



Energy research Centre of the Netherlands

Organosolv pretreatment of olive tree biomass for fermentable sugars

M.J. Díaz¹
W.J.J. Huijgen²
R.R. van der Laan²
J.H. Reith²
C. Cara¹
E. Castro¹

¹Department of Chemical, Environmental and Materials Engineering, Campus Las Lagunillas,
University of Jaén, Jaén, Spain

²Energy Research Centre of the Netherlands (ECN), Biomass, Coal and Environmental Research
Unit, Petten, The Netherlands

Published in Holzforschung, Vol. 65, pp. 177–183, 2011

Organosolv pretreatment of olive tree biomass for fermentable sugars

Manuel J. Díaz¹, Wouter J.J. Huijgen², Ron R. van der Laan², Johannes H. Reith², Cristóbal Cara¹ and Eulogio Castro^{1,*}

¹ Department of Chemical, Environmental and Materials Engineering, Campus Las Lagunillas, University of Jaén, Jaén, Spain

² Energy Research Centre of the Netherlands (ECN), Biomass, Coal and Environmental Research Unit, Petten, The Netherlands

*Corresponding author.

Department of Chemical, Environmental and Materials Engineering, Campus Las Lagunillas, University of Jaén, 23071 Jaén, Spain
Phone: +34-95-3212163
E-mail: ecastro@ujaen.es

Abstract

Olive tree pruning biomass is one of the main agricultural residues available in Mediterranean countries and is currently lacking commercial applications. To take advantage of its sugar content, a pretreatment is necessary to enhance enzyme accessibility of the cellulose fraction of the residue. This paper describes for the first time the use of organosolv pretreatment in this regard. The main process variables such as pretreatment temperature, residence time, and solvent composition (aqueous ethanol) are studied. Results show that organosolv pretreatment causes delignification and hydrolysis of hemicelluloses and improves the enzymatic digestibility of olive tree pruning biomass. A higher pretreatment severity and ethanol content of the solvent were found to increase delignification (up to 64% at 66% w/w aqueous ethanol, 210°C, 60 min). By contrast, xylan hydrolysis was promoted by a lower ethanol content (maximum 92%). The highest enzymatic hydrolysis yield (90% of the structural glucan present in the raw material) has been obtained after pretreatment with 43% w/w aqueous ethanol at 210°C for 15 min. Organosolv pretreatment was found to be the most effective pretreatment for enzymatic hydrolysis of olive tree pruning biomass.

Keywords: enzymatic hydrolysis; fermentable sugars; olive tree pruning biomass; organosolv pretreatment.

Introduction

Agricultural residues have been identified as one of the main renewable resources for fermentable sugars for production of ethanol and other chemicals. Especially in Mediterranean countries, olive tree biomass obtained from pruning is a

major agricultural residue which is thus far lacking practical economic use. It has been estimated that each hectare of olive trees generates two to three tonnes of biomass per year, which must be removed from the fields to prevent spreading of vegetal diseases (Díaz et al. 2009). The standard practice to date, direct burning on the fields, causes economic and environmental concerns.

The production of bioethanol by means of a biochemical process consisting of pretreatment, enzymatic hydrolysis, and fermentation has been proposed. Pretreatment of olive tree pruning biomass by hot water (Cara et al. 2007), dilute acid hydrolysis (Cara et al. 2008a), and steam explosion (Cara et al. 2006, 2008b) has been reported. Following pretreatment, the cellulose fraction of the biomass is enzymatically hydrolysed to glucose. The different pretreatments studied thus far have always left at least approximately 30% of structural glucan in the biomass inaccessible to hydrolytic enzymes (Cara et al. 2007, 2008a,b). Thus, the high potential for ethanol production from this low cost, renewable, and abundant biomass residue is lowered.

It has been reported that the enzymatic digestibility of pretreated biomass can be improved by (partially) removing lignin and hemicelluloses (Hendriks and Zeeman 2009; Kumar et al. 2009). These polymers are linked to the cellulose and hinder its accessibility by enzymes. The organosolv process is a pretreatment that is capable of removing lignin from biomass (Kleinert and Tayenthal 1931; Zhao et al. 2009). In this procedure, biomass is heated in a mixture of water and an organic solvent such as ethanol. As a result, the biomass is partially delignified, hemicelluloses are hydrolysed, and the enzymatic hydrolysis of the lignocellulosic residue is enhanced (Kumar et al. 2009; Zhao et al. 2009). This pretreatment has been applied to various types of biomass including poplar wood (Pan et al. 2006), pine wood (Pan et al. 2005), willow wood (Huijgen et al. 2008), and straw (Huijgen et al. 2010). Organosolv pulping has also been applied previously on olive tree trimmings, but with the aim to produce pulp for paper production (Jiménez et al. 2001). Organosolv pretreatment is more expensive at present than the leading pretreatment processes such as steam explosion and (dilute) acid hydrolysis, but it can provide valuable byproducts including pure lignin (Zhao et al. 2009; Mesa et al. 2010).

