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Liquid Hot Water Pretreatment of Olive Tree Pruning Residues

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Abstract

Olive tree pruning generates an abundant, renewable lignocellulose residue, which is usually burnt on fields to prevent propagation of vegetal diseases, causing economic costs and environmental concerns. As a first step in an alternative use to produce fuel ethanol, this work is aimed to study the pretreatment of olive tree pruning residues by liquid hot water. Pretreatment was carried out at seven temperature levels in the range 170–230°C for 10 or 60 min. Sugar recoveries in both solid and liquid fractions resulting from pretreatment as well as enzymatic hydrolysis yield of the solid were used to evaluate pretreatment performance. Results show that the enzyme accessibility of cellulose in the pretreated solid fraction increased with pretreatment time and temperature, although sugar degradation in the liquid fraction was concomitantly higher.

Index Entries: Biomass; enzymatic hydrolysis; ethanol; liquid hot water pretreatment; olive tree residues; glucose.

Introduction

Pretreatment is an essential operation in lignocellulosic conversion process because of cellulose resistance to enzymatic hydrolysis *(1)*. Physical pretreatments are usually applied to all biomass feedstock to reduce particle size, thus increasing surface area. Then biomass is pretreated by either water or chemicals at different concentration, temperature, or pressure conditions. Diluted acid pretreatment, especially with H_2SO_4, has been extensively applied to lignocellulose raw materials *(2)*. It offers good performance in terms of hemicellulose-derived sugar recoveries but equipment must be acid-resistant, thus increasing costs, and the hydrolyzates must be usually neutralized before using them as a fermentation broth, resulting in a copious solid waste. Water pretreatment, also known as hydrothermal pretreatment, offers several potential advantages compared with diluted acid pretreatment as there is no requirement for purchased acid, for special

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equipment materials or for preliminary feedstock size reduction *(3)*, and hydrolyzate neutralization residues are produced in much lower quantities. Steam explosion and liquid hot water (LHW) are common hydrothermal pretreatments applied to lignocellulose materials. Although both pretreatment methods do improve cellulose susceptibility to enzymatic hydrolysis, LHW pretreatment results in higher hemicellulose sugar recovery and lower fermentation inhibiting hydrolyzates than steam explosion pretreatment *(4,5)*. Typical conditions for LHW pretreatment include temperatures around 200°C for a few minutes.

Olive trees are cultivated especially in Mediterranean countries but in the last few years the culture surface is growing worldwide in countries as different as Argentine, Australia, or the United States, reaching more than 8 million ha *(6)*. In olive tree cultivation, old branches must be cut down to regenerate and prepare trees for the next crop. This action, called pruning, is done every 2 yr after olive harvesting and generates a variable amount of lignocellulose residues that has been estimated at 3000.0 kg/ha, as an annual average *(7)*. A typical olive tree pruning lot includes 70% thin branches (by weight, with approx one-third of leaves) and 30% of wood (thick branches, diameter >5 cm approx). Nevertheless, variable amounts and compositions of pruning are possible, depending on culture conditions, production, and local uses. Disposal of pruning residues is necessary to keep fields clean and to prevent propagation of vegetable diseases; usually they are eliminated by either burning or grinding and scattering on fields, causing economic cost and environmental concerns. Olive tree wood is sometimes separated and put to domestic use as firewood but there are no applications on an industrial scale for these residues. As an alternative, olive tree pruning residues may be used as raw material for ethanol production. Pretreatment by steam explosion of olive tree wood has been reported *(8)* but there is no literature on LHW pretreatment of olive tree pruning residues, to our best knowledge. In this work, LHW pretreatment was applied to olive tree pruning residues at temperatures ranging from 170 to 230°C for 10 or 60 min. The objective of the work is to evaluate the pretreatment performance in terms of sugar yield in the liquid fraction and glucose yield after enzymatic hydrolysis in the solid fraction issued from pretreatment.

Materials and Methods

Raw Material

Olive tree pruning, discarding thick branches (>5 cm diameter), was collected locally after fruit-harvesting, air-dried at room temperature to equilibrium moisture content of about 10%, milled using a laboratory hammer mill (Retsch GmBH, Haan, Germany) to a particle size smaller than 10 mm, homogenised in a single lot, and stored until used.

Pretreatment

LHW pretreatment was performed in a laboratory-scale stirred autoclave (model EZE-Seal, Autoclave Engineers, Erie, PA). The reactor has a total volume of 2 L, with an electric heater and magnetic agitation. The temperature/speed controller is a combination of furnace power control and motor speed control with tachometer. Cooling water was circulated through a serpentine coil to cool the reactor content at the end of each run.

