyl ketone was used as internal standard.

Statistical analysis of results (ANOVA and Optimization by the Desirability function) were performed using Statgraphics Centurion XV software at 95% significance ($p < 0.05$).

2.3. Experimental design

Response surface methodology (RSM), a collection of mathematical and statistical techniques, is usually used for modeling and analyzing systems in which a response of interest is influenced by several variables, allowing the optimization of the processes (Montgomery and Runger, 2002). The influence and optimization of

the studied variables, acid concentration of the solution and heating time, were determined by a Central Composite Design (CCD) as can be seen in Table 1. The second and third columns are the levels of the variables expressed in coded values, whereas the real values are in columns 4 and 5. In this way, the physical values of the factor under study are changed to unit values that are independent of the numeric value of the factors. The value of α ($\alpha = 1.414$, a conventional choice for the CCD) is the distance from the star points to the central point (Barker, 1985).

2.4. Pretreatments

The raw material was treated with sulfuric acid solutions, varying concentrations between 0.3 and 2.4 g H_2SO_4/100 ml. The design included the point corresponding to autohydrolysis (trial 5), in which the catalyst is the acetic acid released by hydrolysis of the acetyl groups present in the raw material (Hendriks and Zeeman, 2009).

The material, at 5% of solids, was placed from 16 to 50 min in a pressure reactor at 5 atmospheres (152 °C). The time to reach the working pressure was 20 min. The pretreatment was finished by a fast decompression followed by a hot vacuum filtration. The liquid fractions were refrigerated to 4 °C whereas the pretreated solids were washed several times with distilled water to remove the remaining acid solution, then they were oven dried at 60 °C for 10 h, and finally they were stored in desiccators.

2.5. Enzymatic hydrolysis

The solid fraction resulting of the pretreatment of rice hulls at the optimums conditions (0.3% acid and 33 min), was saccharified with the commercial enzymes. The hydrolysis reaction was performed by shaking the material at 450 rpm for 48 h at 50 °C and pH 4.8, adjusted with sodium acetate buffer. Similar conditions were used by Fang et al. (2010). The dose was 40 FPU/g of cellulose and the relationship endoglucanase:cellobiase was 10:1. An excess of enzymes was used in order to avoid any limitation of this variable. The samples (0.5 ml) were kept at −16 °C before the HPLC analysis.

2.6. Fermentation

The batch fermentation experiments were carried out in 500 ml fleakers under anaerobic conditions with working volumes of 300 ml. The hydrolysates of the solid fraction resulting of the pretreatment of rice hulls at the optimal conditions were used as substrates, amended with mineral nutrients ($(NH_4)_2SO_4$, KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, $CaCl_2$, $CuSO_4{\cdot}5H_20$, MnCl, $ZnSO_4$ and $CoCl_2$). The fermentation was carried out using *S. cerevisiae* at pH 4.7 and 37 °C for 72 h. Fermentation efficiency was calculated by the relationship:

$$\left(\frac{\text{Practical yield}}{\text{Theoretical yield}}\right) \times 100$$

3. Results and discussion

3.1. Raw material

The chemical composition of rice hulls is shown in Table 2. Rice hulls, as compared with other lignocellulosic materials (Fengel and Wegener, 1984), have relatively low levels of lignin but high levels of ashes and extractives (approximately 23% of the dry material).

This composition agrees quite well with the data published by Hsu et al. (2010), who found that the main sugars from rice straw contain glucose (36.6%) and xylose (16.1%), 14.9% (w/w) of lignin and high content of the ash (14.5% (w/w)) mainly silica. Banerjee

Table 1
Pretreatment conditions of the rice hulls (Central Composite Design, CCD), concentration of the hydrolyzed sugars and of the degradation products in the pretreatment liquid and components of the solid fractions after the pretreatments.

