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EFFECTS OF ALKALINE ORGANOSOLV DELIGNIFICATION ON ENZYMATIC CONVERSION OF CELLULOSE FROM SUGARCANE BAGASSE PRETREATED BY STEAM EXPLOSION

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Recibido 07 Mayo 2013; aceptado 23 Mayo 2013

Disponible en línea: 15 Julio 2013

Abstract: The enzymatic conversion of pretreated and delignificated sugarcane bagasse was evaluated by L₁₈ Taguchi experimental design. The factors selected for the study were: ethanol and caustic soda concentration, temperature, reaction time and fiber/liquor ratio. The NaOH concentration was the only significant factor in the delignification, hemicellulose solubilization and cellulose conversion. Recovery of ethanol was influenced by only the fiber/liquor ratio, for the same level. The lignin and hemicellulose removal from bagasse pretreated by steam explosion has reached over 80% and 55%, respectively. Pulps with the lowest lignin and hemicellulose contents had the best results of the cellulose conversion, almost 70%.

Keywords: Paper pretreatment, bagasse sugarcane, organosolv, enzymatic conversion, second-generation ethanol.

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1. INTRODUCTION

Alternatives to petroleum-derived fuels are being required in order to reduce the world's dependence on non-renewable resources. The most common renewable fuel today is ethanol derived from starch and sucrose, arising on the corn grain and the sugar cane, respectively. The extensive researches to new technologies of ethanol production have been developed in the last two decades ([Mabee and Saddler, 2010](#)) because of the limitations that will probably occur in the near future to supply the raw materials of this mills. Therefore, the conversion of biomass into ethanol has been considered an attractive pathway for future supplies of liquid fuels. These technologies include a low-cost thermochemical pretreatment, highly effective cellulose and hemicellulose hydrolysis and an efficient and robust fermentative microorganism.

Sugarcane bagasse, a waste in the process of sugar extraction, is an abundant and low-cost lignocellulosic material on alcohol and sugar industry. After processing, approximately 280 kg of bagasse remains per ton of sugarcane ([Carrasco et al., 2010](#)). Sugarcane bagasse as well as other types of plant biomass, is composed by cellulose, hemicelluloses, lignin, and small amounts of extractives and mineral salts. The structural components are distributed in a lamellar structure. In addition, the lignocellulosic biomass has also the strong crystalline arrangement of cellulose and the protective effects by lignin and hemicelluloses that difficult the access of enzymes to the β -1 \rightarrow 4 glycosidic bonds of carbohydrates ([Carrasco et al., 2010](#)). Thus, pretreatment and delignification processes are required to disrupt the cellulose-hemicellulose-lignin complex, and they are important technological steps for the fractionation of lignocellulosic biomass.

The steam explosion pretreatment has been claimed as one of the most successful options for fractionation of lignocellulosic biomass ([Ramos, 2003](#)). That consists in a short cooking at high temperature and pressure in saturated steam. Following the cooking, the digester is discharged by a fast decompression. The mechanical effect causes a massive disruption of the fibers ([Rosa et al., 1999](#)) and the high-pressure steam modifies the plant cell wall structure, yielding a dark brown material from which partially hydrolyzed hemicelluloses are easily recovered by water-

washing, leaving a water-insoluble fraction composed of cellulose, residual hemicelluloses and a chemically modified lignin that can be further extracted by alkaline treatments ([Ramos, 2003](#)).

On the other hand, pretreatment alone is not sufficient to extensively remove lignin. The lignin also forms a protective physical barrier against enzymatic attack. So, alkaline extraction or soda process is used for its removal, contributing to fast enzymatic saccharification of biomass with a high sugar yield for both hexoses and pentoses ([Silva et al., 2011](#)).

[Doherty et al. \(2011\)](#) comments that the soda process is currently the predominant method for lignin extraction of non-wood material, such as bagasse, wheat straw, hemp, kenaf and sisal. The organosolv process, which uses an organic solvent in pulping liquor, offers an alternative to conventional delignification processes with significant environment advantage ([El-Sakhawy et al., 1996](#)). This technology is considered a green process, since it reduces production of solids, liquids and gaseous residual. The degradation of carbohydrate is also lower than soda process ([Doherty et al., 2011](#)). The organic solvent on organosolv delignification promotes the dissolution and reduces condensation reactions of lignin ([Sarkanen, 1990](#)).