Olive tree pruning was pretreated at seven temperature levels in the range of 170–230°C for 10 min and for 60 min at five temperature levels ranging from 170 to 210°C. The amount of dry feedstock loaded was 200.0 g and water was added at 1/5 (w/v) solid/liquid ratio. Both water and raw material were initially at room temperature. Agitation was set at 350 rpm. The average heating rate was 3°C/min. Overpressure in the reactor of 3 MPa was kept to prevent formation of an aqueous vapor phase. Pretreatment time (10 or 60 min) was initiated when the selected pretreatment temperature was reached. After treatment, the reactor was removed from the heating jacket and cooling water was charged through the serpentine coil; furthermore, the reactor vessel was introduced in an ice bath. The content of the reactor cooled down to 80°C in approx 5 min. The reactor was kept sealed, and the slurry agitated until the reactor was cooled to about 40°C. Then the wet material was filtered for solid and liquid recovery. The water-insoluble fraction was washed out with water and analyzed for hemicellulosic sugars, glucose, and acid-insoluble lignin (AIL) content and used as substrate in enzymatic hydrolysis tests. Liquid fraction issued from pretreatment was analyzed for sugars, acetic acid, and sugar-degradation products.

Enzymatic Hydrolysis Tests

The washed water-insoluble residue of pretreated olive tree pruning was enzymatically hydrolyzed by a cellulolytic complex (Celluclast 1.5 L) kindly provided by Novozymes A/S (Denmark). Cellulase enzyme loading was 15 filter paper units/g substrate. Fungal β-glucosidase (Novozym 188, Novozymes A/S) was used to supplement the β-glucosidase activity with an enzyme loading of 15 international unit/g substrate. Enzymatic hydrolysis was performed in 0.05 *M* sodium citrate buffer (pH 4.8) at 50°C on a rotary shaker (Certomat-R, B-Braun, Germany) at 150 rpm for 72 h and at 5% (w/v) pretreated material concentration. Samples were taken every 24-h for glucose concentration determination. All enzymatic hydrolysis experiments were performed in duplicate and average results were given.

Analytical Methods

Composition of raw material was determined according to the National Renewable Energy Laboratory (Golden, CO) analytical methods

for biomass *(9)*. Before other determinations, raw material was extracted consecutively with water and with ethanol (two-step extraction procedure). After the first step, the sugar composition of the water-extract was determined by high-performance liquid chromatography (HPLC) in a Waters (Milford, MA) 2695 liquid chromatograph with refractive index detector. An AMINEX HPX-87P carbohydrate analysis column (Bio-Rad, Hercules, CA) operating at 85°C with ultrapure water as a mobile-phase (0.6 mL/min) was used. Free and oligomeric sugar composition were determined before and after a posthydrolysis process consisting in a treatment with sulfuric acid (3% [v/v]) at 121°C and 30 min. The cellulose and hemicellulose content of the extracted solid residue was determined based on monomer content measured after a two-step acid hydrolysis procedure to fractionate the fiber. A first step with 72% (w/w) H_2SO_4 at 30°C for 60 min was used. In the second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121°C for 1 h. This hydrolysis liquid was then analyzed for sugar content by HPLC with the Waters liquid chromatograph. The remaining acid-insoluble residue is considered as AIL.

Following LHW-pretreatment, the composition of solid fraction was determined as described for raw material except that no extraction is used. The sugar content (glucose, xylose, arabinose, mannose, and galactose) of the liquid fraction after pretreatment (prehydrolyzate) was determined by HPLC using the same liquid chromatograph. Furfural and hydroxymethylfurfural content was analyzed by HPLC in a 1100 HP (Hewlett Packard, Palo Alto, CA). Liquid chromatograph, equipped with a 1040A Ultraviolet-diode-array detector (Agilent Technologies, Waldbronn, Germany); the separation was performed with a Bio-Rad HPX-87H column, operating at 65°C with 89% H_2SO_4 (5 m*M*) and 11% acetonitrile as an eluent at a flow rate of 0.7 mL/min. Acetic, formic, and levulinic acids analysis was carried out with the HPLC system with a refractive index detector mentioned above with a Bio-Rad HPX-87H column at 65°C temperature. The mobile phase was 5 m*M* H_2SO_4, at a flow rate of 0.6 mL/min. Glucose concentration from enzymatic hydrolysis samples was measured by an enzymatic determination glucose assay kit (Sigma GAHK-20; Sigma Aldrich Corp., St. Louis, MO). All analytical determinations were performed in duplicate and average results were shown. Relative standard deviations in all cases were less than 5%.

