energies

MDPI

Article

Obtaining Fermentable Sugars from a Highly Productive Elm Clone Using Different Pretreatments

David Ibarra [1], Raquel Martín-Sampedro [1,2], Laura Jiménez-López [1,2], Juan A. Martín [3], Manuel J. Díaz [4] and María E. Eugenio [1,*]

[1] Forestry Products Department, Forest Research Center, INIA, Ctra. de La Coruña, Km 7.5, 28040 Madrid, Spain; ibarra.david@inia.es (D.I.); raquel.martin@inia.es (R.M.-S.); laura.jimenez.lopez@csic.es (L.J.-L.)
[2] Institute of Materials Science of Madrid (ICMM), Spanish National Research Council (CSIC), 28049 Madrid, Spain
[3] Departamento de Sistemas y Recursos Naturales, ETSI Montes, Forestal y del Medio Natural, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain; juan.martin.garcia@upm.es
[4] Research Center in Technology of Products and Chemical Processes, Pro2TecS-Chemical Engineering Department, Campus El Carmen, University of Huelva, 21071 Huelva, Spain; dblanco@diq.uhu.es
* Correspondence: mariaeugenia@inia.es; Tel.: +34-91-347-3948

Abstract: The interest of supplying lignocellulosic materials for producing fermentable sugars has recently emerged in order to diminish the negative environmental effects of fossil fuels. In this study, the *Ulmus minor* clone Ademuz, characterized for its tolerance to Dutch elm disease and its rapid growth, was evaluated as a source of fermentable sugars. For that, different pretreatments, comprising autohydrolysis, dilute acid hydrolysis, acid catalyzed organosolv, and alkaline extraction, were evaluated at two levels of severity (pretreatment temperatures at 160 °C and 180 °C, except for alkaline extraction at 80 °C and 160 °C); and the resulting pretreated materials were enzymatically hydrolyzed for fermentable sugars production. The major extraction of lignin and hemicellulose was achieved during organosolv (48.9%, lignin; 77.9%, hemicellulose) and acid hydrolysis (39.2%, lignin; 95.0%, hemicellulose) at 180 °C, resulting in the major enzymatic digestibility (67.7%, organosolv; 53.5% acid hydrolysis). Contrarily, under the most favorable conditions for autohydrolysis (180 °C) and alkaline extraction (160 °C), lower extraction of lignin and hemicellulose was produced (4.8%, lignin; 67.2%, hemicellulose, autohydrolysis; 22.6%, lignin; 33.1%, hemicellulose, alkaline extraction), leading to lower enzymatic digestibility (32.1%, autohydrolysis; 39.2%, alkaline extraction). Taking into account the sugars produced during enzymatic hydrolysis of pretreated materials and the solubilized sugars from pretreatment liquors, the highest sugars (glucose and xylose) yield production (28.1%) per gram of biomass from *U. minor* clone Ademuz was achieved with acid catalyzed organosolv at 180 °C.

Keywords: Ademuz elm clone; biomass production; enzymatic hydrolysis; fermentable sugars; pretreatment

Citation: Ibarra, D.; Martín-Sampedro, R.; Jiménez-López, L.; Martín, J.A.; Díaz, M.J.; Eugenio, M.E. Obtaining Fermentable Sugars from a Highly Productive Elm Clone Using Different Pretreatments. *Energies* **2021**, *14*, 2415. https://doi.org/10.3390/en14092415

Academic Editor: Dmitri A. Blushev

Received: 21 March 2021
Accepted: 17 April 2021
Published: 23 April 2021

1. Introduction

The urgency of identifying and developing sustainable solutions that respond to society's current and future needs has been clearly demonstrated by the alarming escalation in global energy demand, the need to reduce greenhouse gas emissions, and the exhaustion of fossil fuel reserves. In this scenario, the use of biomass as a basic and renewable raw material implies a transition from an economy based on fossil fuels to one based on biomass, which has been called "bioeconomy". From there arises the biorefinery concept, defined as the use of biomass to produce energy together with a great variety of bioproducts including materials, chemicals, feed, and food [1]. Unlike other biomasses, such as sugar or starch, the lignocellulosic materials are abundant, low cost, and not destined for food. All of

these advantages make lignocellulosic biomass the most suitable raw material for the development of lignocellulosic biorefineries [2].

Among the lignocellulosic forest biomass, several tree species are being evaluated as biomass productive plantations to supply raw materials for diverse applications. Fast-growing resprouting species, such as *Populus*, *Salix*, and *Eucalyptus* spp., are frequently selected for this purpose and managed as short rotation coppice [3,4]. Each tree species has specific environmental requirements that can strongly influence physiological functioning and biomass yield [5,6], such as soil fertility, temperature and precipitation, severity of frost and drought periods, or incidence of diseases and pests [7]. Yet, in practice, few biomass forest plantations can occupy territories with optimal conditions, which are prioritized for more profitable food crops. Therefore, there is a need to explore and design biomass forest plantations able to occupy marginal lands which are not economically sustainable for food crops but can provide profitable wood yields [8,9]. These marginal plantations should also cope with the increasing impact of climate change, which is exacerbating the occurrence of extreme weather events, such as more severe and prolonged droughts in the Mediterranean basin [10], compromising the growth and health of forest stands. Besides the capacity to tolerate a range of sub-optimal environmental conditions, selected tree species or clones should also demonstrate their aptitude to provide valuable products for the forest and/or lignocellulose industry. This knowledge could contribute to promote a wood-based bioeconomy in rural territories in the Mediterranean countries [11].

In this context, some elm (*Ulmus* sp.) species arise as hardwood trees with a potential to cope with sub-optimal environmental conditions in biomass plantations. For instance, the Siberian elm (*Ulmus pumila* L.) is a species native to the Tarim and Gobi deserts which shows a remarkable tolerance to aridity [12], while maintaining outstanding growth rates. These qualities, together with its good tolerance level to Dutch elm disease (DED), a fungal disease which has decimated native elm populations in Europe and North America [13], make Siberian elm a good candidate for bioenergetic crop in short-rotation coppice under Mediterranean climates [14]. As an alternative to the Siberian elm, the field elm (*Ulmus minor* Mill.), native to southern Europe, could offer higher biomass yields than *U. pumila* given its higher growth rates [15]. *U. minor* lacks specific soil type requirements, but prefers calcareous soils, and in spite of its riparian character it is found thriving well in poor soils of inland Spain and in areas located further away from riverbanks, influenced by moderate levels of summer drought [13]. This species tolerates root anoxia and stands out for its capacity to resist winds, recover from mechanical damage, tolerate soil compaction and contaminants, and propagate easily by root and aerial cuttings [13]. Furthermore, it has an excellent ability to resprout from roots and stumps, and therefore can be suitable for short-rotation coppice. However, it is extremely susceptible to DED, which in the last century has prevented its use in forestry. Yet, recent breeding works in Spain have permitted the selection of several DED-resistant *U. minor* clones [16]. Among them, the clone Ademuz has revealed outstanding growth rates and excellent adaptation to a range of environments [16,17]. Furthermore, the use of native instead of exotic elm material in forest plantations would contribute to the conservation of the native elm genetic resources, since exotics of invasive character such as *U. pumila* can pollinate native elms and produce fertile hybrids [18].

In general, the potential of lignocellulosic biomass is based on its high carbohydrate content, mainly cellulose and hemicelluloses. These polymers can be used as such for the production of pulp and paper [19], and more recently novel biomaterials such as nanocellulose [20]; or they can be transformed into fermentable sugars which in turn are converted by fermentation into biofuels, such as bioethanol, or other bioproducts [21]. The production of sugars from the carbohydrates contained in the lignocellulosic biomass is generally carried out through a stage of enzymatic hydrolysis (saccharification) [22]. Cellulase and hemicellulase enzymes access polysaccharide chains and catalyze their degradation until simple sugars are obtained. However, lignocellulose as found in nature is generally a very recalcitrant substrate to enzymatic attack. One of the factors that limit

the maximum recovery of sugars is precisely the presence of lignin, which is a physical barrier that produces non-specific adsorption of hydrolytic enzymes [23,24]. For this reason, biomass must first be pretreated to produce an extensive lignocellulose alteration, mainly through solubilization of hemicelluloses and lignin removal, which increases the accessibility of carbohydrates to hydrolytic enzymes [25].

Among the pretreatment methods that act on lignin, the organosolv process and alkaline extraction can be highlighted. Organosolv process usually uses mixtures of organic solvents such as ethanol, acetone, methanol, and ethylene glycol to solubilize the lignin [26]. Sometimes an acid catalyst is added to increase the xylose yield. This is intended to increase the efficiency of subsequent enzymatic hydrolysis to obtain a higher yield in fermentable sugars [25]. Alkaline extraction is usually carried out with NaOH, which swells and increases the internal surface of the cellulose, reducing the polymerization degree and the crystallinity, which produces the solubilization of the lignin [27] and, as a consequence, increasing the production of fermentable sugars in a subsequent stage of enzymatic hydrolysis [25].

On the other hand, acid hydrolysis and autohydrolysis are pretreatments that stand out for solubilizing hemicellulose, favoring access to hydrolytic enzymes, among other things [25]. Thus, acid pretreatment normally used diluted acid instead of concentrate acid since the former reduces the formation of inhibitors compounds and avoid equipment corrosion. During this process, depending on the conditions used, not only is the hemicellulose solubilized but also the solubilized hemicellulose is converted into fermentable sugars [28]. Autohydrolysis used only water at high temperature to produce a selective hydrolysis of the hemicellulose, therefore it is a simple and environmentally friendly process [29].

In spite of the good properties and characteristics of the Ademuz elm clone biomass, very few studies exist in the literature on its use as raw material of a lignocellulosic biorefinery [30,31]. Therefore, neither the type nor the conditions of the pretreatments able to enhance the production of fermentable sugars from this biomass have been studied. Thus, the aim of this study is to evaluate the effect of four different pretreatments (acid catalyzed organosolv, dilute acid hydrolysis, alkali extraction, and authohydrolysis) at two severity levels on the production of fermentable sugars from *U. minor* clone Ademuz, which can be transformed into different biofuels or bioproducts, and to select the most efficient one.

2. Materials and Methods

2.1. Raw Materials and Chemicals

The *U. minor* clone Ademuz was supplied by the Spanish elm breeding program. This clone eligible for the production of forest reproductive materials and tolerant to Dutch elm disease was registered in Spain. Enzymes used during enzymatic hydrolysis were Celluclast 1.5 L and Novozymes 188 from Novozymes (Bagsvaerd, Denmark) and all chemicals used in this study were acquired from Sigma–Aldrich (Madrid, Spain) or Merck (Barcelona, Spain).