In this study, organosolv pretreatment of olive tree pruning biomass for the production of fermentable sugars is examined for the first time. The influence of pretreatment temperature, residence time, and solvent composition (aqueous ethanol) on fractionation and enzymatic hydrolysis of the biomass was studied. Finally, results are compared to those reported earlier for other pretreatments on the same feedstock.

Materials and methods

Olive tree pruning biomass

Olive tree pruning biomass (OTPB), composed of thin branches (<5 cm diameter) and leaves, was chopped, air-dried, milled to a particle size <10 mm, and stored until use. The same feedstock batch was used in previous studies for other pretreatments (Cara et al. 2007, 2008a,b). The average moisture content of the raw material before organosolv pretreatment was 7.5%.

The composition of OTPB is: glucan, 22.5%; xylan, 9.8%; arabinan, 2.1%; galactan, 1.4%; mannan, 0.7%; acid insoluble lignin, 16.6%; acetyl groups, 2.5%; ash, 3.4%; and extractives, 31.4% (average of five determinations, Cara et al. 2008a). Unless specified otherwise, all % data in this paper are % w/w on dry basis. It is worth noting that the extractives fraction includes 7.9% of non-structural glucose, of which 40% is in monomeric form (Cara et al. 2008a). Unless specified otherwise, the total glucan content (i.e., 29.6%) was the basis for yields evaluation.

Organosolv pretreatment

Organosolv pretreatment was performed in a stirred autoclave reactor (0.5 l Kiloclave, Büchi Glas Uster AG, Uster, Switzerland). The experimental conditions are given in Table 1. Unless specified otherwise, all concentration data of ethanol (EtOH) are given in % w/w. A suspension of OTPB, water, and EtOH (5 ml solvent per 1 g biomass as received) was heated to the reaction temperature, kept at its set-point during the specified reaction time, and subsequently cooled down to below 40°C. The heating time of the reactor was between 44 min (exp. 1) and 50 min (exp. 10) (from 30°C to set-point of reactor). The cooling time was between 32 min (exp. 1) and 39 min (exp. 10) (from set-point of reactor to 40°C). For a typical heating and cooling curve of the autoclave reactor, see Huijgen et al. (2010). After filtration of the resulting slurry, the solid residue was washed, dried at 50°C in a vacuum oven, and weighed to determine the solid recovery ('pulp yield'). Washing was performed at room temperature with a mixture of EtOH-water in sim-

ilar amount and with the same EtOH-water ratio as the solvent applied for the organosolv process itself. From the filtrate (the 'organosolv liquor'), samples were taken for determination of monomeric sugars by HPAEC-PAD analysis (Dionex, ICS3000, Sunnyvale, CA, USA) and other organics, including furfural, hydroxymethylfurfural (HMF), and acetic acid by GC-MS analysis (Thermo Scientific, TRACE GC Ultra DSQII, Waltham, MA, USA). In addition, HPAEC-PAD analysis was performed after post-hydrolysis to determine oligomeric sugars by difference (post-hydrolysis conditions: 3% v/v sulphuric acid, 121°C, 30 min). The dissolved lignin in the organosolv liquor was separated by precipitation upon dilution with water (4°C, H₂O:organosolv liquor = 3:1 w/w). For more details, see Huijgen et al. (2010).

Composition of pretreated material

The composition of the organosolv pretreated materials was determined by a two-step acid hydrolysis procedure (1. 72% H₂SO₄ at 30°C for 60 min; 2. in 4% H₂SO₄ autoclaved at 121°C for 1 h) in triplicate according to the National Renewable Energy Laboratory (1994–1998). The analysis was limited to xylan, glucan, and acid-insoluble lignin (AIL) as representatives of hemicelluloses, cellulose, and lignin, respectively. The monomeric sugars in the hydrolysate were determined by HPLC in a Varian Prostar liquid chromatograph, equipped with an RI detector. HPLC column: AMINEX HPX-87P (Bio-Rad, Hercules, CA, USA). Conditions: 85°C, ultrapure water as mobile phase (0.6 ml min⁻¹).

Enzymatic hydrolysis

Enzymatic hydrolysis was performed (in triplicate and mean results are reported) with a commercial enzyme complex (Celluclast 1.5 l), kindly provided by Novozymes. Loading: 15 Filter Paper Units (FPU)/g substrate. Fungal β -glucosidase (Novozym 188, Novozymes A/S, Kalundborg, Denmark) was added at 15 IU/g substrate to avoid end-product inhibition. Other conditions: 0.05 M sodium citrate buffer (pH 4.8) at 50°C in a rotary shaker at 150 rpm for 72 h and at 5% w/v pretreated material concentration. Sugar con-

Table 1 Solid recovery, composition of pretreated solid material, recovery components in pretreated solid, and 'lignin' precipitate yield as a function of organosolv pretreatment conditions.