Results and Discussion

Raw Material Composition

Table 1 summarizes the composition of olive tree pruning residues. Regarding extractives content, water extraction dissolved 27.5% of the raw material. The second extraction step (with ethanol) dissolved 3.9% of the material. Glucose as monomer in the water extract accounted for 3.2% and

Table 1
Raw Material Composition

Composition	Dry matter (%)
Extractives	31.4 ± 1.6
Glucose	7.9 ± 0.7
Cellulose as glucose	25.0 ± 1.2
Hemicellulosic sugars	15.8 ± 1.2
Xylose	11.1 ± 0.6
Mannose	0.8 ± 0.2
Galactose	1.5 ± 0.2
Arabinose	2.4 ± 0.2
Acid-insoluble lignin	16.6 ± 0.5
Acid-soluble lignin	2.2 ± 0.2
Acetyl groups	2.5 ± 0.1
Ash	3.4 ± 0.1

Mean values and standard deviations of five determinations.

the subsequent posthydrolysis process led to a total glucose content in extractives of 7.9%.

Cellulose (as glucose) and AIL content (25 and 16.6%, respectively) are smaller than that reported for other agricultural residues *(10)*. Considering acid-soluble lignin content, which refers to the small fraction of lignin that is solubilized during the hydrolysis process used to determine AIL, the total lignin value increases up to 18.8%. Hemicellulosic sugars account for 15.8% of raw material with xylose as the main sugar (70%). Acetyl groups, bound through an ester linkage to branched hemicellulose chains, represented about 2.5% of the initial raw material.

LHW Pretreatment

Table 2 shows the total gravimetric recovery (solids remaining after pretreatment divided by original oven-dried weight) and the composition of water-insoluble fiber resulting from LHW pretreatment at the different temperatures and times assayed. No influence of pretreatment temperature or time was detected on total material recovery. Around 45% of original material was solubilized as a consequence of pretreatment, except at the softest conditions assayed (170°C, 10 min) wherein the total gravimetric recovery was a little greater. As expected, the content in hemicellulosic sugar of pretreated material decreased as LHW temperature or time increased, vanishing from 220°C in 10 min runs or 200°C in 60 min experiments. Concomitantly, with hemicellulose content decrease, the content in cellulose of pretreated material increased, reaching a maximum of 45.6% at 200°C, and 10 min; beyond this point, a slight solubilization of cellulose was detected increasing in general with time and temperature.

Concerning AIL content, the maximum value of pretreated material should be around 30% (according to initial lignin content and material

Table 2
Composition (Dry Matter [%]) of Water-Insoluble Fiber Resulting From LHW Pretreatment at Different Conditions

Temperature (°C)	Time (min)	Total gravimetric recovery	Glucose	Xylose	Other sugars[a]	AIL
170	10	62.4	37.4	12.5	1.9	35.1
	60	55.6	42.2	4.6	0.8	45.4
180	10	56.8	40.5	7.2	1.5	42.7
	60	55.2	41.2	3.5	0.5	48.7
190	10	54.7	43.0	4.7	1.0	43.9
	60	57.4	41.9	0.9	0.3	49.7
200	10	54.9	45.6	2.8	0.3	46.1
	60	57.7	41.2	nd	nd	52.3
210	10	56.0	40.7	1.0	nd	49.4
	60	57.1	39.2	nd	nd	54.2
220	10	56.4	40.4	nd	nd	52.4
230	10	56.6	36.3	nd	nd	55.9

nd: not detected.
[a]Galactose, mannose, and arabinose.

recovery); in contrast, Table 2 shows increasing values of AIL as time or temperature of pretreatment increased. Although this fact is in agreement with hemicellulose solubilization, AIL content values are much greater than they should taking into account just hemicellulose solubilization. A similar result is reported by Ballesteros et al. *(11)* who obtained lignin recovery values that exceeded initial concentration in 20–32% when pretreating olive oil extraction residues, a material with a high extractive content. These results could be explained by the formation of "lignin-like" structures obtained as a result of condensation reactions between lignin and carbohydrate degradation products; interferences in the lignin analysis method from compounds present in the extractive fraction have also been reported in an attempt to explain these unusually high lignin contents *(12)*. In order to clarify this fact, an LHW pretreatment experiment was performed using an olive tree pruning residue lot, which had been previously extracted with ethanol for 24 h. Extraction resulted in the solubilization of about 22% of material (out of 31% present in raw material). When this extractive-free material was submitted to LHW pretreatment at 210°C and 10 min, AIL recovery was significantly lower than that of the unextracted material (22.5% AIL content referred to raw material) (27.6% AIL content referred to raw material), supporting the idea of high extractives content interfering the lignin analysis method. Nonetheless, AIL is still greater than the initial content, probably because of the presence of remaining extractives.

In the water-soluble fraction issued from pretreatment (filtrates), sugars were presented in a considerable proportion as oligomers, so that a posthydrolysis step was performed to determine the total amount of sugars.