Pretreatment conditions of CCD design					Hydrolyzed sugars and of the degradation products in the pretreatment liquid[a]					Components of the solid fractions after the pretreatments[b]				
Test no.	H_2SO_4 sol.	Time	H_2SO_4 (% (w/v))	Time (min)	Glucose (%)	Xylose (%)	Arabinose (%)	HMF (%)	Furfural (%)	Glucans (%)	Xylans (%)	Arabinans (%)	Lignin (%)	Ashes (%)
1	−1	−1	0.3	16	10.3	15.6	1.92	0.04	0.11	37.6	4.57	0.30	23.8	22.8
2	+1	−1	1.7	16	14.5	14.0	1.94	0.20	0.99	40.7	0.12	–	30.2	25.0
3	−1	+1	0.3	44	11.1	14.1	1.64	0.15	0.72	44.4	2.38	–	28.5	24.1
4	+1	+1	1.7	44	14.3	8.56	1.50	0.25	1.94	38.4	0.06	–	29.9	25.8
5	−α	0	0.0	30	0.56	0.44	0.21	0.01	0.01	34.6	15.70	1.43	20.5	17.2
6	+α	0	2.4	30	22.4	7.33	1.35	0.36	2.47	41.3	0.03	0.49	29.3	25.0
7	0	−α	1.0	10	13.3	14.7	1.77	0.13	0.50	38.8	1.72	0.19	24.9	23.6
8	0	+α	1.0	50	15.9	9.09	1.52	0.29	1.48	41.7	0.07	–	30.2	25.4
9	0	0	1.0	30	10.7	13.5	1.82	0.30	1.25	43.2	1.02	–	24.8	24.9
10	0	0	1.0	30	13.5	13.0	1.77	0.29	1.13	44.0	0.91	–	27.7	25.5

[a] Percentage over dry weight.
[b] Percentage over dry weight of treated solids.

Table 2
Chemical characterization of rice hulls (% oven dry weight).

Components	% Oven dry weight
Total structural carbohydrates	48.7
– Glucans	34.1
– Xylans	13.1
– Arabinans	1.5
Total lignin	19.0
– Acid insoluble lignin	17.2
– Acid soluble lignin	1.8
Acetyl groups	1.2
Ashes	15.0
Extractives	8.2
Others (waxes, etc., by difference)	7.9

et al. (2009) reported that the untreated rice husks have a high proportion of celluloses but very similar quantities of the others components (42.2% (w/w) of celluloses, 18.5% (w/w) of hemicelluloses, 19.4% (w/w) of lignin and 17.3% (w/w) of ashes).

3.2. Pretreatments

3.2.1. Maximization of xylose and minimization of furfural, both in the liquid

The concentrations of the hydrolyzed sugars (glucose, xylose, and arabinose) and of the degradation products (HMF and furfural) in the pretreatment liquid are shown in Table 1.

The crystalline structure of the cellulose makes impossible its hydrolysis in the mild conditions used in this work. The hemicelluloses, due to their amorphous character, are the portion of the material more easily hydrolyzed (Table 1). The prevalent structural monomer in hemicelluloses of rice hulls is xylose, followed by glucose and arabinose. The most severe treatments have generated a greater dissolution of sugars in the pretreatment liquid. Nevertheless, these conditions also made decrease their concentration in the liquid because of their conversion into degrading products (HMF from glucose and furfural from xylose).

Both the acid concentration and the time had a significant negative effect in the concentration of xylose in the liquid ($p = 0.00$). The highest concentration of xylose in the liquid was obtained in trial 1 (0.3% (w/v) of H_2SO_4, 16 min). On the contrary, the lowest concentration of xylose in the liquid was found at the longest time (trial 4: 1.7% (w/v) of H_2SO_4, 44 min), due to their degradation to furfural. In trial 5 (autohydrolysis, 30 min) a soft hydrolysis took place, producing low concentrations of xylose, glucose and arabinose in the liquid, from which 34% of the glucose and 78% of the xylose are present as oligomers.

Arabinose concentration in the liquid was similar in all treatments (variation of 0.59%). Like in the case of xylose, the treatment carried out without acid produced a very low dissolution of arabinose. Glucans from hemicelluloses and cellulose have been hydrolyzed at the highest concentrations of acid (trial 6: 2.4% (w/v) of H_2SO_4, 30 min). At the same time, this treatment brought about the highest degradation of glucose to HMF.