2.2. Estimation of Wood Biomass Yield

Volume of the main trunk of Ademuz elm clone was estimated by measuring tree height (h) and diameter (d) at the base of the trunk. Elm trees grown in four experimental plots of the Spanish elm breeding program, situated in three different breeding centers and locations: Madrid, Valencia, and Guadalajara (Table 1). The purpose of these plots was to test the resistance level of several elm clones to the Dutch elm disease pathogen through artificial inoculation of pathogen spores, with the exception of the plot "PH-C Ref", whose objective was to serve as clonal bank for conservation of genetic resources and as reference collection for genetic fingerprint. Therefore, it should be highlighted that these plots were not specifically managed for biomass production purposes, and destructive tree sampling in these plots was not possible. However, they are useful for providing a first estimation of the biomass yield potential of this clone. All the plots were watered in spring and summer through drip irrigation, had a border line to avoid side effects, and all

elm genotypes included in each plot (data not shown) were distributed with a complete random design. However, spacing between plants, the year of plant measurement, as well as other cultivation characteristics varied among plots (Table 1).

For trunk volume (v) estimation we used the following Equation (1):

$$v = \frac{\pi h d^2}{4} f \quad (1)$$

where f is a shape factor of the trunk. To estimate wood biomass, we measured wood density (g/cm^3) of elm clone. To this end, five 3-year-old replicates of the clone growing at Puerta de Hierro Forest Breeding Center (Madrid) were cut from the base of the trunk. Basal segments (4 cm in length) were split longitudinally, being soaking in degassed water overnight. Afterwards, their fresh volume was calculated, according to Archimedes' principle, by immersing each sample in a water-filled test-tube placed on a balance [32].

Displacement water weight was transformed to sample volume considering water density (0.9982071 g/cm^3 at 20 °C). Samples were kept at 75 °C for 48 h after which their dry weight was measured. Wood density (g/cm^3) was determined as the ratio of dry weight to fresh volume. The same five tree replicates were also used to calculate the dry weight (DW) of the main trunk, branches, and leaves. Allometric relationships between the dendrometric variables of these five replicates, the wood density, and the DW allowed us to adjust the shape factor (f) of the trunk to 0.33.

For each tree age, the response variables tree height (cm), diameter (cm), trunk volume (cm^3), and trunk biomass yield (t DM/ha/year) were analyzed by a general linear model, with the experimental plot (Table 1) as fixed factor. The relation between tree height and diameter was explored through a Pearson's correlation analysis.

2.3. Pretreatments

Prior to pretreatments, Ademuz elm wood (from Madrid plots) was cut into chips and ground using a Wiley mill. After this, sample was sieved selecting a size of 0.4–0.70 mm. Finally, sample was pre-soaked for 1 h at room temperature in water.

A pressured reactor of 6 L (Autoclave Engineers) was used for all pretreatments, which were carried out with a liquid:solid ratio of 20:1 at two different temperatures. Thus, autohydrolysis at 60 min, 160 °C and at 60 min, 180 °C (denoted AH-160 and AH-180, respectively); diluted acid hydrolysis at 60 min, 160 °C, 3% H_2SO_4 (w/w) and at 60 min, 180 °C, 3% H_2SO_4 (w/w) (denoted AcH-160 and AcH-180, respectively); acid-catalyzed ethanol/water organosolv process at 160 °C, 60 min, 30% ethanol (v/v), 1% H_2SO_4 (w/w) and at 180 °C, 60 min, 30% ethanol (v/v), 1% H_2SO_4 (w/w) (denoted Org-160 and Org-180, respectively); and alkaline extraction at 120 min, 80 °C, 10% NaOH (w/w) and at 60 min, 160 °C, 10% NaOH (w/w) (denoted Alk-160 and Alk-80, respectively).

All pretreatments were conducted in duplicate. The materials resulting from all pretreatments were filtered under vacuum to separate the liquid fractions from the solid fractions. Then, the solid fractions were washed with distilled water resulting in the water insoluble (WIS) fractions, which was subsequently used to perform the enzymatic hydrolysis.

Removal (R) of each component (lignin and the specific carbohydrates), using different pretreatment methods, is calculated using Equation (2).

$$\mathrm{R}\ (\%) = 100 - \left(\frac{\text{component in pretreated material (in \%)}}{\text{component in raw material (in \%)} \times \text{ solid yield of the pretreatment (in \%)}} \right) \times 100 \quad (2)$$

Table 1. Specifications of the experimental plots used to estimate wood yield of Ademuz clone. T = average annual temperature (°C); P = average annual precipation (mm); N = number of replicates; h = height; d = diameter.

Plot	Location in Spain	Climate (Altitude in m.a.s.l.)	T, P	N	Spacing (m)	Soil Type	Fertilization	Weed Management	Plant Propagation (Year)	Monitoring of h and d (Tree Age)
PH-C Ref	Puerta de Hierro, Madrid	Inland Mediter-ranean (597)	14, 450	10	4 × 4	Sandy	Slow release fertilizer in the year of planting	Anti-weed mesh	2012	3 to 8 yr
PH-XXXVII	Puerta de Hierro, Madrid	Inland Mediter-ranean (597)	14, 450	8	1 × 1	Sandy	Slow release fertilizer in the year of planting	Clearing	2013	2 to 4 yr
ES-VI	Guadalajara	Inland Mediter-ranean (685)	13, 450	12	2 × 1.5	Sandy-loam	None	Clearing	2011	3 to 5 yr
AL-I	Alaquàs, Valencia	Coastal Mediter-ranean (42)	17, 445	12	2 × 2	Clay-loam	None	Clearing	2013	2 and 4 yr

2.4. Enzymatic Hydrolysis

All pretreated materials (WIS samples) resulting from the different pretreatments assayed were subjected to enzymatic hydrolysis, which was performed in each case in triplicate. Celluclast 1.5 L (92.5 FPU/mL) and β-glucosidase Novozyme 188 (1274 IU/mL) were added to a 5% (*w/w*) WIS sample suspension using sodium citrate buffer (50 mM, pH 4.8). The enzyme doses were 15 FPU of Celluclast 1.5 L and 15 IU of β-glucosidase per gram of dry sample. A thermostatic rotary shaker was used to perform enzymatic hydrolysis under the following conditions: 50 °C and 120 rpm for 144 h. To evaluate glucose and xylose concentrations by high performance liquid chromatography (HPLC), 1.5 mL of hydrolyzed samples were taken at 24, 48, 72, and 144 h of each hydrolysis. The samples taken were heated (boiling water for 10 min) to stop the enzymatic hydrolysis and filtered (using a 0.45 µm nylon syringe filter) to subsequently determine the sugars present in the samples. The digestibility of glucose (DG), xylose (DX), and total sugars (DT) as well as yields of glucose (YG), xylose (YX), and total sugars (YT) were calculated according to Ibarra et al. [33], using Equations (3) and (4), respectively. Digestibility evaluates the percentage of sugars converted during the enzymatic hydrolysis per grams of sugars in the pretreated material; sugar yield also consider the loss of sugars during the pretreatment, evaluating the yield per gram of dry initial material.

$$\text{DG or DX } (\%) = \frac{\text{g of sugar in liquid phase}}{\text{g of sugar in pretreated material}} \times 100 = \frac{C_h \times V_h}{(m_p \times C_p)} \times 100 \quad (3)$$

$$\text{YG or YX or YT } (\%) = \frac{\text{g of sugar in liquid phase}}{\text{g of dry initial material}} \times 100 = \frac{C_h \times V_h}{(m_p)/Y_p} \times 100 \quad (4)$$

where C_h is the concentration of glucose, xylose or total sugars in the hydrolysate at 144 h of the enzymatic hydrolysis (in g/L); V_h is the volume of hydrolysate in L; m_p are amount of dry pretreated material subjected to enzymatic hydrolysis (in g); C_p is the concentration of glucose, xylose, or total sugars in the pretreated material (in %); and Y_p is the solid yield of the pretreatment (in %).

2.5. Analytical Methods

Chemical composition of raw material and WIS fractions was estimated according to the National Renewable Energies Laboratory procedures [34], being the NREL/TP-510-42618 protocol used. An Agilent Technologies 1260 HPLC (Agilent, Waldbronn, Germany), equipped with a G1362A refractive index (RI) detector, and an Agilent Hi-PlexPb column (at 70 °C) was used for carboydrate content analysis. Ultrapure water was employed as mobile phase (0.6 mL/min). The solid residue that remain after acid hydrolysis is called Klason lignin or acid-insoluble lignin. Both monosaccharides and degradation products (acetic acid, furfural and 5-hydroxymethylfurfural (5-HMF)) of the liquid fractions obtained during the different pretreatments were also determined according to the NREL methodology (NREL/TP-510-42623 protocol). In brief, a 1 mL aliquot was filtered using 0.45 µm nylon syringe membranes and it was used for direct HPLC determination, using an Agilent Hi-PlexH column (at 65 °C). Sulfuric acid (5 mmol/L) was employed as mobile phase (0.6 mL/min) [33]. A second 25 mL aliquot was hydrolysed (quantitative post-hydrolysis: 4% H_2SO_4, 120 °C, 60 min) before HPLC analysis. Increments in the concentrations of monosaccharides and acetic acid produced by post-hydrolysis were used to determine the concentrations of oligomers and acetyl groups bound to oligosaccharides, respectively.

Glucose and xylose concentrations from enzymatic hydrolysis assays were quantified by HPLC, using an Agilent Hi-PlexH column at the conditions described above [33].

Lignin and carbohydrate extraction values according to the type of pretreatment and the temperatures at which they were taken, as well as the digestibility (DG, DX, and DT) of the different pretreated materials obtained, have been statistically analysed. Means and coefficient of variations (CV) among the replicates to represent the extent of the variability have been used. Both parameters have been compared by using analysis of variance

(ANOVA) and Tukey's test, with a significance level of $\alpha = 0.05$. The results of analysis of variance implemented in the figures have been presented. In the figures, the same letters are not significantly different ($p > 0.05$) using Tukey's test. IBM SPSS (version 25.0) software package was used to perform all the statistical analyses.

3. Results and Discussion

3.1. Estimation of Wood Biomass Yield

Average wood density of Ademuz elm clone was 0.54 ± 0.09 g DW/cm^3. Tree diameter and height measured at different tree ages showed a strong linear relationship (r = 0.96; $p < 0.0001$; Figure 1).

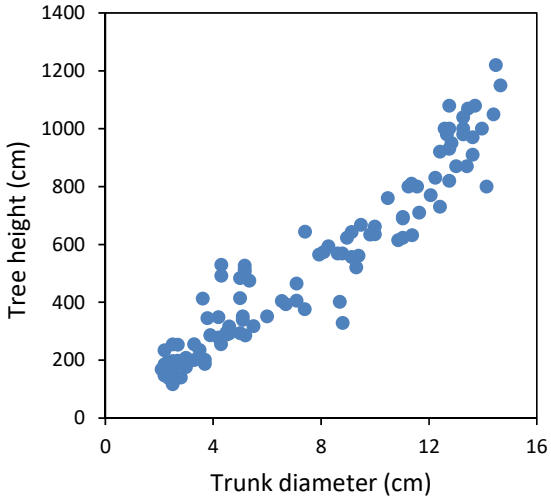

Figure 1. Relation between tree height and diameter measured at the base of the trunk of Ademuz elm clone.

