J Ind Microbiol Biotechnol (2012) 39:439–447
DOI 10.1007/s10295-011-1051-3

BIOENERGY/BIOFUELS/BIOCHEMICALS

Hydrothermal pretreatment of sugarcane bagasse using response surface methodology improves digestibility and ethanol production by SSF

Sandra Helena da Cruz · Bruce S. Dien · Nancy N. Nichols · Badal C. Saha · Michael A. Cotta

Received: 14 July 2011 / Accepted: 19 October 2011 / Published online: 12 November 2011

Abstract Sugarcane bagasse was characterized as a feedstock for the production of ethanol using hydrothermal pretreatment. Reaction temperature and time were varied between 160 and 200°C and 5–20 min, respectively, using a response surface experimental design. The liquid fraction was analyzed for soluble carbohydrates and furan aldehydes. The solid fraction was analyzed for structural carbohydrates and Klason lignin. Pretreatment conditions were evaluated based on enzymatic extraction of glucose and xylose and conversion to ethanol using a simultaneous saccharification and fermentation scheme. SSF experiments were conducted with the washed pretreated biomass. The severity of the pretreatment should be sufficient to drive enzymatic digestion and ethanol yields, however, sugars losses and especially sugar conversion into furans needs to be minimized. As expected, furfural production increased with pretreatment severity and specifically xylose release. However, provided that the severity was kept below a general severity factor of 4.0, production of furfural was below an inhibitory concentration and carbohydrate contents were preserved in the pretreated whole hydrolysate. There were significant interactions between time and temperature for all the responses except cellulose digestion. The models were highly predictive for cellulose digestibility ($R^2 = 0.8861$) and for ethanol production ($R^2 = 0.9581$), but less so for xylose extraction. Both cellulose digestion and ethanol production increased with severity, however, high levels of furfural generated under more severe pretreatment conditions favor lower severity pretreatments. The optimal pretreatment condition that gave the highest conversion yield of ethanol, while minimizing furfural production, was judged to be 190°C and 17.2 min. The whole hydrolysate was also converted to ethanol using SSF. To reduce the concentration of inhibitors, the liquid fraction was conditioned prior to fermentation by removing inhibitory chemicals using the fungus *Coniochaeta ligniaria*.

Keywords Sugarcane bagasse · Bioethanol · Pretreatment

S. H. da Cruz (✉)
Department of Agri-food Industry, Food and Nutrition, "Luiz de Queiroz" College of Agriculture, University of Sao Paulo, Av. Padua Dias, 11, PO Box 9, Piracicaba, Sao Paulo CEP 13418-900, Brazil
e-mail: shcruz@usp.br

B. S. Dien · N. N. Nichols · B. C. Saha · M. A. Cotta
Fermentation Biotechnology Research Unit, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, 1815 N. University Street, Peoria, IL 61604, USA

Introduction

Sugarcane is a major resource for the production of fuel ethanol with production concentrated in Brazil (40%), India (22%), and China (8%). One-third of the sugarcane is recovered as a fibrous residue, sugarcane bagasse, following sucrose recovery [28]. Worldwide, over 500 million metric tons of bagasse is generated each year [19]. Sugarcane bagasse is presently combusted to generate process steam and electricity. However, the use of higher-efficiency boilers [19] should generate an excess of bagasse beyond what is required for heat generation. This excess could be used for biofuels production. Sugarcane bagasse is favored as a potential feedstock for ethanol production because it is centrally located adjacent to fuel ethanol production facilities and is relatively high in carbohydrates and low in ash content [4]. Also, using sugarcane bagasse

would allow expansion of ethanol production without reducing global sugar production.

Conversion of sugarcane bagasse into ethanol is a multi-step process consisting of pretreatment, enzymatic hydrolysis, and fermentation. Often, the enzymatic hydrolysis and fermentation steps are combined for simultaneous saccharification and fermentation (SSF). The pretreatment step is responsible for approximately a third of the processing costs and its efficiency is critical for conversion success. Sugarcane plant cell walls are a composite of hemicellulose, lignin, and cellulose, as well as other minor components [28]. Hemicellulose and lignin protect cellulose fibers from exposure to cellulase enzymes [20], which are needed for conversion of the carbohydrate into fermentable glucose. Pretreatment removes these barriers by solubilizing hemicellulose and displacing the lignin fraction. It also decrystallizes cellulose and allows cellulase enzymes access to individual microfibers. There are numerous pretreatment methods [1, 4, 27] of varying industrial relevance. Hydrothermal pretreatment, where fibers are heated in water, is of particular interest because the only solvent is water [10, 12, 16, 24], which eliminates catalyst costs and reduces process waste streams.