The present study was conducted to determine the effect of ethanol-soda organosolv delignification on the cellulose conversion into fermentable sugars from pretreated sugarcane bagasse. On the section 2.1, the lignocellulosic material was submitted to an industrial steam explosion pretreatment followed by ethanol-soda delignification, shown on the section 2.2, and enzymatic hydrolysis, on 2.4, being characterized on section 2.5. Finally, are shown the results and conclusions of the study.

2. MATERIALS AND METHODS

2.1. Pretreatment of lignocellulosic material

The sugarcane bagasse used in this study was provided by the Usina de Açúcar, Álcool e Biodiesel Vale do Rosário from Orlandia/SP—Brazil. The pretreatment of bagasse was carried out in the industrial reactors of the Mill. This company has three 5 m³ reactors for steam-

explosion pretreatment of bagasse which is used to prepare the animal feed.

The pretreatment was performed under 14.5 – 15.5 atm pressure (temperature near 200 °C) for 7 min. This condition was optimized for production of animal feed and it was not possible to test other conditions. The idea is employing the same equipment and conditions and thus the Mill could have two processes from only one material. Besides, the distance between reactor and cyclone is very large which prevented to carry out tests with lower pressures.

After steam-explosion pretreatment, the bagasse was continuously washed with distilled water to remove the hydrolyzed hemicellulose fraction in order to be used in studies to obtain xylo-oligosaccharides and xylitol by fermentation pathway, within the concept of biorefinery. The washed material was stored in 60 L bags after sundrying.

2.2. Experimental design of organosolv delignification

An organosolv process was used to study the lignin removal of the pretreated bagasse. The organosolv processes is based on the use of organic solvents that promote the dissolution and reduces condensation reactions of lignin ([Hongzhang and Liying, 2007](#)). The ethanol-soda organosolv process was chosen for delignification of the pretreated bagasse, since the presence of sodium hydroxide improves the delignifying ability of ethanol ([Muurinen, 2000](#)).

For this study, a L₁₈ Taguchi array was used to evaluate the parameter effects of the organosolv delignification. This array was chosen because it is able to include several factors in a reduced account of trials. This type of array has 8 maximum numbers of factors, one of them with two levels whereas the others with three levels. For this work, only five columns of the array were used, factors 2, 3, 4, 5 and 6. The factors of experimental design were concentration of ethanol and caustic soda, temperature, reaction time and fiber/liquor ratio and are shown on [Table 1](#). The levels for each factor are shown on [Table 2](#), which were selected according to [Hongzhang and Liying \(2007\)](#). The factors 1, 7 and 8 were used to calculate standard error of the experimental design. The response variables of Taguchi array were delignification rate,

hemicellulose solubilization, ethanol recovery and enzymatic conversion of cellulose.

Table 1. L₁₈ Taguchi array used in the experimental design of organosolv delignification of bagasse

Trial	Factor							
	1	2	3	4	5	6	7	8
1	1	1	1	1	1	1	1	1
2	1	1	2	2	2	2	2	2
3	1	1	3	3	3	3	3	3
4	1	2	1	1	2	2	3	3
5	1	2	2	2	3	3	1	1
6	1	2	3	3	1	1	2	2
7	1	3	1	2	1	3	2	3
8	1	3	2	3	2	1	3	1
9	1	3	3	1	3	2	1	2
10	2	1	1	3	3	2	2	1
11	2	1	2	1	1	3	3	2
12	2	1	3	2	2	1	1	3
13	2	2	1	2	3	1	3	2
14	2	2	2	3	1	2	1	3
15	2	2	3	1	2	3	2	1
16	2	3	1	3	2	3	1	2
17	2	3	2	1	3	1	2	3
18	2	3	3	2	1	2	3	1

Table 2. Factor levels of L₁₈ Taguchi array used in the experimental design to organosolv delignification of bagasse

Factor	Level		
	1	2	3
Ethanol (%)	40	60	80
Temperature (°C)	100	130	160
Time (min)	30	60	90
NaOH (%)	0.1	0.55	1.0
Fiber/Liquor Ratio	1:10	1:7.5	1:5

