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ARTICLE *in* ENERGY & FUELS · OCTOBER 2011

Impact Factor: 2.79 · DOI: 10.1021/ef201189q

energy&fuels

ARTICLE

pubs.acs.org/EF

Second-Generation Bioethanol from Residual Woody Biomass

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ABSTRACT: Olive tree trimmings (OTT) were subjected to sequential stages of autohydrolysis (with hot compressed water) and ethanol−water mixtures (organosolv pulping), to obtain three separate streams containing hemicellulose-derived products (autohydrolysis liquors), lignin fragments (pulping liquors), and autohydrolyzed, delignified solids (denoted ADOTT) mainly made up of cellulose. Up to 42% of the polysaccharides contained in the raw material (accounting for about 25% of the OTT dry mass) were recovered in autohydrolysis liquors as valuable compounds, whereas ADOTT showed high susceptibility toward enzymatic hydrolysis and allowed the formulation of media in which saccharification and ethanol fermentation were carried out simultaneously at a high product yield. Ethanol conversions higher than 90% of the theoretical value (calculated from the ADOTT content of cellulose) were achieved, whereas the ethanol concentration of fermented media reached values up to 39 g/L.

1. INTRODUCTION

The world energy demand is increasing steadily: for example, the expected market of oil-derived fuels is expected to increase by 50% in the next 15 years,[1] owing to the increases in both population and *per capita* consumption.[2] In this context, using new raw materials for manufacturing chemicals and fuels is a strategic goal. For this purpose, lignocellulosic materials (LCM), a clean, renewable, cheap, and largely available source for the production of both chemicals and second generation biofuels, are receiving more and more interest.[3] Second generation biofuels are the only renewable alternative suitable for replacing fossil fuels in a significant extent.[4,5]

LCM are mainly made up of cellulose, hemicelluloses, and lignin, with minor amounts of other compounds (for example, extractives, ashes, and proteins). Processes based on the separation of LCM fractions and the separate utilization of these for defined purposes (following the biorefinery principles) provide an efficient utilization framework.[6,7]

Some fractionation treatments are also suitable as pretreatments for the manufacture of second generation bioethanol by methods based on polysaccharide hydrolysis and the fermentation of the resulting sugars. In most cases, the hydrolysis step is catalyzed by enzymes, whereas fermentation can be carried out either after or during the enzymatic hydrolysis stage. The second approach is known as "simultaneous saccharification and fermentation" (SSF).

In the context of this work, LCM pretreatment must cause chemical and/or structural substrate modification resulting in an increased reactivity toward enzymatic hydrolysis. Owing to its influence on process schemes and costs, it is a key step in biorefineries.[8−10] An ideal pretreatment should meet a number of requirements, including: (a) technological simplicity and economical operation, suitable for scaling up, with limited requirements of energy, water, and chemicals, and resulting in limited generation of wastes; (b) ability for achieving favorable fractionation and structural effects while causing limited losses of polysaccharides; (c) production of a number of added-value fractions (for example, from lignin and hemicelluloses); (d) generation of limited amounts of degradation products (such as furans, organic acids, or phenolic compounds); and (e) obtainment of solids rich in cellulose with high susceptibility toward hydrolytic enzymes.[9−14]

The autohydrolysis reaction (in which an aqueous suspension of LCM is heated to cause the hydrolytic breakdown of hemicelluloses and catalyzed by protons from the dissociation of water and from organics generated from the substrate) may be considered either as a fractionation treatment or as a pretreatment for enzymatic hydrolysis, because hemicelluloses are solubilized selectively by hydrolysis, yielding spent solids mainly composed of cellulose and acid-insoluble lignin.

When the LCM used as autohydrolysis feedstock contain xylan as the major hemicellulosic polymer, the autohydrolysis conditions can be tuned to obtain xylooligosaccharides (XO) and xylose as major hemicellulose-derived products. XO may find applications in the chemical, pharmaceutical, or food industries,[15,16] or they can be used as intermediates for obtaining xylose-based fermentation media suitable for a variety of purposes, including the manufacture of bioethanol.[17,18]

The solids coming from the autohydrolysis stage (here denoted autohydrolyzed solids, AS) show increased proportions of cellulose and may present enhanced susceptibility to cellulolytic enzymes[19,20] or delignification reactions.[21−24] Using highly lignified LCM feedstocks, autohydrolysis conditions leading to AS very susceptible to cellulases may not be compatible with the production of optimal concentrations of valuable soluble saccharides, as it has been reported for *Eucalyptus* wood.[25] For this type of substrate, obtaining susceptible AS entails the utilization of harsh conditions, under which hemicelluloses are decomposed

Received: August 4, 2011
Revised: September 24, 2011
Published: September 25, 2011

Table 1. Composition of Olive Tree Trimmings[a]

	content
glucan	
cellulose	29.0 ± 0.2
glucan in extractives	4.81 ± 0.3
xylan	16.6 ± 0.02
arabinan	2.01 ± 0.07
acetyl groups	2.78 ± 0.02
Klason lignin	24.7 ± 0.1
extractives	13.2 ± 0.3
ash	1.5 ± 0.1

[a] Data in weight percentages on dry basis. Results are expressed as average values ± standard deviations, $n = 4$.