The growth rate of Ademuz elm clone significantly varied among plots and locations. Three-year-old trees growing at the two Madrid's experimental plots showed higher volume than trees growing at Guadalajara's plot ($p < 0.05$) (Figure 2). The same trend is observed in 4-year-old trees. Similarly, 4-year-old trees growing at Madrid showed higher growths than trees growing at Valencia (Figure 2). In spite of the sandier soil at Madrid's plots, trees in Madrid showed higher plant growth than at Guadalajara and Valencia. A priori, a sandy soil is not expected to benefit elm growth due to its low water retention capacity. Although this result should be interpreted with caution due to different dates of measurements in each plot (Table 1), this trend was observed consistently across the different years. The better plant development in Madrid could be explained by several cultivation traits: (i) trees at Madrid received a single input of slow-release fertilizer, but trees at Guadalajara and Valencia did not received any fertilization treatment; (ii) Madrid's plot with 1×1 m spacing was fully protected against weeds by a mesh, which completely inhibited competition from surrounding vegetation, while the rest of the plots received occasional clearing of weeds by means of brush cutters; and (iii) although the three locations have a similar annual precipitation regime (Table 1), trees at Madrid possibly received more water supply through drip-irrigation than at Guadalajara and Valencia. Although we lack specific data about irrigation supplied in each breeding center, the intensity of water regimes is usually higher in Madrid (authors, personal observation). The different plant density among locations did not seem to contribute to the differences in plant development among locations, since the two plant densities in Madrid (4×4, and 1×1 m) resulted in higher growths than in Guadalajara (2×1.5 m) and Valencia (2×2 m) (Figure 2).

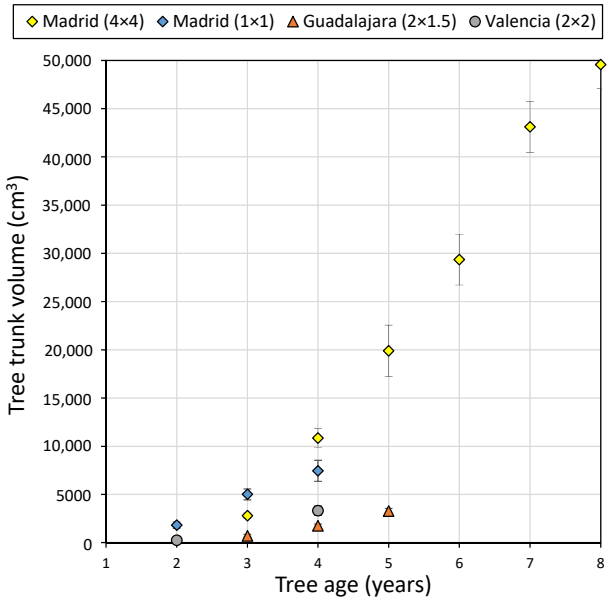

Figure 2. Tree trunk volume estimation for each tree age and experimental plot of Ademuz elm clone. Trunk volume was through direct measurements of diameter at the base and tree height. Vertical bars indicate standard errors.

Higher plant competition between plants limits plant growth rate [35]. However, our results suggest that Ademuz elm clone growing with 1 × 1 m spacing did not suffer from excessive competition when compared with 4 × 4 m spacing during the first three years. The higher growth rate of 3-year-old trees in the plot with 1 × 1 m spacing than in the plot with 4 × 4 m could be due to higher competition from weeds in the last case, as the conservation plot lacked an anti-weed mesh. In 4-year-old plants, the highest plant density in Madrid (1 × 1 m spacing) resulted in lower plant height but similar trunk diameter than in the lowest density (4 × 4 m) (Figure 3).

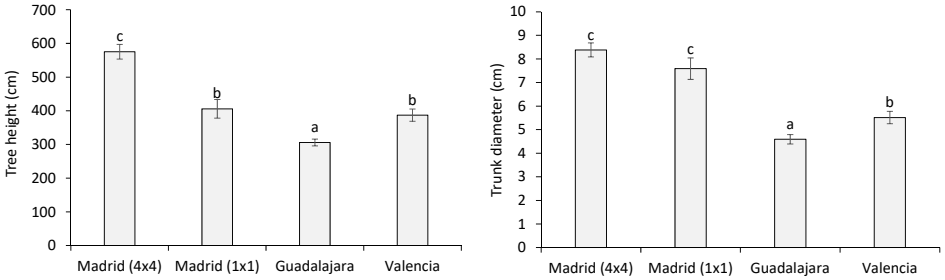

Figure 3. Tree height and diameter at the base of trunk of Ademuz elm clone measured in 4-year-old trees growing at the four experimental plots in Madrid, Guadalajara, and Valencia. Vertical bars indicate standard errors and different letters denote significant differences among plots (LSD test, $p < 0.05$).

According to our estimation, maximum yield of trunk biomass was reached in Madrid in the plots with 1 × 1 m spacing (Figure 4), with an average yield of 9.3 Mg DM/ha/year if harvesting is performed in the third year, and of 9.0 Mg DM/ha/year in the fourth year. In the five trees that were cut for DW estimation, the main stem accounted for 79.4 ± 2.3% (mean ± SE), the secondary branches for 9.4 ± 1.9%, and the leaves for 11.2 ± 0.4% of the total biomass. Therefore, total biomass in the plots with 1 × 1 m spacing could reach a yield of around 11.7 tons of Mg DM/ha/year if harvesting is performed in the third year. This estimation should be considered with caution and taken only as an approximation based on general dendrometric variables. A more specific work should

be done to confirm this estimation, by measuring by destructive sampling the dry mass obtained in a significant sample of trees. However, our estimation situates the Ademuz elm clone in a similar yield potential than the *Populus* clone I-214 growing under deficient short-rotation management scenarios in terms of weed control and irrigation management (10.9 Mg DM/ha/year), but lower than the same clone growing under standard management scenario (15.3 Mg DM/ha/year) [36]. Furthermore, Ademuz elm clone seems to have a higher biomass yield potential than the Siberian elm (*Ulmus pumila*), which yielded around 8 Mg DM/ha/year in Spain [14]. The potential of Ademuz elm clone to be used as source of raw material in short-rotation culture seems therefore interesting, particularly in sandy and poor soils (such as those in Puerta de Hierro Forest Breeding Center, Madrid, Spain), where other tree species are worst adapted.

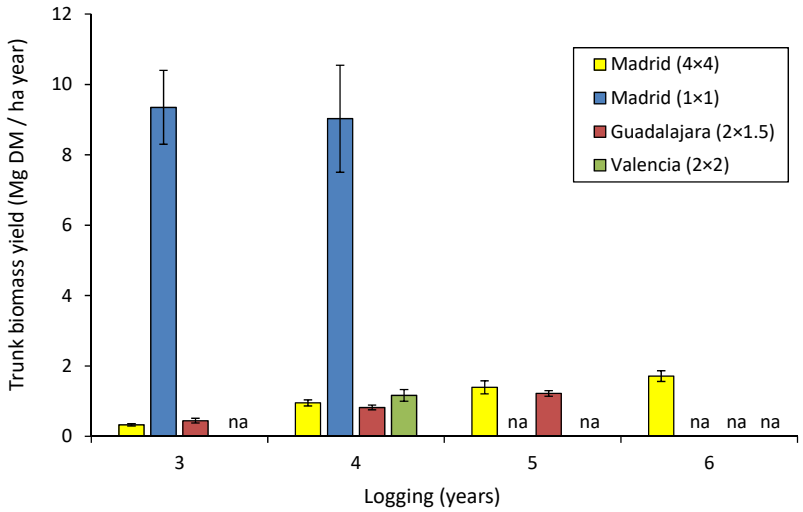

Figure 4. Estimated trunk biomass yield of Ademuz elm clone in the four experimental plots and in different harvesting years. na = data not available. Vertical bars indicate standard errors.

3.2. Composition Analysis of Raw Material and Pretreated Materials

3.2.1. Raw Material

Taking into account the results presented in Section 3.1, Ademuz elm wood (from Madrid plots) was analyzed as a source of fermentable sugars in a biorefinery context. Compared to other elm wood materials described in the literature for sugars production [29,37], Ademuz elm clone displays nearly similar quantity of cellulose, but minor amounts of hemicellulose (mainly xylan) and acid insoluble lignin (Table 2). Nevertheless, the percentage of carbohydrates and lignin differs among different materials of identical species, which depends on the growing conditions (spacing, water, salinity, and fertilizer), the growth age and the tissue type evaluated [38]. Regarding other hardwood materials such as poplar, black locust, and eucalypt [33–40], Ademuz elm clone shows higher cellulose content with lower amounts of hemicelluloses and similar acid insoluble lignin.

Table 2. Yield and chemical composition of the pretreated materials (WIS fraction).

	Pretreated Material Yield (%)	Acid Insoluble Lignin (%)	Acid Soluble Lignin (%)	Glucan (%)	Xylan (%)	Arabinan (%)
Untreated	-	18.0 ± 0.1	2.9 ± 0.1	43.6 ± 0.7	15.6 ±0.1	1.2 ± 0.0
AH-160	71.5	27.2 ± 1.3	1.1 ± 0.1	58.6 ± 1.9	9.4 ± 0.3	0.1 ± 0.1
AH-180	72.4	26.5 ± 1.1	1.1 ± 0.0	58.4 ± 1.7	7.0 ± 0.0	0.0 ± 0.0
AcH-160	54.5	24.8 ± 0.2	0.9 ± 0.0	63.5 ± 0.2	4.7 ± 0.0	0.0 ± 0.0
AcH-180	49.0	25.5 ± 0.2	0.5 ± 0.0	67.7 ± 0.4	1.6 ± 0.0	0.0 ± 0.0
Org-160	76.0	19.9 ± 0.8	2.0 ± 0.3	52.5 ± 0.1	16.8 ± 0.2	0.0 ± 0.0
Org-180	67.3	15.3 ± 0.8	0.6 ± 0.0	64.6 ± 0.6	5.1 ± 0.2	0.0 ± 0.0
Alk-80	81.0	22.4 ± 0.6	2.0 ± 0.1	49.9 ± 0.4	17.1 ± 0.1	0.0 ± 0.0
Alk-160	67.0	22.2 ± 0.5	2.0 ± 0.1	54.6 ± 0.4	15.5 ± 0.2	0.0 ± 0.0

Autohydrolysis (AH) at 160 °C (AH-160) or 180 °C (AH-180); Acid hydrolysis (AcH) at 160 °C (AcH-160) or 180 °C (AcH-180); Organosolv (Org) at 160 °C (Org-160) or 180 °C (Org-180); Alkaline extraction (Alk) at 80 °C (Alk-80) or 160 °C (Alk-160). Mean values and standard deviations were calculated from the triplicates.