The trials of L₁₈ Taguchi array were carried out in a 500 mL stainless steel ampoule heated by silicone bath. 30 g of steam-explosion bagasse were used to all trials of experimental design. After reaction time, the ampoule was quenched by ice bath. After that, the black liquor was separated by filtration, the solid was washed with 2 L distilled water at 70°C and the ethanol in liquor was evaporated in rotary evaporator. A

sample of 5 mL of distillate was added in one bottle properly weighed, with the aid of a volumetric pipette, to determine the mass of the corresponding volume. Thus, it was possible to determine the density of the distillate.

The ethanol concentration in distillate was calculated according to the expression

$$\%_{ETHANOL} (v/v) = 100 \cdot \frac{1 - d_{distilled}}{1 - d_{Ethanol}}, \quad (1)$$

where $d_{distilled}$ is the distilled density (g/mL) and $d_{Ethanol}$ is the density of pure ethanol at 25°C (0.791 g/mL), value obtained in the label of product.

The percentage of solvent recovery was calculated from the ethanol concentration and the volume of liquor. The analysis of the results of experimental design was performed using the software STATISTICA version 7.0 of the StatSoft Company.

2.3. Enzymes

The enzymes used were commercial cellulase concentrates, the Celluclast 1.5 L, produced by *Trichoderma reesei*, supplemented with Novozym 188, the β -glucosidase, produced by *Aspergillus niger*, kindly supplied by Novozymes Latin America Ltd.

Filter paper activity of the commercial cellulase concentrate was measured according to [Mandels et al. \(1976\)](#), and expressed in filter paper units (FPU). The formed reducing sugar was estimated by dinitrosalicylic acid ([Miller, 1956](#)). One unit of FPU is defined as the amount of enzyme required to liberate 1 μ mol of glucose from n° 1 Whatmann filter paper per minute at 50°C. Activity of the β -glucosidase was measured according to [Mongkolthanaruk and Dharmstithi \(2002\)](#).

2.4. Enzymatic hydrolysis

Only one condition of enzymatic hydrolysis was used in order to evaluate only the effect of organosolv delignification parameters on the cellulose conversion of delignified bagasse.

The cellulose, 15 FPU/g substrate, and β -glucosidase, 10 IU/g substrate, were added to

0.05 mol.L⁻¹ sodium citrate buffer pH 4.8 supplemented with 0.02% (w/v) sodium azide to inhibit microbial contamination, and then mixed with the substrate at 1:10 (w/v) fiber/liquor ratio. The experiments were carried out in 125 mL Erlenmeyer flasks containing 30 mL total reaction volume. The flasks were sealed with a plastic film and incubated in a rotary shaker at 100 rpm, 45°C during 72 h.

Pulps with 70% moisture were employed to aid the self soaking. After 72 h reaction, the hydrolysates were heated for 5 min in a boiling water bath to precipitate the protein and prevent further hydrolysis. The fiber residue was separated of hydrolysate by paper filtration and washed with 15 mL of distilled water. Sugars contained in filtrate were quantitatively determined by high performance liquid chromatography (HPLC).

The cellulose conversion ratio was calculated according to the expression

$$CC = \frac{m_{glucose} \times f_h}{m_{initial} \times y_i} \times 100, \quad (2)$$

where CC is the enzymatic cellulose conversion, $m_{glucose}$ is the glucose mass in the hydrolysate (w), $m_{initial}$ is the dry mass of lignocellulosic material, before enzymatic hydrolysis (w), y_i is the cellulose content in the lignocellulosic material and f_h is the hydrolysis factor of cellulose, correspondent to 0.9.

2.5 Chemical characterization of lignocellulosic materials

The chemical composition: cellulose, hemicelluloses and lignin, of the raw material, pretreated bagasse and cellulose pulp, was determined according to Gouveia et al. (2009). Carbohydrates and organic acids were determined by HPLC in a Shimadzu LC-10AD chromatograph equipped with a Shimadzu RID-6A refractive index detector and a Aminex HPX-87H, 300 \times 7.8 mm, Bio-Rad Laboratories Ltd, column. The samples were diluted with deionized water, filtered through Sep-Pak C18 filters, Millipore, and thus injected in the chromatograph under these conditions: 45°C column temperature, 0.005 mol.L⁻¹ sulfuric acid as mobile phase at 0.6 mL.min⁻¹ flow rate.