In this study, sugarcane bagasse was pretreated in water at varying reaction temperatures and times as determined using a 2^2 full factorial design with centered face and five replicates in the central point. Following pretreatment, both the liquid and the solid fractions were quantitatively recovered and analyzed for carbohydrates and Klason lignin as well as furans, produced from destruction of the sugars. The washed solids were further evaluated for enzymatic conversion to sugars and simultaneous saccharification and fermentation to ethanol. These data were statistically analyzed to determine interactions and the optimal reaction time and temperature to maximize yields and minimize sugar degradation products. Subsequently, bagasse was pretreated at the optimal conditions and solids and liquid fractions converted into ethanol using SSF. To minimize the effect of inhibitors on fermentation, the liquid fraction was conditioned prior to fermentation by removing inhibitory chemicals by using the fungus *Coniochaeta ligniaria*.

Materials and methods

Materials and chemicals

Sugarcane bagasse was obtained from sugar mills (Sao Paulo state, Brazil), dried for 2 days at ambient temperature, and further dried for 24 h using a forced-air oven set at 55°C until constant weight. Cellulase Optiflow RC 2.0 was generously donated by Genencor (Rochester, NY). Novo188 β-glucosidase (Novozymes A/S, Denmark) was purchased from Sigma Chemicals (St. Louis, MO). Other reagents and chemicals were of research quality and were purchased from Fisher Scientific (Hampton, NH) or Sigma-Aldrich Chemicals (St. Louis, MO).

Physico-chemical characterization of sugarcane bagasse

The bagasse used for compositional analysis, with 3% moisture content, was ground using a coffee grinder (Smart Grind Coffee, Black & Decker, Towson, Maryland). The soluble extractives-free material was obtained by mixing ground bagasse (3 g) with dH_2O (50 ml) for 1 h at 30°C. The solids were recovered by filtration and subsequently dried at 50°C. Both the extracted liquid and solids were analyzed for composition.

The extracted samples were analyzed for cellulose, xylan, Klason lignin, and the unextracted sample for ash using standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory (NREL) [25, 26]. Moisture content of biomass was analyzed using an automatic infrared moisture analyzer method (IR-60 Moisture Analyzer, Denver Instrument, Bohemia, NY).

Hydrothermal pretreatment of bagasse and analysis of products

Sugarcane bagasse was hydrothermally pretreated in a multi-vessel rotating reactor system equipped with mini 316 SS steel reactors (200-ml working volume) and an infrared heater (Labomat BFA-12 v200, Werner Mathis, Concord, NC). Reactors were routinely rotated at 50 rpm with 60-s clockwise rotations followed by 60-s counter clockwise rotations throughout the reaction. Reaction vessels were water-cooled using tap water following the reaction.

Reactions were prepared by combining 10 g of biomass with 100 ml dH_2O. Reaction conditions were varied from temperatures of 160–200°C and residence times from 5 to 20 min (Table 1). The liquid and solid fractions were separated by vacuum filtration through a glass fiber filter and the solids were washed with water equal to 20 times

Table 1 Range of variables for the central composite design

Variable	Axial point (−1.41)	Low level (−1)	Central level (0)	High level (+1)	Axial point (+1.41)
Temperature, T (°C)	160	165.8	180	194.2	200
Time, t (min)	5	7.2	12.5	17.8	20

the mass of solids before being dried at 50°C, ground, and then weighed.

Experimental responses to interaction between reaction temperature and time by response surface analysis [2] were included post-reaction pH, sugar degradation products (furfural and HMF), composition of liquid and solid hydrolysate phases, cellulose digestibility, and ethanol conversion yield.

The severity factor (SF) was determined following the equation [10]:

$$\mathrm{SF} = \mathrm{Log}[R] = \log_{10}\left(t \times \exp\left[\frac{(T-100)}{14.75}\right]\right)$$

in which t and T are pretreatment time (min) and temperature (°C), respectively. The combined severity factor (CSF) also takes into account pH [22], however, the CSF is customarily used for pretreatments that use an acid catalyst.

Compositional analysis

Solids recovery was measured by weighing the dried washed solids. The composition was analyzed gravimetrically for lignin content, photometrically for soluble lignin, and by HPLC for carbohydrate content using methods reported by NREL for determining biomass carbohydrates, acid insoluble lignin, and acid soluble lignin [24]. Sugars, organic acids, and ethanol were measured using HPLC equipped with a refractive index detector and an organic acid analytical column (Aminex HPX-87H Column, 300 × 7.8 mm, Bio-Rad Laboratories, Inc., Hercules, CA) [6]. Sugars generated from compositional analysis of solids were measured using a sugar analysis column (Aminex HPX-87P Column, 300 × 7.8 mm, Bio-Rad Laboratories, Inc.) according to the standard protocol [25].