3.2.2. Pretreated Materials

In light of the composition analysis and the production of Ademuz elm clone, the potential of this material as a source of fermentable sugars is evidenced. Contrarily, in the same way as other lignocellulosic materials, the complex and recalcitrant structure of elm wood makes necessary a pretreatment step to facility the action of hydrolytic enzymes in the production of fermentable sugars [25]. A mixture of physical factors, such as temperature, and chemical catalysts can be used in the pretreatment step for efficiently increasing the enzymatic hydrolysis of lignocellulose. Then, high temperatures in combination with water (autohydrolysis) or dilute sulfuric acid (acid hydrolysis) largely hydrolyze and solubilize the hemicellulose fraction [41], whereas high temperatures in combination with ethanol (organosolv) or sodium hydroxide (alkaline extraction) extensively remove lignin [41]. In order to increase the accessibility of carbohydrates to hydrolytic enzymes, these four pretreatment technologies (authohydrolysis, acid hydrolysis, organosolv, and alkali extraction) at two different pretreatment temperatures were evaluated on Ademuz elm clone.

Taking into account the chemical composition of the resulting pretreated materials (WIS fraction) and the pretreated material yield (Table 2), lignin and carbohydrates extraction was calculated during the different pretreatments (Table 3). In addition, these extraction values were statistically analyzed according to the type of pretreatment and the temperatures at which they were carried out (Figure 5).

Table 3. Removal of lignin and the specific carbohydrates using different pretreatment methods per gram of each component in raw material.

	Total Lignin (%)	Glucan (%)	Xylan (%)	Arabinan (%)
AH-160	3.4	3.8	56.8	92.2
AH-180	4.8	2.9	67.2	100.0
AcH-160	33.1	20.5	83.6	100.0
AcH-180	39.2	23.8	95.0	100.0
Org-160	20.3	8.5	17.8	100.0
Org-180	48.9	0.2	77.9	100.0
Alk-80	5.6	7.2	11.2	100.0
Alk-160	22.6	15.9	33.1	100.0

Autohydrolysis (AH) at 160 °C (AH-160) or 180 °C (AH-180); Acid hydrolysis (AcH) at 160 °C (AcH-160) or 180 °C (AcH-180); Organosolv (Org) at 160 °C (Org-160) or 180 °C (Org-180); Alkaline extraction (Alk) at 80 °C (Alk-80) or 160 °C (Alk-160).

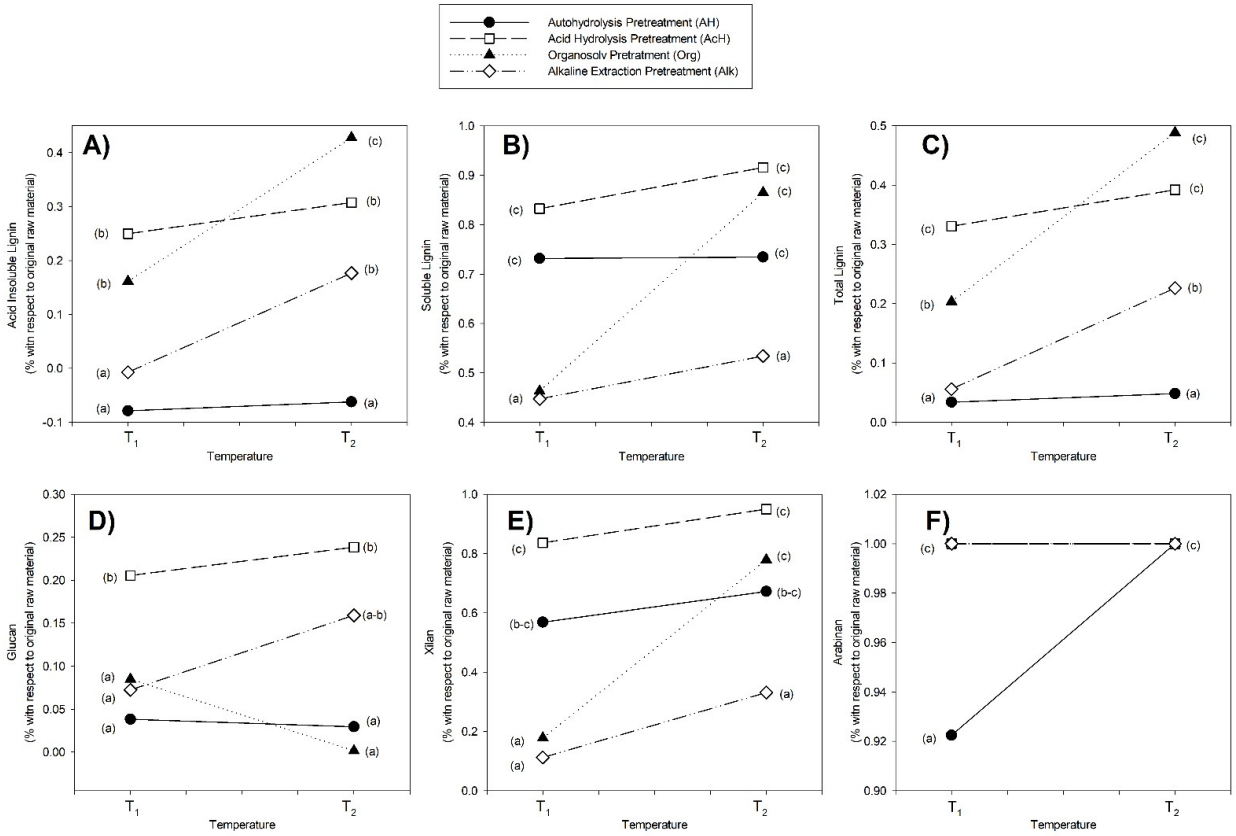

Figure 5. Analysis of lignin and carbohydrate extraction values per gram of each component in raw material according to the type of pretreatment and the temperatures at which they were taken: (**A**) acid insoluble lignin; (**B**) soluble lignin; (**C**) total lignin; (**D**) glucan; (**E**) xylan; (**F**) arabinan. T_1, pretreatment temperatures at 160 °C, except for alkaline extraction at 80 °C. T_2, pretreatment temperatures at 180 °C, except for alkaline extraction at 160 °C. Lines with the same letter for initial and final temperature are not significantly different (Tukey test, $p < 0.05$).

Regarding acid insoluble lignin (Figure 5A), Tukey test showed that the pretreatments extractions were significantly different among them. At the lowest temperature (T_1), no significant differences between autohydrolysis and alkaline extraction have been found, in the same way that between acid hydrolysis and organosolv pretreatments. In that form, among autohydrolysis-alkaline extraction and acid hydrolysis-organosolv, significant values (F = 73.2 and $p = 0.0344105$) have been obtained. As $p < 0.05$, the null hypothesis of no significant difference between pretreatments has been rejected. Taking into account that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05 among both groups, it is suggested that the one or more pretreatments are significantly different. Having carried out these pretreatments at highest temperature (T_2), autohydrolysis and acid hydrolysis pretreatments have not significantly increased their initial values, although alkaline extraction and organosolv pretreatments have had a significant and positive increase in their extraction capacity. In this way, the organosolv pretreatment at 180 °C is the one that most acid insoluble lignin has extracted. Moreover, soluble lignin encountered has been very similar for organosolv and alkaline pretreatments (Figure 5B), being significantly higher for autohydrolysis and acid hydrolysis pretreatments at the lowest temperature (T_1). At the highest temperature (T_2), the organosolv pretreatment was the only one that had significantly increased its soluble lignin extraction capacity with respect to the other studied pretreatments. Finally, for the total lignin (Figure 5C), very similar trends to those found for acid insoluble lignin have been found. Consequently, at the highest temperature (T_2) the acid hydrolysis and organolsov pretreatments were the most effective in the extraction of lignin from Ademuz elm clone, although the pretreated material recovery yield (%) value for acid hydrolysis was rather low compared to organosolv.

Previous studies have also shown the ability of acid hydrolysis pretreatment to extract lignin from different hardwoods, which is produced by the lignin fragmentation and solubilization due to the aryl-ether linkages breakdown [42]. At the highest severity of acid hydrolysis pretreatment, a delignification of 39.2% was found for Ademuz elm clone, whereas at similar conditions (i.e., 60 min, 180 °C, 3% H_2SO_4) Martín-Sampedro et al. [43] reported a slightly higher delignification degree (43%) with white poplar, and Jiménez-López et al. [40] described lower lignin extraction (33%) with black locust. Ibarra et al. [33] obtained lower values for lignin extraction (3–11%) when different poplar clones were exposed to acid hydrolysis at similar pretreatment conditions but lower temperature (i.e., 130 °C). Organosolv pretreatment also produces delignification by α- and β-aryl ether linkages cleavage in lignin due to the action of acetic acid released from xylan during this pretreatment [44]. In addition, the external catalyst (sulfuric acid) used together with ethanol in organosolv pretreatment also promotes the lignin depolymerization and solubilization [45]. The delignification degree (49%) observed for Ademuz elm clone at the highest severity, i.e., 60 min, 180 °C, 30% ethanol, and 1% H_2SO_4 is quite similar to the values observed by Martín-Sampedro et al. [43] and Jiménez-López et al. [40] for white poplar (46%) and black locust (50%) at the same pretreatment conditions. These values are higher compared to lignin extraction (27–42%) reported in different elm wood materials subjected to similar organosolv pretreatment parameters but with major ethanol concentration (75% *v/v*) [46,47]. Compared to the other pretreatment technologies analyzed in this study, the main benefit of organosolv technology is the high purity of solubilized lignin for its subsequent valorization [48]. Alkaline extraction also leads to lignin depolimerization and solubilization by aryl-ether linkages cleavage [49]. Noori and Karimi [37] assayed an alkaline pretreatment at 120 min, 80 °C, and 8% NaOH (*w/v*) on elm wood, observing a similar lignin extraction (22.6%) that the lignin removal observed for Ademuz elm clone at the highest severity, i.e., 60 min, 160 °C, and 10% NaOH (*w/v*). Finally, the capacity of lignin extraction by autohydrolysis pretreatment is more limited. Then, lignin extraction around 4.8% was achieved with Ademuz elm clone subjected to autohydrolysis at the highest pretreatment parameters, i.e., 180 °C and 60 min, a quantity significantly lower compared to the 16.9% of lignin extraction reported by Amiri and Karimi [29] with elm wood pretreated with autohydrolysis at equal severity (60 min at 180 °C).

