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Citric waste saccharification under different chemical treatments

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ABSTRACT. Second generation ethanol from lignocellulose materials has been used in applications for food processing wastes. Since Brazil has a leading position in orange juice exports, the influence of acid and alkali pretreatments on liquor saccharification, solubilization of solid fraction and mass yield was evaluated. Time and C_{acid} or $C_{alkaline}$ at different concentrations of solids (low to moderate, 1 to 9%) and high catalyst concentrations were analyzed. A hydrothermal pretreatment was conducted under the same conditions of acid and alkaline treatments to investigate the relative selectivity increase in using the catalysts. The chemical analyses of wastes indicated a 70% total carbohydrate level denoting a promising raw material for bioethanol production. Pretreatment caused acid saccharifications between 25 and 65% in total reducing sugars (TRS) and mass yields (MY) between 30 and 40%. In alkaline pretreatment, these rates ranged between 2 and 22.5% and between 30 and 80, respectively. In hydrothermal pretreatment, solubilized TRS varied between 3 and 37%, whereas MY remained between 45 and 60%, respectively. $C_{biomass}$ strongly influenced the three variables; in the same way, time affected MY.

Keywords: pretreatment, biomass, experimental design, orange.

Sacarificação de resíduo cítrico sob diferentes tratamentos químicos

RESUMO. Etanol de segunda geração a partir de materiais lignocelulósicos tem sido abordado como uma das aplicações de resíduos de processamento de alimentos. Sendo o Brasil líder na exportação de suco de laranja, avaliou-se a influência de pré-tratamentos ácidos e alcalinos na sacarificação do licor, na solubilização da fração sólida e no rendimento mássico. Para isso, estudou-se tempo e $C_{ácido}$ ou $C_{álcali}$ em diferentes concentrações de sólidos (baixo a moderado, 1-9%) e altas concentrações de catalisador. Um pré-tratamento hidrotérmico foi realizado, tendo as mesmas condições dos tratamentos ácido e alcalino, a fim de investigar o aumento da seletividade em relação ao uso desses catalisadores. Análises químicas de resíduos indicaram um nível total de carboidratos de 70%, denotando uma matéria-prima promissora para a produção de bioetanol. O pré-tratamento conduziu a sacarificações ácidas de 25 a 65% em açúcares redutores totais (ART) e rendimentos mássicos (RM) entre 30 e 40%. Para o pré-tratamento alcalino, esses valores foram de 2 a 22,5%, e de 30 a 80%, respectivamente. No pré-tratamento hidrotérmico, o ART solubilizado variou entre 3 e 37%, enquanto que o RM manteve-se entre 45 e 60%. A $C_{biomassa}$ influenciou fortemente as três variáveis, bem como o tempo utilizado com o RM.

Palavras-chave: pré-tratamento, biomassa, planejamento experimental, laranja.

Introduction

Biofuels play an important role in reducing changes in global climate. Their impact depends upon several aspects related to novel technologies, legal restrictions, international trade, land use as well as the choice of raw materials and management techniques (WORLDWATCH INSTITUTE, 2007). During the last two decades, second generation ethanol has been proposed as an alternative for biofuel production, though hydrolysis and fermentation of lignocellulosic materials have been known and implemented since the late nineteenth century. Several studies have been executed particularly in the USA and in Europe, albeit still within laboratory scale, and aim at an efficient biofuel capable of being produced worldwide. Moreover, all biomass wastes derived from agribusiness, agro-industry residues and urban waste have high lignocellulosic contents (MACEDO et al., 2008).

Biomass production costs in Brazil are considered the lowest in the world, with further possibilities in achieving more promising results. The production of lignocellulosic ethanol is expected to increase up to 50% ethanol production without the need of expanding the area of current plantations (SILVA, 2012).

With approximately 35% of global production, estimated at 47,010 thousand tons, Brazil is the leading orange producer worldwide, followed by USA, China, India, Mexico, Egypt and Spain. The Brazilian 2013 crop produced about 16.3 million tons of oranges with an expected increase of 1% in 2014 (IBGE, 2014). As a result of this large-scale orange processing, great amounts of waste are generated since orange bagasse corresponds to 50% of its fresh weight. On a dry basis, orange biomass features nearly 16% hemicellulose, 28% cellulose and 9% lignin (RETORE et al., 2010), denoting an alternative for the production of cellulose or second generation ethanol (LENNARTSSON et al., 2012).

The technology for obtaining bioethanol from lignocellulosic materials comprises the hydrolysis of biomass polysaccharides into fermentable sugars and further fermentation, standing out as a feasible energetic alternative to meet global demands. Bellido et al. (2011) pointed out five unit operations required for an efficient conversion of lignocellulosic biomass into ethanol: (1) biomass size reduction to increase surface area and uniformity; (2) pretreatment to break lignin and hemicellulose structures, reducing cellulose crystallinity while increasing biomass porosity; (3) enzymatic hydrolysis to convert polymeric sugars into monomeric ones; (4) fermentation, to produce ethanol from monomeric sugars; (5) ethanol recovery by distillation or any other separation technique.