Table 3
Composition (Raw Material g/100.0 g) of the Filtrate Resulting From LHW Pretreatment at Different Conditions

Pretreatment temperature (°C)	170		180		190		200		210		220	230
Pretreatment time (min)	10.0	60.0	10.0	60.0	10.0	60.0	10.0	60.0	10.0	60.0	10.0	10.0
Glucose	10.1	9.2	8.9	6.2	8.2	2.6	5.9	0.5	2.8	0.1	0.8	0.2
Xylose	4.0	7.0	6.3	3.5	6.6	0.6	4.1	0.0	0.7	0.0	0.0	0.0
Galactose	1.4	1.9	1.4	0.9	1.2	0.6	1.0	0.0	0.3	0.0	0.0	0.0
Arabinose	2.6	1.8	2.1	0.5	1.9	0.1	0.6	0.0	0.1	0.0	0.0	0.0
Mannose	0.2	0.8	0.3	0.4	0.5	0.2	0.7	0.1	0.2	0.0	0.0	0.0
Acetic acid	0.4	1.6	0.7	2.5	1.2	3.2	2.3	3.6	2.8	4.1	3.5	3.9
Formic acid	0.4	0.6	0.6	0.8	0.6	0.7	0.7	1.1	0.8	0.7	0.9	0.7
Levulinic acid	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0
Furfural	0.1	0.4	0.2	1.4	0.5	1.7	1.2	1.3	1.6	0.7	1.7	1.2
HMF	0.2	0.5	0.3	1.1	0.6	1.8	1.0	1.9	1.4	1.1	1.7	1.5
pH	3.8	3.8	3.7	3.7	3.5	3.7	3.7	3.4	3.5	3.3	3.3	3.4

The composition of filtrates after LHW pretreatment and posthydrolysis is shown in Table 3. It is worth noting that glucose is the most abundant sugar in the liquid fraction at any conditions; in fact, the highest glucose content (10.1 g/100.0 g raw material, equivalent to a concentration of 20.2 g/L) is found in the filtrate obtained from pretreated material at the softest conditions (170°C, 10 min). Considering that glucose was present at high proportion in the extract fraction of raw material (Table 1), it is likely that the most part of this component was transferred to liquid fraction after pretreatment. This result is in agreement with that reported by Ballesteros et al. *(11)* using olive oil extraction residues. Glucose content of filtrates decreased as a consequence of sugar degradation as either temperature or pretreatment time increased. In contrast, xylose content in the filtrates rose progressively with pretreatment temperature until 190°C (10-min experiments), reaching a maximum value of 13.3 g xylose/L or 6.6 g xylose/100.0 g raw material, and then a decrease was detected; a similar trend was found for the rest of hemicellulosic-derived sugars. Only at the lowest pretreatment temperature (170°C) the content in xylose increased when pretreatment time was changed from 10 to 60 min.

The sugar degradation process observed when the severity of pretreatment was increased is in agreement with the increasing proportion of non-sugar compounds found in the filtrates (Table 3). Acetic acid, furfural, hydroxymethylfurfural (HMF), formic, and levulinic acid are known to act as inhibitors for yeast growth under selected conditions *(13,14)*. For example, the filtrate content in furfural and HMF increased as pretreatment temperature increased, except at 230°C. For a given pretreatment temperature, increasing the process time from 10 to 60 min resulted in an increase of furfural and HMF contents, except at the highest pretreatment (210°C) with 60 min, which showed a decrease, probably owing to further transformation of furfural and HMF into other degradation products, as stated by other authors *(11)*. Regarding other products, organic acids (acetic and formic acid) were observed at all pretreatment conditions; levulinic acid was only detected at 60-min experiments for any pretreatment temperature (except at 170°C). As a whole, the content of filtrates in compounds other than sugars was similar to that reported in the LHW-pretreatment of other lignocellulosic residues as poplar *(15)*, although the proportion of HMF found in olive tree pruning hydrolyzates is somewhat higher, in agreement with a higher glucose composition in filtrates. Moreover, heat-up times (lasting from 40 to 60 min, depending on final process temperature) may also be responsible for the formation of degrading products, especially from extractive soluble glucose.