Following pretreatment, liquid fraction was recovered by filtration. The pH was then recorded and furfural and HMF were measured using reverse-phase HPLC with an Econosphere C18 column (Alltech, Deerfield, Ill.) and a UV1000 ultraviolet detector (277 nm; Thermo Finnigan, San Jose, CA) [17]. Total soluble carbohydrates were analyzed by HPLC after being hydrolyzed by treating with 2 M trifluoroacetic acid (TFA) for 1 h at 100°C.

Enzymatic digestibility of pretreated bagasse

Cellulose digestibility was determined using a modified version of a NREL procedure [23] according to Dien et al. [8]. The solids were weighed and brought up to a final volume of 10 ml by the addition of citric acid buffer (50 mM, pH 4.5) and the antibacterial agent thymol (500 mg/l). The enzymatic digestion was initiated by adding Optiflow RC 2.0 cellulase (60 FPU/g cellulose) and Novo188 β-glucosidase (64 *p*NPGU/g cellulose). The reaction was carried out at 50°C and 4 rpm for 72 h in a constant-temperature incubator (Hybaid hybridization chamber, Thermo Scientific, CA). Digestion reactions were clarified by centrifuging in a micro-centrifuge and the supernatant was analyzed for soluble carbohydrates and monosaccharides. Total soluble carbohydrates were analyzed by HPLC after being hydrolyzed by treating with 2 M trifluoroacetic acid for 1 h at 100°C.

SSF of pretreated bagasse solids

Ethanol conversion yields were determined using simultaneous saccharification and fermentation (SSF) using cellulases in combination with xylose-fermenting *S. cerevisiae* YRH400 [11] as described by Dien et al. [8]. The recovered solid fraction was diluted with 22 ml of sodium citrate buffer (50 mM, pH 4.5) and 2.5 ml of yeast extract and peptone solution (YP, 10 g/l yeast extract and 20 g/l peptone final concentrations). After adding Optiflow cellulase (15 FPU/g cellulose) and Novo 188 β-glucosidase (40 U/g cellulose) enzymes, the culture was inoculated with *S. cerevisiae* YRH400 [11] to a beginning OD_{600} of 0.5 (0.30 g/l).

The fermentation flask was capped with a rubber stopper and the SSF culture was incubated at 30°C for 72 h while shaking at 100 rpm using an Innova shaker/incubator (New Brunswick, NJ). The inoculum was prepared according to Dien et al. [8]. Samples for HPLC analysis of fermentation products at the beginning and end of incubation were frozen.

SSF of hydrolyzate bagasse solids combined with the bioabated liquid fraction

It was desirable to also ferment the whole hydrolysate so as to capture the carbohydrate released by pretreatment that are lost by the washing step included in the earlier experiments. However, the liquid fraction also contains the microbial inhibitors. So, the liquid and solid fractions were separated following pretreatment, the liquid portion conditioned using biological abatement, and recombined with the solids prior to SSF. Specific details follow.

Bagasse samples were hydrolyzed at 194.2°C for 7.2 min as described above. Solids were removed by centrifugation (20 min, 25°C, and 15,000 × *g*) and washed with a 10% volume of sterile water. The supernatant was combined with wash liquid and the pH was adjusted to 6.5 with $Ca(OH)_2$. Solids remaining after pretreatment were stored at 10°C for later saccharification and fermentation.

A volume of *C. ligniaria* NRRL30616 [17] cells equal to 10% inoculum was washed with an equal volume of mineral medium [13] and then added to one-half of the

Table 2 Chemical composition of sugarcane bagasse

Composition[a]	Milled sugarcane bagasse	
	Average (%)	SD (%)
Glucan	40.9	0.359
Xylan	26.9	0.734
Galactan	1.4	0.146
Arabinan	2.3	0.123
Total carbohydrates	71.5	1.03
Acid insol lignin	19.4	0.294
Acid sol lignin	5.3	0.076
Total lignin	24.8	0.365

[a] %wt/wt reported on a dry basis

hydrolysate along with 0.1% (w/v) $(NH_4)_2SO_4$ and incubated for 24 h with shaking 200 rpm at 30°C. The other half of the hydrolysate, which served as an uninoculated (unabated) control, was incubated at 30°C in parallel with each bioabatement experiment. *C. ligniaria* cells were not removed from inhibitor-abated hydrolysates prior to fermentation.