The lignocellulosic biomass is composed of cellulose (a polysaccharide formed by glucose molecules linked by β-1.4-glycoside bonds) chains joined by hydrogen interactions. These long cellulose fibers are coated with hemicelluloses which are branched polysaccharides mainly consisting of D-xylose and small amounts of L-arabinose, D-glucose, D-mannose, D-galactose, glucuronic acid, mannuronic acid and lignin (WYMAN et al., 2005).

The main pretreatment technologies are chemical pretreatments, including acid, alkaline and oxidative treatments. Most pretreatments differ in chemical structure and in the mechanisms of cell wall chemical and structural modification, which in turn leads to improved enzyme accessibility and increased yields (OGEDA; PETRI, 2010). Table 1 presents the activities of different pretreatment types as to lignocellulosic structure chemical and conformational modifications.

Table 1. Effect of various pretreatment methods on the structure of lignocellulosic biomass.

Pretreatment	Available surface area	Cellulose de-crystallization	Hemicellulose removal	Lignin removal	Changes to lignin structure
Diluted acid	■	ND	■	-	■
Alkali	■	ND	▨	■	■
Hydrothermal	■	ND	■	-	▨

■ Higher effect. ▨ Lower effect. ND: Not determined. Source: Adapted from Mosier et al. (2005).

Pretreatment with diluted sulfuric acid thoroughly hydrolyzes the hemicellulose fraction to the medium which, depending on the acid concentrations employed, also releases cellulose to a greater or lesser extent, and other components such as pectin and water-soluble proteins (CORTEZ, 2010). Contrastingly, milder operating conditions (temperature and pressure) are employed in alkaline processes whose main effect is the removal of lignin from the biomass, enhancing higher fiber reactivity (MOSIER et al., 2005; ROCHA et al., 2009). The alkali, usually soda, tends to cause the swelling of the biomass decreasing cellulose crystallinity and increasing surface area and porosity (PITARELO et al., 2012). The hydrothermal pretreatment consists in the combination of water and biomass for 15 min. at 230°C where between 40 and 60% of total biomass is dissolved in water, generating 4-22% cellulose, 30-60% lignin, coupled to complete hemicellulose removal (MOSIER et al., 2005).

Some authors have evaluated orange residue pretreatment by using diluted acid as a catalyst to saccharify hemicellulose from the biomass, with low acid (< 1%) and high biomass concentrations. Vaccarino et al. (1989) employed diluted sulfuric acid and $C_{biomass}$ ranging from 7.4 to 18.5% at 100°C for a long heating time (1.5 hour). Talebnia et al. (2008) used C_{acid} between 0 and 1%, temperatures from 100 to 132°C, $C_{biomass}$ from 2 to 18%, and heating times ranging from 5 to 25 min. in citrus residues, with maximum saccharification of 45%. Miller et al. (2012) studied the saccharification of orange waste using 6% sulfuric acid for 15 to 120 min. and employing $C_{biomass}$ of 10% biomass, with a yield of about 30% of total reducing sugars. Employing a hydrothermal process with orange residue, Pitarelo et al. (2012) applied temperatures between 195 and 210°C and short heating times (4, 6, and 8 min.) and reported that the degradation of sugars in the liquor was enhanced by increased temperatures. Grohann et al. (1995) used hydrothermal process at milder temperatures (100-140°C) and $C_{biobass}$ of 1% and obtained 55-65% saccharifications.

Current analysis evaluates the severity of acidic and alkaline pretreatments on liquor saccharification, solubilization of solid fraction and mass yield, by studying the binomial time and C_{acid} or C_{alkali} at different solid concentrations (low to moderate, 1 to 9%) and high catalyst concentrations. A hydrothermal pretreatment was performed under the same conditions of acidic and alkaline processes to investigate the relative increase in selectivity by using the above-mentioned catalysts.

Material and methods

The methodology consisted of waste preparation, physicochemical characterization, and acidic, alkaline or hydrothermal pretreatment, resulting in pretreated biomass and liquor.

Waste, obtained after juice/pulp extraction at the processing plant (COOPLAL, in Santana do Mundaú AL Brazil), was collected in plastic bags, ice-cold stored and taken to the laboratory where it was thawed, sanitized with a solution containing 100 ppm of sodium hypochlorite for 15 min., kiln-dried at 55°C until constant weight was reached, crushed in Wyllie mill with a 30 mesh sieve, and stored airtight in plastic bottles at room temperature.

Moisture, ash, protein, lipid, fiber, sugar and pectin contents were determined in waste samples, following analytical procedures by the Adolfo Lutz Institute (IAL, 2005) and AOAC (2002). The percentage of total carbohydrates was calculated by the difference of the analyses described above. Reducing (RS) and total reducing (TRS) sugars were determined by colorimetry (MILLER, 1959) with 3.5-dinitrosalicylic (DNS) acid. For the determination of TRS, samples were treated with H_2SO_4 1.5 M in boiling water for 20 min. with occasional stirring to hydrolyze polysaccharides and other non-reducing sugars.