The regression equations relating the organic components in the liquid fraction with the treatment conditions were:

$$\%\,\text{Xylose} = 12.57 - 2.37 \times C - 1.92 \times t - 0.91 \times C \times t \quad R^2 = 0.91 \tag{1}$$

$$\%\,\text{Glucose} = 10.18 + 1.88 \times C + 0.61 \times t + 2.35 \times t^2 \quad R^2 = 0.96 \tag{2}$$

$$\%\,\text{Arabinose} = 1.71 - 0.11 \times C - 0.13 \times t \quad R^2 = 0.68 \tag{3}$$

$$\%\,\text{Furfural} = 1.73 + 0.97 \times C + 0.66 \times t \quad R^2 = 0.93 \tag{4}$$

$$\%\,\text{HMF} = 0.18 + 0.09 \times C + 0.05 \times t \quad R^2 = 0.86 \tag{5}$$

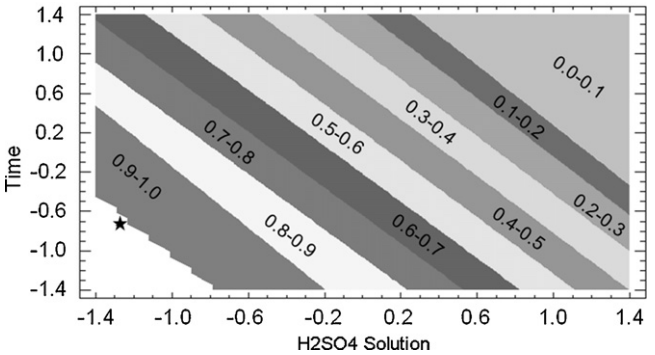

Fig. 1. Contour plot for the maximization of xylose concentration and the minimization of furfural in the liquid (the star denotes the optimum of the Desirability function).

$$\%\,\text{Acetic acid} = 1.84 + 0.33 \times C - 0.41 \times C^2 \quad R^2 = 0.80 \tag{6}$$

$$\%\,\text{Formic acid} = 0.49 + 0.25 \times C + 0.21 \times t \quad R^2 = 0.83 \tag{7}$$

where C is the concentration of sulfuric acid and t is heating time (both as coded variables).

Only the variables with coefficients having a level of significance of 95% were considered in the equations. All the presented equations show significant adjustments at 95% of significance. In the cases of compounds found in very low quantities, experimental errors can influence the quantification (case of the arabinose, Eq. (3)). Moreover, there is a continuous degradation of the compounds to others of lower molecular weight, even some unidentified, which could also affect the fit.

Through the coefficients of the equations it can be observed that the effect of the acid concentration is more important than the effect of the heating time. The only exception is arabinose, but, as it was already explained, the measured quantities were so low that the equation can be affected by experimental errors.

Both factors produce a decrease in xylose and arabinose and an increase of glucose and degradation products in the liquid. Only xylose is affected by the interaction between factors. Quadratic effects on glucose and acetic acid indicate that the values of the variables stabilized, i.e. there is a point from which not greater modification occurs due to that factor.

The Desirability function is the most popular method for the solution of multiresponse optimization problem. This approach to simultaneously optimize multiple equations, translates the functions to a common scale ([0,1]), and combines them using the geometric mean and optimizing the overall metric. This function was used to maximize the extraction of hemicelluloses, minimizing the generation of degradation products during the pretreatment. Fig. 1 shows the Contour Plot of the Desirability function that maximizes xylose concentration in the liquid whereas minimizing furfural (Eqs. (1) and (4)).

As shown in Fig. 1, the optimal conditions (Desirability 0.9–1.0) correspond to low concentrations of sulfuric acid and time. The best point (star in Fig. 1) corresponds to 0.1% of sulfuric acid concentration (coded variable −1.28) and 20 min (coded variable −0.74).

3.2.2. Maximization of glucans and minimization of xylans, both in the solid

The percentages of each component in the pretreated solids, expressed on dry base, can be observed in Table 1.