Exp.	Pretreatment conditions			Yield of solid material after pretreatment (%) ^b	Composition of pretreated solid material			Recovery component in pretreated solid			'Lignin' precipitate (%) ^{b,d}
	Temp. (°C)	Time (min)	EtOH (%) ^a		Glucan (%)	Xylan (%)	Lignin ^c (%)	Glucan (%)	Xylan (%)	Lignin (%)	
OTPB ^c					29.6	9.8	16.6				
1	190	15	66	61.7	46.4	16.8	20.7	96.6	105.3	76.7	8.9
2	190	60	66	53.3	48.7	14.6	19.7	87.4	79.3	63.3	13.3
3	190	15	43	48.0	50.0	11.3	24.4	80.9	54.9	70.4	10.3
4	200	15	66	54.3	53.7	15.3	18.7	98.3	84.6	61.2	13.1
5	200	60	66	42.9	54.5	12.9	19.4	78.9	56.4	50.1	17.0
6	200	15	43	40.9	54.0	7.9	26.4	74.5	33.0	65.1	13.0
7	210	15	66	46.4	54.5	13.4	17.6	85.3	63.5	49.3	15.4
8	210	60	66	35.9	57.5	9.5	16.8	69.5	34.6	36.3	20.0
9	210	15	43	39.6	45.5	4.2	34.2	60.9	16.9	81.7	13.7
10	210	60	43	39.0	42.4	2.2	41.3	55.7	8.5	97.1	14.6

^aAqueous ethanol concentrations of 43% and 66% w/w correspond to 49% and 71% v/v, respectively.

^bThe reproducibility was estimated to be $\pm \sim 0.5\%$ based on experience with other feedstocks.

^cAcid insoluble lignin.

^dBased on dry raw material.

^eOTPB, olive tree pruning biomass.

centration was determined every 24 h by HPLC (see above). The maximum glucose concentration obtained for untreated OTPB was 2.8 g l^{-1} (after 72 h enzymatic hydrolysis, at identical enzyme dosage) (Cara et al. 2007).

Results and discussion

Yield and composition of organosolv pretreated materials

The solid recovery of the OTPB after organosolv treatment and its composition are presented in Table 1. The yield of OTPB after pretreatment ranged from 62% to 36% and decreased, as expected, with increasing pretreatment severity. Severity is expressed as the combined effect of both temperature and residence time (Heitz et al. 1994); for example, exp. 2 (190°C for 60 min) and exp. 4 (200°C for 15 min) gave similar results in terms of solid recovery. Even at the mildest pretreatment conditions, almost 40% of the raw material is dissolved, probably due to the high extractives content of OTPB.

Organosolv pretreatment resulted in a glucan-enriched material at all conditions (Table 1). This effect can be attributed to partial dissolution of the extractives, soluble ash fractions, and other non-structural components, as well as partial hydrolysis of the hemicelluloses and delignification.

The effect of the pretreatment temperature on solids composition can be discussed on the basis of exp. 1, 4, and 7, performed at 190°C, 200°C, and 210°C, respectively (15 min, 66% EtOH). A temperature increase resulted in a higher glucan content of the pretreated material, whereas the xylan and lignin contents decreased (Table 1). Similar effects were observed at longer pretreatment times (60 min, 66% EtOH) (exp. 2, 5, and 8). However, for the lower EtOH concentration series (43% EtOH, 15 min; exp. 3, 6, and 9), the lignin content increased with temperature (xylan content decreased as in other series).

The influence of pretreatment time can be illustrated by comparison of exp. 1 and 2, 4 and 5, or 7 and 8 (66% EtOH). Longer pretreatment times led to higher glucan and lower xylan and lignin contents. By contrast, exp. 9 and 10 show that for 43% EtOH, the lignin content increased with time.

Finally, for all experiments performed with 43% EtOH, the xylan content is lower than for the corresponding experiments with 66% EtOH. In other words, lowering the EtOH concentration results in a better hemicelluloses solubilisation, as was also observed for other types of biomass (Huijgen et al. 2008). At the same time, the lignin content of the pretreated material increased. Apparently, a lower EtOH concentration leads to lower delignification, which will be discussed in more detail in the following section.

Fractionation efficiency

The yields of lignin, xylan, and glucan in the organosolv pretreated material are given in Table 1. The fractionation efficiencies (i.e., delignification, xylan hydrolysis, and glucan recovery) are presented in Figure 1 as a function of pretreatment conditions.