Three pretreatments were performed: (1) acidic, with diluted sulfuric acid (H_2SO_4) solutions; (2) alkaline, with sodium hydroxide (NaOH) solutions; and (3) hydrothermal, with distilled water only. Response surface methodology (RSM) was used to optimize the pretreatment conditions. For the acidic and alkaline pretreatments, the independent variables were time, solution concentration (C_{acid} or C_{alkali}), and biomass concentration ($C_{biomass}$), whereas for hydrothermal pretreatment, the variables were time and $C_{biomass}$ only. Data were treated with Statistica 7.0, and the response variables were mass yield (MY), TRS content released in the pretreatment liquor, and total soluble solids (TSS) in the liquor.

Response surfaces were built from significant variables. Analysis of variance (ANOVA) was employed to validate the model proposed by Statistica, according to Equation 1.

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i,j=1}^{k}\beta_{ij} X_i X_j + \sum_{j=1}^{k}\beta_j X_j^2 \qquad (1)$$

where:
Y is the response variable;
β_0 is a constant;
β_i, β_j and β_{ij} are the linear, quadratic and interaction coefficients, respectively.

The central composite design (CCD) was used to acquire data to fit the above equation. In acidic and alkaline pretreatments, a 2^3 full factorial design was used, whereas a 2^2 full factorial design was employed in the hydrothermal pretreatment, both of which including three replicates at the central point, resulting in eleven and nine experiments, respectively, to investigate the selected variables.

The environmental conditions were 121°C and 1 atm, monitored in autoclave. After pretreatment in the autoclave reactor, samples were filtered and their liquors were analyzed as to TRS by DNS method (MILLER, 1959), according to Equation 2. The solid fraction was dried at 37°C for 24 hours and then weighed.

$$\%TRS = 100.\frac{C\ (g.L^{-1}).V\ (L)}{Biomass\ (g)} \qquad (2)$$

where:
C (g L^{-1}) is the TRS concentration obtained from a glucose standard curve;
V (L) is the volume of the extracted liquor;
Biomass (g) is the waste mass.

Equation 3 gives the calculation of mass yield (*MY*) which considers the biomass´s initial and final weights (before and after the pretreatment, respectively), whereas Equation 4 considers solubilization by a relationship between TSS (°Brix) and waste mass.

$$\%MY = 100.\frac{m_{final}(g)}{m_{initial}(g)} \qquad (3)$$

$$TSS = \frac{°Brix}{Biomass\ (g)} \qquad (4)$$

Results and discussion

The waste´s drying process took 18 hours, with approximately 85% moisture loss. The physicochemical characterization indicated high carbohydrate contents (Table 2) and showed itself compatible with the characterizations reported elsewhere, suggesting that the residue is a potential source for second generation ethanol production.

Table 2. Physicochemical characterization of grinded and dehydrated orange waste.

Crude fiber (%)	Moisture (%)	Ash (%)	Protein (%)	Lipids (%)	Total carbohydrate (%)	Pectin (%)	Reference
12.23±2.95	10.05±0.10	3.46±0.07	3.38±0.69	1.74±0.28	69.14	12.03±0.55	Current assay
11.04	12.16	4.92	4.85	2.16	70.08	-	Ruviaro et al. (2008)
7.17	0.96	-	11.08	6.00	-	-	Clemente et al. (2012)
-	13.36	8.57	7.93	3.44	68.85	-	Retore et al. (2010)

Table 3 gives results of the acidic pretreatment. It may be observed that sugar contents released to the liquid fraction after pretreatment remained between 27 and 62%, underscoring the great influence of the studied variables on the saccharification process. In soluble solid and TRS (Total Reducing Sugars) release profiles, due to material hydrolysis as a result of heating in acidic medium, it may be observed that, regardless of heating time, low biomass and high acid concentrations resulted in high quantities of dissolved substances when compared to the biomass used in each experiment.

Table 3. Experimental design of the acidic pretreatment with orange residue.

Assay	Time (min.)	C_{acid} (%)	$C_{biomass}$ (%)	TRS* (%)	MY** (%)	TSS*** (%)
1	15	1.0	1.0	52.29 ± 2.03	41.43 ± 4.82	1.65 ± 0.07
2	120	1.0	1.0	65.52 ± 14.50	36.02 ± 4.34	2.23 ± 0.05
3	15	1.0	9.0	26.37 ± 1.08	41.15 ± 1.48	0.59 ± 0.02
4	120	1.0	9.0	39.59 ± 5.82	40.14 ± 5.06	0.70 ± 0.00
5	15	5.0	1.0	62.53 ± 11.63	41.16 ± 3.13	6.62 ± 0.11
6	120	5.0	1.0	56.70 ± 2.79	32.90 ± 3.61	7.06 ± 0.19
7	15	5.0	9.0	32.89 ± 6.66	39.60 ± 0.07	0.97 ± 0.02
8	120	5.0	9.0	33.95 ± 9.24	34.73 ± 1.29	2.11 ± 0.01
9	67.5	3.0	5.0	26.40 ± 5.79	34.83 ± 0.15	1.73 ± 0.02
10	67.5	3.0	5.0	28.68 ± 1.45	35.82 ± 0.19	1.72 ± 0.06
11	67.5	3.0	5.0	29.30 ± 0.57	30.62 ± 6.22	1.71 ± 0.07

Results are expressed as mean standard followed by standard deviation. *TRS – Total reducing sugars; **MY – Mass yield; ***TSS – Total soluble solids. The three studied variables influenced sugar and total solid solubilizations, though their effects were not significant in the studied intervals for mass yield. Positive values in the Pareto charts indicate positive contributions of increased variable within the studied interval, whereas negative values indicate negative contributions.