to unwanted degradation products. This situation can be avoided by using processing schemes based on sequential stages of autohydrolysis and pulping, which allow the separation of components into three different streams containing mainly hemicellulose-derived products (autohydrolysis liquors), lignin-derived compounds (pulping liquors), and cellulose (solids resulting from autohydrolysis−pulping), respectively. In this approach, soluble sulfur-free, lignin fragments can be obtained when the pulping stage is carried out using a suitable organosolv medium such as ethanol−water.[26,27] In comparison with AS, the solid phase from autohydrolysis−pulping presents higher cellulose content and improved enzymatic susceptibility,[7] whereas the soluble lignin fragments can be used for a variety of purposes.[28]

When biorefineries use LCM or residual nature as raw materials, additional environmental benefits can be reached. This is the case of olive tree trimmings (OTT), which are generated in Spain at a rate of about 7×10^6 metric tons/year.[29,30] Currently, OTT lack added value and, in many cases, are burned *in situ*, causing environmental problems which could be alleviated by their utilization as a feedstock for the pulp industry or biorefineries.[31−34]

This work deals with the production of second-generation bioethanol from OTT using a biorefinery scheme based on sequential stages of autohydrolysis (performed under optimal conditions for the generation of valuable hemicellulose-derived compounds in liquors) and organosolv delignification of AS. The influence of the selected variables defining the chemical processing conditions on the production of bioethanol by SSF is assessed.

2. MATERIAL AND METHODS

2.1. Raw Material. OTT (*Olea europaea* trimmings) were collected in Córdoba (South of Spain), milled to pass an 8 mm screen, air-dried, homogenized in a single lot, and stored in a dark and dry place until use.

2.2. Analysis of the Raw Material. OTT samples to be used for analytical purposes were milled to pass a 0.5 mm screen, and then, they were assayed for extractives (TAPPI T-264-cm-97 method), moisture (T-264-cm-97 method), and ashes (T 211 om-93 method). Extractive-free OTT samples were subjected to quantitative acid hydrolysis (T-249-cm-85 method), and the liquid phase was analyzed by HPLC (Refractive Index detector, Aminex HPX-87H column eluted with 0.01 M H_2SO_4, 0.6 mL/min flow rate) for glucose, xylose, arabinose, and acetic acid. The solid residue from the T-249-cm-85 assay was considered as Klason lignin. Extractives were analyzed by HPLC (before and after acidic posthydrolysis at 121 °C for 30 min in the presence of 4% w/w H_2SO_4) to assess the presence of glucose and glucose-containing oligomers. The OTT compositional results are shown in Table 1.

2.3. Non-Isothermal Autohydrolysis of OTT and Analysis of Resulting Phases. In hydrothermal fractionation stages, OTT and water were mixed at the desired proportions (8 kg water/kg wood) and reacted in a stainless steel reactor of 1 gallon volume (Parr Instruments Company, Moline, IL) fitted with two six-blade turbine impellers. The vessel was heated with an external fabric mantle to reach the target temperature and then cooled immediately. The temperature profiles in heating and cooling stages correspond to the standard operational conditions.[35] The solid and liquid phases from treatments were separated by filtration. The solid phases (denoted as autohydrolyzed olive tree trimmings, AOTT) were washed with distilled water and employed for the gravimetric determination of solid yield in the autohydrolysis stage (denoted Y_A and defined as g AOTT/100 g raw material, on oven-dry basis).

The combined effects of temperature and time in autohydrolysis experiments were assessed by means of the severity (S_o),[36] defined as

$$S_o = \log R_o = \log[R_{o_{HEATING}} + R_{o_{COOLING}}] = \log\left[\int_0^{t_{MAX}} \exp\left(\frac{T(t) - T_{REF}}{\omega}\right) dt + \int_{t_{MAX}}^{t_F} \exp\left(\frac{T'(t) - T_{REF}}{\omega}\right) dt\right] \quad (1)$$

where R_o is the severity factor (considering the heating and cooling stages), t_{MAX} (min) is the time needed to achieve the target temperature T_A (°C), t_F (min) is the time needed for the whole heating−cooling period, and $T(t)$ and $T'(t)$ represent the temperature profiles of heating and cooling, respectively. Calculations were made assuming the values reported for ω and T_{REF} (14.75 and 100 °C, respectively).

The values considered for T_A (196, 203, or 210 °C, corresponding to S_o values of 3.67, 3.88, and 4.09, respectively) were chosen from the literature[37] as the operational conditions defining the optimum experimental domain, in which the recovery of maximal amounts of hemicellulose-derived saccharides is expected.

AOTT samples were subjected to chemical analysis using the same methods indicated for the raw material in section 2.2. Samples of liquid phases were filtered through 0.45 μm membranes and used for direct HPLC determination of glucose, xylose, arabinose, acetic acid, hydroxymethylfurfural (HMF), furfural (F), formic acid, and levulinic acid, using the same method cited in section 2.2. Samples of liquid phases were also subjected to posthydrolysis (4% w/w sulfuric acid, 121 °C, 30 min), filtered through 0.45 μm membranes, and analyzed by HPLC to assess the increases in the concentrations of monosaccharides and acetic acid caused by posthydrolysis, which measured the content of oligomers and their degree of substitution by acetyl groups.[9] The liquor content of nonvolatile compounds (NVC, g nonvolatile compounds in liquid phase/100 g raw material, on dry basis) was measured by oven-drying at 105 °C until constant weight.