3. RESULTS AND DISCUSSION

The chemical characterization of the used bagasse is shown on [Table 3](#). The data are similar to those reported by the literature ([Silva et al., 2011](#); [Rocha et al., 2012](#)).

Table 3. Chemical composition in percentage, of the in natura bagasse and the pretreated bagasse.

Component	<i>In natura</i> bagasse	Pretreated bagasse
Yield		68,0%
Cellulose	44,0 ± 1,0	51,7 ± 0,6
Hemicellulose	25,8 ± 0,8	8,9 ± 0,1
Lignin	28,2 ± 0,8	34,3 ± 0,3
Ash	1,4 ± 0,2	5,5 ± 0,2
Total	99,2 ± 0,8	100,3 ± 0,4

The analysis of [Table 3](#) revealed that most of the hemicellulose is solubilized once the concentration was reduced to 8.9% after the pre-treatment, while in sugarcane bagasse represented approximately 26% of the composition of the material. These data show, beyond the chemical composition, that pre-treatment with steam explosion has the characteristic of preferentially removing the hemicellulose in the cellulose.

A L_{18} Taguchi array was used to evaluate influence of ethanol and caustic soda concentration, reaction time, temperature and fiber/liquor ratio on organosolv delignification of steam-explosion pretreated bagasse. The response variables of Taguchi array were: delignification rate, hemicellulose solubilization, ethanol recovery and enzymatic conversion of cellulose. The results are depicted in the [Table 4](#).

Delignification rates for pretreated bagasse obtained in the design of experiments ranged from 40 to 82%. The best result was trial number 10, since it caused 82% of delignification of lignocellulosic material, shown on [Table 3](#). However, the prediction condition suggested by the analysis of main effects that maximizes the delignification rate is different from trial number 10. This analysis indicates the condition: ethanol concentration and fiber/liquor ratio at level 2, 60% (v/v) and 1:7.5 (w/v), respectively, NaOH concentration and temperature at level 3, 1% (w/v) and 160 °C, respectively, and reaction time at both 1 and level 2, since these levels have the same influence on organosolv delignification, [Fig. 1](#). The shortest time was chosen, 30 min, as

the prediction condition. None of the trials in the design of experiments have this combination of factor levels.

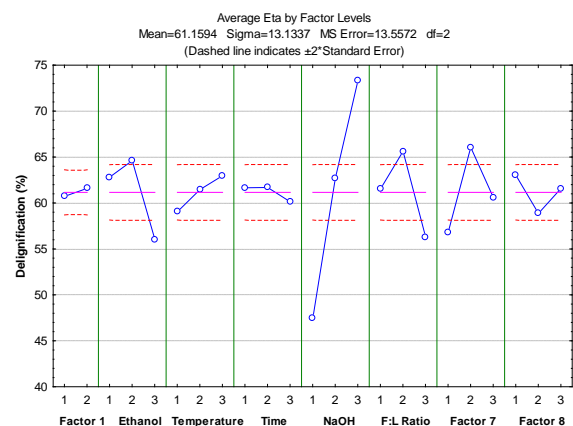


Fig. 1. Graphic generated from the analysis of main effects on the response variable delignification rate.

The analysis of variance (ANOVA) for the delignification rate pointed out only the caustic soda concentration as a significant factor in organosolv delignification, NaOH p-value < 0.05 or F value greater than $F_{\text{tabulated}}$, indicated on [Table 5](#).

Other factors have p-values higher than 0.05 and F value less than $F_{\text{tabulated}}$, on [Table 5](#) F_{tab} is 4.74 for a confidence level of 95%. Their factor square sums were also similar to the empty column square sums, the errors. This indicates these factors have no influence on response variable, the delignification rate, for a confidence level of 95%, on [Fig. 1](#) is also possible to verify this observation. Only the NaOH concentration factor caused a change in the dependence variable higher than limits of red dashed lines; this line represents the deviation of two standard errors about the mean value. The variation of other factors was less than limits of the red dashed lines. [Sarkanen \(1990\)](#), in his organosolv pulping study comments that the solvent would not be likely to change the reactivity of lignin with respect to NaOH significantly. The beneficial effect of solvent may be ascribed to either improved lignin solubility or its reduced tendency towards condensation. The results on [Fig. 1](#) show that ethanol solvent has an influence on the delignification rate. The increase of solvent concentration from 40 to 60% caused an increase of delignification in the lignocellulosic material, but its influence was not considered statistically significant for a confidence level of 95%.