For simultaneous saccharification and fermentation (SSF) of biomass hydrolysates, 5 g (dw) of thawed pretreated solids and 50 mM citric acid were added to the liquid portion of the pH adjusted hydrolysate. Optiflow RC 2.0 cellulase (15 Filter Paper U/g (dw) solids; Genencor, Beloit, WI), Novo188 β-glucosidase (40 U/g (dw) solids; Novozymes A/S, Denmark), and a 2% (v/v) inoculum of washed *Saccharomyces cerevisiae* YRH400 cells [11] were then added to initiate fermentations in 80 ml of total volume. Fermentations were stirred with magnetic stir bars and sampled for HPLC analysis of fermentation products at the beginning and end of incubation. Progress of fermentations was followed by measuring CO_2 production, associated with ethanol fermentation, at 15-min intervals. Gas production was monitored by measuring cumulative gas pressure accumulation using Ankom (Macedon, NY) gas production systems and converting to CO_2 using the ideal gas law. Fermentations of unabated hydrolysates were carried out in parallel with the bioabated samples. For pressure accumulation, most fermentations were carried out in duplicate.

Statistical analysis

Each result is expressed as the mean of at least two replicates, except the central point. The obtained data were subjected to a one-way analysis of variance (ANOVA) using Design-Expert 6. Differences between means at the $p < 0.05$ level were considered significant.

Results and discussion

Chemical composition of bagasse

Sugarcane bagasse polysaccharide composition is presented in Table 2. Both milled sugarcane bagasse and

Table 3 pH, inhibitors, and ethanol yield after simultaneous saccharification and fermentation of hydrothermal pretreated bagasse

T (°C)	Time (min)	SF	pH	Furfural (g/l)	HMF (g/l)	Final ethanol (g/l)[a]	Yield efficiency (% of max)[b]
−1	−1	2.79	4.20	0.0362	0.00214	3.74	22.55
+1	−1	3.63	3.80	1.15	0.0876	12.23	55.40
−1	+1	3.19	4.15	0.0521	0.00561	2.88	12.55
+1	+1	4.02	3.54	2.04	0.179	12.18	68.10
+1.41	0	4.04	3.44	3.37	0.230	14.71	68.95
−1.41	0	2.86	4.48	0.0255	0.00	2.69	10.85
0	+1.41	3.66	3.91	0.423	0.0496	7.92	33.95
0	−1.41	3.05	4.01	0.117	0.0164	5.06	21.20
0	0	3.45	4.10	0.217	0.0228	5.74	24.30
0	0	3.45	3.72	0.478	0.0496	7.71	37.80
0	0	3.45	3.74	0.382	0.0415	7.49	30.80
0	0	3.45	3.66	0.315	0.0310	8.34	36.90
190[c]	20	3.95	3.68	0.1976	0.01073	12.44	50.07

Reaction temperatures and times are coded as presented in Table 2

The values given were the average of duplicate experiments, except central point

[a] SSF Basal medium (see Methods); 10% (w/v) substrate; 10% (v/v) yeast inoculum (*Saccharomyces cerevisiae)*

[b] % of theoretically possible ethanol yield obtained based upon beginning biomass carbohydrate content

[c] Assay performed at the selected conditions

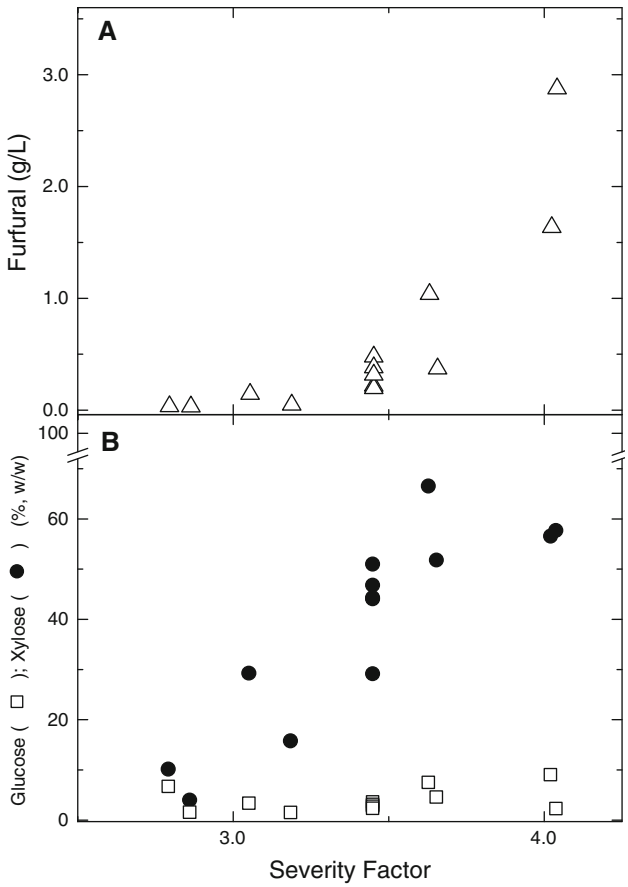

Fig. 1 **a** Furfural, **b** glucose, and xylose released in the hydrolysates prepared from hydrothermal pretreated sugarcane bagasse as a function of severity factor. Following the pretreatment, liquid fraction was recovered by filtration. Total glucose and xylose is the sum of mono-and polymeric sugars obtained after TFA digestion

soluble extractives-free bagasse contained nearly (wt/wt, dry basis) 40% cellulose, 30% hemicelluloses, and 25% lignin. These results are consistent with those previously published for sugarcane bagasse [3, 9].

