ORGANOSOLV PRETREATMENT OF OLIVE TREE BIOMASS FOR FUEL AND CHEMICALS PRODUCTION

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ABSTRACT: Olive tree biomass is an agricultural, lignocellulosic, lacking of alternatives residue largely available especially in Mediterranean countries. The use of this biomass as raw material for producing ethanol and other chemicals has been proposed. The conversion process of the sugar components of any lignocellulosic material requires a pretreatment step in order to break down the fibrous structure, thus improving the access of enzymes to hydrolyse cellulose. In the organosolv pretreatment, a mixture of biomass, water and an organic solvent (e.g., ethanol) are thermochemically treated at typically 160-200°C. As a result, biomass is partially delignified, hemicelluloses are solubilised, and the enzymatic hydrolysis of the lignocellulosic resulting residue is enhanced. This work examines the influence of the main operation variables for organosolv pretreatment of olive tree biomass (i.e., pretreatment temperature, process time and the ratio ethanol/water). The pretreated residue was submitted to enzymatic hydrolysis using a commercial enzymatic complex. The pretreatment performance was calculated in terms of enzymatic hydrolysis yields, hemicellulose solubilisation and residual lignin content (delignification).
Keywords: agricultural residues, ethanol, olive tree, pretreatment

1 INTRODUCTION

Biomass has been recognized as one of the most promising renewable energy sources, which can contribute to alleviate global warming concerns and partially replace fossil fuels. Among the different types of biomass, agricultural residues benefit from the fact of needing to be eliminated from fields and, hence, their use as raw material for biofuel and other chemicals production means also an economic alternative to usual disposal methods.

Olive tree cultivation is becoming widely spread all over the world, mainly due to beneficial health effects of olive oil consumption. In Spain, land surface dedicated to olive tree cultivation is more than two million hectares of the more than eight million hectares worldwide. As an essential culture operation, olive tree pruning is performed every two years to eliminate old, unproductive branches and to prepare trees for the next crop. This action generates yearly between two and three tones of biomass per hectare, which must be eliminated from fields to prevent spreading of vegetal diseases. Until date, no economic application for this biomass has been found, and residues are usually burnt in the culture fields.

Alternatively, based upon the lignocellulosic composition of olive tree biomass, the production of ethanol by means of a biochemical process, including pretreatment, enzymatic hydrolysis and fermentation, has been proposed.

Pretreatment of olive tree biomass by steam explosion [1], liquid hot water [2] or dilute acid hydrolysis [3] has been reported. The pretreated residue has been enzymatically hydrolyzed [4] and fermented [5], while the liquid fractions issued from pretreatment can be directed also to ethanol production [6]. In addition, other ways for taking advantage of them have been reported, such as the presence of compounds exhibiting antioxidant properties [7]. The possibility of using biomass as raw material for producing a variety of compounds, including biofuels, antioxidants, food additives and others, represents an option termed as the 'biorefinery concept', in a similar way as many products can be derived from oil crude in a traditional refinery.

This work examines for the first time the use of organosolv pretreatment on olive tree biomass. In this procedure, a mixture of biomass, water and an organic solvent (e.g., ethanol) are thermochemically treated at typically 160-200°C [8-9]. As a result, biomass is partially delignified, hemicelluloses are solubilised, and the enzymatic hydrolysis of the lignocellulosic resulting residue is enhanced.

The influence of pretreatment temperature, process time and concentration of ethanol on enzymatic hydrolysis is studied.

2 MATERIALS AND METHODS

2.1 Raw material

Olive tree pruning residues, composed of thin branches (< 5 cm diameter) and leaves, were chopped, air-dried, milled to a particle size smaller than 10 mm, and stored until used. The moisture content of the raw material as received for the organosolv experiments was 7.5wt%.

2.2 Organosolv pretreatment

Lab scale experiments were performed in an autoclave reactor (0.5 L, kiloclave, Büchi Glas Uster AG). The experimental conditions applied are given in Table I. A suspension of biomass-water-ethanol (5ml solvent per 1g biomass) was heated to the reaction temperature, while being stirred. The suspension was kept at its set-point during the specified reaction time and subsequently cooled down.