The above suggests that, besides pectin and hemicellulose fractions, a portion of another carbohydrate, most likely cellulose, was also released during the process. This occurred because the experiment with a greater saccharification degree, featuring the shorter pretreatment (15 min.), the lowest biomass concentration (1 g 100 mL^{-1}) and the highest acid concentration (5 mL 100 mL^{-1}), resulted in a saccharification degree of 62% (70% of the total theoretical carbohydrates).

The acidic pretreatment showed that $C_{biomass}$ negatively affected sugar solubilization, probably due to the low solid/liquid ratio interfering with structure breakdown. In their study on the pretreatment of orange peel in diluted sulfuric acid (0.2 to 0.6%) with $C_{biomass}$ between 7.4 and 18.5%, at 100°C for 1.5 hour, Vaccarino et al. (1989) reported that $C_{biomass}$ increase caused higher mass yield (from 39 to 62%). This behavior was similar to the observations in current assay, although $C_{biomass}$ was not significant at 95% as to mass yield in the studied range (1-9%). It has also been observed that TRS released to the liquor varied between 21.3 and 45.8%, which are the highest rates obtained at higher C_{acid} and lower $C_{biomass}$, according to current results.

C_{acid} positively influenced waste matrix solubilization at higher acid concentrations, resulting in lower mass yield and indicating a major biomass solubilization. Grohann et al. (1995) used sulfuric acid at concentrations 0.06 and 0.5%, temperatures between 100 and 140°C, and pretreatments for 10-40 min. and achieved mass yields between 18 and 30%, with lower yields being obtained for longer times, as observed in current study. At 120°C and heating times between 10 and 40 min., there was a TRS dissimilation of 30 to 37% and of 11% for C_{acid} of 0.5 and 0.06%, respectively. These rates were lower than those found in the current analysis, probably due to lower C_{acid} employed. The above indicates the positive influence of acid for sugar dissimilation, as shown in the Pareto chart (Figure 1), even though its influence was not significant, at 95%, within the studied range, unlike its interaction with time.

However, C_{acid} negatively affected sugar solubilization since, as previously enhanced, at a certain time there was a severity that led to sugar degradation, provided that mass yield was not significantly influenced by the assessed variables, i.e., $p < 0.05$, between 30 and 40%. The R^2 coefficient shows that regression models adjusted for TRS (0.9953), mass yield (0.9965), and biomass solubilization (0.8906) were suitable.

Reduced amount of sugars with increasing time and higher catalyst concentration may be due to degradation of these compounds. In fact, sugar degradation is influenced by acids whilst high temperatures and long exposure times also generated compounds capable of inhibiting further fermentation, including hydroxymethylfurfural (HMF) and furfural (MOSIER et al., 2005; CARA et al., 2008).

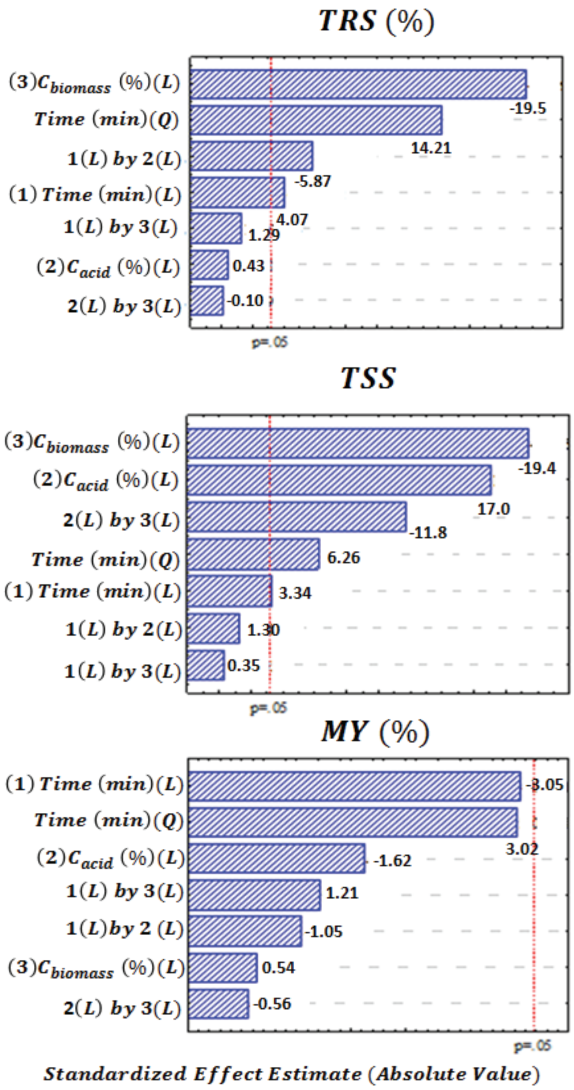

Figure 1. Sugar saccharification, mass yield, and solubilization after acidic pretreatment.