On the other hand, the glucan extracted (Figure 5D) has been very similar for the tested pretreatments under the lowest temperature (T_1) with the exception of the acid hydrolysis pretreatment which has been significantly higher. In this way, between acid hydrolysis and the other pretreatments, F = 7 and $p = 0.027$ values have been found. As $p < 0.05$, we reject the null hypothesis that there is no significant difference between groups. In the studies carried out at the highest temperature (T_2), slight and non-significant increases in glucose extraction has been observed for the acid hydrolysis and alkaline extraction. Between (a) and (b) pretreatments, F = 13.5 and $p = 0.021312$ values have been obtained. As $p < 0.05$, we reject the null hypothesis that there is no significant difference between groups. However, a decrease in glucose extraction was observed for organosolv and authohydrolysis pretreatments when severity was increased. This fact could be due to glucose degradation, since the glucose removed during the evaluated pretreatments on Ademuz elm clone could be found in the pretreatment liquors in the form of monosaccharides, oligosaccharides or their degradation compounds (5-HMF) (Table 4). The highest glucose removal was obtained when Ademuz elm clone was subjected to acid hydrolysis (23.8%). Lower glucose removal (18%) was reported by Jiménez-López et al. [40] when black locust was exposed to acid hydrolysis at the same pretreatment parameters (60 min, 180 °C, 3% H_2SO_4). Ibarra et al. [33] also reported lower values for glucose extraction (10–12%) with different poplar clones pretreated with acid hydrolysis at equal pretreatment conditions but lower temperature (i.e., 130 °C).

Table 4. Sugars and degradation compounds (g/L) of the liquid fractions from the different pretreatments of Ademuz elm clone.

	Glucose	Xylose	Arabinose	Acetic Acid	5-HMF	Furfural
AH-160	3.70 ± 0.3 (2.90)	6.45 ± 0.6 (5.35)	0.70 ± 0.1 (0.30)	2.35 ± 0.3 (1.80)	0.10 ± 0.0	0.05 ± 0.0
AH-180	3.65 ± 0.3 (3.10)	3.20 ± 0.6 (1.85)	1.10 ± 0.0 (0.55)	1.00 ± 0.2 (0.00)	0.30 ± 0.1	0.40 ± 0.3
AcH-160	3.45 ± 0.4 (0.40)	5.55 ± 0.1 (0.50)	0.90 ± 0.0 (0.00)	1.85 ± 0.3 (0.10)	0.30 ± 0.1	0.30 ± 0.4
AcH-180	2.70 ± 0.0 (0.40)	3.10 ± 0.3 (0.30)	0.50 ± 0.1 (0.00)	1.55 ± 0.3 (0.10)	0.35 ± 0.1	0.90 ± 0.0
Org-160	2.75 ± 0.3 (2.0)	1.95 ± 0.4 (1.05)	0.60 ± 0.0 (0.20)	0.30 ± 0.3 (0.10)	0.05 ± 0.0	0.02 ± 0.0
Org-180	3.50 ± 0.3 (1.55)	6.35 ± 0.1 (3.60)	0.80 ± 0.2 (0.50)	0.70 ± 0.3 (0.00)	0.35 ± 0.1	0.35 ± 0.2
Alk-80	0.90 ± 0.0 (0.55)	0.75 ± 0.3 (0.25)	0.30 ± 0.1 (0.25)	2.30 ± 0.3 (0.10)	-	-
Alk-160	1.20 ± 0.5 (0.95)	1.30 ± 0.3 (0.95)	0.75 ± 0.0 (0.75)	2.55 ± 0.3 (0.20)	-	-

Autohydrolysis (AH) at 160 °C (AH-160) or 180 °C (AH-180); Acid hydrolysis (AcH) at 160 °C (AcH-160) or 180 °C (AcH-180); Organosolv (Org) at 160 °C (Org-160) or 180 °C (Org-180); Alkaline extraction (Alk) at 80 °C (Alk-80) or 160 °C (Alk-160). Oligomers and acetyl groups bound to oligosaccharides are given between parentheses. Mean values and standard deviations were calculated from the triplicates.

Significant differences for the extracted xylan with the different pretreatments are shown in Figure 5E. Hence, organosolv and alkaline extraction pretreatments showed the lowest extraction values. The acid hydrolysis pretreatment displayed significantly higher extractions at the lowest temperature (T_1). Therefore, for both pretreatments groups, F = 13.5 and $p = 0.0213$ values have been found. As $p < 0.05$, we reject the null hypothesis of no significant difference between groups. Additionally, the acid hydrolysis pretreatment did not substantially increase its extraction capacity at the highest temperature (T_2), while the organosolv pretreatment showed a larger significant extraction capacity as the temperature is being raised. Regarding arabinan, Figure 5F shows low significant differences for the different pretreatments at the lowest temperature (T_1). In that form, F = 13.5 and $p = 0.32$ as $p\ (x \leq F) = 0.676551$ values have been found. At the highest temperature (T_2) this fact has not been observed, yielding very similar values for this compound. In spite of the previously marked delignification observed by acid hydrolysis pretreatment on Ademuz elm clone, this technology is more effective on the solubilization of hemicelluloses by hydrolysis of glycosidic linkages [41]. Similar elimination of hemicellulose than that observed for Ademuz elm clone at the highest severity conditions (95% xylose removal at 60 min, 180 °C and 3% H_2SO_4) was described when white poplar (90% xylose elimination) and black locust (85% xylose removal) were subjected to acid hydrolysis with the same pretreatment conditions [40,43]. A complete hemicellulose removal was achieved by Foston and Ragauskas [50] with poplar at comparable pretreatment severity. Ibarra et al. [33] described hemicellulose elimination values between 76–87% with different poplar clones pretreated with acid hydrolysis at comparable conditions but lower temperature (i.e., 130 °C). The external catalyst acid used in organosolv pretreatment is also the main responsible for the high removal of hemicellulose (78% at the highest severity conditions, i.e., 60 min, 180 °C and 3% H_2SO_4) observed in Ademuz elm clone. Nevertheless, solvents employed in the organosolv process can also solubilize a part of sugars [41]. A lower hemicellulose elimination (62% for white poplar and 67% black locust) was described during organosolv process at equivalent pretreatment conditions [40,43]. Other studies with different elm clones also showed lower hemicellulose removal values with similar pretreatment parameters but major ethanol concentration (75% *v/v*) [46,47]. Hemicellulose is also extensively solubilized during autohydrolysis pretreatment by the random attack of hydronioum ions (from autoionization of water) to xylan [41]. At similar autohydrolysis pretreatment conditions (at the highest severity, i.e., 60 min at 180 °C), Amiri and Karimi [29] described

a lower hemicellulose removal (51%) with elm wood compared to the value reported for Ademuz elm clone (67%). In the same way, da Silva Morais [51] also solubilized lower quantities of hemicellulose, between 30% and 32%, when different species of eucalypt were subjected to autohydrolysis pretreatment at 170–190 °C. Finally, alkaline extraction in addition to removing lignin can produce a significant reduction in hemicellulose. In fact, Noori and Karimi [37] reported a higher hemicellulose removal (57% xylan elimination) when elm wood was pretreated with alkaline extraction at 120 min, 80 °C and 8% NaOH (*w/v*), compared to the hemicellulose removal (33% xylan elimination) of Ademuz elm clone with alkaline extraction at 60 min, 160 °C and 10% NaOH (*w/w*). Mirahmadi et al. [52] also described an important reduction in hemicellulose content in birch wood by alkali pretreatment with 7% NaOH (*w/w*) at different temperatures for 2 h. In the same way as glucose, the xylose eliminated during the evaluated pretreatments on Ademuz elm clone could be also recovered in the pretreatment liquors in the form of monosaccharides (especially during acid hydrolysis at 160 °C), oligosaccharides (mainly during autohydrolysis at 160 °C and organosolv at 180 °C), or their degradation compounds (acetic acid and furfural) (Table 4).

3.3. Production of Fermentable Sugars

As mentioned above, the potential of lignocellulosic biomass is based on its high carbohydrate content from which fermentable sugars are extracted to produce an extensive variety of biofuels and bioproducts [21]. For that, the enzymatic hydrolysis of lignocellulose is the desired choice for the extraction of fermentable sugars since it is a selective technology, it needs low energetic demands, and it does not generate inhibitory compounds [22]. Then, the pretreated materials (WIS fraction) resulting from the different pretreatments of Ademuz elm clone underwent enzymatic hydrolysis for 144 h (Figure 6), being compared to the enzymatic hydrolysis of the untreated material. The production of glucose, xylose, and total sugars at the end of enzymatic hydrolysis was used to calculate sugars digestibility and yield values (Table 5), being statistically analyzed according to the type of pretreatment and the temperatures at which they were carried out (Figure 7).

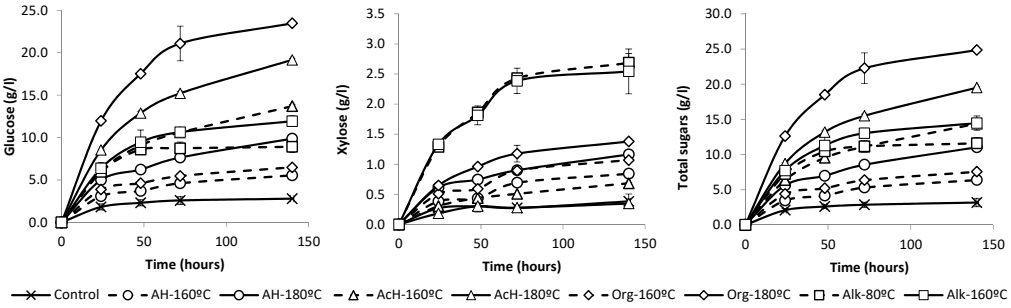

Figure 6. Time course of sugars production during enzymatic hydrolysis of the pretreated materials (WIS fraction) resulting from the different pretreatments of Ademuz elm clone. AH, autohydrolysis pretreatment; AcH, acid hydrolysis; Org, organosolv pretreatment; Alk, alkaline extraction. Mean values and standard deviations were calculated from the triplicates.

Table 5. Sugar production (g/L), yield (%) and digestibility (%) values of Ademuz elm clone (untreated) and their materials (WIS fraction) resulting from the different pretreatments at 144 h of enzymatic hydrolysis.