After filtration of the resulting slurry, the solid residue was washed with ethanol-water, dried at 50°C in a vacuum oven and weighted to determine the solid recovery. From the filtrate and the washing solution, samples were taken for GC-MS analysis (e.g., furfural, HMF and acetic acid) and HPAEC-PAD analysis (monomeric sugars, Dionex ICS3000). The dissolved lignin in the remaining organosolv filtrate was separated by precipitation upon dilution with water (4°C, 3:1 ratio).

Table I: Experimental conditions of organosolv pretreatment

Exp.	Temperature, ºC	Time, min	Ethanol, % (w/w)
A	190	15	71.4
B	190	60	71.4
C	200	15	71.4
D	190	15	48.6
E	200	60	71.4

2.3 Biomass composition

Composition of raw material was determined according to the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass [10]. Prior to 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 HPLC in a Varian Prostar liquid chromatograph, equipped with refractive index detector. An AMINEX HPX-87P carbohydrate analysis column (Bio-Rad, Hercules, CA) operating at 85 ºC with ultrapure water as a mobile-phase (0.6 mL/min) was used. Free and oligomeric sugar compositions were determined before and after a posthydrolysis process consisting in a treatment with sulphuric acid (3% v/v) at 121 ºC 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 ºC for 60 minutes was used. In a second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121 ºC for 1 hour. This hydrolysis liquid was then analyzed for sugar content by HPLC as described above. The remaining acid- insoluble residue is considered as acid-insoluble lignin (AIL).

Following pretreatment, the composition of solid fraction was determined as described for raw material except that no extraction was used.

2.4 Enzymatic hydrolysis

The water insoluble, washed pretreated material was further subjected to enzymatic hydrolysis by a commercial enzyme complex (Celluclast 1.5L), kindly provided by Novozymes. Cellulase enzyme loading was 15 Filter Paper Units (FPU)/g substrate. Fungal ß-glucosidase (Novozym 188, Novozymes A/S), at an enzyme loading of 15 International Unit (IU)/g substrate, was added to avoid end-product inhibition. Enzymatic hydrolysis was performed in 0.05 M sodium citrate buffer (pH 4.8) at 50 ºC on a rotary shaker at 150 rpm for 72 h and at 5% (w/v) pretreated material concentration. Samples were taken every 24 h for sugar concentration determination. Enzymatic hydrolysis experiments were performed in triplicate and mean results are reported.

3 RESULTS AND DISCUSSION

3.1 Raw material composition

The composition of the raw material was (% w/w, dry basis): glucan, 22.7; xylan, 9.8; arabinan, 2.1; galactan, 1.4; lignin, 18.8; acetyl groups, 2.5; ash, 3.4; extractives, 31.4. It is worth noting that extractive content includes 7.9% of non structural glucose, with 40% in monomeric form. This unusually high extractives content has been taken into account for yields evaluation.

3.2 Pretreated materials

The composition of pretreated materials together with the solid recovery is shown in Table 2. As expected, solid recovery decreased as the pretreatment severity increased; experiments B and C gave similar results, so 190ºC for 60 min produces almost the same effect in terms of solid recovery than 200ºC for 15 min.

Pretreatment resulted in a glucan-concentrated material at any pretreatment conditions. This fact can be attributable to some extractive dissolution, as well as partial solubilization of the hemicellulosic fraction and delignification.

Table II: Solid recovery (% dw based) and composition of organosolv pretreated materials (% dry matter)

Exp.	Solid recovery	Glucan	Xylan	Lignin
A	61.7	46.9	16.8	20.7
B	53.3	49.1	14.6	19.7
C	54.3	54.2	15.3	18.7
D	48.0	50.5	11.3	24.4
E	42.9	55.0	12.9	19.4

For a better understanding of the organosolv pretreatment, Figures 1 and 2 show the delignification that took place in the pretreated materials and the hydrolysis of glucan and xylan as a consequence of the pretreatment.

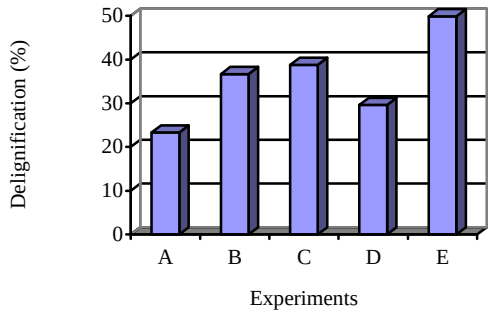

Figure 1: Delignification of pretreated materials

The pretreatment resulted in a variable delignification degree, depending on operational conditions, ranging from 23.3 to 49.9% of the original lignin content. It seems clear that the use of ethanol as a solvent produces lignin solubilization, in contrast with other results reported on different pretreatment methods and the same raw material. For example, steam explosion pretreatment on olive tree biomass resulted in a pretreated material with higher lignin content than that of the original lignocellulosic residue, which was attributed to condensation reactions between extractives and carbohydrates [3]; similar results were obtained when using liquid hot water as a pretreatment on the same raw material [2].