Table 3 shows also the pH value of hydrolyzates, ranging from 3.3 to 3.8. In general, a slight decrease of pH as a function of pretreatment temperature was detected. Figure 1 illustrates the sugar recovery yield (either referred to glucose or hemicellulosic sugars) in both the water-insoluble fibers and in the filtrates for all experiments performed. This yield is expressed as sugars in the water-insoluble fiber or in the filtrate (referred to

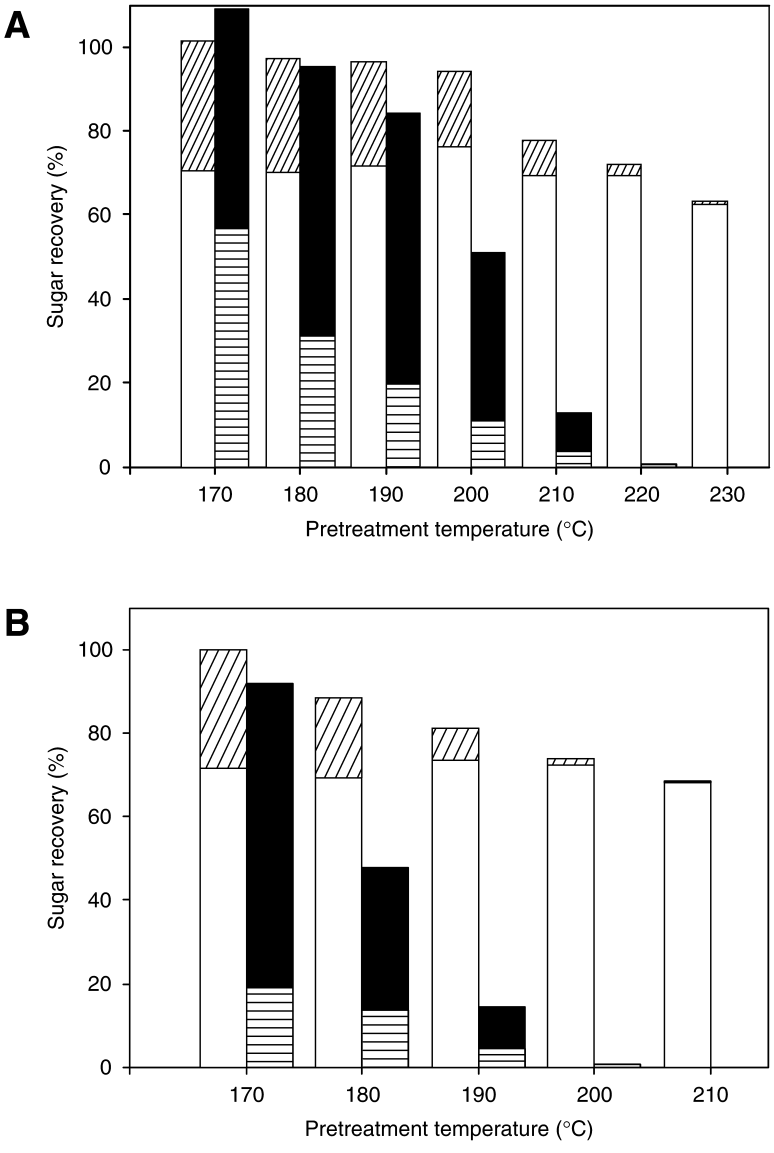

Fig. 1. Sugar recovery at different LHW temperatures, expressed as a percentage of the initial content in the raw material. Glucose recovery in solid (▫) and liquid (▨) fractions and hemicellulose-derived sugars recovery in solid (▤) and liquid (■) fractions, **(A)** pretreatment time 10 min and **(B)** pretreatment time 60 min.

original raw material) divided by potential sugars in the raw material. Potential glucose in the raw material (32.9 g/100.0 g, *see* Table 1) has been evaluated taking into account both glucose from cellulose (accounting for 76% of the total glucose) and glucose in the extractive fraction (24%). Glucose recovery yields ranging from 63 to 76% were obtained for pretreated material. However, if just the glucose from cellulose were considered as potential glucose, recovery yields above 90% would be obtained

(regardless of process time) except for the experiment performed at the highest pretreatment temperature (230°C) whereby 82% cellulose recovery yield in solid fraction was determined. This is attributable to cellulose solubilization at these conditions. Regarding the filtrates, glucose recovery yields as high as 30.6% (at the softest conditions) were obtained, owing to glucose from extractives fraction. The higher the pretreatment temperature or time the lower glucose recovery yield. Beyond a pretreatment temperature of 200°C, maintained for 60 min, there is scarcely any glucose left in the filtrates.

Concerning hemicellulose recovery yield in solids, it came down progressively as the pretreatment temperature increased; in the same way, keeping pretreatment temperature for 60 min resulted in a deep decrease of hemicellulose recovery yield, vanishing at a pretreatment temperature of 200°C or above. In the filtrates, a similar drop of hemicellulose recovery yield with pretreatment temperature or time is evidenced for long-time experiments, in agreement with results reported by Laser et al. *(4)*. In the case of 10-min trials, the hemicellulose recovery yield in the liquid fractions rose as a function of pretreatment temperature until 190°C and then it came down; this indicates that sugars released from lignocellulose structure are getting degraded, and hence, poor recoveries in the filtrates are obtained. A different process configuration that better preserves dissolved hemicellulosic sugars, for example, by removing them through continuous percolation, may improve recoveries *(16)*. Although total mass balance (sum of the solubilized and residual solid fractions) moved away from 100% with increasing both pretreatment temperature and time, the susceptibility of the recovered cellulose to enzymatic hydrolysis is also a key aspect in optimizing the overall process and ensuring maximum substrate utilization.