The delignification degree ranged from 3% to 64% of the original lignin, depending on operational conditions. It seems clear that the use of EtOH as a solvent leads to lignin solubilisation, in contrast to results reported on other pretreatment methods applied on OTPB. For example, steam explosion pretreatment on OTPB led to apparently higher 'lignin' content (i.e., acid insoluble material) than present in the original OTPB. This seems to be caused by condensation reactions between extractives and carbohydrates (Cara et al. 2006). Similar results were obtained with hot water pretreatment on OTPB biomass (Cara et al. 2007). From this point of view, organosolv pretreatment is more effective.

Except for exp. 1 (190°C, 15 min), all experiments with 66% EtOH resulted in higher delignification than the experiments with 43% EtOH (Figure 1a). In addition, delignification improved with pretreatment severity when 66% EtOH was used. With 43% EtOH, delignification slightly increases by elevating the temperature from 190°C to 200°C (15 min). However, a further temperature increment to 210°C (and extension of pretreatment time from 15 to 60 min at 210°C) is detrimental (Table 1). This effect is probably due to the formation of condensation products between lignin and, for example, carbohydrates (Cara et al. 2006) or furfural (Huijgen et al. 2010). It might well be that lignin is first extracted during the organosolv process, reacts with carbohydrates (derivatives), and subsequently reprecipitates on the pretreated material (e.g., during cooling of the reactor) (Xu et al. 2007).

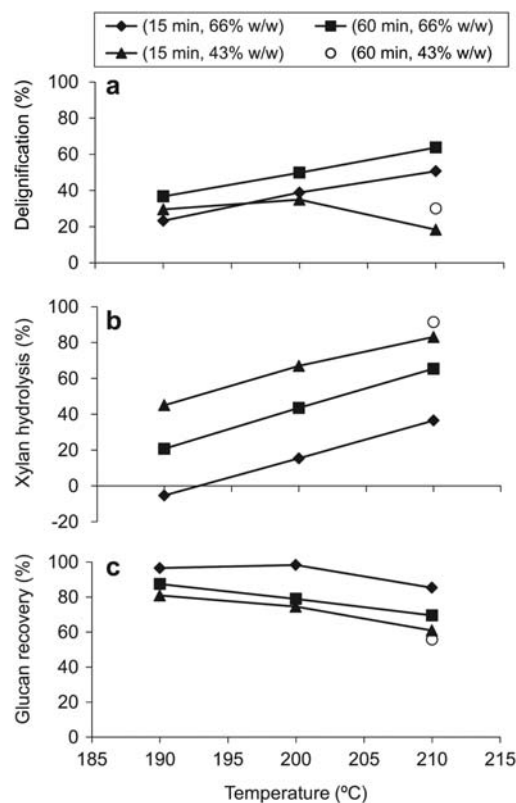


Figure 1 Fractionation efficiency as function of organosolv process conditions.

The dissolved lignin extracted from the biomass was separated from the filtrate by precipitation upon dilution with water. The amount of precipitate (Table 1) shows a similar dependency on the process conditions as the delignification. However, the amount of precipitate is typically twice as large as the delignification degree as illustrated in Figure 1a. Accordingly, the precipitated product contains only approximately 50% lignin (the precipitate was not characterised). Extractives from bark and wood, proteins from leaves and thin twigs, and their humification products could explain this observation. Typically, the purity of the lignin isolated by organosolv processes from lignocellulosic biomass is high, for example, a purity of 96.5% was reported for Alcell™ lignin (Gosselink et al. 2004). The precipitate obtained from OTPB was very dark compared to organosolv lignins from other types of wood. For exp. 9 and 10 (43% EtOH, 210°C), an even higher ratio between 'lignin' precipitate and delignification based on AIL content pretreated material was found supporting the hypothesis of formation of pseudo-lignin at these conditions.

Concerning hydrolysis of (hemi)cellulose(s), it can be deduced that at the mildest pretreatment conditions (exp. 1, 190°C for 15 min, 66% EtOH) xylan is practically unaltered, whereas just 3% of glucan is hydrolysed (Table 1). It is probable that this glucan has its origin in non-structural sugars. Both xylan and glucan hydrolysis increased with pretreatment severity and a decrease of the EtOH content (Figure 1b,c). At the most severe conditions with 43% EtOH (exp. 10), 44% total glucan (or 27% structural glucan) and 92% xylan hydrolysis occurred. Previously, similar observations were made: hot water pretreatment at 190°C for 10 min resulted in 64% hemicellulose hydrolysis (Cara et al. 2007), whereas only 49% hydrolysis was observed at 180°C.

