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ORIGINAL

Optimisation study of *Ulmus pumila* woody biomass fractionation by steam explosion for bioproducts production

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Abstract

Ulmus pumila represents a promising lignocellulosic biomass source for biofuels and bioproducts production since it can grow in low rainfall and extreme temperature zones. A first step in the conversion process is biomass fractionation to enhance the performance of the hydrolysis and subsequent biological conversion steps. The aim of this work is to optimise the main variables (temperature, residence time and the addition or not of sulphuric acid) of steam explosion to pretreat *Ulmus pumila* biomass. The optimal conditions to maximise both glucose and xylose recovery were 204.8 °C and 30 mg H_2SO_4/g biomass, obtained through a multilevel factorial design of experiments. Additionally, enzymatic hydrolysis using high solid loads (15% and 20% (w/w)) and different enzyme doses was studied. As a result, steam explosion at optimal conditions followed by enzymatic hydrolysis with 20% solid loading and 60 mg protein/g cellulose of enzyme allow the recovery of 70% of the potential sugars.

Introduction

Nowadays, fossil fuel shortages, environmental concerns, increasing energy demand and energy security have driven the transition from a fossil-based economy towards a bio-based economy. In this context, biomass has a key role to play in this essential transition. Sustainably sourced biomass, and its efficient use in energy applications (bioenergy), are of vital importance for any climate mitigation strategy and for boosting energy security and price stability at a global level. According to REN statistics (Ren21 2022), bioenergy use reached 12.3% of worldwide total final energy consumption (TFEC), with traditional use of biomass for cooking and heating in developing and emerging countries (6.7% of TFEC) being the main contributor. The

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non-traditional or modern bioenergy (supplying heat for industry and buildings, electricity and biofuels for transport) reached 5.6% of TFEC. In terms of renewable energy consumption, bioenergy accounted for 47% of global final energy consumption in 2020. In spite of these significant figures, it will definitely be necessary to expand the use of sustainable bioenergy to meet the increasing demand for renewable energy and also to achieve greater energy independence.

Besides bioenergy, the transition towards a bio-based economy is reflected in the growing demand for bio-based products, such as chemicals and plastics, pharmaceuticals, paper and paper products, and textiles. The co-production of these bioproducts together with biofuels and heat is the basis of the biorefinery concept, which integrates bioenergy applications and the production of other marketable products of interest from the same source of biomass (Lindorfer et al. 2019). In this context, the use of lignocellulosic biomass (LB) can play an important role in the production of sustainable energy and valuable products through biological processes since it presents significant advantages compared to other types of biomass: wide availability, high variety of sources (cultivated and residues of different origins) and a valuable composition in terms of major and minor components (carbohydrates, lignin, extractives, etc.).

A critical factor in the large-scale implementation of lignocellulose-based industries (biorefineries) is having a secure and sustainable supply of LB, while avoiding competition with the use of land for food production. The European Union has made a strong effort to define a series of sustainability and GHG emission criteria in the Renewable Energy Directive II (RED II) (Eu 2018) that biofuels, bioliquids and biomass fuels must fulfil to be considered within the national renewable energy targets for each country by 2030 (the overall EU target for renewable energy sources consumption by 2030 of 32%; specifically for the road and rail transport this value should be a minimum of 14%). Moreover, RED II lists a series of non-food feedstock for the production of advanced biofuels such as agricultural and agro-industrial residues, forestry residues and other lignocellulosic materials. A possibility of increasing interest for the production of these types of non-waste feedstock is based on the cultivation of LB on abandoned lands, contributing to rural development and reducing energy dependence. The restoration of these lands for biomass energy crops will contribute to expanding the cultivable area in a specific locale, without needing to use traditionally cultivated areas. Besides, these new cultivated areas could also contribute to diminishing some of the detrimental effects that the use of biomass exerts on traditional forest system, such as the decrease in soil fertility produced by nutrient extraction in whole-tree harvesting, as well as the modification of habitat and biodiversity in easily damaged sites (Alesso et al. 2021).

Short-rotation woody crops have several intrinsic logistic benefits and economic advantages compared to other lignocellulosic energy crops. Trees cultivated in short rotation can typically be harvested year-round and continue growing year after year providing a long-term source of available biomass. Although there are a great variety of woody species that could be envisaged as potential energy crops, not all have been grown and studied in a short rotation regime. Woody species such as poplar and willow are probably the most short-rotation crops studied (Pažitný et al. 2020), but they need plenty of water to grow and would not be adequate for marginal lands with low water availability during the summer season. For these reasons, alternative fast-growing species with lower water requirements such as *Ulmus pumila* (UP) (Siberian elm) are being considered. UP trees can grow in zones with average rainfall less than 400 mm and extreme temperatures, and so, they are well adapted to inland areas of Spain.

However, for the UP biomass to be considered an adequate feedstock for biorefining, the first step to be addressed is its pretreatment, aimed at breaking up the structure of the biomass fibres. The lignocellulosic nature of woody biomass, made up of carbohydrate polymers and lignin tightly interwoven in a complex structure, makes the pretreatment a necessary step prior to the hydrolysis of carbohydrates. The pretreatment required for this intricate and recalcitrant biomass structural matrix usually represents one of the most costly and energy consuming steps of the whole biomass conversion process. Among the different pretreatment technologies already developed and tested, steam explosion (SE) is a well-known technique that has been proven to break down the biomass fibres effectively and promote carbohydrate hydrolysis in a large variety of lignocellulosic substrates (Bonfiglio et al. 2019; Brenelli et al. 2022; Duque et al. 2016; Moreno et al. 2019; Pažitný et al. 2022). SE uses saturated steam at high pressure, injected into a batch or continuous reactor where lignocellulosic biomass has been previously placed, for a short (a few minutes) time. It is considered a very promising technique for a biomass biorefinery due to its low environmental impact, moderate energy consumption and demonstrated potential to be used at industrial scale (Moreno et al. 2019; Ziegler-Devin et al. 2021). SE has been reported to cause particle size reduction, increase in surface area and porosity, changes in lignin structure, partial depolymerisation and solubilisation of hemicelluloses and a marked enhancement of enzymatic digestibility of pretreated substrates (Hassan et al. 2018; Malik et al. 2022; Ruiz et al. 2020). On the other hand, it has been shown that SE can augment the efficiency of the process in certain biomasses by using catalysts such as sulphuric acid, sulphur dioxide, phosphoric acid and carbon dioxide (Fockink et al. 2018). In the present study, the addition of sulphuric acid as catalyst is evaluated.