Concerning sugar hydrolysis, Figure 2 shows that at the lowest pretreatment conditions (experiment A, 190ºC for 15 min), xylan is practically unaltered, while just 3.4% of glucan is hydrolysed; moreover, it is likely that this glucan solubilisation comes from the extractive glucan content in the raw material. Hemicellulose solubilization increased as severity did and more than 40% xylan hydrolysis is detected in experiments D and E.

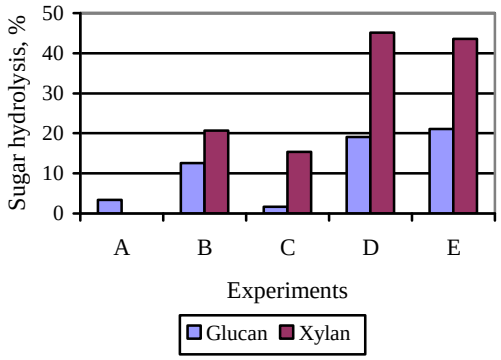

Figure 2: Glucan and xylan hydrolysis

Comparison of experiments A and D, differing only in the proportion of ethanol used as a solvent, shows that increasing water ratio in the solvent mixture results in a deeper hemicelluloses solubilisation, as was also observed for other types of biomass [9]. This result also agrees with those found when using other pretreatment methods on the same raw material. For example, liquid hot water pretreated materials at 190ºC for 10 min produced 64% hemicellulose hydrolysis [2] and almost total dissolution when the raw material was dilute acid pretreated with 0.6% sulfuric acid at the same temperature [3]. In addition, a lower ethanol concentration in the solvent mixtures resulted in a higher hemicellulose hydrolysis; as a consequence, the concentration of sugar degradation products such as furfural and HMF was also higher than that obtained in experiments performed at increasing ethanol proportion (data not shown).

3.3 Enzymatic hydrolysis

Figure 1 shows the concentration of glucose (g/L) obtained by enzymatic hydrolysis as a function of time at all assayed pretreatment conditions. Most of glucose is released in the first 24 h of enzymatic attack; in experiment A, corresponding to the lowest severe conditions (190ºC, 15 min), 88% of the glucose obtained after 72 h is already released in the first 24 h period, while these percentages are 75-82% for the rest of experiments. This result is in accordance with that obtained when olive tree biomass was pretreated by other pretreatment methods, as steam explosion [1], with more than 75% of the glucose released in the first 24 h of enzymatic hydrolysis.

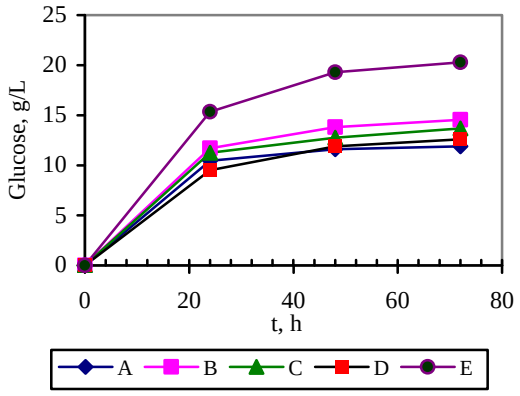

Figure 3: Glucose concentration as a function of enzymatic hydrolysis time.

As far as glucose concentration is concerned, it can be clearly deduced from Fig. 3 that experiment E, performed at the most severe conditions, produced the pretreated residue most suitable for enzymatic hydrolysis, with a glucose concentration of 20 g/L at the end of the process.

The analysis of the enzymatic hydrolysis broth evidenced that the enzyme complex exhibited also xylan hydrolysis capacity, which resulted in sugar solutions containing xylose in a range 2.50-5.39 g/L as a function of pretreatment conditions (Fig. 4). Following a similar pattern to that described for glucose, most of the xylose was also released in the first 24 h of enzymatic hydrolysis. Referred to xylan content in the raw material, the above mentioned xylose concentrations represent an enzymatic xylose yield varying from 32.1 to 43.2%; the lowest value corresponds to experiment D, that performed at the lowest ethanol proportion, where the xylan hydrolysis was found to be the highest one (Fig. 2).