Hydrolysate composition

Table 2 summarises the pH and composition of the organosolv liquors. The sugars were mainly present in oligomeric form, similar to what has been reported for liquid fractions derived from hydrothermal pretreatment of biomass (Caparós et al. 2008; Ruiz et al. 2008). The largest amount of oligomeric sugars was found in exp. 3, in which the lowest EtOH proportion in the organosolv mixture and the mildest pretreatment conditions were applied. Solubilisation of hemicelluloses is promoted by the presence of water, not only because it is required for the hydrolysis reaction but also as it is the carrier of protons (resulting mainly from acetic acid formation) needed for acid hydrolysis. The pH of the organosolv liquor is lower for exp. 3 than for the other experiments performed at 190°C with 66% EtOH (exp. 1 and 2), although the concentration of acetic acid is not the highest. Similar effects have been obtained for the experiments at 200°C and 210°C (exp. 6 and 9, Table 1). Clearly, the formation of acetic acid catalyses the organosolv process more strongly when a higher proportion of water is present. This effect also explains the higher hydrolysis degree of hemicelluloses with 43% EtOH.

At 66% EtOH concentration, the total concentration of sugars shows an increase with pretreatment severity at low

Table 2 Composition of organosolv liquors.

Exp.	Pretreatment conditions			Composition (g kg liquor ⁻¹)									
	Temp. (°C)	Time (min)	EtOH (%)	pH	Monomeric sugars		Oligomeric sugars		Sugar derivatives			Organic acids	
					Glucose	Xylose	Glucose	Xylose	Furfural	HMF	Levulinic acid	Acetic acid	Formic acid
1	190	15	66	4.9	0.94	0.02	1.54	0.99	0.20	0.21	<DTL ^a	2.23	0.78
2	190	60	66	4.8	0.21	0.06	2.35	1.53	0.48	0.30	<DTL	3.78	1.26
3	190	15	43	4.4	0.13	0.20	8.45	6.21	0.60	0.47	<DTL	2.56	1.12
4	200	15	66	4.8	0.29	0.05	1.83	1.66	0.33	0.25	<DTL	3.28	1.05
5	200	60	66	4.7	0.13	0.06	3.37	1.35	0.94	0.42	<DTL	4.72	1.55
6	200	15	43	4.2	0.65	0.46	7.64	3.71	1.42	0.91	<DTL	4.46	1.45
7	210	15	66	4.7	0.21	0.08	1.02	0.53	0.80	0.39	<DTL	4.73	1.48
8	210	60	66	4.6	0.12	0.04	3.45	0.96	0.88	0.48	0.12	5.21	1.83
9	210	15	43	4.1	0.68	0.24	4.09	1.02	1.76	1.37	<DTL	5.59	2.00
10	210	60	43	4.2	0.11	0.02	0.97	0.09	1.32	1.27	0.17	5.74	1.65

^a<DTL, below detection limit.

severity level (exp. 4 vs. 1 and 5 vs. 2) due to increased hemicellulose hydrolysis. However, the concentration of oligomeric xylose starts to decrease in the case of exp. 5 and this decrease is continued at 210°C. Apparently, the subsequent reaction of xylose into furfural becomes dominant over the formation of xylose oligomers by hemicellulose hydrolysis. The yields of sugar derivatives and the organic acids in the organosolv liquor increased with pretreatment severity and water proportion of the organosolv solvent. A detectable concentration of levulinic acid was measured only at the most severe pretreatment conditions.

Material balances based on Tables 1 and 2 show that hemicellulose and cellulose fractions have been partly converted into components for which the organosolv liquor was not analysed. This unknown fraction increases with pretreatment severity and is much larger for xylan than for glucan. Most probably, this fraction includes the formation of 'humins' (insoluble sugar derivatives based polymers) and components formed by reactions between sugars or sugar derivatives and lignin fragments (Huijgen et al. 2010). In addition, some sugar degradation might occur during the post-hydrolysis before oligomeric sugar analysis, possibly causing a slight underestimation of the oligomeric sugar concentrations.

Enzymatic digestibility of organosolv pretreated OTPB

Enzymatic hydrolysis Figure 2a shows the concentration of glucose obtained by enzymatic hydrolysis of fresh and pretreated OTPB as a function of hydrolysis time. Organosolv pretreatment was found to substantially enhance enzymatic cellulose hydrolysis at all process conditions assessed resulting in higher glucose concentrations (13–28 g l⁻¹ after 72 h) compared to fresh OTPB (3 g l⁻¹). Experiments 9 and 10 (most severe conditions and the lower EtOH concentration) produced pretreated OTPB most suitable for enzymatic hydrolysis, with a glucose concentration of 28 and 27 g l⁻¹ at the end of the process, respectively (Figure 2a).

Most glucose is released in the first 24 h of enzymatic conversion. For example, in exp. 1, corresponding to the least severe conditions (190°C, 15 min), 88% of the glucose obtained after 72 h is already released in the first 24 h period. For the other experiments, these percentages are 72–92%. This result is in accordance with that obtained when OTPB was pretreated by other pretreatment methods, such as steam explosion (Cara et al. 2006), with more than 75% of the glucose released in the first 24 h. A similar behaviour has been described for other substrates such as softwoods (Xiao et al. 2004).