2.4. Organosolv Delignification of AOTT. AOTT samples were subjected to organosolv delignification with ethanol−water

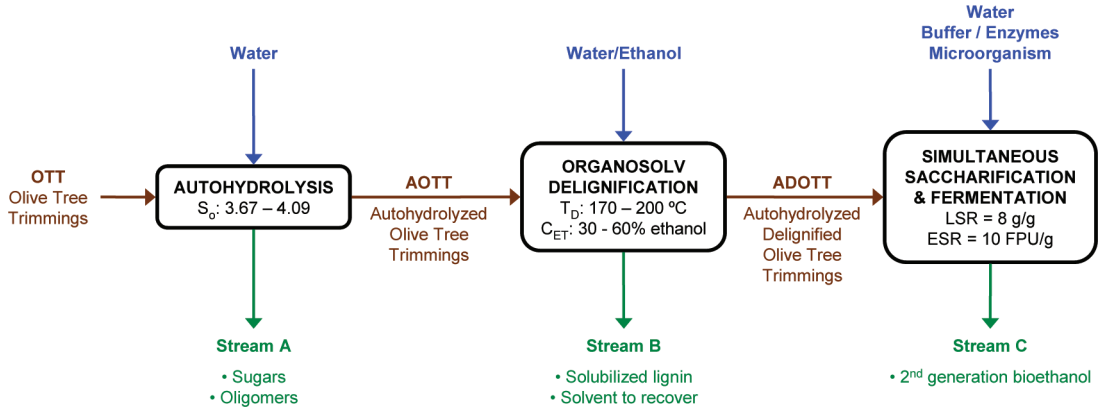

Figure 1. Conceptual flowchart of the biorefinery scheme considered in this work. (S_o, autohydrolysis severity (see eq 1); T_D, delignification temperature; C_{ET}, ethanol concentration in pulping liquor; LSR, liquid to solid ratio; and ESR, enzyme to substrate ratio.)

solutions in the Parr reactor cited in section 2.3, using a liquid to solid ratio (LSR) = 8 g liquid/g AOTT (on dry basis, considering the AOTT moisture as water) for 1 h. Time zero was taken when the system reached the preset temperature. At the end of treatments, the media were cooled, and the autohydrolyzed-delignified olive tree trimmings (denoted ADOTT) were recovered by filtration and washed (first with ethanol−water and then with distilled water). After washing, ADOTT samples were employed for the gravimetric determination of the solid yield corresponding to the delignification stage (Y_D, defined as kg of ADOTT/100 kg AOTT, on oven-dry basis). The total yield (Y_T) was defined as kg of ADOTT/100 kg OTT, on dry basis. ADOTT samples were subjected to chemical analysis using the same methods indicated for the raw material.

2.5. Yeast Cultivation and Inocula Preparation. The strain *Saccharomyces cerevisiae* CECT-1170 (obtained from the Spanish Collection of Type Cultures, Valencia, Spain) was employed for fermentation. Cells were grown at 32 °C for 24 h in a medium containing 10 g glucose/L, 5 g peptone/L, 3 g malt extract/L, and 3 g yeast extract/L.

2.6. Simultaneous Saccharification and Fermentation of ADOTT. ADOTT samples obtained under different conditions of autohydrolysis-delignification were used as substrates for SSF experiments. Except for the type of substrate, SSF assays were performed under fixed conditions to assess the comparative suitability of the various ADOTT for ethanol manufacture. SSF assays were performed under the following conditions: LSR = 8 g liquid/g ADOTT (oven-dry basis); enzyme to solid ratio = 10 FPU/g (oven-dry basis); and β-glucosidase to cellulase activity ratio = 5 IU/FPU. The commercial enzyme concentrates employed in this work ("Celluclast 1.5 L" cellulases from *Trichoderma reesei* and "Novozym" β-glucosidase from *Aspergillus niger*) were kindly provided by Novozymes (Madrid, Spain). The cellulase activity of "Celluclast 1.5 L" concentrates was measured by the Filter Paper assay, and the activity was expressed in Filter Paper Units (FPU).[38] The β-glucosidase activity of "Novozym" concentrates was measured in International Units (IU).[39] The activities of "Celluclast 1.5 L" and "Novozym" were 70.1 FPU/mL and 630 UI/mL, respectively.

The SSF assays were carried out in 250 mL Erlenmeyer flasks placed in orbital shakers at 120 rpm (pH = 5). Suspensions containing water and ADOTT were autoclaved (121 °C, 15 min) separately from the nutrients and thermostatted at 35 °C. The SSF experiments started with yeast inoculation and enzyme addition. Preparing 100 mL of media required 10 mL of inocula (leading to an initial yeast cell concentration about 1.0 g/L) and 10 mL of nutrient solution (containing 5 g peptone/L, 3 g yeast extract/L, and 3 g malt extract/L). At given times, samples were withdrawn from the media and centrifuged (5000 rpm, 5 min). Supernatants were assayed for sugars, acetic acid, and ethanol by HPLC, using the method indicated in section 2.2.

2.7. Fitting of Data. The experimental data were fitted to the proposed models using commercial software (Microsoft Excel spreadsheet).

3. RESULTS AND DISCUSSION

3.1. Autohydrolysis. The biorefinery approach developed in this work involves two sequential treatments: autohydrolysis and organosolv delignification. Autohydrolysis was carried out under non-isothermal conditions to yield soluble hemicellulose-derived saccharides in amounts as high as possible but resulting in limited participation of sugar-degradation reactions and in minimal effects on cellulose and acid-insoluble lignin. Organosolv delignification was performed in media containing uncatalyzed ethanol−water solutions, aiming to recover both solids enriched in cellulose highly susceptible to enzymatic hydrolysis and liquors containing soluble lignin fragments. This approach (see Figure 1) enables the separation of the three structural LCM components (untouched or as soluble products) in separate streams, which can be employed for specific purposes. The cellulose-enriched solids resulting from processing (ADOTT) were assayed as substrates for bioethanol production by SSF (carried out under fixed operational conditions).