Table 4. Results of response variables of the L18 Taguchi array used in the design of experiments to organosolv delignification of the bagasse pretreated by steam explosion

Trial	Ethanol % (v/v)	Temperature (°C)	Time (min)	NaOH % (w/v)	F:L Ratio (w:v)	Delignif. Rate (%)	Hemicellu. Solubiliz. (%)	Ethanol Recovery (%)	Cellulose Conversion (%)
1	40	100	30	0.1	1:10	46.4	23.3	28.2	45.9 ± 0.9
2	40	130	60	0.55	1:7.5	73.3	46.2	27.5	62 ± 1
3	40	160	90	1.0	1:5	71.8	56.1	31.2	67.1 ± 0.1
4	60	100	30	0.55	1:7.5	66.9	41.6	55.3	62.9 ± 0.7
5	60	130	60	1.0	1:5	68.3	41.1	21.7	57 ± 2
6	60	160	90	0.1	1:10	52.7	19.3	63.7	42 ± 2
7	80	100	60	0.1	1:5	40.9	14.3	21.7	38 ± 3
8	80	130	90	0.55	1:10	58.2	43.5	66.8	55 ± 1
9	80	160	30	1.0	1:7.5	68.0	55.8	59.2	57.6 ± 0.3
10	40	100	90	1.0	1:7.5	82.0	50.8	60.3	69.9 ± 0.4
11	40	130	30	0.1	1:5	41.2	15.1	24.9	44.8 ± 0.7
12	40	160	60	0.55	1:10	62.2	39.1	42.9	54 ± 1
13	60	100	60	1.0	1:10	74.7	55.9	63.4	63.8 ± 0.8
14	60	130	90	0.1	1:7.5	52.7	25.8	38.0	50 ± 1
15	60	160	30	0.55	1:5	72.4	46.2	35.7	70 ± 1
16	80	100	90	0.55	1:5	44.3	24.3	25.2	44 ± 2
17	80	130	30	1.0	1:10	75.0	56.3	69.4	68 ± 2
18	80	160	60	0.1	1:7.5	50.8	24.9	49.8	33.9 ± 0.9

Table 5. ANOVA data of the factors for delignification rate from experimental design. (SS stands for sum of square. SM symbolizes mean of square)

Effect	SS	Degree free	SM	F	p-value
Factor 1	3.47	1	-	-	-
Ethanol	244.96	2	122.48	2.50	0.151
Temperature	47.11	2	23.55	0.48	0.637
Time	9.55	2	4.77	0.10	0.908
NaOH	2026.15	2	1013.08	20.69	0.001
F:L ratio	261.98	2	130.99	2.68	0.137
Factor 7	258.75	2	-	-	-
Factor 8	53.32	2	-	-	-
Residual	342.66	7	48.95	-	-
Total	2932.41	18	-	-	-

Another component of lignocellulosic that could be affected by organosolv delignification is hemicellulose. The solubilization of this component in the design of experiment ranged 14.3 to 56.3%. The best results, shown on Table 4, were obtained from the experiments 3, 9, 13 and 17, causing one solubilization of above 55%. The ANOVA and main effects indicated only the NaOH concentration as significant factor for the

hemicellulose solubilization, as shown in [Fig. 2](#) and [Table 6](#).