Many investigations on SE as a pretreatment for LB have been carried out in recent decades under different process conditions and scales. Among woody biomass species, Asada et al. (2018) proposed a complete biorefinering strategy for poplar biomass by steam explosion (2 L batch system) followed by extraction and separation processes to obtain cellulose nanofibers, epoxy resin from lignin and high added value products from the water extract. Also in poplar biomass, Tang et al. (2018) carried out a study of the effect of SE pretreatment using a 2L reactor under different SE strategies (autohydrolysis, deacetylation with NaOH, SO_2, acidic sulphonation and neutral sulphonation) on the subsequent pelletising of pretreated material. The authors analysed pellet durability and the enzymatic digestibility of pelletised biomasses and found different responses in relation to the potential thermochemical or biochemical application of pellets. At a larger scale (10 L semicontinuous reactor), Cebreiros et al. (2021) have reported on the SE pretreatment of eucalyptus biomass for xylooligosccharides and butanol production. The authors highlight the good performance of SE that results in a reasonably high xylooligosaccharide recovery of 50% and even higher values of enzymatic hydrolysis (EH) yield (80%).

Regarding the pretreatment of elm biomass, little work has been reported in the literature. For instance, Ibarra et al. (2021) compared different pretreatments, specifically autohydrolysis, dilute acid hydrolysis, acid catalysed organosolv, and alkaline extraction. But no references regarding the pretreatment of UP biomass by SE have been found in the literature.

This study helps to fill this gap in the literature by investigating the effectiveness of the SE process in pretreating UP biomass in order to identify the conditions leading to the maximum recovery of sugars from carbohydrates, which may be used in a later step of biological conversion. The effects of temperature, residence time and the addition or not of sulphuric acid as a catalyst on pretreatment effectiveness are tested in a batch, pilot scale SE plant. Moreover, the release of sugars by EH of the steam exploded UP biomass is studied in laboratory-scale experiments using commercial enzymes. A relevant contribution of this study to the state-of-the-art is that the EH experiments are carried out under high substrate load conditions, mimicking conditions closer to those of a large-scale process. This is valuable information for establishing the feasibility of producing a certain bioproduct from the sugar stream derived from the pretreated biomass, since it allows envisaging the final bioproduct production yield that could be obtained.

Materials and methods

Raw material

Ulmus pumila (UP) biomass was provided by the Centre for the Development of Renewable Energy Sources (CEDER, Soria, Spain). The biomass was crushed to a particle size of about 8 mm and had a moisture content of 12.3%. The biomass was stored and used as it was delivered.

Material characterisation

The chemical composition of the UP biomass and the water insoluble solids (WIS) fraction of SE pretreated materials was determined through the methods for analysing biomass described by Sluiter et al. (2010). These methods allow quantifying the main components (cellulose, hemicellulose, lignin, extractives, and ash) of LB samples. The quantity of structural sugars, cellulose and hemicellulose, was obtained by taking into account the amount of monomers solubilised after the acid hydrolysis stage in two steps, first with 72% (w/w) sulphuric acid (30 °C, 60 min) and then at 4% (w/w) sulphuric acid (121 °C, 60 min). The sugar content of the hydrolysis liquors was measured by high performance liquid chromatography (HPLC) as described below in point 2.6. The insoluble solid remaining unaltered after acid hydrolysis corresponds to acid-insoluble lignin, whereas the acid-soluble lignin content was evaluated by ultraviolet–visible spectroscopy. The ash content was also determined as the solid remaining after combustion at 550 °C.

Steam explosion pretreatment: main SE operating conditions

The main variables in the SE process studied in the present work are temperature and residence time. Also, the effectiveness of the addition of an acid catalyst during SE is evaluated. To determine the operation conditions that have the greatest impact on the effectiveness of SE pretreatment, a series of SE trials were carried out as follows: (i) as a starting point some experiments of autohydrolysis under different temperatures (200 °C, 210 °C and 220 °) and at a fixed residence time of 5 min to determine whether autohydrolysis is effective in the fractionation of the raw UP or if the addition of an acid catalyst is necessary as reported in the literature (Semwal et al. 2019); (ii) experiments with an acid catalyst concentration of 20 mg of H_2SO_4 /g dry biomass, at 5 and 7.5 min residence time to evaluate the effect of residence time under acidic conditions (in this case, 190 and 200 ° were tested); and (iii) experiments with 20 and 30 mg of H_2SO_4/g dry biomass at 190, 200 and 210 °C, at a fixed residence time of 5 min, to evaluate the effect of temperature under acidic conditions. This last set of experiments was performed according to a design of experiments (DOE) model.

All SE pretreatment trials were performed in a 2L SE pilot unit based on Mansonite technology. After each SE trial, the material was recovered in a cyclone and the wet material (slurry) was cooled to about 40 °C and filtered to recover two fractions: (i) the water insoluble solids fraction (WIS) and (ii) the filtrate or prehydrolysate (PH). After separating, the WIS fraction was thoroughly washed with water and dried at 45 °C for storage. The WIS fraction was analysed for carbohydrates and acid-insoluble lignin content as described in the section "Enzymatic hydrolysis at standard conditions". The PH fraction was analysed for its content of monomeric and oligomeric sugars by HPLC as described in section "Enzymatic hydrolysis at high solid loading". Next, each WIS fraction generated was submitted to EH tests under standard conditions (see below) to determine the enzymatic digestibility of the pretreated substrates as an indicator of pretreatment effectiveness.

The pretreatment conditions were statistically evaluated through a multilevel factorial design of experiments (DOE) model, further described in section "Factorial design of experiments (DOE) model".

Enzymatic hydrolysis at standard conditions

EH trials were performed in 100 ml Erlenmeyer flasks with 5% (w/w) dry material load in 0.05 M sodium citrate buffer (pH 4.8), and agitation at 150 rpm in a rotary shaker (Certomat-R B-Braun, Germany) at 50 °C for 72 h. A commercial cellulase cocktail (SAE0020, Sigma-Aldrich, Darmstadt, Germany) was added in a dosage of 22 mg protein/g dry material. Samples were taken at 72 h, and sugar concentration in EH media was measured by HPLC as described below.