The enzyme complex also exhibited xylan hydrolysis activity, which resulted in sugar solutions containing 1.4–5.4 g l⁻¹ xylose as a function of pretreatment conditions (Figure 2b) (or xylose yields varying from 10% to 44% based on the xylan content of the raw material). Following a similar pattern as described for glucose, most of the xylose was released in the first 24 h of enzymatic hydrolysis. Enzymatic cellulose hydrolysis can potentially be improved by supplementing the enzyme mixture used with xylanase to further improve xylan hydrolysis, thereby improving the access of enzymes to cel-

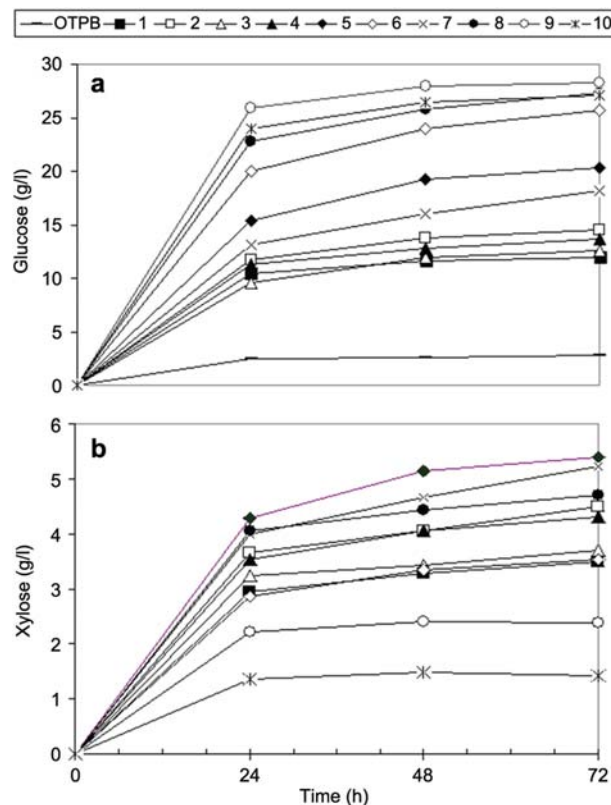


Figure 2 Glucose (a) and xylose (b) concentrations as function of enzymatic hydrolysis time. Mean values of triplicate experiments (standard deviation for all individual data points <8%). OTPB, olive tree pruning biomass. For numbering of samples see Table 1.

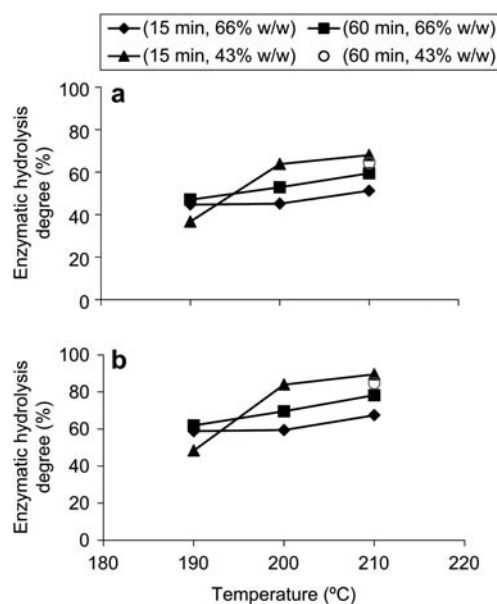


Figure 3 Effect of pretreatment conditions on enzymatic hydrolysis yields based on (a) total glucan content raw material (i.e., structural and extractable) and (b) structural glucan content raw material.

Table 3 Optimum pretreatment conditions for enzymatic hydrolysis of OTPB.

Pretreatment method	Pretreatment conditions	Glucose yield (%) ^a	Reference
Hot water	200°C, 60 min	71.2	Cara et al. (2007)
Steam explosion	240°C, 10 min, water-impregnation	51.6	Cara et al. (2008b)
Dilute acid hydrolysis	210°C, 0.6% H ₂ SO ₄	55.2	Cara et al. (2008a)
Organosolv	210°C, 15 min, 43% EtOH	89.6	This study

^aEnzymatic hydrolysis yield based on structural glucan present in the raw material.

lulose. For example, Öhgren et al. (2007) reported near-theoretical glucose yield (96–104%) from acid-catalysed steam pretreated corn stover when xylanases are used to supplement cellulases during hydrolysis.