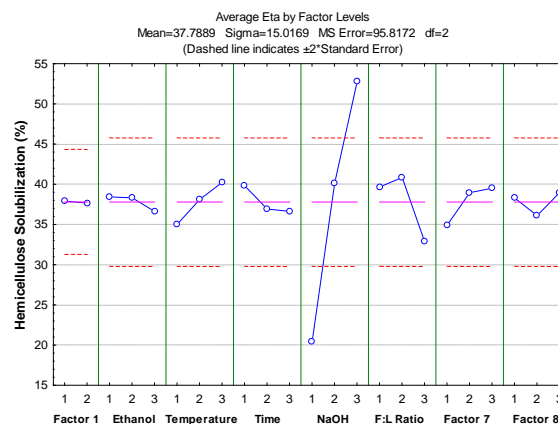


Fig. 2. Graphic generated from the analysis of main effects on the response variable hemicellulose solubilization.

P-value of this factor was less than 0.05 and its F value was greater than $F_{\text{tabulated}}$, which on [Table 6](#) is 4.74 for a confidence level of 95%. The other factors had the F value lower than $F_{\text{tabulated}}$ indicating to have no influence on the response variable, the hemicellulose solubilization, for a confidence level of 95%. The square sums for these factors were lower or similar than the error factors, except to the fiber:liquor ratio factor. The best condition for the NaOH concentration factor

was 1%, level 3 on [Fig. 2](#). The average of hemicellulose solubilization at this condition on [Fig. 2](#) was 53%, close to the values obtained in the trials 3, 9, 13, and 17.

Table 6. ANOVA data of the factors for hemicellulose solubilization from experimental design. (SS stands for sum of square. SM symbolizes mean of square)

Effect	SS	Degree free	SM	F	p-value
Factor 1	0.27	1	-	-	-
Ethanol	12.41	2	6.20	0.15	0.8657
Temperature	81.99	2	41.00	0.97	0.4239
Time	37.25	2	18.62	0.44	0.6596
NaOH	3183.27	2	1591.64	37.77	0.0001
F:L ratio	223.74	2	111.88	2.65	0.1387
Factor 7	76.07	2	-	-	-
Factor 8	27.00	2	-	-	-
Residual	294.98	7	42.14	-	-
Total	3833.64	18	-	-	-

One advantage of organosolv delignification is the solvent recovery used in the process, and the ability to produce substantial amounts of useful by-products, like lignin, sugars, furfural and acetic acid, as well as pulp ([Hongzhang and Liying, 2007](#)). The ethanol recovery in the experiment design ranged from 22% to 70%. The ANOVA and main effects only pointed out the fiber/liquor ratio as a significant factor for ethanol recovery. Its P-value was less than 0.05 and F value was greater than $F_{\text{tabulated}}$, which on [Table 7](#) is 4.74 for a confidence level of 95%.

The other factors had the square sums similar than the empty columns, errors, indicating the other factors have no influence on response variable, the ethanol recovery, for a confidence level of 95%. The best condition for the fiber/liquor ratio factor was 1:10, level 1 on [Fig. 3](#), which had a solvent recovery average of 56%, value considered insufficient to make feasible a solvent recovery unit.

The higher fiber/liquor ratio used in our experiments was only 1:10, a ratio lower than the values employed in the literature. [Hongzhang and Liying \(2007\)](#) evaluated the clean fractionation of wheat straw using the steam explosion pretreatment and ethanol-water delignification. In this study, the authors used a fiber/liquor ratio of 1:50 (w/v), giving an ethanol recovery of 85% in

the process. At higher fiber/liquor ratios, solvent loss is reduced due to the great liquor volume available in this process.

In our study, the fiber/liquor ratio of 1:10 was chosen as the largest in the design of experiments in order to reduce the solvent consumption. At this condition, the solvent loss was 2.6 L per kilogram of lignocellulosic, taking into account a solvent recovery average of 56% and ethanol concentration average of 60%, while for [Hongzhang and Liying \(2007\)](#) the ethanol loss was 3.0 L per kilogram of lignocellulosic, which used a 1:50 fiber/liquor and that had 40% ethanol concentration and 85% solvent recovery. Therefore, since using a fiber/liquor lower, our study has an absolute loss 15% lower than those the authors.