EH conversion yield of cellulose (EHY_C) and xylan (EHY_X) was calculated according to Eqs. (1) and (2). The amount of potential glucose and xylose is what could be obtained if the glucan and xylan carbohydrates present in the WIS fraction were fully hydrolysed, respectively.

$$EHY_C = \frac{g\,\text{glucose in EH liquid} - g\,\text{glucose in enzyme solution}}{g\,\text{of potential glucose in WIS}} \times 100 \tag{1}$$

$$EHY_X = \frac{g\,\text{xylose in EH liquid}}{g\,\text{of potential xylose in WIS}} \times 100 \tag{2}$$

Enzymatic hydrolysis at high solid loading

The WIS fraction generated was submitted to an EH test at high solid loading to evaluate the sugar production yield in conditions more similar to those required in a conversion process at larger scale. With the aim of maximising the production of sugars, several EH assays were carried out to evaluate and optimise the main EH reaction conditions. The experiments were carried out in 250 mL Erlenmeyer flasks with 15 and 20% (w/w) dry material loads in 0.05 M sodium citrate buffer (pH 4.8), and agitation at 150 rpm in a rotary shaker (Certomat-R B-Braun, Germany) using different enzyme doses (30 and 60 mg protein/g of cellulose) of the commercial enzyme cellulase cocktail. Tween-20 (25 µL/g dry material) was added in order to enhance the accessibility of enzymes to the material under high solid loading conditions. Samples were taken at 24, 48 and 72 h and sugar concentration in EH media measured by HPLC as described in section "Analytical methods".

Analytical methods

The monomeric sugar content of the liquors from the two-step hydrolysis process for PH fractions and EH sugar-containing media was analysed by HPLC using a liquid chromatograph with refractive index detector Waters 2695 (Waters, Mildford, MA) according to Padilla-Rascón et al. (2020). A carbohydrate column CARBOSep CHO-782 Pb, Transgenomic, Inc., Omaha, NE with ultrapure water as eluent at a flow rate of 0.6 mL/min and a column temperature of 70 °C was used.

Factorial design of experiments (DOE) model

In this study, a multilevel factorial DOE model was performed, using the software Statgraphics XVII CenturionTM (Statpoint Technologies, Inc., Warrenton, VA, USA), to evaluate the influence of the main operation conditions, i.e. temperature, residence time and catalyst addition. The value of these operating variables determines the SE pretreatment severity, which is well described in the severity factor (SF) through a formula (Eq. 3) widely used in the literature that was proposed by Overend and Chornet (1987).

$$\mathrm{SF} = \log\left(t_S \times e^{\frac{T_r-100}{14.75}}\right) \tag{3}$$

where t_s is the residence time in minutes and T_r the temperature in °C.

Although this formula provides an estimation of the effect of SE pretreatment on hemicellulose-derived sugar solubilisation and the level of accessibility of enzymes to the carbohydrates in the pretreated substrates, it is not applicable when an acid or basic catalyst is added. In these cases, the pH of the medium strongly influences the mechanisms underlying the disorganisation of the LB structure and thus, a modification of the formula (Eq. 4) was proposed by Chum et al. (1990) that includes pH in the combined severity factor (CSF).

$$\mathrm{CSF} = \mathrm{SF} - \mathrm{pH} \tag{4}$$

In the present work, the influence of different levels of temperature (190/200/210 °C) and acid concentration (20 and 30 mg of H_2SO_4/g dry biomass) were studied, while the residence time was held constant at 5 min. Table 1 shows the different operation conditions evaluated and the CSF of each of them.

To assess SE pretreatment effectiveness under the different conditions tested, the overall glucose (OYG) and xylose (OYX) yields were calculated according to Eqs. (5) and (6), respectively. OYG and OYX are based on the amount of sugars released in the PH fraction after pretreatment and from the WIS fraction by EH, expressed as a percentage of the sugar content in the biomass feedstock, using the solid recovery yield (SRY) parameter (Eq. 7). SRY indicates the amount of dry WIS recovered per 100 g of dry biomass feedstock introduced into the SE unit. The EH yield measures the amount of sugars released from the pretreated materials by EH, and is expressed as a percentage of the maximum theoretical yield that could be achieved if the cellulose and xylan carbohydrates present in the pretreated material were fully hydrolysed. These key parameters allow evaluation of the effects of the different pretreatment conditions tested on both the fractionation of main carbohydrates (cellulose and xylan) and the enzymatic digestibility of each biomass material.

$$\mathrm{OYG} = \frac{\left(\mathrm{EHY}_C \times \mathrm{SRY} \times \text{potential glucose in raw biomass}\right) + g \text{ of glucose in prehydrolysate}}{g \text{ of potential glucose in raw biomass}} \times 100 \tag{5}$$

Table 1 Operation conditions for SE pretreatment of *Ulmus pumila* evaluated in the DOE

Assay	Temperature,°C	Acid load, mg/g dry biomass	pH	CSF
1	190	20	1.91	1.4
2	200	20	1.96	1.7
3	210	20	1.97	2.0
4	190	30	1.27	2.1
5	200	30	1.27	2.4
6	210	30	1.20	2.7

$$\mathrm{OYX} = \frac{\left(\mathrm{EHY_X} \times \mathrm{SRY} \times \text{potential xylose in raw biomass}\right) + \text{g of xylose in prehydrolysate}}{g \text{ of potential xylose in raw biomass}} \times 100 \qquad (6)$$

$$\mathrm{SRY} = \frac{g \text{ dry WIS}}{g \text{ dry raw biomass}} \times 100 \qquad (7)$$

Results and discussion

Raw material

Table 2 presents the chemical composition of the UP biomass as a percentage of its dry weight (dry weight basis, dwb). The table indicates that UP contains approximately 35% cellulose and 19% hemicellulose, which accounts for close to 55% of overall carbohydrate composition (dwb). This value is quite similar to that reported by Martín-Sampedro et al. (2019) for *Ulmus minor* species, but lower than the amount described for other non-specified elm species that can reach more than 65% total carbohydrates (Amiri and Karimi 2015). In relation to hemicelluloses content, xylan is the main constituting polymer (71% of total hemicelluloses), as expected in a species belonging to the hardwood group. Minor amounts of other hemicellulose-derived sugars such as galactose, arabinose and mannose were found, but the discussion in the present work will be focussed only on the monomer xylose derived from xylan. Acetyl groups, which play an important role in xylan depolymerisation, are also quantified in the analysis and represent around 5% of UP biomass. Acid insoluble lignin content, which accounts for 24.6% of the dry weight basis, is within the range of values found in the papers cited above and also in other hardwood species such as poplar (18.4%) (Antczak et al. 2022). Other minor components such as

Table 2 Composition in g/100 g dry weight basis (dwb) of *Ulmus pumila* biomass. Data show mean values ± standard deviation