Effect of pretreatment conditions on enzymatic hydrolysis yields Enzymatic hydrolysis yields were evaluated as the percentage of glucose released referred to both the total glucan content of the raw material (i.e., including glucose present in the extractives) (Figure 3a) and the structural glucan content of the raw material (Figure 3b). Enzymatic hydrolysis yields were found to increase with temperature, reaction time (except for 43% EtOH at 210°C), and lower EtOH content (except at 190°C). Pretreatment at 210°C, 15 min, and 43% EtOH (exp. 9) resulted in maximum enzymatic glucose yields of 68% and 90% based on total and structural glucan content of OTPB, respectively. The glucose present in the extractives fraction seems to be removed from the biomass during organosolv pretreatment at higher pretreatment severity and at higher EtOH content (see also glucan recovery, Figure 1c). This effect limits the maximum enzymatic glucose yield. Further increasing the severity of the pretreatment is not expected to improve the enzymatic digestibility because the glucan recovery (Figure 1c) will probably decrease further (cf. exp. 9 and 10).

Irrespective of the EtOH concentration used, hydrolysis of hemicelluloses clearly promoted enzymatic cellulose hydrolysis. Removing hemicellulose improves accessibility of enzymes to the cellulose fraction. For delignification, results were less straightforward. For the 66% EtOH experiments, the pretreated materials showing higher enzymatic digestibility were also the ones with the lowest lignin contents (Table 1) and those with the highest delignification (Figure 1a). This result is in agreement with other reports showing that lignin removal improves enzymatic hydrolysis (Öhgren et al. 2007; Mussatto et al. 2008). However, the experiments in which 43% EtOH was used showed an opposite trend (the higher the AIL content of the pretreated material, the better the enzymatic digestibility). Possible explanations include the overestimation of the AIL content of these materials due to the formation of pseudo-lignin and a difference in biomass recalcitrance caused by native lignin and recondensated lignin. Examples of pretreatment studies can also be found in the literature, in which no direct relation could be found between a lower lignin content of the pretreated biomass and a better enzymatic digestibility [e.g., EtOH-water pretreatment of pine (Pan et al. 2007) and acetone-water pretreatment of *Pinus radiata* (Araque et al. 2007)].

Comparison of pretreatment methods for OTPB Table 3 compares the enzymatic hydrolysis results at optimum pretreatment conditions from this study with results obtained in earlier studies on different pretreatment methods for OTPB (Cara et al. 2007, 2008a,b). Organosolv pretreatment was found to be the best pretreatment method for enzymatic hydrolysis of OTPB reported to date. In other words, organosolv pretreatment leads to a better use of the glucan content of the raw material.

Conclusions

Organosolv pretreatment was found to fractionate OTPB and to enable enzymatic sugar production from this biomass. Delignification increased by a higher EtOH content of the solvent and more severe pretreatment (up to 64% at 66% EtOH, 210°C, 60 min). By contrast, xylan hydrolysis is promoted by a lower EtOH content (maximum 92% at 43% EtOH, 210°C, 60 min). Enzymatic digestibility of the organosolv pretreated OTPB is enhanced at all pretreatment conditions tested compared to the raw material. In the range of variables assayed, the optimum enzymatic hydrolysis yield (90% of the structural glucan present in the raw material) was obtained when OTPB was subjected to organosolv pretreatment at 210°C for 15 min, using 43% EtOH. Organosolv was found to be the most effective pretreatment method for enzymatic hydrolysis of OTPB studied thus far. Further research efforts should focus on extraction of the significant amount of non-structural glucose and the hemicellulose sugars without degradation to further improve the production of fermentable sugars from OTPB.

Acknowledgments

Financial support from the Spanish Ministerio de Ciencia e Innovación (Project ENE2008-06634-C02-02/ALT) including Fondo Europeo de Desarrollo Regional (FEDER) funds is gratefully acknowledged by the University of Jaén. The Energy Research Centre of the Netherlands (ECN) part of this research was funded by the Dutch Ministry of Economic Affairs as part of the biomass research program of ECN.

References

- Araque, E., Parra, C., Freer, J., Contreras, D., Rodríguez, J., Mendonça, R., Baeza, J. (2007) Evaluation of organosolv pretreatment for the conversion of *Pinus radiata* D. Don to ethanol. *Enzyme Microb. Technol.* 43:214–219.