There are enough glucans in bagasse to theoretically produce 276.7 l/ton of ethanol and enough total carbohydrates to produce 474.0 l/ton., which is comparable with those determined for other grass type residues, including corn stover (427.75 l/ton) and rice straw (416.0 l/ton) [5, 7].

Hydrothermal pretreatment

The samples were hydrothermally pretreated and the solid and liquid fractions analyzed for compositions. The reaction conditions and severity factor (SF) are listed in Table 3. The extent of sugar decomposition incurred by the pretreatments is indicated by furfural and HMF production (Table 3). Furfural and HMF are sugars degradation

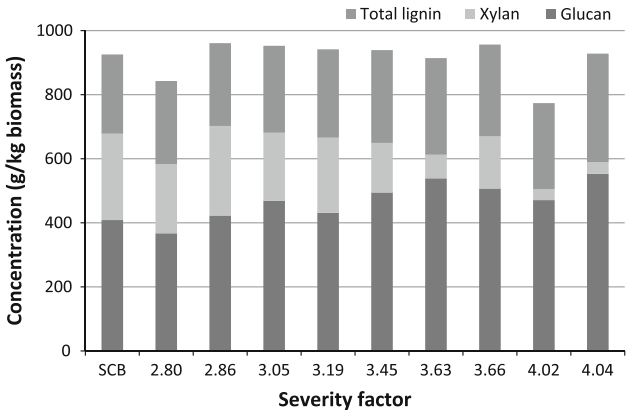

Fig. 2 Composition of residual solids recovered from hydrothermal pretreatment of sugarcane bagasse (g/kg, ODB)

products. The concentrations were generally low as expected because hydrothermal pretreatment does not generate a large amount of monosaccharides, which are susceptible to conversion to furans. Likewise, furfural production exceeded HMF because the xylans are more readily hydrolyzed to xylose than cellulose is to glucose (Fig. 1). Except for the most severe pretreatments (194.2 and 200°C), the amount of inhibitors generated are modest, and below the concentration that is expected to be inhibitory to yeast [17]. From an operational viewpoint, it would be ideal to keep the SF below 4.0 to minimize furfural formation.

The pretreated and washed solids were analyzed for carbohydrate and Klason lignin composition (Fig. 2). As the severity of the pretreatment increased, the samples became enriched for cellulose and Klason lignin with a corresponding reduction in xylan. These results reflect the expected chemistry, where xylan is partially hydrolyzed releasing soluble xylan oligomers, while the hydrophobic lignin and less reactive cellulose remain with the solids. Other components that would have been expected to be extracted are the water-soluble extractables.

As a response surface design had been used to select pretreatment conditions, the post-pretreatment data was also analyzed by linear regression (Table 4). Correlations for fitting the data were 0.86–0.96. The pH and glucan were fit with a linear model, suggesting time and temperature acted independently, and all other factors required quadratic models. The correlations were calculated separately for temperature and time and temperature had a much more significant effect compared to time, which is reflected in the calculation of the severity factor where temperature is raised to an exponential value. While our emphasis is on cellulose digestibility and ethanol SSF yields (discussed below) these models could be useful for adjusting other processing parameters.

Table 4 Final equation for furfural, cellulose digestibility and ethanol yield responses resulting from the complete 2^2 factorial design