Component	Composition (%)
Extractives	6.4 ± 0.8
Cellulose	35.2 ± 0.9
Hemicelluloses:	19.1 ± 0.2
Xylose	15.4 ± 0.3
Galactose	3.2 ± 0.0
Arabinose	1.5 ± 0.0
Mannose	1.4 ± 0.0
Acetyl groups	5.3 ± 0.0
Lignin:	26.2 ± 1.0
Acid insoluble lignin	24.6 ± 0.9
Acid soluble lignin	1.7 ± 0.1
Ash	2.7 ± 0.2

Table 3 WIS composition (in % dry weight basis of WIS) and EH yields of cellulose and xylan (EHY_C, and EHY_X, in % of the maximum theoretical) at the different SE temperatures and a fixed residence time of 5 min

Assay	SE conditions	SF	Cellulose	Hemicellulose	Lignin	Ash	EHY_C, %	EHY_X, %
1	200 °C	3.6	42.2 ± 0.4	11.9 ± 0.2	31.7 ± 0.7	5.3 ± 0.8	5.4 ± 0.0	23.0 ± 0.7
2	210 °C	3.9	47.6 ± 1.3	9.2 ± 0.2	36.5 ± 0.3	1.4 ± 0.1	26.8 ± 0.4	58.1 ± 0.3
3	220 °C	4.2	52.4 ± 1.3	6.0 ± 0.2	39.0 ± 1.0	1.1 ± 0.1	41.4 ± 0.1	60.1 ± 0.5

Table 4 Sugar release into prehydrolysate fraction (in g/ 100 g dwb of biomass feedstock) obtained in the SE trials at the different SE temperatures and a fixed residence time of 5 min

Assay	SE conditions	CSF	Glucose	Xylose	Galactose + Arabinose + Mannose	Furfural	HMF	Acetic Acid
1	200 °C	3.6	2.8 ± 0.1	5.0 ± 0.3	3.0 ± 0.2	0.04 ± 0.0	0.02 ± 0.0	0.7 ± 0.0
2	210 °C	3.9	2.9 ± 0.1	6.7 ± 0.3	2.9 ± 0.1	0.13 ± 0.0	0.04 ± 0.0	1.4 ± 0.1
3	220 °C	4.2	2.8 ± 0.1	5.8 ± 0.2	2.5 ± 0.1	0.33 ± 0.0	0.11 ± 0.0	2.2 ± 0.1

extractives and ash are also measured to complete the UP biomass composition. In brief, the high carbohydrate content of UP makes it an excellent raw material for the production of biofuels and bioproducts. Moreover, the lignin component, mostly present in the residue left after the enzymatic hydrolysis process, can be also considered an interesting source of valuable chemicals and products.

Non-catalysed steam explosion pretreatment: Autohydrolysis

As described above, a series of SE trials were carried out under different operation conditions to evaluate their influence on SE effectiveness. Firstly, a series of autohydrolysis SE trials was performed to evaluate the effectiveness of autohydrolysis by SE. For this purpose, several tests were carried out under different temperature conditions (200, 210 and 220 °C) and a fixed residence time of 5 min, resulting in increasing levels of pretreatment severity from 3.6 to 4.2. After the autohydrolysis trials, WIS and PH fractions were recovered by filtration and analysed. As described in section "Steam explosion pretreatment: main SE operating conditions", the WIS fraction was submitted to EH under standard laboratory conditions. Tables 3 and 4 present the chemical composition of the WIS fraction and the release of sugars into the PH fraction, respectively. The results of the EH conversion yield of cellulose and xylan of pretreated materials are also included in Table 3.

As shown in Table 3, the hemicellulose content of the WIS obtained varies from approximately 12% to 6% dwb, which decreases as the pretreatment severity increases. As expected, these values are lower than that of the raw lignocellulosic material (19.1%), indicating a partial solubilisation of this component. As a consequence of hemicellulose solubilisation, the WIS fraction obtained under all conditions tested has higher cellulose and lignin content than the raw UP biomass, reaching values in the intervals of 42–52% and 32–39%, respectively, depending on pretreatment severity. Cellulose enrichment in the pretreated materials in relation to the raw biomass is a common characteristic of hydrothermal and chemical pretreatment (Bonfiglio et al. 2019; Cebreiros et al. 2021; Kumar et al. 2023).

The EH yields of cellulose and xylan obtained after the non-catalysed SE pretreatment were rather low, reaching the highest values (41.4% and 60.1% for cellulose and xylan, respectively) at 220 °C. With regard to the SE temperature, it was found that better EH yields are obtained as the temperature increased. This effect occurred at the same time that hemicellulose content in the pretreated material diminished, which supports the early finding by Mosier et al. (2005). These authors stated that hemicellulose removal contributes to the increased digestibility of the solids as a result of the presence of hemicellulose in the substrate hindering the enzymatic attack and negatively affecting the enzymatic digestibility of substrates. Later work also corroborates this hypothesis, for example the studies of Chen et al. (2014) and Bonfiglio et al. (2019) carried out in bamboo pretreated by dilute sulphuric acid pretreatment and switchgrass pretreated by steam explosion, respectively.

These results obtained in autohydrolysis experiments indicate the possibility of improving yields by using, for example, an acid catalyst. Kataria et al. (2017) have shown the good performance of steam explosion at bench scale in sulphuric acid-impregnated elephant grass biomass, which caused significant modifications in biomass structure that in turn improved the saccharification yield by sevenfold, in comparison to untreated biomass. This approach was also used by Semwal et al. (2019) in an SE pretreatment optimisation study using rice straw as the LB feedstock, obtaining EH yields between 15 and 30% higher when the LB was pretreated using an acid catalyst.

Regarding the sugar release into the PH fraction shown in Table 4, the production of furfural, HMF and acetic acid clearly increases as the pretreatment severity rises, reaching values of 0.33, 0.11 and 2.2 g/100 g raw biomass, respectively, at the highest SF of 4.2. It is well-known that in hydrothermal pretreatments like SE the presence of acetic acid is due to deacetylation of hemicellulose polymers contained in hardwoods like elm, while furfural and HMF originate from sugar degradation (Duque et al. 2021; Padilla-Rascón et al. 2020; Semwal et al. 2019). However, the variations found among the sugars released to PH are more evident in the case of xylose, which is most affected by the increase in the SF. An increment of SF from 3.6 to 3.9 results in xylose yield increasing from 5.0 to 6.7 mg/100 g raw elm, although a further rise of SF to 4.2 provokes a drop to 5.8 mg/100 g, which indicates xylose degradation under this severity condition.