Approximately 30% of the cellulose was saccharified at 170°C when olive wood was used (CARA et al., 2008). In fact, under severe pretreatment conditions, part of the cellulose was solubilized in the pretreatment liquor, i.e., some cellulose was actually lost prior to the enzymatic hydrolysis process. Additionally, hemicellulose recovery in the liquor remarkably dropped to 5% with longer times and higher temperatures, which also suggesting pentose degradation.

In the studied range (15 to 120 min.), time played a significant role in biomass saccharification because its interaction with C_{acid} was significantly influential. Saccharification of about 30% was obtained in the liquor of orange waste after hydrolysis with diluted sulfuric acid at 122°C, using C_{acid} of 6 mL 100 mL^{-1} and $C_{biomass}$ of 10 g 100 mL^{-1}, without much variation for the pretreatment times 15 to 120 min. (MILLER et al., 2012). This yield was similar to that in current assay for 9% of $C_{biomass}$ and 15 min and showed that at higher $C_{biomass}$ (~ 10%), 15 min. was the most suitable pretreatment time for saccharification.

Talebnia et al. (2008) studied orange bagasse hydrolysis in diluted sulfuric acid with $C_{biomass}$ between 2 and 18% (w v^{-1}), C_{acid} from 0 to 1% (w v^{-1}), temperatures at 100, 108, 116, 124, and 132°C, and heating times in autoclave between 5 and 25 min., and observed that the best saccharification (approximately 45% of saccharification in TRS) occurred after the use of 116°C, 0.5% sulfuric acid, 6% $C_{biomass}$, and 15 min. heating, suggesting mild heating (116°C) and C_{acid} 0.5% as optimal parameters. The authors also reported that HMF formation and saccharified sugar degradation were first detected at the highest temperatures (≥ 124°C), heating times (> 15 min.), C_{acid} (≥ 0.75%) and $C_{biomass}$ of 4%.

The models obtained for TRS and TSS (°Brix.$g_{biomass}^{-1}$) are given in Equations 5 and 6, respectively. The MY model was not significant and might have been affected by the acidic pretreatment conditions.

$$\%TRS_{acid} = 64.153 - 0.7441.time + 0.0066.time^2 - 3.5086.C_{biomass} - 0.0372.time.C_{acid} \quad (5)$$

$$TSS_{acid} = 1.317 - 0.0486.time + 0.0004.time^2 + 1.2792.C_{acid} - 0.0465.C_{biomass} - 0.1252.C_{acid}.C_{biomass} \quad (6)$$

In the alkaline pretreatment, lower mass yields (about 30%) were obtained, except for Experiments 3 and 4, due to the fact that the two experiments generated a gum and impaired pretreatment efficiency because of too much biomass (9%) for pretreatment time. The results are presented in Table 4.

The removal of lignin and hemicellulose fractions is a characteristic of alkaline treatments (SUN; CHENG, 2002; MOSIER et al., 2005) and maybe only a small fraction of these as well as a cellulose fraction may have remained. In current assay, high TSS and, consequently, higher solubilization of fractions were obtained, as well as lower saccharification when compared to acidic pretreatment. It is worth mentioning that sugar dissimilation alone in the liquor during the pretreatment does not evaluate the process efficiency because it could be affected by the saccharification of the hexoses present, mainly in the cellulosic fraction, indicating the need of further enzymatic hydrolysis or characterization of the resulting solid fraction.

Table 4. Experimental design from alkaline pretreatment of orange residue.

Assay	Time (min.)	C_{alkali} (%)	$C_{biomass}$ (%)	TRS* (%)	MY** (%)	TSS*** (%)
1	15	0.5	1.0	17.07 ± 6.34	31.14	1.80 ± 0.07
2	120	0.5	1.0	23.08 ± 6.59	25.81	1.70 ± 0.05
3	15	0.5	9.0	1.27 ± 0.34	81.30	1.00 ± 0.02
4	120	0.5	9.0	1.51 ± 0.22	69.91	0.10 ± 0.00
5	15	2.5	1.0	12.00 ± 5.44	28.09	2.80 ± 0.11
6	120	2.5	1.0	19.26 ± 7.26	29.42	2.30 ± 0.19
7	15	2.5	9.0	8.36 ± 1.40	27.24	6.60 ± 0.02
8	120	2.5	9.0	14.88 ± 2.79	27.67	5.80 ± 0.01
9	67.5	1.5	5.0	14.31 ± 6.83	30.30	2.80 ± 0.02
10	67.5	1.5	5.0	14.07 ± 4.82	29.00	2.80 ± 0.06
11	67.5	1.5	5.0	11.29 ± 4.70	30.78	3.40 ± 0.07

Results are expressed as mean standard followed by standard deviation. *TRS – Total reducing sugars; **MY – Mass yield; ***TSS – Total soluble solids. The three studied variables influenced sugar and total solid solubilizations, though their effects were not significant in the studied intervals for mass yield. Positive rates in the Pareto charts indicate positive contributions of increased variable within the studied interval, whereas negative rates indicate negative contributions.