At the end of the pretreatment, the solid consisted mostly of cellulose, lignin and ashes. The studied variables (acid concentration and time) had no significant impact on the delignification at these levels. Ashes also remained in the solid. Arabans are practically missing in pretreated solid. The regression equations of these

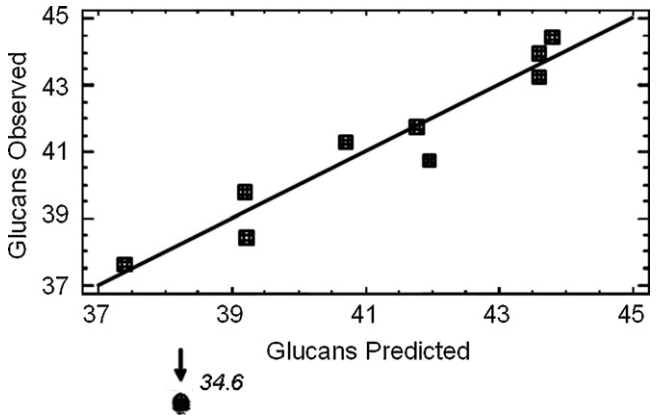

Fig. 2. Observed vs. predicted values of glucans in the solid fractions, highlighting (circle) the approximate position of the pretreatment without acid.

three parameters are no shown, as they do not illustrate relevant results.

The regression equations for glucans and xylans in the solid fraction were:

$$\%\,\text{Glucans} = 43.6 + 0.91 \times t - 1.45 \times C^2 - 2.29 \times C \times t - 1.56 \times t^2 \quad R^2 = 0.92 \tag{8}$$

$$\%\,\text{Xylans} = 0.93 - 1.71 \times C - 0.58 \times t + 0.81 \times C^2 + 0.54 \times C \times t \quad R^2 = 0.99 \tag{9}$$

The autohydrolysis treatment (trial 5, without acid for 30 min) is a special case, since it produced a very low chemical attack, resulting in anomalous quantities of the products in the liquid fractions compared to the other treatments. As mentioned above, rice hulls have an outer epidermis coated with a thick cuticle layer of highly silicified cells and a sclerenchyma with a thick lignified and silicified wall (Sun, 2010). In absence of acid, conditions are too weak to penetrate these layers, becoming a drawback for hydrolysis. A demonstration of the foregoing emerges from Fig. 2. Facing the excellent correlation between glucans measured and calculated by Eq. (8), the point corresponding to the autohydrolysis is a clear outlier. This behavior means that the treatment had no effect, i.e. the content of glucans as well as other components in the resulting solid was similar to that of the raw material.

The difference in glucans between the acid treatments which maximize (0.3% of acid, 44 min) and minimize (0.3% of acid and 16 min) glucans in the solid was 7%. Severe conditions (high concentration of acid combined with long heating times) almost completely hydrolyze hemicelluloses, increasing the porosity of the solid and therefore its superficial area. It is expected that this will improve the subsequent enzymatic hydrolysis for bioethanol production.

The application of the Desirability function, combining the maximization of glucans (Eq. (8)) and the minimization of xylans in the solid fraction (Eq. (9)) are presented in Fig. 3. There is a clear optimum (star in the figure) at 1.3% sulfuric acid and 33 min approximately (in coded variables: acid 0.38 and time 0.21).

3.2.3. Maximization of glucans in the solid and of xylose in the liquid

The optimization of the pretreatment of rice hulls with diluted acid by means of the Desirability function based on the maximization of glucans in the solid fraction (Eq. (8)) and of xylose in the liquid fraction (Eq. (1)) is show in Fig. 4.

Following these criteria, the optimal pretreatment process can be achieved with a concentration of sulfuric acid of 0.3% and a heating time of 33 min (star in Fig. 4). Others studies indicate that a treatment involving 1.0% (v/v) H_2SO_4, 121 °C for 15 min gave good yields of sugars after enzymatic saccharification without excessive formation of sugar degradation products (Saha et al., 2005). Karimi et al. (2006) found good results (xylose maximum yield of 80.8%) using an acid concentration of 0.5%, a pressure of 15 bar, 10 min retention time. The optimal conditions for the pretreatment of rapeseed straw for ethanol production were 1% sulfuric acid for 10 min at 180 °C (Lu et al., 2009).