Acid-catalysed steam explosion pretreatment

Effect of residence time and temperature

The effects of residence time and temperature were examined in SE trials using sulphuric acid as catalyst. The experiments were performed at 5 and 7.5 min and

Table 5 WIS composition (in % dwb of WIS) and EH yields of cellulose and xylan (EHY$_C$, and EHY$_X$, in % of the maximum theoretical) at the different SE conditions and acid concentration of 20 mg of H_2SO_4/g dry biomass

Assay	SE conditions	CSF	Cellulose	Hemicellulose	Lignin	Ash	EHY$_C$, %	EHY$_X$, %
1	190 °C , 5 min	1.4	42.7 ± 0.6	7.6 ± 0.1	36.8 ± 0.3	1.96 ± 0.1	35.3 ± 0.8	50.9 ± 0.9
2	190 °C , 7.5 min	1.6	49.7 ± 0.3	6.5 ± 0.1	37.8 ± 0.2	1.5 ± 0.2	33.6 ± 0.7	59.7 ± 1.1
3	200 °C , 5 min	1.7	44.0 ± 1.0	6.5 ± 0.3	38.5 ± 0.2	2.0 ± 01	42.7 ± 0.5	83.3 ± 3.1
4	200 °C , 7.5 min	1.9	48.6 ± 0.3	4.6 ± 0.1	40.6 ± 4.5	1.7 ± 0.0	42.4 ± 0.7	81.9 ± 2.2

Table 6 Sugar release into prehydrolysate fraction (in g/ 100 g dwb of biomass feedstock) obtained in the SE trials at the different SE conditions and acid concentration of 20 mg of H_2SO_4/g dry biomass

Assay	SE conditions	CSF	Glucose	Xylose	Galactose + Arabinose + Mannose	Furfural	HMF	Acetic Acid
1	190 °C , 5 min	1.4	3.3 ± 0.4	7.3 ± 0.8	4.1 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.1
2	190 °C , 7.5 min	1.6	3.3 ± 0.2	7.1 ± 0.5	3.9 ± 0.3	0.2 ± 0.0	0.1 ± 0.0	1.3 ± 0.1
3	200 °C , 5 min	1.7	3.9 ± 0.3	8.5 ± 0.6	4.7 ± 0.3	0.3 ± 0.0	0.2 ± 0.0	1.4 ± 0.1
4	200 °C , 7.5 min	1.9	4.0 ± 0.2	8.6 ± 0.5	4.8 ± 0.3	0.4 ± 0.0	0.2 ± 0.0	1.8 ± 0.1

temperatures of 190 and 200 °C. In all cases, the acid catalyst concentration was set at 20 mg of H_2SO_4/g dry biomass. The chemical composition of the WIS fraction and the release of sugars into PH fraction obtained in the different trials are presented in Table 5 and 6, respectively. Table 5 also includes the results of the EH conversion yields of cellulose and xylan.

As in the non-catalysed SE trials, the hemicellulose content of the WIS fraction decreases from 7.6% to 4.6% as the pretreatment severity increases (Table 5), indicating a partial solubilisation of that component. It results in a solid material with a higher cellulose and lignin content than the raw UP biomass, reaching values between 42–48% and 36–41%, respectively, depending on pretreatment severity. Regarding the PH fraction, as expected, the release of hemicellulosic sugars increases as the pretreatment severity increases (Table 6), reaching the highest production value of 13.4 g/100 g raw UP at 200 °C and 7.5 min (CSF of 1.9). In these cases, although the concentration of inhibitor compounds (furfural, HMF and acetic acid) was also higher than in the non-catalysed cases (Table 4), the values achieved can be considered low enough to not affect the efficacy of any subsequent sugar transformation process (van der Pol et al. 2014).

As presented in Table 5, the EH yields of cellulose and xylan obtained after SE pretreatment at different residence times were very similar, thus indicating a minor influence of the residence time on the pretreatment effectiveness for the specific conditions evaluated in this work. In all tested conditions, the values obtained for cellulose and xylan EH yield were substantially higher than those obtained in autohydrolysis, achieving values up to 7.9 and 3.6 times higher at the same temperatures (200 °C and 5 min), respectively. These results support the positive effect of acid addition in SE. The results show a clear influence of the SE temperature in cellulose and xylan EH yield, reaching the highest values in the trial at 200ºC and 5 min (approximately 43% and 83%, respectively). Considering these results, in the present work the optimisation of SE pretreatment was focussed on the evaluation of the combined effect of temperature and acid catalyst concentration. Since the residence time showed a minor effect on the efficacy of SE pretreatment, the shorter time tested (5 min) was used.

Factorial design of experiments (DOE) model: optimisation of SE pretreatment conditions

Based on the results above, for the DOE model the variables were restricted to temperature and acid loading at a fixed time of 5 min. Tables 7 and 8 show the composition of WIS fraction and the sugar release into PH fraction obtained under the different severity conditions tested, respectively. The EH yields of cellulose and xylan reached under each condition tested are also shown in Table 7.

As shown in Table 7 and 8, in general, as the CSF parameter increases, the hemicellulose content in the WIS fraction decreases, indicating a higher solubilisation of hemicellulose component and, therefore, a higher concentration of cellulose in the WIS material. As a consequence of hemicellulose removal, enhanced cellulose and xylan HE yields are achieved as the severity of the process increases, reaching the highest values (72.9% and 98.5% for cellulose and xylan, respectively) at 210 °C and 30 mg H_2SO_4/g dry UP (CSF of 2.7). However, as can be noted in Table 8, the increase in severity of the pretreatment also results in an increase in the release of glucose into the PH fraction, indicating the partial solubilisation of the cellulose

Table 7 WIS composition (in % dry weight basis of WIS) and EH yields of cellulose and xylan (in % of the maximum theoretical) at the different SE conditions and a fixed residence time of 5 min

Assay	SE conditions	CSF	Cellulose	Hemicellulose	Lignin	Ash	EHY_C, %	EHY_X, %
1	190 °C , 20 mg H_2SO_4/g dry UP	1.4	42.7	7.6	36.8	1.6	35.3	51.0
2	200 °C , 20 mg H_2SO_4/g dry UP	1.7	44.0	6.5	38.5	2.0	42.7	83.3
3	210 °C , 20 mg H_2SO_4/g dry UP	2.0	47.1	3.0	42.2	2.1	65.3	96.4
4	190 °C , 30 mg H_2SO_4/g dry UP	2.1	44.7	4.3	39.2	1.0	49.0	80.8
5	200 °C , 30 mg H_2SO_4/g dry UP	2.4	46.3	3.9	40.3	1.6	60.2	77.9
6	210 °C , 30 mg H_2SO_4/g dry UP	2.7	45.0	2.5	45.0	1.6	72.9	98.5