Studies with banana stem showed that, for enzymatic hydrolysis, the alkaline pretreatment with NaOH 1% (m v^{-1}) for 1 h and at 100ºC was more efficient, reaching glucose yields of about 61%. If compared with that of the acidic pretreatment, such a high yield has to do with delignification and cellulose loss, the latter being around 30% for the acidic pretreatment and only 1.2% for the alkaline one (GONÇALVES FILHO et al., 2013). Approximately 80% of lignin was removed after delignification of previously steam-exploded sugarcane straw with 1% NaOH 1%, for 1 hour, at 100ºC (OLIVEIRA et al., 2013).

Figure 2 presents the Pareto charts for each response variable. Lower $C_{biomass}$ and C_{alkali} influenced sugar extraction in the liquor, where 9 g of biomass and 15 min. of reaction were not enough to make the suspension liquid; a gelatinous mixture was yielded. As to mass yield, lower C_{alkali} and time favored the solubilization of the lignocellulosic fraction, suggesting $C_{biomass}$ 5% and 67.5 min. as the best conditions. One hour at 100ºC has been reported as effective for the delignification of biomass from agro-industrial residues (GONÇALVES FILHO et al., 2013; OLIVEIRA et al., 2013).

In the case of TSS, the interaction between C_{alkali} and $C_{biomass}$ appears to have played an important role in the solubilization of lignocellulosic matrix substances. This reflects the susceptibility of the lignocellulosic matrix, mainly lignin and hemicellulose, to NaOH (SUN; CHENG, 2002; MOSIER et al., 2005). In the treatment of agro-industrial residues, 1% of NaOH at 100ºC may be effective in the preparation of biomass to enzymatic hydrolysis (GONÇALVES FILHO et al., 2013; OLIVEIRA et al., 2013), revealing the influence of almost all the variables of the experimental design and their interactions in mass yield.

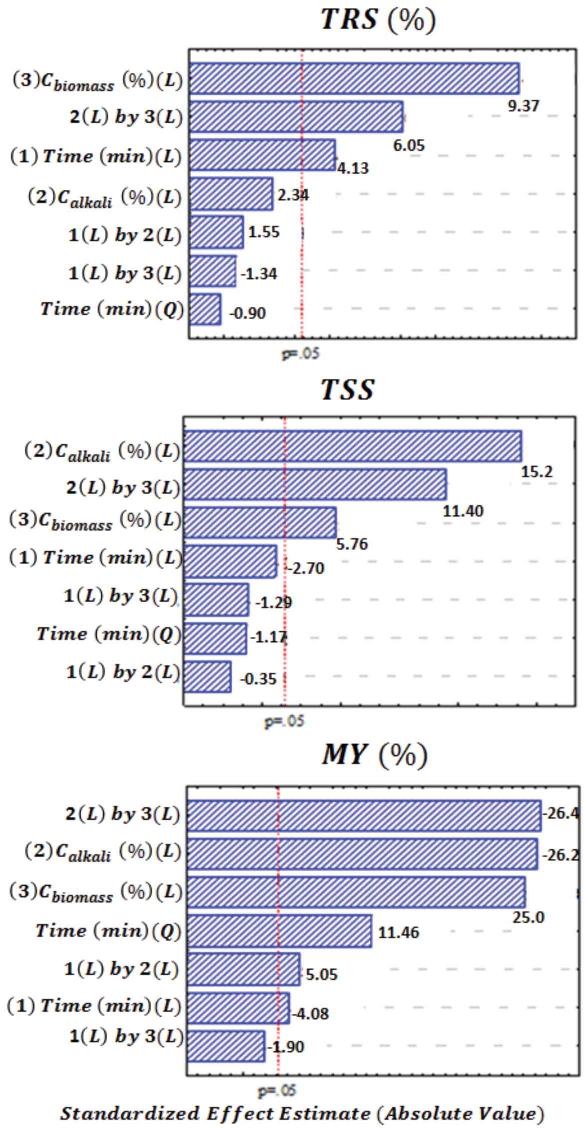

Figure 2. Sugar saccharification, mass yield, and solubilization after alkaline pretreatment.