Response	Final equation in terms of actual factors	Model	R^2	Correlation	
				T	t
Analysis post-pretreatment					
Liquid fraction					
pH	$+6.784 - 0.01552T - 0.007539t$	L	0.7883	−0.873	−0.159
Furfural	$+43.51 - 0.5147T - 0{,}1931t + 0{,}001404T\,t + 0{,}001512T^2 - 0.001253t^2$	Q	0.9590	0.821	0.140
Xylose	$-309.01 + 3.135T + 3.224t - 0{,}01555Tt - 0{,}007594T^2 - 0.01676t^2$	Q	0.8792	0.742	0.022
Solid fraction					
Glucan	$+6.31434 + 0.2188T + 0.1607t$	L	0.5905	0.704	0.194
Total lignin	$-25.41 + 0.2985T + 2.735t - 0.01255T \times t - 6.486T^2 - 0.01801t^2$	Q	0.8575	0.762	0.061
Analysis post enzymatic digestion or SSF					
Cellulose digestibility	$-1.406 - 0.4654T - 1.784t\ 0.01180T \times t + 0.004084T^2 - 0.01527t^2$	Q	0.8861	0.937	−0.012
Xylose recovery	$+1337.5 - 14.69T - 18.56t + 0.06584T \times t + 0.04221T^2 + 0.2688 \times t^2$	Q	0.9344	0.855	0.001
Final ethanol (g/l)	$+73.29\ -1.040\ T\ -0.07618t + 0.002691T \times t + 0.003647T^2 - 0.01337t^2$	Q	0.9581	0.958	0.086
SSF Yield	$+340.08 - 4.114\ \mathrm{T} - 5.348t + 0.03392T \times t + 0.01306T^2 - 0.01715t^2$	Q	0.9552	0.929	0.113

T temperature, t time, R^2 coefficient of determination, L linear, Q quadratic

As hydrothermal pretreatments do not rely on an external catalyst (e.g., mineral acid), pretreatment reaction conditions only needed to be optimized for reaction temperature and time. However, adjusting these variables is complicated because of the possible interactions and because multiple responses need to be optimized. The severity of the pretreatment needs to be sufficient to drive enzymatic digestion and ethanol yields. Also sugars loss, and especially sugar conversion into furans, needs to be minimized. Furans are potent inhibitors of fermentation and are expensive to remove. A central composite was sought as an efficient mechanism for optimizing reaction conditions. As expected, furfural production increased with pretreatment severity and specifically xylose release. However, it was observed that, provided the severity was kept below 4.0, production of furfural was below an inhibitory concentration and carbohydrate contents were preserved in the pretreated whole hydrolysate.

Enzymatic saccharification of washed pretreated bagasse solids

The washed solids were digested with cellulases at low solids loading and monitored for glucose and xylose yields. Glucose and xylose yields increased with pretreatment severity (Fig. 3). The maximum glucose yield efficiency (% of max) was >80% with a severity of 4.0. Statistical analysis of the experimental data showed that both independent variables (temperature and reaction time) significantly influenced enzymatic glucose yields (Table 4; Fig. 4). The data was best fit with a linear model (Table 5), suggesting that time and temperature effects acted independently. This may be in part because temperature had a much stronger effect than time on determining cellulose digestibility as demonstrated by comparison of their independent correlations (Table 4), similar to what has been observed previously [3].

Simultaneous saccharification and fermentation of washed pretreated bagasse

Ethanol yields were also directly measured for the washed pretreated solids using xylose-fermenting *S. cerevisiae* YRH400. The biomass was converted using a simultaneous saccharification and fermentation (SSF) scheme, where the hydrolytic enzymes for hydrolyzing cellulose and xylan are co-added with the yeast. While the xylose-fermenting *S. cerevisiae* uses glucose much faster than xylose, the xylose remaining in the final SSF represented less than 20% of the beginning xylan content. Yields for both sets of experiments are listed in Table 3. Most notably, the final ethanol yield was close to 70% of the theoretical maximum. This value exceeds that reported by Sasikumar and Viruthagiri [21], which was only 13.5% for acid pretreated sugarcane bagasse. Statistical analysis of the ethanol yields in regard to reaction time and temperature indicated a quadratic model was required to adequately fit the data ($R^2 = 0.9552$) (Tables 4, 5). However, as observed with cellulose digestibility, ethanol yield continued to increase with greater reaction time and temperature (Fig. 4).

Fermentation of whole hydrolysate

While SSF of the washed solids recovered following pretreatment is one option for producing ethanol, another option would be to ferment the whole hydrolysate, that is,