Table 8 Sugar release into prehydrolysate fraction (in g/100 g dwb of biomass feedstock) obtained in the SE trials at the different SE conditions and a fixed residence time of 5 min

Assay	SE conditions	CSF	Glucose	Xylose	Galactose + Arabinose + Mannose	Furfural	HMF	Acetic Acid
1	190 °C , 20 mg H_2SO_4/g dry UP	1.4	3.3	7.3	4.1	0.1	0.1	0.9
2	200 °C , 20 mg H_2SO_4/g dry UP	1.7	3.9	8.5	4.7	0.3	0.2	1.4
3	210 °C , 20 mg H_2SO_4/g dry UP	2.0	4.3	8.4	4.7	0.6	0.4	2.2
4	190 °C , 30 mg H_2SO_4/g dry UP	2.1	4.1	8.7	4.6	0.3	0.2	2.8
5	200 °C , 30 mg H_2SO_4/g dry UP	2.4	5.0	8.8	4.8	0.6	0.3	3.4
6	210 °C , 30 mg H_2SO_4/g dry UP	2.7	5.8	7.9	4.5	0.6	0.4	3.7

component, and in the production of higher levels of inhibitory compounds (such as furfural, HMF and acetic acid) in comparison to milder conditions. The formation of inhibitory compounds during SE pretreatment indicates that sugar degradation is taking place, leading to a loss of sugars. Although it is possible to minimise these degradation reactions by using less severe pretreatment conditions, this would affect the pretreatment effectiveness and thus the yield of the subsequent EH (Bonfiglio et al. 2021). Considering these contradictory effects of the severity of the SE pretreatment, the optimal SE conditions to recover the maximum amount of sugars possible in both fractions needed to be determined. For this purpose, the amount of the sugars recovered in both fractions were evaluated by calculating the overall glucose (OYG) and xylose (OYX) yields. Figure 1a,b shows the results of overall yield for glucose and xylose in the liquid fraction (LF) after pretreatment, in the solid fraction (SF) by enzymatic hydrolysis and the total overall yield, respectively. As expected, the maximum glucose recovery was obtained under the harsher conditions (210 °C and 30 mg H_2SO_4/g biomass) reaching a total value (OYGT) of approximately 72% and being mainly recovered in the solid fraction. In the case of xylose component, the highest OYXT (75%) was obtained at milder conditions, 200 °C and 20 mg H_2SO_4/g biomass and was mainly recovered in the liquid fraction.

In order to determine the optimal pretreatment conditions to maximise both glucose in the solid fraction and xylose in the liquid fraction, a multilevel factorial design of experiments (DOE) model was carried out using Statgraphics software. Figure 2 shows the Pareto charts obtained in this statistical analysis to assess the independent and interaction effects of the operation conditions under study. In these figures, the vertical line indicates which effects are statistically significant at the 95.0% confidence level, i.e. bars that do not reach the vertical line are not statistically significant. In Fig. 2a, it can be noted that the temperature (A) exhibited a significant positive effect on OYGS parameter. The acid load parameter and the interaction between both operation conditions did not have a

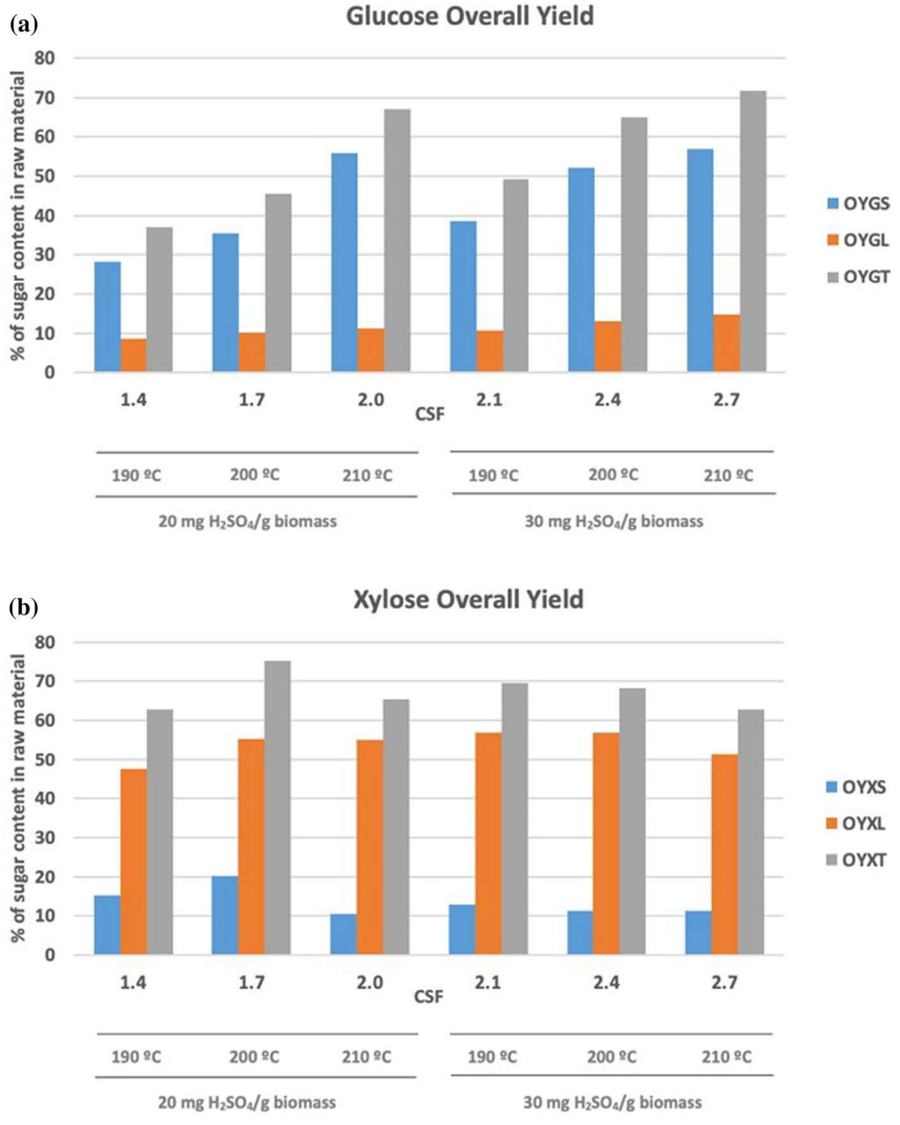

Fig. 1 Overall glucose (**a**) and xylose (**b**) yield in the liquid fraction (OYL), in the solid fraction (OYS), and total yield (OYT) at different SE conditions for *Ulmus pumila*

significant effect on this parameter. Similar results showing the temperature as the main parameter affecting glucose recovery in SE pretreatment have been found by other authors using different lignocellulosic biomasses such as poplar (Antczak et al. 2022), elephant grass (Kataria et al. 2017) and switchgrass (Bonfiglio et al. 2019). With regard to the OYXL parameter (Fig. 2b), no significant effect was found for any of the operating conditions in the range studied.