For pretreating biomass of fruit residues, several authors have considered the use of alkaline pretreatment for the removal and/or breakdown of lignocellulosic structures after using diluted sulfuric acid to remove hemicellulose at moderate temperature (100°C). The literature reports a relative efficiency in the association of these two processes. In an acidic pretreatment to delignify cashew bagasse, approximately 75% of mass yield was achieved after pretreatment with low sulfuric acid concentration (0.8 mol L^{-1}) and C_{alkali} of about 4%, for 1 hour and at 100°C (ROCHA et al., 2009). When cashew bagasse was pretreated with 0.5 to 3.5% of acid and 1.5% of alkali, glucose conversions of up to 60% were attained (ROCHA et al., 2014). Although the combination of these processes might be efficient in agro-industrial waste treatment, studies on cost and feasibility must be undertaken to this end.

The use of alkaline solutions has the drawback of their neutralization and disposal, coupled to making the liquor useless due to numerous lignin-derived inhibitors (JORDAN et al., 2012). This would probably be the function of the process with diluted acid: initial removal of hemicelluloses, favoring lignin breakage by NaOH and, consequently, improving the enzymatic process.

ANOVA parameters indicated R^2 coefficients related to the response variations better than those adjusted to the acidic pretreatment, especially to mass yield (0.9986). The models obtained for TRS, biomass solubilization and mass yield are given in Equations 7 to 9, respectively.

$$\%TRS_{alkali} = 20.5866 + 0.0913.time - 2.5327.C_{biomass} + 0.9172.C_{alkali}.C_{biomass} \qquad (7)$$

$$TSS_{alkali} = 1.7728 + 0.1451.C_{alkali} - 0.2574.C_{biomass} + 0.3031.C_{alkali}.C_{biomass} \qquad (8)$$

$$\%MY_{alkali} = 33.0219 - 0.5729.time + 0.0036.time^2 + 0.1969.C_{alkali} + 7.6843.C_{biomass} + 0.044.time.C_{alkali} - 3.0269.C_{alkali}.C_{biomass} \qquad (9)$$

In the case of hydrothermal pretreatment, divergences were noted in the results (Table 5), chiefly regarding TRS and TSS contents released by the biomass in the liquor, with rates between 3 and 37 and between 0.30 and 0.95, respectively. In fact, they are the highest rates obtained when lower biomass concentrations were used.

Table 5. Conditions and results obtained in the hydrothermal pretreatment of orange residue.

Assay	Time (min.)	$C_{biomass}$ (%)	TRS* (%)	MY** (%)	TSS*** (%)
1	15	1.0	37.35 ± 5.49	51.50	1.80 ± 0.07
2	15	5.0	6.41 ± 0.50	56.00	1.70 ± 0.05
3	15	9.0	3.43 ± 0.08	59.46	1.00 ± 0.02
4	67.5	9.0	25.05 ± 0.84	47.92	0.10 ± 0.00
5	67.5	1.0	5.75 ± 0.27	52.87	2.80 ± 0.11
6	67.5	5.0	3.08 ± 0.13	55.55	2.30 ± 0.19
7	120	1.0	37.02 ± 4.65	46.11	6.60 ± 0.02
8	120	5.0	8.92 ± 0.81	52.37	5.80 ± 0.01
9	120	9.0	10.97 ± 1.50	55.76	2.80 ± 0.02

Results are expressed as mean standard followed by standard deviation. *TRS – Total reducing sugars; **MY – Mass yield; ***TSS – Total soluble solids.

The Pareto charts in Figure 3 demonstrate that, among the studied factors, the lowest $C_{biomass}$ was significant in both responses and suggested that high biomass causes lower self-catalytic action and lessens the biomass water/surface ratio or sugar degradation due to prolonged exposure.

Pitarelo et al. (2012) hydrothermally processed bagasse and sugar cane straw for 4, 6, and 8 min., at temperatures of 195, 202.5, and 210°C, and with moisture contents of 8, 33, and 50%. Higher residence times in the reactor, as well as elevated temperatures, led to greater losses in the pretreatment process. These losses may be attributed to the prolonged exposure of the material to high temperatures and to the further increase in the degradation rates of the carbohydrates present in the bagasse (decomposition).