Table 2 shows experimental results concerning the fractionation effects and the compositions of solid and liquid phases from autohydrolysis performed under selected, non-isothermal conditions (corresponding to S_o of 3.67, 3.88, and 4.09). According to reported results,[33] this operational domain is expected to include the conditions leading to optimal recovery of soluble saccharides derived from hemicelluloses.

The autohydrolysis solid yield (Y_A) varied in the range 52.1−56.9 g AOTT/100 g raw material, on an oven-dry basis. These values are close to the weight percent of the raw material corresponding to the joint contributions of cellulose and lignin (53.7 wt %), suggesting that both fractions were not significantly affected by the treatments. This idea was confirmed by the compositional AOTT data: about 90 wt % of samples corresponded

Table 2. Operational Conditions Employed in the Non-Isothermal Autohydrolysis of OTT and Experimental Concerning Fractionation and Composition of Solid and Liquid Phases[a]

S_o (dimensionless)	3.67	3.88	4.09
Y_A (kg AOTT/100 kg oven-dry OTT)	56.9	56.7	52.1
NVC (kg nonvolatile compounds/100 kg oven-dry OTT)	34.8	33.2	30.1
Solid Phase Composition (kg/100 kg oven-dry OTT)			
cellulose	28.3	28.2	27.8
xylan	5.7	4.4	3.1
arabinan	0.22	0.31	0.31
acetyl groups	0.69	0.41	0.06
Klason lignin	25.5	24.3	25.3
Liquid Phase Composition (g/L)			
glucose	1.95	1.72	1.93
xylose	1.25	2.43	3.85
arabinose	1.55	0.96	1.18
formic acid	0.91	0.50	0.81
acetic acid	0.62	0.72	2.33
levulinic acid	0.05	0.01	0.04
hydroxymethylfurfural	0.08	0.34	0.46
furfural	0.20	0.11	1.51
gluco-oligomers	9.02	6.90	6.32
xylo-oligomers	15.28	14.16	9.70
arabino-oligomers	0.50	0.52	0.16
acetyl groups linked to oligomers	2.96	2.83	1.93

[a] Oligomers are expressed as monomer equivalents.

to cellulose and lignin, and the combined amounts of these fractions matched the ones contained in raw material. The rest of AOTT corresponded to the contribution of residual hemicelluloses (3.4−6.6 g/100 g OTT) and to other compounds, which are of minor importance for the purposes of this study.

Regarding the liquid phase composition, three different types of compounds were quantified: hemicellulosic oligomers, monosaccharides, and sugar-decomposition products (furans). Oligomers were the most abundant solutes, reaching a maximum concentration of 27.8 g/L (which corresponded to more than 20 g oligomers/100 g oven-dry OTT) in the experiment performed at the lowest S_o (3.67). Oligomers were made up of glucooligomers (mainly coming from the extractive fraction, which contained soluble glucan) and hemicellulose-derived oligomers (which presented a fairly constant xylose/acetyl groups molar ratio of 2:1, together with limited amounts of arabinosyl substituents). The concentrations of monosaccharides (mainly xylose and glucose) varied from 4.74 g/L (in the experiment performed at S_o = 3.67) up to 6.96 g/L (in the assay carried out at S_o = 4.09). The concentrations of acetic acid (generated by the cleavage of acetyl groups in hemicelluloses) increased with severity from 0.62 up to 2.33 g/L. Sugar dehydration products (furfural generated from xylose and arabinose, and hydroxymethylfurfural produced from hexoses) showed similar variation patterns, reaching concentrations in the ranges 0.11−1.51 and 0.08−0.46 g/L, respectively. Other organic acids detected in the medium include formic acid (which reached concentrations in the range 0.50−0.91 g/L) and levulinic acid (with concentrations below 0.1 g/L).

Considering the possible formulation of sugar-containing fermentation media from autohydrolysis liquors, it can be noted that, in the experiment performed at S_o = 3.67, sugars and sugar oligomers can be recovered at yields up to 22.0 g/100 g oven-dry raw material (expressing the oligomers as monomer equivalents and neglecting the contribution of acetyl groups). The saccharides solubilized in the best autohydrolysis stage accounted for 42% of the total amount of carbohydrates contained in the raw material.

The NVC content of autohydrolysis liquors varied in the range 30.1−34.8 g/100 g of oven-dry OTT. Material balances showed that 72−81% of NVC corresponded to identified compounds (mainly oligomers, which accounted by 44−58% of NVC) and the rest to non-saccharide components (including extractive and acid soluble lignin-derived compounds).

3.2. Assessment of Delignification and Fermentation. AOTT samples (obtained under the various conditions selected for the manufacture of soluble saccharides) were subjected to organosolv treatment with ethanol−water mixtures. The operational variables considered in this part of the study and their respective variation ranges were selected on the basis of our own experience and preliminary experiments (data not shown) as follows:

- S_o (dimensionless), in the range 3.67−4.09
- delignification temperature (T_D, in the range 170−200 °C), and
- ethanol concentration in organosolv media (C_{ET}, in the range 30−60 g ethanol/100 g solution).