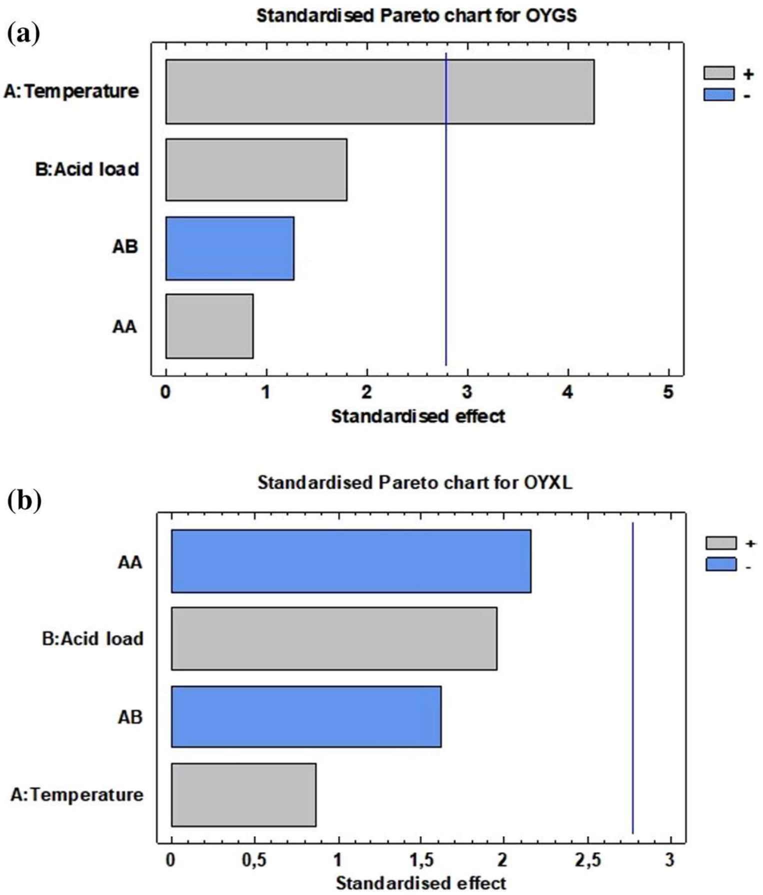

Fig. 2 Pareto chart for the effect of temperature and H_2SO_4 load on the overall glucose yield in the solid fraction (**a**) and xylose yield in the liquid fraction (**b**)

Finally, with the aim of obtaining the optimal pretreatment conditions to maximise overall yields of both glucose and xylose, a multi-response optimisation based on response surface analysis was carried out. To this end, the established criterion was to reach the maximum possible value for both OGY and OXY. Figure 3 shows the estimated contour response surface obtained. This statistical model determined the optimal pretreatment conditions to be 204.8 °C and 30 mg H_2SO_4/g biomass, reaching values of OYGS and OYXL of 48.5% and 59.0%, respectively. In order to validate the optimisation of the DOE model, three trials were carried at optimal conditions obtaining averages for OYGS and OYXL of 46.3% and 61.0%, respectively, which support the validity of the model. Therefore, 204.8 °C and 30 mg H_2SO_4/g biomass were selected as the best operation conditions to pretreat UP biomass by SE.

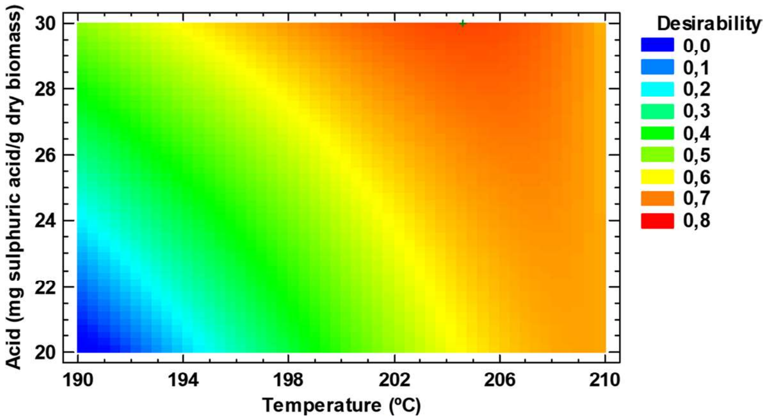

Fig. 3 Estimated contour response surface of DOE model for SE pretreatment of *Ulmus pumila*

Enzymatic hydrolysis at high solid loading

The pretreated material of UP obtained at the optimal conditions was used as substrate in EH experiments under high solid loading conditions and two enzyme doses. These trials allowed the assessment of the sugar production yield under conditions close to those that would be used in a real production process. In these assays, Tween-20 was added to improve EH yield under high solid loading conditions. Several studies have proved that the use of surfactants, such as polyethylene glycol and Tween, enhances the action of enzymes on the substrate thus improving enzymatic hydrolysis yields (Hou et al. 2022; Sánchez-Muñoz et al. 2022). The results of EH yields and final glucose concentration are shown in Table 9. These results show that better EH yields are obtained by increasing the enzyme doses for both solid loadings. With regard to solid loading the EH yield decreased at 20% loading mainly due to the increase in the viscosity of the mixture. In all cases, except for the test with the 15% solid load and 30 mg protein/g cellulose of enzyme dose, the increase between 48 and 72 h is not significant. The highest EH yield was achieved at 15% solid load and 60 mg protein/g cellulose of enzyme dose, reaching 70.3% of theoretical yield, with a glucose concentration of 70.8 g/L. On the other hand, although the EH yield achieved was slightly lower (68.1%) using the 20% solid load and 60 mg protein/g cellulose of enzyme dose, a higher glucose concentration (96.1 g/L) was reached under these conditions.