	Sugars (g/L)			Yield (%)			Digestibility (%)		
	G	X	T	G	X	T	G	X	T
Untreated	2.80	0.38	3.18	5.3	0.7	6.0	12.2	4.7	10.2
AH-160	5.55	0.84	6.39	7.5	1.1	8.7	18.0	17.0	17.9
AH-180	9.88	1.16	11.04	13.6	1.6	15.2	32.1	31.3	32.1
AcH-160	13.71	0.68	14.39	14.2	0.7	14.9	41.0	27.7	40.1
AcH-180	19.17	0.35	19.52	17.9	0.3	18.2	53.8	41.3	53.5
Org-160	6.49	1.07	7.56	9.4	1.5	10.9	23.5	12.1	20.7
Org-180	23.48	1.38	24.86	30.0	1.8	31.8	69.0	51.2	67.7
Alk-80	8.92	2.68	11.60	13.7	4.1	17.9	34.0	29.9	32.9
Alk-160	11.93	2.54	14.47	15.2	3.2	18.4	41.5	31.1	39.2

Autohydrolysis (AH) at 160 °C (AH-160) or 180 °C (AH-180); Acid hydrolysis (AcH) at 160 °C (AcH-160) or 180 °C (AcH-180); Organosolv (Org) at 160 °C (Org-160) or 180 °C (Org-180); Alkaline extraction (Alk) at 80 °C (Alk-80) or 160 °C (Alk-160). Oligomers and acetyl groups bound to oligosaccharides are given between parentheses. G: glucose; X: xylose; T: glucose + xylose.

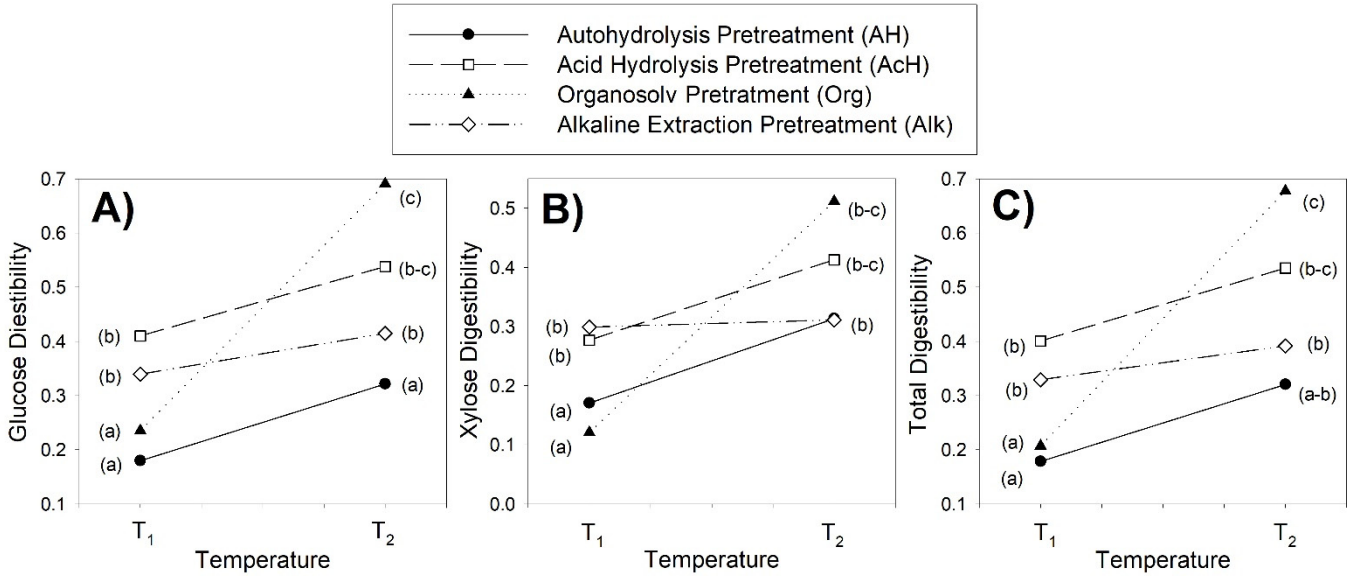

Figure 7. Analysis of digestibility of the different pretreated materials: (**A**) glucose digestibility; (**B**) xylose digestibility; and (**C**) total digestibility. T_1, pretreatment temperatures at 160 °C, except for alkaline extraction at 80 °C. T_2, pretreatment temperatures at 180 °C, except for alkaline extraction at 160 °C. Lines with the same letter for initial and final temperature are not significantly different (Tukey test, $p < 0.05$).

Only two statistically different groups have been found for glucose digestibility (Figure 7A) in the evaluated samples at the lowest temperature (T_1). A significant difference between groups has been found (F = 13.5 and $p = 0.76$). Hence the digestibility encountered for acid hydrolysis and alkaline extraction pretreatments were similar as well as the digestibility found for autohydrolysis and organosolv pretreatments. These trends have not been observed at the highest temperature (T_2). Only the organosolv pretreatment underwent a significant increase under the highest temperature ($p = 0.0031$ and p ($x \leq F$) = 0.9968). The rest of the pretreatment showed a slightly but no significant increase in digestibility when temperature raised.

A similar trend to that found in glucose digestibility data has been found for xylose (Figure 7B). Only organosolv and autohydrolysis pretreatments experienced a significant increase in xylose digestibility when temperature raised ($p = 0.586$ and p ($x \leq F$) = 0.413). The acid hydrolysis and alkaline extraction suffered only slight increases. Accordingly, the total digestibility (Figure 7C) also showed the same trend, being more important the

influence of glucose digestibility with respect to xylose due to its higher concentration. Therefore, the variations for total digestibility were similar to the variations found for glucose. The highest digestibility found (67.7%) was for organosolv pretreatment at 180 °C.

In general, the enhancement of glucose digestibility by the different analyzed pretreatments on Ademuz elm clone can be due to lignin and hemicellulose removal produced by them. As it is well-known, lignin constitutes a physical obstacle restricting the accessibility of hydrolytic enzymes and promoting their non-specific adsorption, decreasing the enzymes concentration during the enzymatic hydrolysis [23]. Furthermore, the hemicellulose removal by these pretreatment technologies is also associated with the enhancement of glucose digestibility [41], which is ascribed to an improved accessibility to cellulose and the breakdown of linkages between hemicellulose and cellulose. According to this, the major combined extraction of lignin and hemicellulose achieved during acid catalyzed organosolv (48.9% of lignin and 77.9% of hemicellulose) and dilute acid hydrolysis (39.2% of lignin and 95.0% of hemicellulose) pretreatments at the highest severity conditions, i.e., 180 °C, resulted in the major glucose digestibility values (69.0% for organosolv and 53.8% for acid hydrolysis) of Ademuz elm clone (Table 5). In the same way, the major glucose yield values were obtained for organosolv (30.0%) and acid hydrolysis (17.9%) pretreatments, producing 23.48 g/L and 19.17 g/L of glucose at the end of enzymatic hydrolysis, respectively (Table 5). Moreover, considering the glucose solubilized in the pretreatment liquors of organosolv (3.50 g/L) and acid hydrolysis (2.70 g/L) pretreatments (Table 4), the overall glucose production by organosolv pretreatment followed by enzymatic hydrolysis was increased to 42.0% glucose yield (Table 6), whereas for acid hydrolysis and the subsequent enzymatic hydrolysis was 27.2% glucose yield (Table 6). Several studies about glucose production by enzymatic hydrolysis from different hardwood materials pretreated with organosolv and acid hydrolysis have been described. Jiménez-López et al. [40] obtained slightly lower glucose digestibility and yield values than that described in this study for Ademuz elm clone when white poplar (65.5% and 25.8% for glucose digestibility and yield, respectively) and black locust (64.1% and 25.9% for glucose digestibility and yield, respectively) were subjected to organosolv pretreatment and enzymatic hydrolysis at the same pretreatment severities and enzyme dosages. It also resulted in glucose production being slightly lower (20.7 g/L for white poplar and 21.8 g/L for black locust). The same study also analyzed the influence of acid hydrolysis pretreatment on enzymatic hydrolysis at similar operational conditions, observing that glucose digestibility and yield for black locust (53.5% and 18.7%, respectively) were comparable to the values showed for Ademuz elm clone, resulting in 17.0 g/L of glucose at the end of enzymatic hydrolysis. However, when white poplar was used as raw material, rather high values for glucose digestibility and yield values (83.8% and 31.8%, respectively) were obtained compared to Ademuz elm clone, resulting in a final glucose production of 29.4 g/L. Amiri and Kamiri [46] described lower glucose yield (55.5%) and glucose production (15.6 g/L), when elm wood was subjected to organosolv pretreatment at the same conditions than those used herein but with higher ethanol concentration (75% *v/v*).

Table 6. Total sugars yield per gram of carbohydrates contained in raw material, total sugars yield per gram of raw material and total sugars yield per hectare/year of Ademuz elm clone.

	Total Sugars Yield Per Gram of Carbohydrates Contained in Raw Material (%) [1]		Total Sugars Yield Per Gram of Raw Material (%) [2]		Total Sugars Yield Per Hectare/Year (Mg DM/ha/Year)	
	G	X	G	X	G	X
Untreated	5.3	0.7	2.3	0.1	0.21 (0.21)	0.01 (0.01)
AH-160	20.3	63.4	8.8	9.9	0.30 (0.82)	0.02 (0.92)
AH-180	26.1	32.2	11.4	5.0	0.55 (1.06)	0.02 (0.46)
AcH-160	26.1	54.1	11.3	8.4	0.57 (1.05)	0.01 (0.78)
AcH-180	27.1	30.3	11.8	4.7	0.72 (1.09)	0.00 (0.43)
Org-160	18.8	20.4	8.2	3.2	0.37 (0.76)	0.02 (0.29)
Org-180	42.0	62.8	18.3	9.8	1.22 (1.70)	0.02 (0.91)
Alk-80	16.8	11.3	7.3	1.8	0.55 (0.67)	0.06 (0.16)
Alk-160	19.3	15.8	8.4	2.5	0.62 (0.78)	0.05 (0.23)

Autohydrolysis (AH) at 160 °C (AH-160) or 180 °C (AH-180); Acid hydrolysis (AcH) at 160 °C (AcH-160) or 180 °C (AcH-180); Organosolv (Org) at 160 °C (Org-160) or 180 °C (Org-180); Alkaline extraction (Alk) at 80 °C (Alk-80) or 160 °C (Alk-160). Oligomers and acetyl groups bound to oligosaccharides are given between parentheses. Total sugars yield (%): percentage of sugars converted during the enzymatic hydrolysis together with the sugars solubilized in pretreatment liquors expressed per gram of carbohydrates in raw material [1] and expressed per gram of raw material [2]. Total sugars yield per hectare/year (Mg DM/ha/year): percentage of sugars produced during the enzymatic hydrolysis (together with the sugars solubilized in pretreatment liquors between parenthesis) taking into account the biomass production (9.3 Mg DM/ha/year) of Ademuz elm clone. G: glucose; X: xylose.