Table 7. ANOVA data of the factors for ethanol recovery from experimental design. SS stands for sum of square. SM symbolizes mean of square

Effect	SS	Degree free	SM	F	p-value
Factor 1	65.59	1	-	-	-
Ethanol	561.89	2	280.94	2.33	0.167
Temperature	112.25	2	56.12	0.47	0.646
Time	313.56	2	156.78	1.30	0.331
NaOH	537.62	2	268.81	2.23	0.178
F:L ratio	2723.06	2	1361.53	11.30	0.006
Factor 7	554.26	2	-	-	-
Factor 8	2.64	2	-	-	-
Error	843.26	7	120.47		
Total	5091.64	18	-		

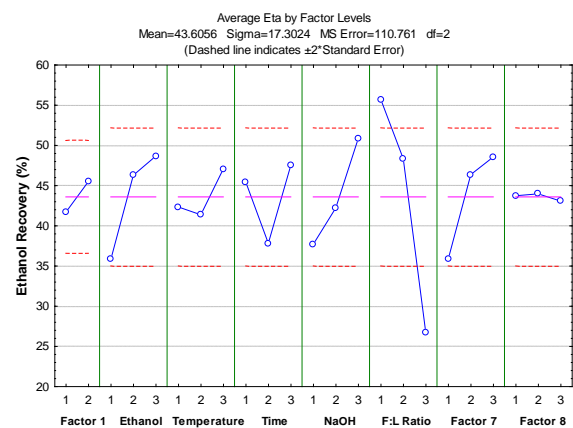


Fig. 3. Graphic generated from the analysis of main effects on the response variable ethanol recovery.

The amount of hemicellulose and lignin in the biomass is the main influence factors of enzymatic hydrolysis yield (Öhgren et al., 2007). This information can be confirmed by the linear correlation among cellulose conversion and solubilization of lignin and hemicelluloses, shown on Fig. 4. The higher cellulose conversions were obtained to the pulps with more solubilization of lignin and hemicellulose, trials 3, 10, 13 and 17 on Table 4. Hemicellulose forms a physical barrier around the cellulose hindering enzyme access to cellulose. Lignin also forms a protective physical barrier to enzymatic attack and it causes inhibition of hydrolysis by non-specific hydrophobic binding of lignin to the cellulose binding domain of the enzymes (Berlin et al., 2006; Gray et al., 2006; Öhgren et al., 2007). The correlations obtained in this work could be used to estimate the cellulose conversion of sugarcane bagasse pretreated by steam explosion and delignified by alkaline organosolv, provided that same conditions of pretreatment, organosolv delignification and enzymatic hydrolysis of cellulose are used.

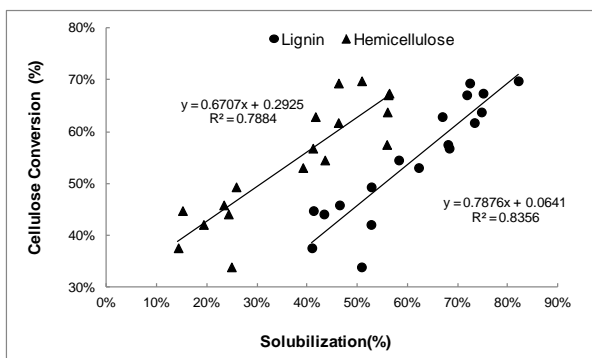


Fig. 4. Linear correlation among cellulose conversion and solubilization of lignin and hemicellulose from the design of experiments.

Data from experiment design show the removal of lignin and hemicellulose was affect by only the NaOH concentration in the experiment design. Therefore, it is expected that cellulose conversion will also be influenced by the same factor. The analysis of main effects indeed shows this effect on the cellulose conversion. Only the NaOH concentration caused a variation of the results higher than limits of red dashed lines, this line represents the deviation of two standard errors about the mean value on Fig. 5. The ANOVA also pointed out this behavior. Only the NaOH concentration has a p-value less than 0.05 and F value greater than the $F_{\text{tabulated}}$, which is 4.74 for a confidence level of 95% on Table 8. Other factors

had p-value higher than 0.05 and F value smaller than $F_{\text{tabulated}}$ and their square sums were similar than the square sums of the empty columns, the errors. This indicates that these factors have no influence on response variable, the cellulose conversion, for a confidence level of 95%. El-Sakhawy et al. (1996), in his study of alcoholic-alkaline pulping for wheat straw, also noted that concentration of alkali had a major influence on delignification of lignocellulosic material.