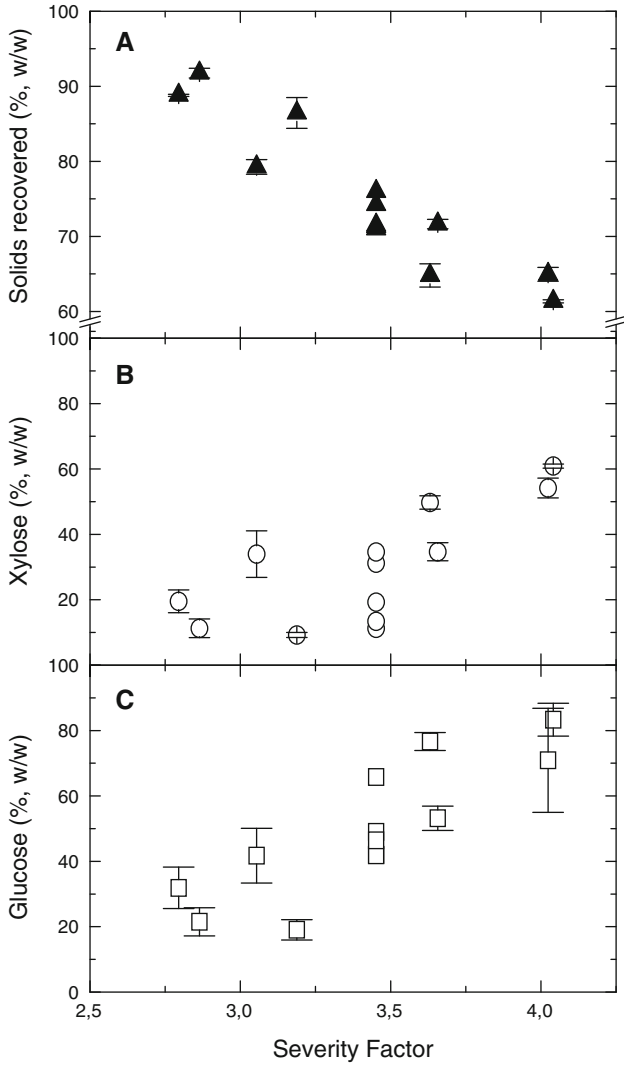

Fig. 3 Solids recovered following pretreatment and glucose and xylose recovered enzymatic saccharification of washed solids (%wt recovered per wt of beginning biomass, oven-dried basis)

Table 5 Analysis of variance (ANOVA) for the squared model of furfural, cellulose digestibility, and the squared model of xylose and ethanol yield

Source	SS	DF	MS	*F* value	Prob > *F*	
Furfural						
Model	10.04	5	2.01	27.84	0.0004	Significant
Residual	0.43	6	0.072			
Lack of fit	0.40	3	0.13	10.97	0.0399	Significant
Pure error	0.036	3	0.012			
Total	10.47	11				
Cellulose digestibility						
Model	3621.02	5	724.20	25.68	0.0006	Significant
Residual	169.23	6	28.20			
Lack of fit	77.02	3	25.67	0.84	0.5571	Not significant
Pure error	92.21	3	30.74			
Total	3790.25	11				
Xylose recovery						
Model	3617.87	5	723.57	17.10	<0.0017	Significant
Residual	253.93	6	42.32			
Lack of fit	13.88	3	4.63	0.058	0.9787	Not significant
Pure error	240.05	3	80.02			
Total	3871.80	11				
Ethanol yield						
Model	157.92	5	31.58	52.27	<0.0001	Significant
Residual	6.90	6	1.15			
Lack of Fit	3.18	3	1.06	1.44	0.2916	Not significant
Pure error	3.72	3	1.24			
Total	164.82	11				

SS sum of squares, *DF* degrees of freedom, *MS* mean square

hydrolyzate bagasse solids combined with the liquid fraction. This later would be advantageous as it would dispense with a separation, and associated equipment costs, as well as allow for conversion of the soluble carbohydrates. However, SSF of whole hydrolysates is more challenging because inhibiting chemicals generated during pretreatment (discussed above) reside in the liquid phase. Our laboratory has had success at conditioning hydrolysates by using biological abatement to metabolize the inhibitory furans and phenolics [13–15]. The hydrolysates are abated using a specific isolate of *C. ligniaria* that mineralizes a wide spectrum of inhibitor-associated organic compounds, including phenolics and furans, and does so preferentially to sugars. Furthermore, treatment with *C. ligniaria* has been shown to improve fermentations of a wide variety of dilute acid-treated feedstocks hydrolysates [13–15]. In this study, bioabatement with *C. ligniaria* was evaluated on hydrothermally pretreated sugarcane bagasse (Fig. 5).

Significant concentrations of furfural, from xylose degradation, were observed in the hydrolysates; HMF concentrations were minor (Table 3). Conditioning the pretreated slurry with *C. ligniaria* successfully removed 46.0% of the furfural present after pretreatment. SSF of both the conditioned and unconditioned slurries were followed by continuously measuring production of CO_2. Fermentations commenced immediately, which indicates that even the unconditioned hydrolysate had a relatively low amount of inhibitors. Frequently for biomass treated under more severe conditions, lag phases are observed in excess of 24 h. However, conditioning the slurry significantly improved the final ethanol concentration from 1.59 to 1.72% (w/v), a yield improvement of 3 g/l ethanol/g