- Caparrós, S., Ariza, J., López, F., Nacimiento, J.A., Garrote, G., Jiménez, L. (2008) Hydrothermal treatment and ethanol pulping of sunflower stalks. *Bioresour. Technol.* 99:1368–1372.
- Cara, C., Ruiz, E., Ballesteros, I., Negro, M.J., Castro, E. (2006) Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline-peroxide delignification. *Process Biochem.* 41:423–429.
- Cara, C., Romero, I., Oliva, J.M., Sáez, F., Castro, E. (2007) Liquid hot water pretreatment of olive tree pruning residues. *Appl. Biochem. Biotechnol.* 137–140:379–394.
- Cara, C., Ruiz, E., Oliva, J.M., Sáez, F., Castro, E. (2008a) Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification. *Bioresour. Technol.* 99:1869–1876.
- Cara, C., Ruiz, E., Ballesteros, M., Manzanares, P., Negro, M.J., Castro, E. (2008b) Production of fuel ethanol from steam-explosion pretreated olive tree pruning. *Fuel* 87:692–700.
- Díaz, M.J., Huijgen, W.J.J., van der Laan, R.R., Reith, J.H., Cara, C., Castro, E. (2009) Organosolv pretreatment of olive tree biomass for fuel and chemicals production. In: *Proceedings of the 17th European Biomass Conference and Exhibition, Hamburg, Germany*. Eds. Santi, G.F., Dallemand, J.F., Ossenbrink, H., Grassi, A., Helm, P. ETA-Florence Renewable Energies, Florence. pp. 1970–1973.
- Gosselink, R.J.A., Abächerli, A., Semke, H., Malherbe, R., Käuper, P., Nadif, A., van Dam, J.E.G. (2004) Analytical protocols for characterisation of sulphur-free lignin. *Ind. Crops Prod.* 19:271–281.
- Heitz, M., Brown, A., Chornet, E. (1994) Solvent effects on liquefaction: solubilization profiles of a Canadian prototype wood, *Populus deltoides*, in the presence of different solvents. *Can. J. Chem. Eng.* 72:1021–1027.
- Hendriks, A.T.W.M., Zeeman, G. (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* 100:10–18.
- Huijgen, W.J.J., van der Laan, R.R., Reith, J.H. (2008) Modified organosolv as a fractionation process of lignocellulosic biomass for co-production of fuels and chemicals. In: *Proceedings of the 16th European Biomass Conference and Exhibition, Valencia, Spain*. Eds. Schmid, J., Grimm, H.P., Helm, P., Grassi, A. ETA-Florence Renewable Energies, Florence. pp. 1651–1655.
- Huijgen, W.J.J., Reith, J.H., den Uil, H. (2010) Pretreatment and fractionation of wheat straw by an acetone-based organosolv process. *Ind. Eng. Chem. Res.* 49:10132–10140.
- Jiménez, L., Pérez, I., García, J.C., Rodríguez, A. (2001) Influence of process variables in the ethanol pulping of olive tree trimmings. *Bioresour. Technol.* 78:63–69.
- Kleinert, T., Tayenthal, K. (1931) Separation of cellulose and incrusting substances. *Ztschr. Angew. Chem.* 39:788–791.
- Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res.* 48:3713–3729.
- Mesa, L., González, E., Ruiz, E., Romero, I., Cara, C., Felissia, F., Castro, E. (2010) Preliminary evaluation of organosolv pre-treatment of sugar cane bagasse for glucose production: application of 2³ experimental design. *Appl. Energy* 87:109–114.
- Mussatto, S.I., Fernandes, M., Milagres, A.M.F., Roberto, I.C. (2008) Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. *Enzyme Microb. Technol.* 43:124–129.
- National Renewable Energy Laboratory (NREL). Chemical analysis and testing laboratory analytical procedures. LAP-002 (1996). LAP-003 (1995). LAP-004 (1996). LAP-005 (1994). LAP-010 (1994) and LAP-017 (1998). NREL, Golden, CO, USA. Available at: http://www.nrel.gov/biomass/analytical_procedures.html.
- Öhgren, K., Bura, R., Saddler, J., Zacchi, G. (2007) Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Bioresour. Technol.* 98:2503–2510.
- Pan, X., Arato, C., Gilkes, N., Gregg, D., Mabey, W., Pye, K., Xiao, Z., Zhang, X., Saddler, J. (2005) Biorefining of softwoods using ethanol organosolv pulping: preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnol. Bioeng.* 90:473–481.
- Pan, X., Gilkes, N., Kadla, J.F., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., Saddler, J.N. (2006) Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields. *Biotechnol. Bioeng.* 94:851–861.
- Pan, X., Xie, D., Yu, R.W., Lam, D., Saddler, J.N. (2007) Pretreatment of lodgepole pine killed by mountain pine beetle using the ethanol organosolv process: fractionation and process optimization. *Ind. Eng. Chem. Res.* 46:2609–2617.
- Ruiz, E., Cara, C., Manzanares, P., Ballesteros, M., Castro, E. (2008) Evaluation of steam explosion pre-treatment for enzymatic hydrolysis of sunflower stalks. *Enzyme Microb. Technol.* 42:160–166.
- Xiao, Z., Zhang, X., Gregg, D.J., Saddler, J.N. (2004) Effects of sugar inhibition on cellulases and β -glucosidase during enzymatic hydrolysis of softwood substrates. *Appl. Biochem. Biotechnol.* 115:1115–1126.
- Xu, Y., Li, K., Zhang, M. (2007) Lignin precipitation on the pulp fibers in the ethanol-based organosolv pulping. *Colloids Surface A* 301:255–263.
- Zhao, X., Cheng, K., Liu, D. (2009) Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* 82:815–827.

Received July 7, 2010. Accepted October 27, 2010.
Previously published online February 7, 2011.