Table 3 shows the experimental conditions corresponding to the various experiments performed, as well the results achieved for the following set of experimental variables:

- total yield after autohydrolysis and delignification (Y_T, g ADOTT/100 g raw material, oven-dry basis)
- variables employed to measure the recovery of key feedstock components in processed samples (expressed as the percentages of cellulose, xylan, arabinan, acetyl groups, and Klason lignin recovered in respect to the amounts present in the raw material)
- a single variable measuring the comparative fermentability of substrates (the maximum ethanol concentration, denoted E_{MAX} (g ethanol/L).

In order to perform a surface response analysis of data, the independent variables were converted into dimensionless, normalized parameters with a variation range (−1, +1) using the following equation:

$$x_i = 2\,\frac{X_j - X_{me}}{X_{max} - X_{min}} \qquad (2)$$

where the subscript i refers to the independent variable considered (x_1 for S_o, x_2 for T_D, and x_3 for C_{ET}), j refers to the experiment considered, me refers to the mean value of the variation range, min refers to the minimum value considered, and max refers to the maximum value of the considered variable.

The interrelationship between dependent and independent variables was established by empirical models following the generalized polynomial expression:

$$y_j = b_{0j} + \sum_i b_{ij}x_j + \sum_i \sum_k b_{ikj}x_j x_k \qquad (3)$$

where y_j is the dependent variable considered (j: 1 to 7, see Table 3), x_i or x_k (i or k: 1 to 3, $k \geq i$) are the dimensionless, independent variables defined by eq 2, and $b_{0j}....b_{ikj}$ are the

Table 3. Operational Conditions Considered[a] and Experimental Results Obtained for Dependent Variables[b]

	independent variables												
	dimensional			dimensionless			solid yield	recovery percentages in ADOTT (in respect to the amounts in OTT), g in ADOTT/100 g in OTT					bioethanol concn
expt	S_o	T_D, °C	C_{ET}, w/w	x_1	x_2	x_3	Y_T (y_1), kg ADOTT/100 kg OTT	cellulose (y_2)	xylan (y_3)	arabinan (y_4)	acetyl groups (y_5)	Klason lignin (y_6)	E_{MAX} (y_7), g ethanol/L
1	4.09	200	60	1	1	1	40.4	96.1	9.4	2.4	9.5	40.2	36.7
2	4.09	200	30	1	1	−1	47.2	95.6	8.1	2.9	4.3	60.7	34.4
3	4.09	170	60	1	−1	1	42.1	84.8	9.5	2.9	8.5	55.7	30.6
4	4.09	170	30	1	−1	−1	44.8	91.2	10.4	0.0	6.0	57.3	31.4
5	3.67	200	60	−1	1	1	43.4	97.7	18.1	0.0	14.8	43.7	32.0
6	3.67	170	60	−1	−1	1	46.4	89.1	21.1	1.7	18.7	50.4	21.3
7	3.67	170	30	−1	−1	−1	49.7	89.5	16.3	0.8	6.0	64.7	21.1
8	3.67	200	30	−1	1	−1	49.0	102.1	13.4	0.0	3.7	53.5	32.2
9	4.09	185	45	1	0	0	44.5	90.6	9.2	0.0	10.3	58.7	31.8
10	3.67	185	45	−1	0	0	44.5	94.8	17.9	0.7	14.1	42.0	30.7
11	3.88	200	45	0	1	0	38.7	89.9	12.0	0.0	8.6	35.7	38.7
12	3.88	170	45	0	−1	0	45.2	89.4	15.7	0.0	18.8	55.3	24.7
13	3.88	185	60	0	0	1	44.4	93.4	15.1	0.0	12.3	46.6	28.3
14	3.88	185	30	0	0	−1	44.3	87.5	13.1	0.0	9.3	53.9	33.9
15	3.88	185	45	0	0	0	40.4	88.5	13.3	0.0	8.8	39.4	33.6
16	3.88	185	45	0	0	0	41.4	86.6	13.5	0.0	7.1	45.0	32.7
17	3.88	185	45	0	0	0	41.8	86.0	12.4	0.0	12.1	44.3	29.8

[a] expressed in terms of dimensional and dimensionless independent variables. [b] S_o: severity, T_D: delignification temperature, C_{ET}: ethanol concentration in pulping liquor.

Table 4. Values Calculated for the Regression Coefficients and Statistical Parameters Measuring the Correlation and Significance of the Models

param.	Y_T (y_1)	cellulose (y_2)	xylan (y_3)	arabinan (y_4)	acetyl groups (y_5)	Klason lignin (y_6)	E_{MAX} (y_7)
b_{0j}	41.89[a]	88.25[a]	13.54[a]	−0.228	11.38[a]	44.47[a]	32.030[a]
b_{1j}	−1.40[b]	−1.49	−4.020[a]	0.511[b]	−1.881[c]	1.832	2.758[a]
b_{2j}	−0.95[c]	3.741[a]	−1.201[a]	−0.0111	−1.710[c]	−4.952[b]	4.487[a]
b_{3j}	−1.83[b]	−0.479	1.197[a]	0.348[c]	3.440[b]	−5.367[b]	−0.424
b_{11j}	2.11[c]	3.544[c]	−0.343	0.737[c]	−0.695	4.701	−0.770[c]
b_{12j}	0.54	0.428	−0.0305	0.399	0.782	−0.198	−1.602
b_{13j}	−0.05	1.280	0.211	0.399	−2.133[c]	4.592	0.194
b_{22j}	−0.45	−0.687	0.432[c]	0.613[c]	0.704	0.718	−0.366
b_{23j}	−0.80	−0.142	−1.145[a]	0.189	−2.008[c]	0.254	0.334
b_{33j}	1.94[c]	0.374	0.271	−0.548[c]	−0.133	−1.811	−0.943
R^2	0.78	0.74	0.98	0.84	0.75	0.76	0.86
F	2.8	2.2	36.5	4.0	2.3	2.4	4.7
sig. level (%)	>96	>93	>99	>98	>93	>94	>99