To the best of authors' knowledge, there is limited research in the literature using elm as the raw material to obtain fermentable sugars, and not one using SE as a pretreatment. For instance, Ibarra et al. (2021) evaluated different methods to pretreat the *Ulmus minor* clone Ademuz (autohydrolysis, dilute acid hydrolysis, acid catalysed organosolv, and alkaline extraction), and the resulting pretreated materials were subjected to EH at 5% (w/w) for fermentable sugars production. In that study, the highest EH yield obtained (69%) was achieved with acid catalysed organosolv at 180 °C, obtaining results very similar to those achieved in the present work.

Table 9 EH yield and final glucose concentration obtained at high solid load conditions of pretreated *Ulmus pumila*

Assay	Solid load, %	Enzyme dose, mg protein/g cellulose	Time, h	EHY_C, %	Final glucose concentration, g/L
1	15	30	24	49.7 ± 1.0	48.4 ± 1.0
			48	54.0 ± 0.3	52.4 ± 0.3
			72	59.7 ± 0.2	57.7 ± 0.2
2	15	60	24	62.9 ± 1.2	63.0 ± 1.2
			48	70.3 ± 0.4	70.8 ± 0.4
			72	70.0 ± 0.2	70.0 ± 0.2
3	20	30	24	44.6 ± 0.9	61.9 ± 1.2
			48	56.0 ± 0.3	76.9 ± 0.3
			72	56.7 ± 0.2	77.8 ± 0.3
4	20	60	24	54.4 ± 1.1	78.2 ± 1.1
			48	68.1 ± 0.4	96.1 ± 0.5
			72	68.2 ± 0.2	96.3 ± 0.3

However, it is important to note that the EH yield reported herein was obtained under considerably higher solids loads (20%) and therefore, the glucose concentration achieved (96.1 g/L) is significantly higher than that reported from *Ulmus minor* (23.5 g/l). On the other hand, considering other hardwood biomasses, several studies about sugar production by SE pretreatment and EH have been described. Dou et al. (2017) evaluated the performance of sugar production from poplar biomass by SE at 195 °C for 5 min with SO_2 impregnation and subsequent EH at 5% solid load. The authors reported a slightly higher EH yield of cellulose (72.7%) than that obtained in this study for UP, but at lower solid loading (5%). This comparison proves the good result obtained in the present work even when using high loads. With regard to EH at high solid loading, Di Risio et al. (2011) evaluated the effect of increased solid loading (20% and 30%) on enzymatic hydrolysis using as a substrate the whole slurry from poplar pretreated by SE under different temperatures (200–208 °C) and residence times (5–9 min). In that study, the best results were achieved in poplar biomass pretreated at 200 °C and 8 min, using a solid load of 20%, obtaining an EH yield of 44%, which is considerably lower than that obtained in the ongoing work using UP biomass. The better results obtained in the present work may be due to higher pretreatment effectiveness and better hydrolysis performance when only the WIS fraction is used instead of the whole slurry.

In brief, these results indicate the suitability of SE pretreatment for obtaining fermentable sugars from UP biomass, representing a step forward in the conversion process of this biomass into biofuels and bioproducts. The main outcome obtained in this work shows that an SE pretreatment strategy at 204.8 °C with 30 mg H_2SO_4/g biomass followed by EH with 20% solid loading and 60 mg protein/g cellulose of enzyme dose allows recovery of the highest amount of fermentable sugars possible from the raw UP biomass among the different conditions tested.

To provide a global overview of the process studied, Fig. 4 shows a mass balance including the different streams generated and the sugar content of each of them under those conditions. As can be seen, this strategy allows the production of two sugar-rich streams: a PH fraction rich in hemicellulosic sugars, which contain mainly xylose and glucose, and the enzymatic hydrolysate fraction rich in glucose. Overall, a total of 43.2 kg of sugars per 100 kg of raw UP are recovered, which represents 70% of the sugars recovered by this methodology. These results can be considered a novel and significant contribution to the use of UP biomass as feedstock in a potential biorefinery facility, since it establishes the bases for future developments in conversion processes aimed at maximising the generation of bio-based compounds from this particular biomass.

Conclusion

From the results of this work, it can be inferred that UP offers great potential as a raw material for the production of biofuels and high added-value bioproducts, based on its high content of carbohydrates (close to 55% dwb), which can be solubilised and recovered from the raw material through a combined SE pretreatment and EH process. Successful optimisation of the operation conditions of SE pretreatment has permitted identification of the process conditions (204.8 °C and 30 mg H_2SO_4/g biomass) that lead to a reasonably high xylose recovery in the liquid fraction or prehydrolysate and a large amount of fermentable sugars (mostly glucose) obtained by EH of the pretreated solid fraction (up to 70% potential sugars recovery). The results of this study prove the need to use an acid catalyst to achieve successful biomass fractionation, while temperature is identified as the main parameter affecting glucose recovery in SE pretreatment. Finally, this study shows that the combination of SE pretreatment and EH at high solid loadings is an efficient strategy that allows obtaining a sugar-rich media with close to 100 g/L of glucose that could be used as broth for fermentation for bioethanol or other bioproducts of interest, such as lactic acid, in the context of an integrated biorefinery based on UP biomass.

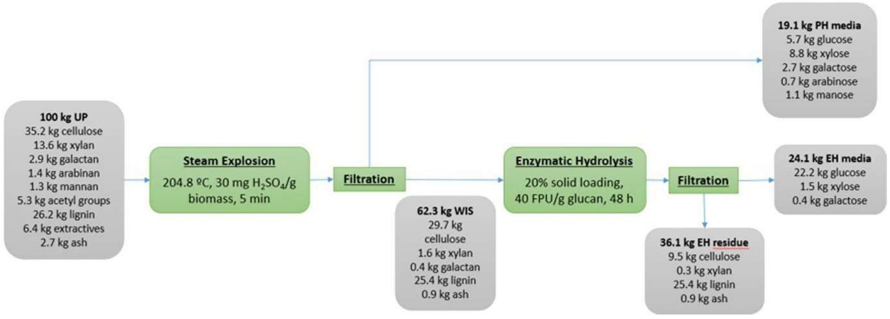

Fig. 4 Mass balance of 100 kg of UP biomass submitted to SE pretreatment and enzymatic hydrolysis under the best conditions tested

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Declarations

Conflict of interest The authors report there are no competing interests to declare.

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