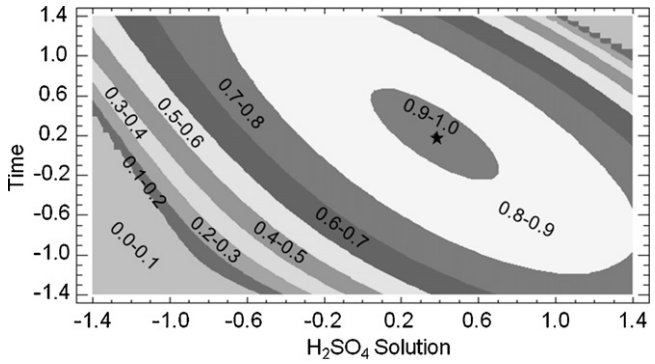

Fig. 3. Contour plot for the maximization of glucans and the minimization of xylans in the solid fraction (the star denotes the optimum of the Desirability function).

An additional set of diluted acid pretreatment experiments at these specific conditions was performed to corroborate the predicted values. The experimental value for glucans concentration in the solid was 52.8% whereas the predicted value was 47.8%. Moreover, the experimental value for xylose extracted and solubilized in the liquid was 14.7% and the predicted value was 14.7%.

3.3. Enzymatic saccharification of pretreated rice hulls and fermentation of the hydrolysates

The solid fraction resulting of the pretreatment of rice hulls at 0.3% acid and 33 min was enzymatically saccharified. The performance of the enzymatic hydrolysis was determined by measuring the yield of glucose from the cellulose conversion, expressed as the percentage of glucose released in relation to the total amount of glucose in the solid. The performance of the reaction was about 50% over a period of 48 h of reaction.

After the pretreatment with diluted acid and the enzyme saccharification of the rice hull, the fermentation of the hydrolysates produced 4.42 g/l of bioethanol. The efficiency of the conversion was 84%. This means 0.43 g bioethanol/g of sugars and 0.11 g bioethanol/g of rice hulls pretreated and 0.06 g bioethanol/g of crude rice hull. These results agree with those of other researchers.

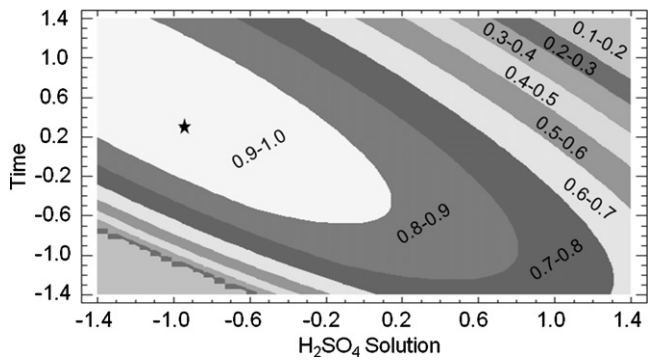

Fig. 4. Contour plot for the maximization of glucans in the solid fraction and of xylose in the liquid fraction (the star denotes the optimum of the Desirability function).

Saha and Cotta (2008) found a yield of 0.49 g bioethanol/g sugar, whereas Saha et al. (2005) found a 60% of hydrolysis conversion, 0.40 g of bioethanol/g of sugars and 0.11 g of bioethanol/g of rice hull.

4. Conclusions

The optimization of the pretreatments was performed following three scenarios. The highest purity of glucans in the solid fraction of the acid pretreatment of rice hulls was achieved with an acid concentration of 1.3% and 33 min of heating time, but these conditions inevitably result in a great degradation of xylose to furfural. As a compromise to maximize the utilization of sugars present in the raw material, the optimum was established as the conditions that maximize glucans in the solid, and xylose in the liquid (0.3% of sulfuric acid and a 33 min). In these last conditions, 50% of the glucans remained in the solid fraction. None of the combinations produced significant delignification or ashes removal.

The performance of the enzymatic hydrolysis of the pretreated rice hull in the selected conditions was about 50% (25% of total sugars present) over a period of 48 h of reaction, and the efficiency of conversion of dissolved sugars to bioethanol was 84%.

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