[a] Coefficients significant at the 99% confidence level. [b] Coefficients significant at the 90% confidence level. [c] Coefficients significant at the 80% confidence level.

regression coefficients, calculated from the experimental data by multiple regression using the least-squares method (see Table 4 for results). The same table provides information on the statistical significance of the coefficients (based on the Student's t test) and on the statistical parameters measuring the correlation (R^2) and significance of models (based on the Fisher's F-test).

The experimental data determined for Y_T (or y_1) varied in the range 38.7−49.7 kg ADOTT/100 kg raw material, on a dry basis. According to the values calculated for the regression coefficients, it can be inferred that the major variations of Y_T were caused by S_o and C_{ET}. Figure 2 shows the response surface calculated from the empirical model, which predicted higher Y_T for experiments performed at lower C_{ET}, with a minimum value at S_o = 3.92.

ADOTT samples were mainly composed of cellulose (50.9−67.4 g cellulose/100 g oven-dry ADOTT), corresponding to about 85−100% recovery of the cellulose present in the native OTT feedstock (average recovery, 91.3%). Figure 3 shows the response surface predicted by the empirical model for the

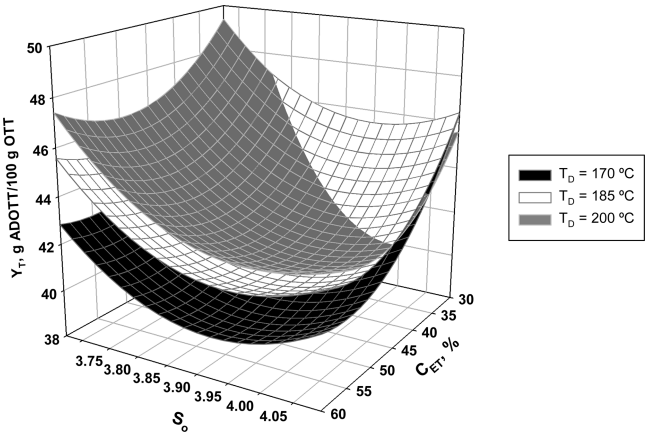

Figure 2. Calculated dependence of Y_T (total solid yield, g ADOTT/100 g OTT, oven-dry basis) on S_o (severity, dimensionless, see eq 1) and C_{ET} (ethanol concentration in pulping liquor, g ethanol/100 g liquor).

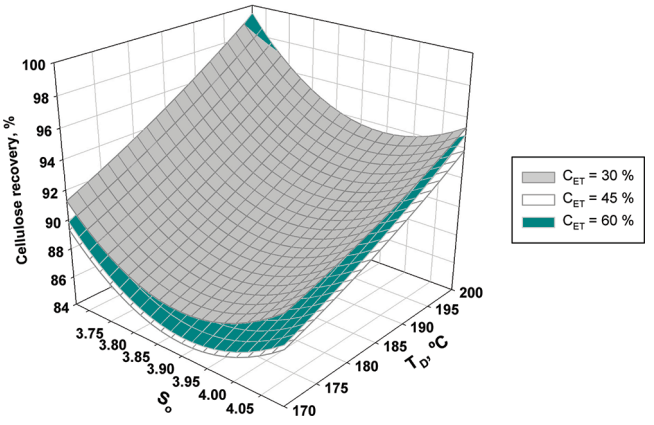

Figure 3. Calculated dependence of cellulose recovery (g cellulose in ADOTT/100 g cellulose in OTT, oven-dry basis) on S_o (severity, dimensionless, see eq 1) and T_D (delignification temperature, °C).

percentage of cellulose recovery in ADOTT samples as a function of the most influential operational variables (T_D and S_o) for the assayed values of C_{ET}. The recovery of cellulose improved with T_D, with limited influence of S_o and negligible effects associated to C_{ET}.

Small increases in of cellulose recovery caused by T_D were noticed, which are ascribed to experimental and fitting errors related to the limited variation range observed for this variable. As a general trend, it can be concluded that the high cellulose recovery observed along the whole experimental domain is a very favorable feature of the processing scheme proposed in this work.

Hemicelluloses were extensively solubilized by the chemical stages: the recovery of xylan varied in the range 8.1−21.1 g xylan in ADOTT/100 g xylan in OTT (average value, 13.4%), whereas arabinan was almost totally removed, with recoveries in the range 0.0−2.9 g arabinan in ADOTT/100 g arabinan in OTT (average value, 0.7%). Acetyl groups were also split off from saccharides (with recoveries in the range 3.7−18.8 g acetyl groups in ADOTT/100 g acetyl groups in OTT and an average recovery of 10.2%). As a general trend, the recoveries of hemicellulose-derived compounds decreased when S_o increased, whereas the rest of the independent variables were slightly influential. This behavior is consistent with the well-known ability of autohydrolysis treatments