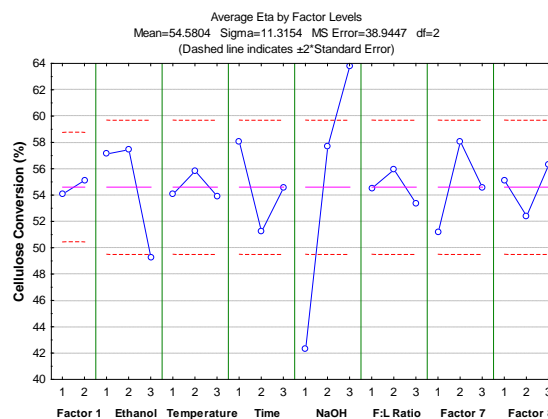


Fig. 5. Graphic generated from the analysis of main effects on the response variable cellulose conversion.

Table 8. ANOVA data of the factors for cellulose conversion from experimental design. (SS stands for sum of square and SM symbolizes mean of square)

Effect	SS	Degree free	SM	F	p-value
Factor 1	4.98	1	-	-	-
Ethanol	259.13	2	129.57	3.32	0.097
Temperature	13.66	2	6.83	0.18	0.843
Time	139.73	2	69.86	1.79	0.235
NaOH	1470.97	2	735.49	18.85	0.002
F:L ratio	20.01	2	10.01	0.26	0.781
Factor 7	142.34	2	-	-	-
Factor 8	47.93	2	-	-	-
Residual	273.13	7	39.02		
Total	2176.63	18	-	-	-

The prediction condition of organosolv delignification for efficiently conversion of cellulose from lignocellulosic material was: ethanol concentration, temperature and fiber:liquor ratio at level 2, 60% (v/v), 130 °C and 1:7.5 (w/v), respectively, reaction time at first level - 30 min - and NaOH concentration at level 3 -1% (m/v). None of 18 points tested in the L_{18}

Taguchi array contained these conditions. As a result, a trial of organosolv delignification under prediction conditions was carried out in order to validate the model used in the analysis of the results from design of experiments. The results of this test were displayed in [Table 9](#).

Table 9. Data of variable responses for the prediction condition test of the organosolv delignification. Ethanol concentration: 60% (v/v), temperature: 130 °C, time: 30 min, NaOH concentration: 1% (w/v) and fiber/liquor ratio: 1:7.5 (w:v)

Response Variable	Value
Delignification rate	74.1 %
Hemicellulose solubilisation	56.1 %
Ethanol recovery	45.0 %
Cellulose conversion	62 ± 1 %

Value of delignification rate obtained for test trial on [Table 9](#) was 9.8% lower than trial number 10, which had the best results of the experiment design – [Table 4](#). The reduction of delignification rate caused a decline in the cellulose conversion, -11,3%, despite the hemicellulose removal has increased in the trial test - 10.2%. The inhibitory effect of lignin on cellulases was more significant with respect to increase the cellulose accessibility by reduction of hemicellulose content.

The cellulose conversion of 62% obtained for the test trial is lower than the expected value from the experiment design analysis. We conclude that the array model used in the design of experiments is not suitable to depict the behavior of the response variable in relation to the organosolv delignification factors. The interactions among factors could be occurred in the experimental design and the model used to maximize the conversion of cellulose could be deficient, since the L18 Taguchi arrays did not allow to evaluate effects of the factor interactions on the response variables ([Ross, 1991](#)). New experiments to confirm the possibility of the interaction among factors will be carried out in the future works.

4. CONCLUSION

The results from experiment design for steam-explosion pretreated bagasse showed that the ethanol-soda delignification is an effective

process to fractionate the bagasse pretreated by steam explosion. NaOH concentration was the only significant factor of experiment design. The removal of lignin and hemicellulose was essential to increase cellulose digestibility of the bagasse pretreated by steam explosion at 200°C/7min. The pulps with higher solubilization of lignin and hemicellulose had the best results of cellulose conversion, almost 70%.

ACKNOWLEDGEMENT

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors are grateful to Novozymes Latin America Ltd by supplying of the enzymes and to Usina de Açúcar, Álcool e Biodiesel Vale do Rosário by providing the steam-pretreated sugarcane bagasse.

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