Enzymatic Hydrolysis

To evaluate pretreatment performance the water-insoluble residues were submitted to enzymatic hydrolysis using a cellulose complex (Celluclast 1.5L) supplemented with β-glucosidase (Novozyme 188) and 5% solids concentration. Enzymatic hydrolysis was monitored by sampling every 24 h for a 72-h period. Samples were analyzed for glucose concentration. Results are shown in Table 4. For comparison purposes, enzymatic hydrolysis was also conducted on untreated raw material, with and without enzyme addition. Even when no enzyme was added, 1.6 g glucose/L are solubilized at the hydrolysis conditions (50°C, 72 h), which corresponds to 9.6% of the total potential glucose or 3.2 g/100.0 g raw material, in accordance to the amount detected as free glucose in the first extraction step (*see* Raw Material Composition Section). This amount of glucose will not be available in water-insoluble residues because it is readily solubilized to liquid fractions during pretreatment. When untreated raw material was submitted to enzymatic hydrolysis at the same conditions, the concentration of glucose released after 72 h was greater than that obtained from pretreated material at 170°C for 10 min, because of the

Table 4
Glucose Concentrations (g/L) Obtained by Enzymatic Hydrolysis of Olive Tree Pruning Residues at Varying Pretreatment Conditions

Pretreatment conditions		Enzymatic hydrolysis time (h)		
Temperature (°C)	Time (min)	24	48	72
170	10	2.4	2.6	2.7
	60	4.3	5.5	6.6
180	10	3	3.2	3.4
	60	7	9.5	10.6
190	10	4.1	4.3	4.9
	60	8.4	11.6	12.7
200	10	6.6	8.2	9.3
	60	11.1	13.5	15.4
210	10	9.8	12.8	14.3
	60	10.1	13.7	15.1
220	10	11.7	14.1	15
230	10	11.6	13.5	14
Untreated, no enzyme		1.1	1.4	1.6
Untreated + enzyme		2.5	2.6	2.8

aforementioned free glucose present in untreated material. For this reason, comparisons based on enzymatic hydrolysis yields will be done taking into account the difference between glucose concentration obtained from untreated material with and without added enzyme.

Figure 2 shows the enzymatic hydrolysis yields determined from the glucose concentration values at each sampling time (Table 4) and the glucose potential content in the pretreated material (Table 2). As an average, 75% of the enzymatic hydrolysis yield (referred to the yield value at 72 h) was attained within the first 24 h of enzymatic attack. The cellulose digestibility increased as a function of pretreatment temperature and time. This seems to be related to the solubilization of hemicellulosic sugars from the solid (Fig. 1) *(17)*. In fact, the highest enzymatic hydrolysis yields (around 75%) were obtained using solids in which the hemicellulose fraction was completely solubilized, e.g., more than 220°C and 10 min (Fig. 1A) and more than 200°C for 60-min runs (Fig. 1B).

Comparing with untreated material, pretreatment temperatures below 190°C and short times (10 min, Fig. 2A) led to just a slight improvement on enzymatic cellulose accessibility. The pretreatment effect was evidenced from 200°C pretreatment temperature. Enzymatic hydrolysis yields greater than 70% (at 72 h hydrolysis time) were obtained from solids pretreated at 210°C or higher. For a given pretreatment temperature and pretreatment time lasting for 60 min, the enzymatic hydrolysis yields were always higher than those corresponding to short experiments, especially at low pretreatment temperatures. For example, a threefold higher yield was obtained at 180°C for 60 min comparing with 180°C for 10 min.