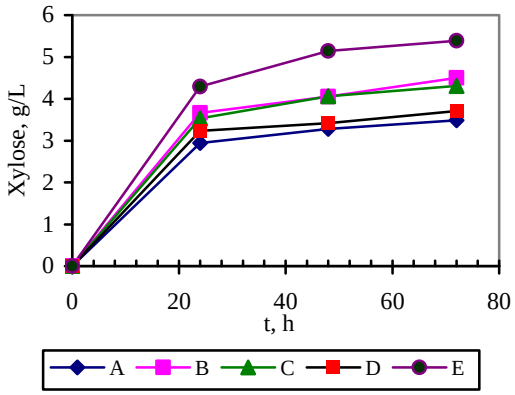

Figure 4: Xylose concentration as a function of enzymatic hydrolysis time

Enzymatic hydrolysis yields were evaluated as the percentage of glucose released referred to the glucose content in either the pretreated or raw material.

The highest yields were obtained for experiment E and account for 67% of the glucose present in the pretreated material or 53% of that in the raw biomass (Fig. 5). The enzymatic hydrolysis yields can probably be further improved by increasing the severity of the pretreatment (e.g., higher temperature or the addition an acid catalyst [9]). This will be subject of further study.

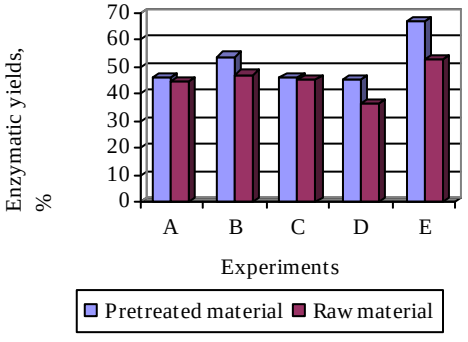

Figure 5: Glucose enzymatic hydrolysis yields referred to either raw or pretreated materials at different pretreatment conditions (see Table 1 for details).

3.4 Effect of pretreatment temperature and process time on enzymatic hydrolysis yields.

Comparisons on data obtained from Figure 5 show that pretreatment temperature and process time exert a different influence on enzymatic hydrolysis yields. As can be observed in Figure 6, which displays yields after 72 h enzymatic hydrolysis for experiments A, B, C and E, pretreatment time has a greater impact on yields than temperature; for example, if experiments A and C are compared, increasing pretreatment temperature from 190 to 200ºC results in 1.1% improvement of enzymatic hydrolysis yields (from 44.7% to 45.2%). Moreover, the greater effect of time depends also on the level of the temperature; at the lower level (190ºC), extending the pretreatment time from 15 to 60 min resulted in 5.4% increase of enzymatic yields; referred to the higher level of temperature (200ºC), the gain increased to 17%.

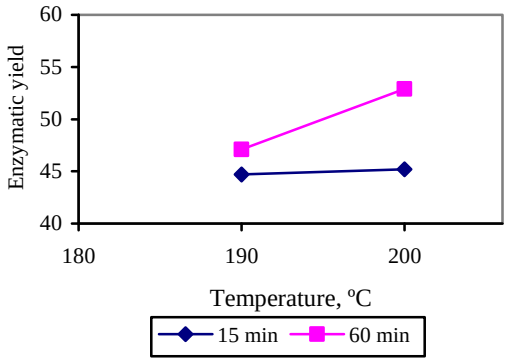

Figure 6: Effect of pretreatment variables on enzymatic hydrolysis yields

Experiments A and D can be used to compare the effect of ethanol proportion as pretreatment solvent. In terms of enzymatic hydrolysis yields, referred to pretreated material, little influence of this variable can be observed. If enzymatic yield are referred to raw biomass, taking into account also the glucose content in the extractive fraction, increasing the proportion of ethanol in the solvent mixture resulted in yield improvement by 22% after 72 h enzymatic hydrolysis.

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7 ACKNOWLEDGEMENTS

Financial support from Spanish Ministerio de Ciencia e Innovación (Project ENE2008-06634-C02-02/ALT) including FEDER-funds is gratefully acknowledged.