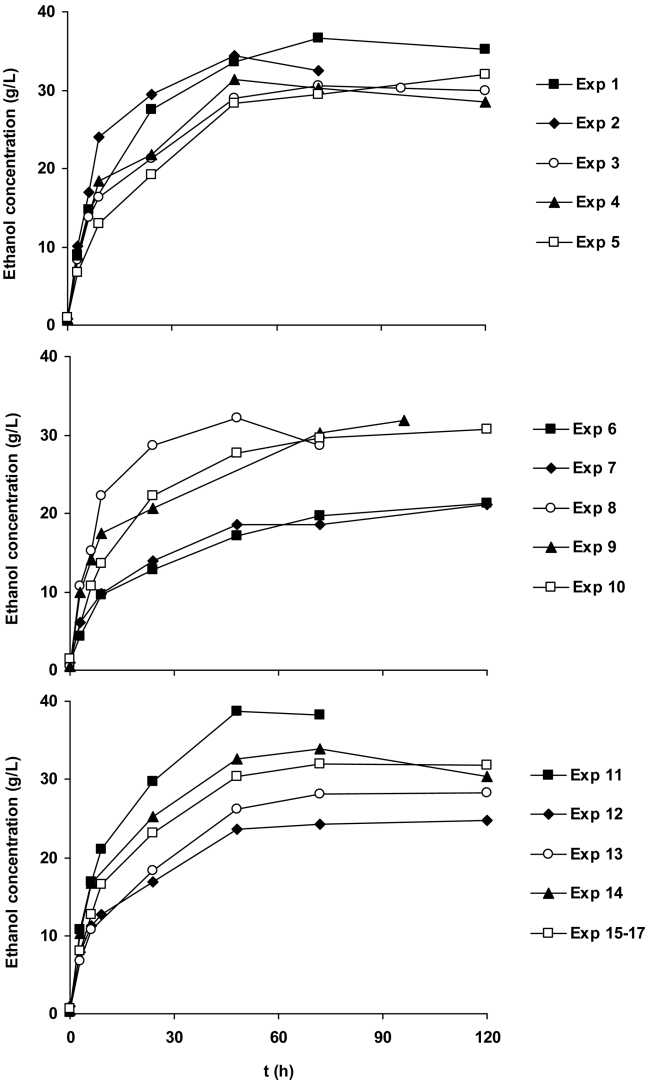

Figure 4. Time course of ethanol concentration in simultaneous saccharification and fermentation media.

for solubilizing hemicelluloses, as the AOTT content of this polymer was low. Under these conditions, the delignification effects (measured by T_D and C_{ET}) were limited.

The recovery of Klason lignin in ADOTT varied in the range 35.7−64.7%, and it decreased when T_D and/or C_{ET} increased, whereas the effects associated with the variable S_o were of limited importance.

3.3. Simultaneous Saccharification and Fermentation (SSF). SSF experiments using ADOTT samples as substrates were carried out to assess the potential of the process depicted in Figure 1 for bioethanol production. Mild operational conditions were chosen for the enzymatic hydrolysis stage (liquor to solid ratio = 8 g liquid/g oven-dry ADOTT; enzyme to substrate ratio = 10 FPU/g oven-dry ADOTT) in order to assess the behavior of the substrates on a comparative basis.

Figure 4 shows the ethanol concentration profiles determined in representative experiments in which typically maximum concentrations were achieved after 48−72 h. Besides ethanol, the fermentation media contained glucose (in the initial reaction stages), xylose (at concentrations that increased steadily up to

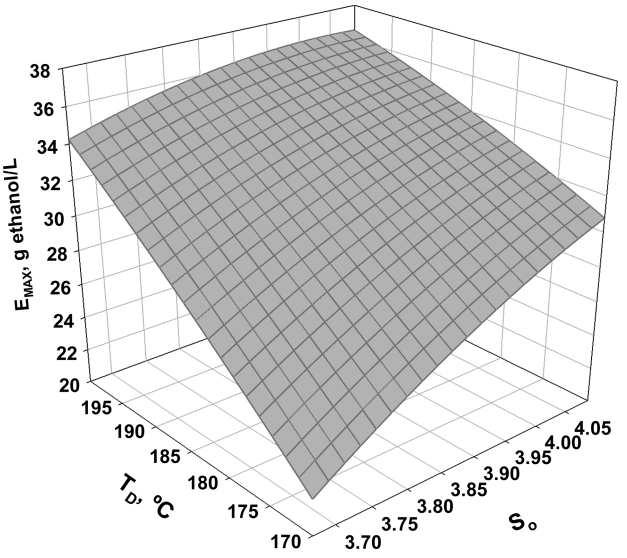

Figure 5. Calculated dependence of E_{MAX} (maximum ethanol concentration achieved in SSF, g ethanol/L) on S_o (severity, dimensionless, see eq 1) and T_D (pulping temperature, °C) for C_{ET} = 45%.

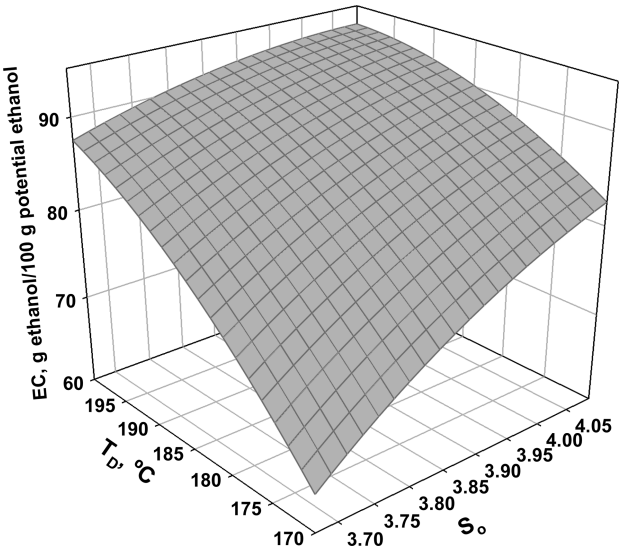

Figure 6. Calculated dependence of EC (ethanol conversion achieved in SSF, measured as percentage in respect to the theoretical yield) on S_o (severity, dimensionless, see eq 1) and T_D (delignification temperature, °C) for C_{ET} = 30%.

reach constant values in the range 1−3 g/L), and minor amounts of acetic acid and glycerol.