Contrarily, lower combined extraction of lignin and hemicellulose were produced during autohydrolysis (4.8% of lignin and 67.2% of hemicellulose) and alkaline extraction (22.6% of lignin and 33.1% of hemicellulose) even at the highest severity conditions (i.e., 180 °C and 160 °C, respectively), resulting in lower glucose digestibility values (32.1% for autohydrolysis and 41.5% for alkaline extraction) of Ademuz elm clone (Table 5). Similarly, lower glucose yield values were obtained for autohydrolysis (13.6%) and alkaline extraction (15.2%), generating 9.88 g/L and 11.93 g/L of glucose at the end of enzymatic hydrolysis, respectively. Taking into account the glucose solubilized in the pretreatment liquors of autohydrolysis (3.65 g/L) and alkaline extraction (1.20 g/L) pretreatments (Table 4), the overall glucose production by both pretreatments followed by enzymatic hydrolysis was 27.1% and 19.3% glucose yield for autohydrolysis and alkaline extraction, respectively (Table 6). Amiri and Kamiri [29] reported a higher glucose yield (40%) and glucose production (12.5 g/L) when elm wood was subjected to autohydrolysis (60 min and 180 °C) and subsequently hydrolyzed with higher enzymes dosages (25 FPU cellulose and 40 IU β-glucosidase per g of substrate). Moreover, the overall glucose production was increased to 15 g/L in view of the glucose solubilized during autohydrolysis. Noori and Karimi [37] described a rather high glucose yield (79.8%) when elm wood was subjected to alkaline extraction at 120 min, 80 °C and 8% NaOH (*w/v*) followed by enzymatic hydrolysis (30 FPU cellulose and 60 IU β-glucosidase per g of substrate).

In the same way as glucose digestibility, xylose digestibility was also higher for Ademuz elm clone pretreated with organosolv and acid hydrolysis at 180 °C (51.2% and 41.3%, respectively); whereas lower xylose digestibility values (around 31%) were obtained with autohydrolysis (180 °C) and alkaline extraction (160 °C) (Table 5). Nevertheless, xylose production at the end of enzymatic hydrolysis was very low in all pretreated materials (Table 5). This fact is mainly due to the minor hemicellulose amount existing in these pretreated materials (Table 2) as well as the scarce xylanase activity contained in the hydrolytic enzymes used in this study. However, in view of the xylose solubilized during the different pretreatments (Table 4), the overall xylose production increased in those pretreatments with the greatest ability to extract hemicelluloses under optimal conditions that do not lead to its degradation. Then, xylose production yield of 63.4% for autohydrolysis at 160 °C, 54.1% for acid hydrolysis at 160 °C and 62.8% for organosolv pretreatment at 180 °C were obtained (Table 6). Jiménez-López et al. [40] also described similar xylose digestibility values when white poplar and black locust were subjected to organosolv and acid hydrolysis pretreatments at the same severity conditions (46.8% and 57.6% xylose digestibility for

white poplar pretreated with organosolv and acid hydrolysis, respectively; and 41.7% and 43.9% xylose digestibility for black locust pretreated with organosolv and acid hydrolysis, respectively). Nevertheless, xylose production also resulted in very low concentrations at the end of enzymatic hydrolysis, being augmented with these xylose productions in view of the xylose solubilized in the pretreatment liquors.

Taking into account the biomass production previously estimated (Section 3.1), i.e., a maximum yield of trunk biomass of 9.3 Mg DM/ha/year reached in Madrid elm trees, the highest glucose yield production per hectare was observed when Ademuz elm clone was pretreated with organosolv process at 180 °C followed by enzymatic hydrolysis (1.22 Mg DM/ha/year). Nevertheless, this value increased to 1.70 Mg DM/ha/year, taking into account the glucose solubilized in the liquid fraction of organosolv pretreatment (Table 6). Acid hydrolysis at 180 °C and subsequent enzymatic hydrolysis resulted in a glucose yield production per hectare of 0.72 Mg DM/ha/ year, increasing to 1.10 Mg DM/ha/year with the glucose solubilized in the liquid fraction of acid hydrolysis pretreatment. Alkaline extraction at 160 °C followed by enzymatic hydrolysis resulted in a glucose yield production of 0.62 Mg DM/ha/year (0.78 Mg DM/ha/year taking into account the glucose solubilized during alkaline extraction). Finally, autohydrolysis at 180 °C and subsequent enzymatic hydrolysis produced 0.55 Mg DM/ha/year, increasing to 1.06 Mg DM/ha/year with the glucose solubilized during autohydrolysis pretreatment. Regarding xylose yield production, taking into account the xylose produced during enzymatic hydrolysis of pretreated material and the amount of xylose solubilized during the corresponding pretreatment, the highest xylose yield production per hectare was achieved for both organosolv at 180 °C and autohydrolysis at 160 °C (0.92 Mg DM/ha/year). Nevertheless, glucose and xylose yield production values per hectare from Ademuz elm clone could increase, taking into account the total biomass production estimated, i.e., 11.7 tons of Mg DM/ha/year including the main stem, the secondary branches and the leaves. Therefore, Ademuz elm clone can be used as a possible source for fermentable sugars production using different pretreatment technologies according to the type of sugar desired and its subsequent valorization route (e.g., glucose for 3-hydroxypropionic acid, succinic acid, lactic acid, L-lysine, butanol, and polyhydroxyalkanoates, among others; xylose for itaconic acid and xylitol; and both glucose and xylose for bioethanol). From the different pretreatments evaluated, acid-catalyzed ethanol/water organosolv process at 180 °C, 60 min, 30% ethanol (*v/v*), 1% H_2SO_4 (*w/w*) followed by enzymatic hydrolysis makes it possible to obtain the highest glucose and xylose production from Ademuz elm clone. Moreover, the main benefit of organosolv pretreatment compared to other pretreatments evaluated is the high purity of solubilized lignin for its subsequent valorization in an extensive variety of high added-value compounds (e.g., aromatic chemicals, resins, polymers and composites, carbon fibers), making the production of fermentable sugars more sustainable and competitive.

Author Contributions: Conceptualization, D.I., R.M.-S., J.A.M. and M.E.E.; methodology, D.I., R.M.-S., J.A.M. and M.E.E.; formal analysis, R.M.-S., L.J.-L., J.A.M. and M.J.D.; investigation, D.I., R.M.-S., L.J.-L., J.A.M., M.J.D. and M.E.E.; data curation, R.M.-S., L.J.-L., J.A.M. and M.J.D.; writing—original draft preparation, D.I., J.A.M., M.J.D. and M.E.E.; writing—review and editing, D.I., R.M.-S., J.A.M. and M.E.E.; supervision, D.I. and M.E.E.; project administration, D.I., J.A.M., M.J.D. and M.E.E.; funding acquisition, D.I., J.A.M., M.J.D. and M.E.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Comunidad de Madrid and MCIU/AEI/FEDER, UE via grant number SUSTEC-CM S2018/EMT-4348 and RTI2018-096080-B-C22, respectively. Raquel Martín-Sampedro acknowledges an IJCI contract (IJCI-2016-28403).

Acknowledgments: We thank the personnel at Puerta de Hierro Forest Breeding Centre (Ministerio para la Transición Ecológica y el Reto Demográfico) and the Spanish Elm Breeding Program for providing the elm material.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. De Jong, E.; Higson, A.; Walsh, P.; Wellisch, M. *Bio-Based Chemicals Value Added Products from Biorefineries*; IEA Bioenergy: Paris, France, 2012; Available online: https://www.ieabioenergy.com/wp-content/uploads/2013/10/Task-42-Biobased-Chemicals-value-added-products-from-biorefineries.pdf (accessed on 1 December 2020).
2. Arevalo-Gallegos, A.; Ahmad, Z.; Asgher, M.; Parra-Saldivar, R.; Iqbal, H.M.N. Lignocellulose: A sustainable material to produce value-added products with a zero waste approach—A review. *Int. J. Biol. Macromol.* **2017**, *99*, 308–318. [CrossRef]
3. Hall, J.P. Sustainable production of forest biomass for energy. *For. Chron.* **2002**, *78*, 391–396. [CrossRef]
4. García-Morote, A.; López-Serrano, F.R.; Martínez-García, E.; Andres-Abellan, M.; Dadi, T.; Candel, D.; Rubio, E.; Lucas-Borja, M.E. Stem Biomass Production of *Paulownia elongata x P. fortunei* under Low Irrigation in a Semi-Arid Environment. *Forests.* **2014**, *5*, 2505–2520. [CrossRef]
5. Ghezehei, S.B.; Wright, J.; Zalesny, R.S., Jr.; Nichols, E.G.; Hazel, D.W. Matching site-suitable poplars to rotation length for optimized productivity. *For. Ecol. Manag.* **2020**, *457*, 117670. [CrossRef]
6. Rodríguez-García, A.; Martín, J.A.; Lopez, R.; Mutke, S.; Pinillos, F.; Gil, L. Influence of climate variables on resin yield and secretory structures in tapped Pinus pinaster Ait. in central Spain. *Agric. For. Meteorol.* **2015**, *202*, 83–93. [CrossRef]
7. Fernández, M.; Alaejos, J.; Andivia, E.; Vázquez-Piqué, J.; Ruíz, F.; López, F.; Tapias, R. Eucalyptus x urograndis biomass production for energy purposes exposed to a Mediterranean climate under different irrigation and fertilisation regimes. *Biomass Bioenergy* **2018**, *111*, 22–30. [CrossRef]
8. Mehmood, M.A.; Ibrahim, M.; Rashid, U.; Nawaz, M.; Ali, S.; Hussain, A.; Gull, M. Biomass production for bioenergy using marginal lands. *Sustain. Prod. Consum.* **2017**, *9*, 3–21. [CrossRef]
9. Fernández, M.J.; Barro, R.; Pérez, J.; Ciria, P. Production and composition of biomass from short rotation coppice in marginal land: A 9-year study. *Biomass Bioenergy* **2020**, *134*, 105478. [CrossRef]
10. IPCC. AR5 Synthesis Report: Climate Change 2014. Geneva (Switzerland). Available online: https://www.ipcc.ch/report/ar5/syr/ (accessed on 1 December 2020).
11. Martínez de Arano, I.; Muys, B.; Topi, C.; Pettenella, D.; Feliciano, D.; Rigilot, E.; Lefevre, F.; Prokofiva, I.; Labidi, J.; Carnus, J.-M.; et al. *A Forest-Based Circular Bioeconomy for Southern Europe: Visions, Opportunities and Challenges*; European Forest Institute: Joensuu, Finland, 2018; p. 124. Available online: https://espas.secure.europarl.europa.eu/orbis/sites/default/files/generated/document/en/EFI-Reflections%20on%20the%20bioeconomy%20-%20Synthesis%20Report%202018%20%28web%29_0.pdf (accessed on 1 December 2020).
12. Wang, C.W. *The Forests of China, with a Survey of Grassland and Dessert Vegetation*; Harvard University: Cambridge, MA, USA, 1961. [CrossRef]
13. Martín, J.A.; Sobrino-Plata, J.; Rodríguez-Calcerrada, J.; Collada, C.; Gil, L. Breeding and scientific advances in the fight against Dutch elm disease—Will they allow the use of elms in forest restoration? *New For.* **2019**, *50*, 183–215. [CrossRef]
14. Pérez-García, I. Evaluación de Ulmus Pumila L. y Populus Spp. Como Cultivos Energéticos en Corta Rotación. Ph.D. Thesis, E.T.S.I. Agrónomos. Universidad Politécnica de Madrid, Madrid, Spain, 2016. [CrossRef]
15. Solla, A.; Martín, J.A.; Corral, P.; Gil, L. Seasonal changes in wood formation of *Ulmus pumila* and *Ulmus minor* and its relation with Dutch elm disease. *New Phytol.* **2005**, *166*, 1025–1034. [CrossRef]
16. Martín, J.A.; Solla, A.; Venturas, M.; Collada, C.; Domínguez, J.; Miranda, E.; Fuentes, P.; Burón, M.; Iglesias, S.; Gil, L. Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. *iForest.* **2015**, *8*, 172–180. [CrossRef]
17. Martínez-Arias, C.; Sobrino-Plata, J.; Ormeño-Moncalvillo, S.; Gil, L.; Rodríguez-Calcerrada, J.; Martín, J.A. Endophyte inoculation enhances Ulmus minor resistance to Dutch elm disease. *Fungal Ecol.* **2015**, *50*, 101024. [CrossRef]
18. Cogolludo-Agustín, M.A.; Agundez, D.; Gil, L. Identification of native and hybrid elms in Spain using isozyme gene markers. *Heredity.* **2000**, *85*, 157–166. [CrossRef] [PubMed]
19. Brännvall, E. Overview of pulp and paper processes. In *Pulp and Paper Chemistry Technology*; Ek, M., Gellerstedt, G., Henriksson, G., Eds.; De Gruyter: Berlin, Germany, 2009; pp. 1–12. [CrossRef]
20. Li, T.; Chen, C.; Brozena, A.H.; Zhu, J.Y.; Xu, L.; Driemeier, C.; Carlos, D.; Dai, J.; Rojas, O.J.; Isogai, A.; et al. Developing fibrillated cellulose as a sustainable technological material. *Nature* **2021**, *590*, 47–56. [CrossRef]
21. Susmozas, A.; Martín-Sampedro, R.; Ibarra, D.; Eugenio, M.E.; Iglesias, R.; Manzanares, P.; Moreno, A.D. Process Strategies for the Transition of 1G to Advanced Bioethanol Production. *Processes* **2020**, *8*, 1310. [CrossRef]
22. Alvira, P.; Ballesteros, M.; Negro, M.J. Progress on enzymatic saccharification technologies for biofuels production. In *Biofuel Technologies*; Gupta, V., Tuohy, M., Eds.; Springer: Berlin, Germany, 2013; pp. 145–169. [CrossRef]
23. Martín-Sampedro, R.; Rahikainen, J.L.; Johansson, L.S.; Marjamaa, K.; Laine, J.; Kruus, K.; Rojas, O.J. Preferential adsorption and activity of monocomponent cellulases on lignocellulose thin films with varying lignin content. *Biomacromol* **2013**, *14*, 1231–1239. [CrossRef]
24. Rahikainen, J.L.; Martín-Sampedro, R.; Heikkinen, H.; Rovio, S.; Marjamaa, K.; Tamminen, T.; Rojas, O.J.; Kruus, K. Inhibitory effect of lignin during cellulose bioconversion: The effect of lignin chemistry on non-productive enzyme adsorption. *Bioresour. Technol.* **2013**, *133*, 270–278. [CrossRef]
25. Alvira, P.; Pejó-Tomás, E.; Ballesteros, M.; Negro, M.J. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* **2010**, *101*, 4851–4861. [CrossRef] [PubMed]