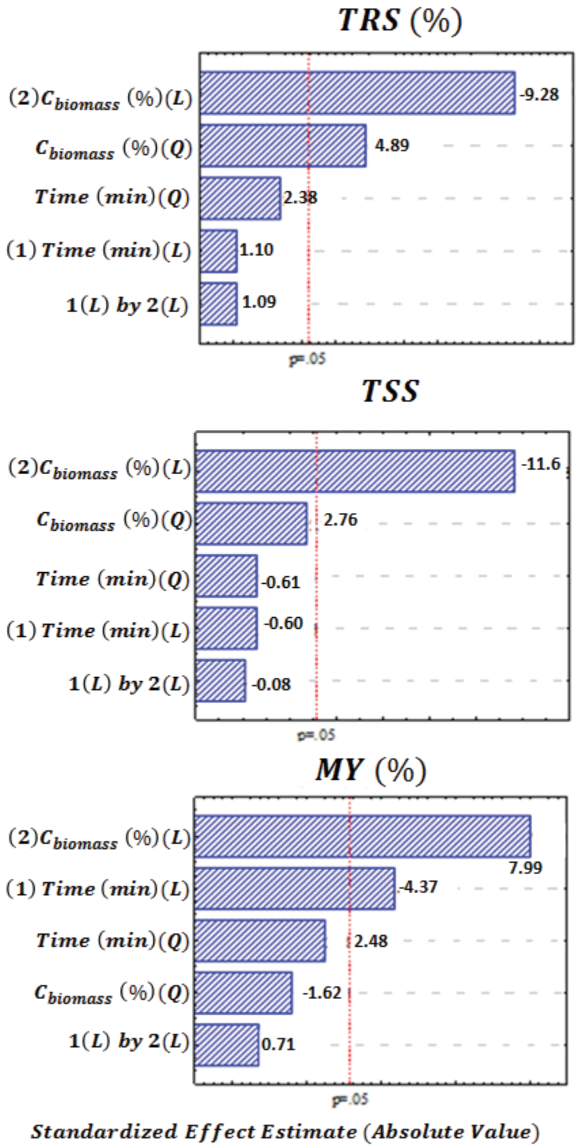

Figure 3. Sugar saccharification, mass yield, and solubilization after hydrothermal pretreatment.

Mass yield ranged between 45 and 60%, with increased $C_{biomass}$ (%) and reduced time being significant variables. In the hydrothermal treatment of 1% citrus waste, temperatures of 100, 120, and

140°C and reaction times between 5 and 40 min., mass yields ranging from 55 to 65% were obtained at 120°C for 40 min., with the longest time resulting in the lowest mass yield (GROHANN et al., 1995).

It is likely that the temperature employed was not enough to breakdown the lignocellulosic structure, since in the absence of chemical catalysts (acid or alkali), the catalytic action was reduced. Many authors reported temperatures between 175 and 225°C as the most effective for this pretreatment (SUN; CHENG, 2002; MOSIER et al., 2005). Given the low saccharification in the liquor, enzymatic hydrolysis tests may confirm the efficiency of this process. However, biomass reactivity was greater in the previous pretreatments.

The models obtained for TRS, biomass solubilization and mass yield are given in Equations 10 to 12, respectively.

$$\%\mathrm{TRS_{hydrothermal}} = 50.6145 - 11.8320.\,\mathrm{C_{biomass}} + 0.7785.\,\mathrm{C_{biomass}}^2 \tag{10}$$

$$\mathrm{TSS_{hydrothermal}} = 1.0326 - 0.1609.\,\mathrm{C_{biomass}} - 0.0082.\,\mathrm{C_{biomass}}^2 \tag{11}$$

$$\%\mathrm{MY_{hydrothermal}} = 51.5573 - 0.1425.\,\mathrm{time} + 1.82021.\,\mathrm{C_{biomass}} \tag{12}$$

Table 6 presents a comparison between the different pretreatments that, albeit distinct, had similar influences on lower $\mathrm{C_{biomass}}$ and sugar saccharification, the positive significance for time when alkali or acid were present, and the fact that $\mathrm{C_{acid}}$ or $\mathrm{C_{alkali}}$ alone was not significant, but only together with other variables.

Table 6. Summary of the significant factors for each pretreatment.

Pretreatment	Variable	Time	$\mathrm{C_{acid/alkali}}$	$\mathrm{C_{biomass}}$
Acidic	%TRS	positive	NS	negative
	%MY*	NS	NS	NS
	°Brix/$\mathrm{g_{biomass}}$	positive	positive	negative
Alkaline	%TRS	positive	NS	negative
	%MY*	negative	negative	positive
	°Brix/$\mathrm{g_{biomass}}$	NS	positive	positive
Hydrothermal	%TRS	NS	-	negative
	%MY*	negative	-	positive
	°Brix/$\mathrm{g_{biomass}}$	NS	-	negative

*MY- mass yield (%); NS - not significant.

Based on these data and on the discussions above, it is believed that long reaction times, low acid or alkali concentrations and low biomass concentrations should be used for high saccharification rates. However, the operating costs are high, encouraging the resort to shorter times and higher $\mathrm{C_{biomass}}$. Further studies on enzymatic hydrolysis and composition of the resulting solid fraction must be undertaken to better support these hypotheses. It should be also noted that saccharification is considerably high in the pretreatment where cellulose is probably lost.

Conclusion

Interactions between the variables (time, $\mathrm{C_{acid}}$ and $\mathrm{C_{biomass}}$) have been analyzed. The sugar range was much larger and indicated that time and $\mathrm{C_{acid}}$ might contribute towards sugar liquor degradation when the most severe conditions are used. The use of higher $\mathrm{C_{biomass}}$, lower $\mathrm{C_{acid}}$, and shorter times may be effective. In alkaline pretreatment, $\mathrm{C_{alkali}}$ did not affect mass yield; however, time affected it negatively, suggesting the use of lower $\mathrm{C_{alkali}}$, treatment times between 67.5 and 120 min. and $\mathrm{C_{biomass}}$ of 5%. The hydrothermal pretreatment produced the highest mass yields (60%), recommending lower pretreatment times and higher $\mathrm{C_{biomass}}$.

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