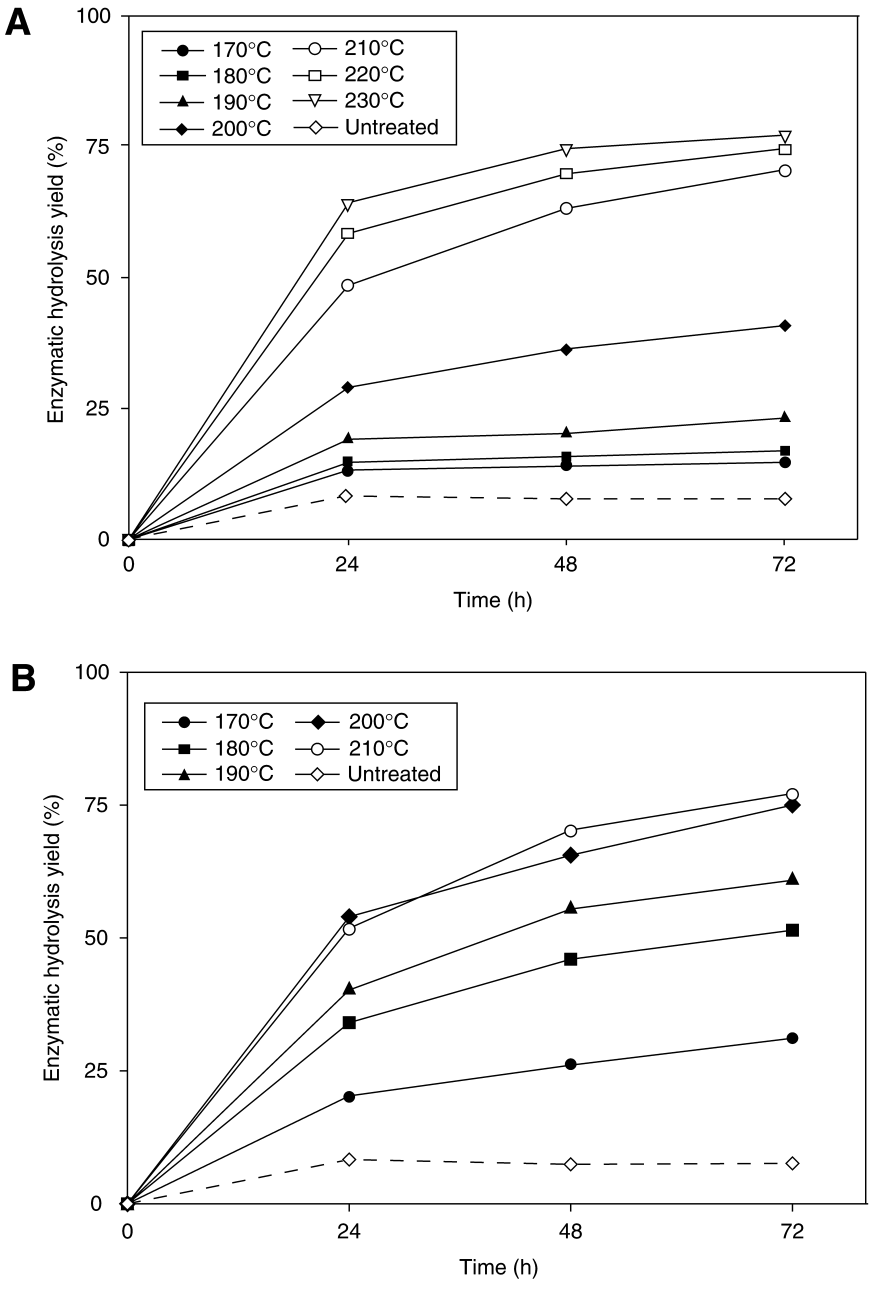

Fig. 2. Enzymatic hydrolysis yield expressed as glucose obtained in the enzymatic hydrolysis divided by the potential glucose in pretreated material (%). **(A)** pretreatment time 10 min and **(B)** pretreatment time 60 min.

Although enzymatic hydrolysis yields improved by 10-fold comparing the best results with untreated material, there is still some 20% of cellulose in pretreated materials that is not hydrolyzed after 72 h hydrolysis time. This fraction of unaltered cellulose did not significantly vary even in

7-d hydrolysis experiments. In an attempt to understand whether or not it is possible to attack this cellulose fraction (and to get further improvement on enzymatic yields), a new experimental series was performed by milling previously pretreated solids from 10-min runs until particle sizes decreased to below 1 mm. A slight improvement ranging from 6 to 11% in enzymatic hydrolysis yields was obtained for solids pretreated at low temperatures (170–200°C), whereas no increase on yields was detected when hydrolyzing milled solids pretreated above 210°C. Thus, it seems that there is a recalcitrant cellulose fraction that is not possible to hydrolyze under the assayed operational conditions, probably because of cellulose structural rearrangements that can occur during high-temperature pretreatments *(18)*. Further research on alternative pretreatments or enzymatic hydrolysis configurations should be necessary to improve both glucose yields and raw material utilization.