26. Zhao, X.; Cheng, K.; Liu, D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 815–827. [CrossRef]
27. Taherzadeh, M.J.; Karimi, K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int. J. Mol. Sci.* **2008**, *9*, 1621–1651. [CrossRef]
28. Wyman, C.E. *Handbook on Bioethanol: Production and Utilization*; Taylor Francis: Washington, DC, USA, 1996; p. 417.
29. Amiri, H.; Kamiri, K. Autohydrolysis: A promising pretreatment for the improvement of acetone, butanol, and ethanol production from woody materials. *Chem. Eng. Sci.* **2015**, *137*, 722–729. [CrossRef]
30. Jiménez-López, L.; Eugenio, M.E.; Ibarra, D.; Darder, M.; Martín, J.A.; Martín-Sampedro, R. Cellulose Nanofibers from a Dutch Elm Disease-Resistant *Ulmus minor* Clone. *Polymers* **2020**, *12*, 2450. [CrossRef]
31. Martín-Sampedro, R.; Eugenio, M.E.; Fillat, U.; Martín, J.A.; Aranda, P.; Ruiz-Hitzky, E.; Ibarra, D.; Wicklein, B. Biorefinery of lignocellulosic biomass from an elm clone: Production of fermentable sugars and lignin-derived biochar for energy and environmental applications. *Energy Technol.* **2019**, *7*, 277–287. [CrossRef]
32. Hacke, U.G.; Sperry, J.S.; Pittermann, J. Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. *Basic Appl. Ecol.* **2000**, *1*, 31–41. [CrossRef]
33. Ibarra, D.; Eugenio, M.E.; Cañellas, I.; Sixto, H.; Martín-Sampedro, R. Potential of different poplar clones for sugar production. *Wood Sci. Technol.* **2017**, *51*, 669–684. [CrossRef]
34. National Renewable Energy Laboratory, NREL. *Chem. Anal. Test. Lab. Anal. Proced.* **2010**. Available online: http://www.eere.energy.gov/biomass/analyticalprocedures.html (accessed on 23 February 2021).
35. Das, A. The effect of size and competition on tree growth rate in old-growth coniferous forests. *Can. J. Forest Res.* **2012**, *42*, 1983–1995. [CrossRef]
36. Pérez-Cruzado, C.; Sanchez-Ron, D.; Rodríguez-Soalleiro, R.; Hernández, M.J.; Sánchez-Martín, M.M.; Cañellas, I.; Sixto, H. Biomass production assessment from Populus spp. short-rotation irrigated crops in Spain. *GCB Bioenergy* **2014**, *6*, 312–326. [CrossRef]
37. Noori, M.S.; Karimi, K. Detailed study of efficient ethanol production from Elmwood by alkali pretreatment. *Biochem. Eng. J.* **2016**, *105*, 197–204. [CrossRef]
38. Pauly, M.; Keegstra, K. Plant cell wall polymers as precursors for biofuels. *Curr. Opin. Plant Biol.* **2010**, *13*, 305–312. [CrossRef]
39. Ballesteros, M.; Oliva, J.M.; Negro, M.J.; Manzanares, P.; Ballesteros, I. Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces marxianus CECT 10875. *Process Biochem.* **2004**, *39*, 1843–1849. [CrossRef]
40. Jiménez-López, L.; Martín-Sampedro, R.; Eugenio, M.E.; Santos, J.I.; Sixto, H.; Cañellas, I.; Ibarra, D. Co-Production of soluble sugars and lignin from short rotation white poplar and black locust crops. *Wood Sci. Technol.* **2020**, *54*, 1617–1643. [CrossRef]
41. Moreno, A.D.; Olsson, L. Pretreatment of Lignocellulosic Feedstocks. In *Extremophilic Enzymatic Processing of Lignocellulosic Feedstocks to Bioenergy*; Sani, R., Krishnaraj, R., Eds.; Springer International Publishing AG: New York, NY, USA, 2017; pp. 31–52. [CrossRef]
42. Zhang, L.; Yan, Z.; Wang, D.D.; Laskar, M.S.; Swita, J.R.; Cort, J.R.; Yang, B. Characterization of lignin derived from water-only and dilute acid flowthrough pretreatment of poplar wood at elevated temperatures. *Biotechnol. Biofuels* **2015**, *8*, 203. [CrossRef] [PubMed]
43. Martín-Sampedro, R.; Santos, J.I.; Fillat, Ú.; Wicklein, B.; Eugenio, M.E.; Ibarra, D. Characterization of lignins from *Populus alba* L. generated as by-products in different transformation processes: Kraft pulping, organosolv and acid hydrolysis. *Int. J. Biol. Macromol.* **2019**, *126*, 18–29. [CrossRef]
44. Guo, Y.; Zhou, J.; Wen, J.; Sun, G.; Sun, Y. Structural transformation of triploid of *Populus tomentosa* Carr. lignin during auto-catalyzed etanol organosolv pretreatment. *Ind. Crops Prod.* **2015**, *76*, 522–529. [CrossRef]
45. Hage, R.E.; Brosse, N.; Sannigrahi, P.; Ragauskas. Effect of process severity on the chemical structure of mischanthus ethanol organosolv lignin. *Polym. Degrad. Stab.* **2010**, *95*, 997–1003. [CrossRef]
46. Amiri, H.; Kamiri, K. Improvement of acetone, butanol, and ethanol production from woody biomass using organosolv pretreatment. *Bioprocess Biosyst. Eng.* **2015**, *38*, 1959–1972. [CrossRef]
47. Mirmohamadsadeghi, S.; Karimi, K.; Zamani, A.; Amiri, H.; Horváth, I.S. Enhanced solid-state biogás production from lignocellulosic biomass by organosolv pretreatment. *Biomed Res. Int.* **2014**, *2014*, 350414. [CrossRef]
48. Martín-Sampedro, R.; Santos, J.I.; Fillat, Ú.; Eugenio, M.E.; Wicklein, B.; Jiménez-López, L.; Ibarra, D. Chemical and thermal analysis of lignin streams from *Robinia pseudoacacia* L. generated during organosolv and acid hydrolysis pre-treatments and subsequent enzymatic hydrolysis. *Int. J. Biol. Macromol.* **2019**, *140*, 311–322. [CrossRef]
49. Santos, J.I.; Fillat, Ú.; Martín-Sampedro, R.; Eugenio, M.E.; Negro, M.J.; Ballesteros, I.; Rodríguez, A.; Ibarra, D. Evaluation of lignins from Side-Streams generated in an olive tree Pruning-Based biorefinery: Bioethanol production and alkaline pulping. *Int. J. Biol. Macromol.* **2017**, *105*, 238–251. [CrossRef]
50. Foston, M.; Ragauskas, A.J. Changes in lignocellulosic supramolecular and ultrastructure during dilute acid pretreatment of Populus and switchgrass. *Biomass Bioenergy* **2010**, *34*, 1885–1895. [CrossRef]

51. da Silva Morais, A.P.; Sansígolo, C.A.; de Oliveira Neto, M. Effects of autohydrolysis of *Eucalyptus urograndis* and *Eucalyptus grandis* on influence of chemical components and crystallinity index. *Bioresour. Technol.* **2016**, *214*, 623–628. [CrossRef] [PubMed]
52. Mirahmadi, K.; Kabir, M.M.; Jeihanipour, K.; Karimi, K.; Taherzadeh, M.J. Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production. *Bioresources* **2010**, *5*, 928–938.