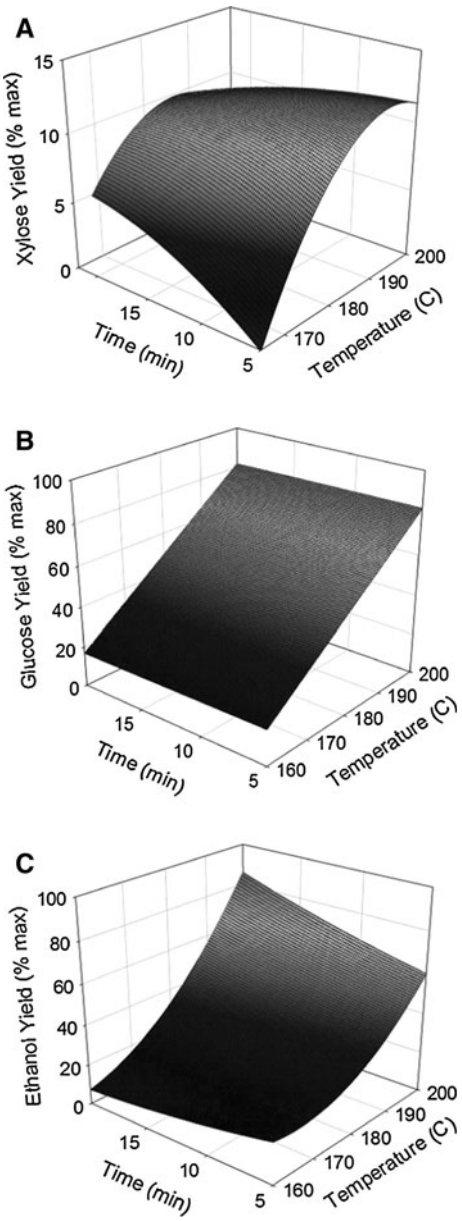

Fig. 4 Response surface plots showing the influence of temperature and residence time on **a** % xylose extracted following enzymatic saccharification, **b** cellulose digestibility following enzymatic saccharification, and **c** ethanol conversion yield from cellulose simultaneous saccharification and fermentation

biomass. Therefore, condition still appears worthwhile, because the solids produced 6.15 g/l of ethanol/g biomass in the SSF.

Conclusions

Hydrothermal pretreatment was evaluated for use in converting sugarcane bagasse for enzymatically extracting sugars and producing ethanol. Both reaction time and temperature were studied using a response surface methodology. Sugarcane bagasse "as received" and the liquid and solid fractions following pretreatment were analyzed for composition. This allowed for a complete mass balance to be formulated around the pretreatment step. The effect of the pretreatment was determined by measuring enzymatic digestibility and ethanol, the later using SSF.

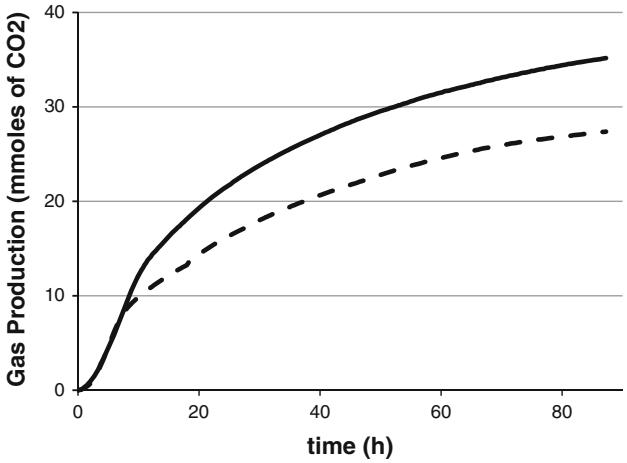

Fig. 5 Fermentation by *S. cerevisiae* YHR400 of hydrolysate that was treated (*solid line*) or untreated (*dashed line*) with *C. ligniara* to ameliorate fermentation inhibitors prior to fermentation

Enzymatic extraction of glucose and xylose and production of ethanol were all observed, as expected, to be influenced by reaction time and temperature. These data were successfully described by response surfaces, as a convenient graphical method to observe the influences of reaction time and temperature. There were significant interactions between time and temperature for all the responses except cellulose digestion. The models were highly predictive for cellulose digestibility ($R^2 = 0.8861$) and for ethanol production ($R^2 = 0.9581$), but less so for xylose extraction. Both cellulose digestion and ethanol production increased with severity. As furfural production increased as well, this suggests the final pretreatment conditions will need to be compromised between that which gives a favorable yield while minimizing furfural concentration below an inhibiting concentration. The data here suggests this can be done by targeting a reaction temperature around 190°C and 17.2 min.

Acknowledgments The authors gratefully acknowledge the financial support of the Conselho Nacional de Desenvolvimento Cientıfico e Tecnologico (CNPq). The authors thank Gregory Kennedy, Patricia O'Bryan, and Sarah Frazer for excellent technical help.

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