Overall Process Yield

In order to optimize the overall process, attention must be paid to several partial objectives, e.g., sugars recovery in the filtrate (readily solubilized glucose and hemicellulose-derived sugars), cellulose recovery in the solid residue, and enzymatic hydrolysis performance. Table 5 summarizes the main results achieved when pretreating olive tree pruning residues by LHW. Results are expressed as yields referred to 100.0 g of raw material. Data in liquid-column show yields in sugars (sum of glucose, xylose, mannose, arabinose, and galactose) recovered in filtrates; the highest values are obtained at soft pretreatment conditions (temperatures in the range 170–190°C for 10 min and 170°C for 60 min). It is worth noting that these yields are equivalent to sugars concentrations of around 40.0 g/L in solutions available for fermentation with relatively low concentration of inhibitors (Table 3). Glucose in solid-column in Table 5 includes glucose remaining in solids after pretreatment; significant cellulose losses occurs only at the highest temperature. The subsequent enzymatic hydrolysis performed on pretreated solids led to glucose yields shown in the next column in Table 5. These values take into account both saccharification performance and glucose recovery in pretreated solids (referred to raw material). In contrast to results of liquid-column, the highest glucose yields are obtained at harsh pretreatment conditions. Moreover, enzymatic hydrolysis yields increased as a function of pretreatment temperature and time (Fig. 2), whereas glucose yields in Table 5, which take into account also glucose losses in solids, show a decrease at the experiments performed at the highest pretreatment temperature (230°C, 10 min and 210°C, 60 min). Because of this, no higher values of pretreatment temperatures were assayed. This glucose, released by enzymatic action, is readily fermentable to ethanol, so based on this parameter, best results are obtained in the range of pretreatment temperature of 210–220°C for 10 min or 200–210°C for 60 min.

Table 5
Sugar Yields (Raw Material [g sugar/100.0 g]) From LHW-Pretreatment of Olive Tree Pruning Residues at Varying Pretreatment Conditions

Temperature (°C)	Time (min)	Liquid[a]	Glucose (in solid)[b]	Glucose from enzyme hydrolysis[c]	Overall glucose yield[d]	Overall sugar yield[e]
170	10	18.3	23.3	3.4	13.4	21.7
	60	20.7	23.5	7.3	16.5	28
180	10	19	23	3.8	12.7	22.8
	60	11.6	22.7	11.7	17.9	23.3
190	10	18.3	23.5	5.4	13.6	23.8
	60	4.1	24.1	14.6	17.2	18.7
200	10	12.2	25	10.2	16.1	22.4
	60	0.6	23.7	17.8	18.3	18.4
210	10	4.2	22.8	16.0	18.8	20.2
	60	0.1	22.4	17.2	17.3	17.3
220	10	0.9	22.8	16.9	17.8	17.9
230	10	0.2	20.6	15.8	16.1	16.1

[a]Total sugar yield (sum of glucose, xylose, arabinose, mannose, and galactose) in the liquid fraction.

[b]Glucose recovery in solid fractions referred to raw material. Cellulose (as glucose) content in raw material is 25.0 g/100.0 g.

[c]Glucose from 72-h enzymatic hydrolysis at 5% (w/v) pretreated material concentration.

[d]Sum of glucose from enzymatic hydrolysis and glucose in liquid fraction (total glucose content in raw material is 32.9 g/100.0 g).

[e]Sum of glucose from enzymatic hydrolysis and total sugars in liquid fraction (total sugar content in raw material is 48.6 g/100.0 g).

Values in overall glucose yield-column represent the sum of glucose obtained by enzymatic hydrolysis and glucose contained in liquid fractions. Finally, the overall sugar yield-column takes into account all sugars available, e.g., those coming from liquid fractions and glucose from enzymatic hydrolysis. Although relatively good results in terms of overall sugar yields are obtained at soft conditions (170°C for 60 min), the bioconversion of hemicellulose-derived sugars is more difficult as pentose-fermenting microorganisms are required *(19,20)*. Moreover, the pretreated solids would be used in a very limited extension.

To make use of most of the sugars in this raw material under the assayed conditions, some other process configurations might be considered. For example, a two-step process could be an interesting option. In the first one, conducted at low pretreatment temperature, hemicellulosic sugars, and the fraction of glucose easily solubilized would be recovered in the filtrate. The second step, at more severe conditions, would improve cellulose digestibility by enzymatic hydrolysis. Another alternative approach is the use of dilute acids in the pretreatment. Further research on these points is needed.

Conclusion

A characteristic feature of olive tree pruning residues is the presence of a readily soluble glucose proportion that accounts for up to 7.9% of raw material. When pretreating these residues by LHW this glucose will enter the liquid fraction together with hemicellulose sugars. Up to 57.6% of total sugars (most of them solubilized in the liquid fraction) in raw material may be available at 170°C pretreatment temperature for 60 min. Nevertheless, the resulting solid fraction offers poor digestibility to enzymatic hydrolysis. In contrast, 57.1% of total glucose, easier to convert into ethanol than a sugar mixture, is made available by pretreatment at 210°C for 10 min. The huge amount of olive tree pruning residues yearly generated, the need of disposal, their low cost, and the lack of economic alternatives make these residues deserve a deeper study for ethanol conversion.

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