Even if the main objective of this work was to provide an overview on the suitability of autohydrolysis−pulping processes for bioethanol production (and not to reach maximum ethanol concentrations), it can be highlighted that E_{MAX} reached 38.7 g ethanol/L in experiment 11 (carried out at S_o = 3.88, T_D = 200 °C, and C_{ET} = 45% w/w), which compares favorably with the literature reported for ethanol production from this feedstock and is close to the threshold reported for economic feasibility.[40] Figure 5 shows the response surface calculated for E_{MAX} as a function of T_D and S_o. E_{MAX} increased with the variables defining the intensity of treatments, whereas the effects caused by C_{ET} were of limited importance.

The dependence of E_{MAX} on T_D and S_o can be justified on the basis of the following ideas: (a) the higher structural alteration caused by harsher treatments, which is expected to increase the kinetics and yields of the enzymatic hydrolysis; (b) the increased cellulose content of samples treated and high T_D, and/or S_o; and (c) the fact that the enhanced delignification observed under harsh conditions results in the limitation of the barrier effects caused by lignin in the enzymatic hydrolysis of treated solids. An additional benefit of enhanced delignification lies in that higher cellulose contents of SSF substrates increase the potential ethanol concentration for fixed operational conditions.

The ethanol conversion (denoted EC and defined as ethanol/100 g potential ethanol) is a key parameter to assess the efficiency of a given process. EC can be calculated as a function of E_{MAX} using the equation:

$$EC = 100 \frac{E_{max}}{\frac{Cel_{ADOTT}}{100}\frac{92}{162}\frac{\rho}{LSR + 1 - KL_{ADOTT}/100}} \quad (4)$$

where E_{MAX} is the maximum ethanol concentration achieved in the experiment (or variable y_7 in Table 3), Cel_{ADOTT} is the cellulose content of ADOTT (expressed as g cellulose/100 g oven-dry ADOTT), 92/162 is the stoichiometric factor for ethanol production from cellulose (g ethanol/g cellulose), ρ is the density of liquors (average value, 1005 g/L), LSR is the liquid-to-solid ratio (fixed at 8 g/g) and KL_{ADOTT} is the Klason lignin content of ADOTT (expressed as g Klason lignin/100 g oven-dry ADOTT). For a generalized interpretation of data, the values of variables Y_T, Cel, KL and E_{MAX}, already defined, can be obtained (for defined operational conditions) using eq 3 and the parameters listed in Table 4; then, variables Cel_{ADOTT} and KL_{ADOTT} can be calculated using the following expressions:

$$Cel_{ADOTT} = Cel\ Cel_{RM}/Y_T \quad (5)$$

$$KL_{ADOTT} = KL\ KL_{RM}/Y_T \quad (6)$$

where Cel_{RM} and KL_{RM} give the raw material content of cellulose and Klason lignin.

This operational procedure allowed the calculation of the surface response plotted in Figure 6, which shows the calculated dependence of EC on T_D and S_o. The variation pattern presented features similar to the ones already described for E_{MAX}, whereas the results confirmed that EC > 90% could be obtained operating at $T_D \geq$ 182 °C and C_{ET} = 30%. These results compare well with the literature and confirmed the efficiency of the scheme autohydrolysis−organosolv pulping for the development of second-generation ethanol biorefineries.

Most experiments led to EC > 80%, whereas remarkably high values where obtained under the following conditions: experiment 14 (carried out at S_o = 3.88, T_D = 185 °C, and C_{ET} = 30% w/w), EC = 92.4; experiment 2 (carried out at S_o = 4.09, T_D = 200 °C, and C_{ET} = 30% w/w), EC = 91.2%; and experiment 11 (carried out at S_o = 3.88, T_D = 200 °C, and C_{ET} = 45% w/w), EC = 90.6%.

4. CONCLUSIONS

The autohydrolysis−ethanol pulping approach resulted in the selective fractionation of hemicelluloses, lignin, and cellulose in three separate streams. Up to 42% of the polysaccharides contained in the raw material (accounting for about 25% of the OTT

dry mass) were recovered in autohydrolysis liquors as valuable compounds. The pulping stage, carried out with ethanol−water mixtures, resulted in partial delignification, and the solid phases subjected to sequential steps of autohydrolysis and pulping (ADOTT) showed high susceptibility toward enzymatic hydrolysis. SSF media from ADOTT enabled the production of ethanol at a high yield. Remarkable ethanol conversions (higher than 90% of the theoretical maximum) were achieved at concentrations in the vicinity of 40 g/L. These data confirm the potential of the approach considered in this work for the development of biorefineries having second-generation ethanol as one of the target products.

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ACKNOWLEDGMENT

The authors are grateful to Xunta de Galicia for the financial support of this work, in the framework of Research Project Reference No. 08REM002383PR.

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