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Evaluation of strategies for second generation bioethanol production from fast growing biomass Paulownia within a biorefinery scheme

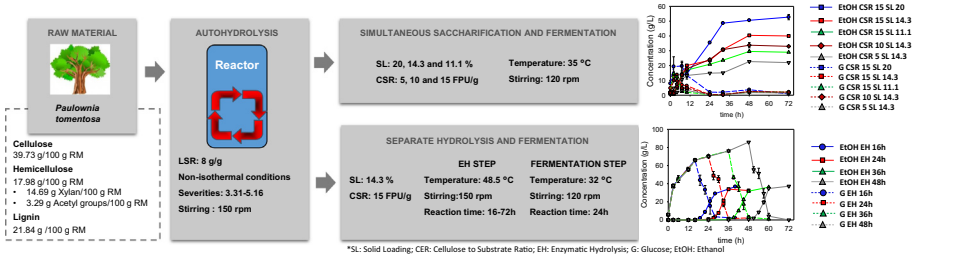
Elena Domínguez [a,b], Aloia Romaní [a,b,c], Lucília Domingues [c], Gil Garrote [a,b,*]

[a] Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), As Lagoas, 32004 Ourense, Spain
[b] CITI-Tecnopole, San Ciprian de Viñas, 32901 Ourense, Spain
[c] CEB-Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal

HIGHLIGHTS

- Autohydrolysis of Paulownia biomass was studied as first step of a biorefinery.
- At S_0 = 4.19, 78.9% of xylan was recovered as xylose and xylooligosaccharides.
- At S_0 = 4.19, 47% higher ethanol concentration was achieved by SHF than SSF.
- At S_0 = 4.72, 52.7 g/L of ethanol (80% of conversion) was obtained by SSF.
- An energy production of 648,074 MJ/ha·year could be produced from this process.

GRAPHICAL ABSTRACT



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ABSTRACT

Fast-growing and short-rotation biomass is identified as glucan-rich feedstock to be used for bioenergy purposes. For the first time to our knowledge, fast growing biomass (*Paulownia tomentosa*) was evaluated for bioethanol production in a biorefinery scheme. For that, Paulownia wood was subjected to autohydrolysis pretreatment under severity (S_0) conditions in the range of 3.31–5.16. The effect of this treatment on its fractionation was evaluated by means of hemicelluloses solubilization as hemicellulose-derived compounds in liquid phase and enzymatic hydrolysis of glucan (remained in the solid phase) into glucose. A xylose and xylooligosaccharides concentration of 17.5 g/L was obtained at S_0 = 3.99 which corresponds to complete xylan solubilization. On the other hand, glucose yield of enzymatic hydrolysis increased up to reach 99% at S_0 = 4.82. In addition, separate and simultaneous saccharification and fermentation assays (SHF and SSF) of autohydrolyzed Paulownia were compared for ethanol production. An increase of 47% in ethanol concentration was obtained by SHF in comparison with results achieved by SSF for Paulownia treated at S_0 = 4.19. In SSF, Paulownia was successfully converted into ethanol (52.7 g/L which corresponded to 80% of ethanol yield) operating at 20% solid loadings and S_0 = 4.72. Energy analysis of results obtained in this work showed that 83% of energy respect to raw material can be recovered considering the ethanol and the combustion of residual lignin. This work provides a feasible process for bioethanol production using fast growing specie which could enrich the feedstock needs for biofuels sector.

* Corresponding author at: Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), As Lagoas, 32004 Ourense, Spain.
E-mail address: gil@uvigo.es (G. Garrote).

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Nomenclature

Abbreviations

EH	enzymatic hydrolysis
F	furfural
FPU	Filter Paper Units
HMF	hydroxymethylfurfural
IU	International Unit
SHF	separate hydrolysis and fermentation
SSF	simultaneous saccharification and fermentation

Parameters and constants

ρ	density (g/L)
ω	empirical constant (14.75 °C)
[Biomass]	dry biomass concentration at the beginning of the fermentation (g/L)
[Cellobiose]	cellobiose concentration (g/L)
$[EtOH]_f$	ethanol concentration at the end of the fermentation (g/L)
$[EtOH]_0$	ethanol concentration at the beginning of the fermentation (g/L)
[Glucose]	glucose concentration (g/L)
C_{EtMAX}	maximal ethanol concentration (g/L)
CSR	cellulase to Substrate Ratio (FPU/g)
E_{EG}	energy produced from ethanol from glucan (MJ)
E_{ELP}	energy produced from ethanol from liquid phase (MJ)
E_{LIGNIN}	energy produced from lignin (MJ)
f	cellulose fraction of dry biomass (g/g)
Gn	glucan content (g glucan/100 g spent solid, oven dry basis)
HHV	Higher Heating Value (MJ/kg)
KL	Klason Lignin content (g Klason lignin/100 g spent solid, oven dry basis)
LSR	liquid to solid ratio (g/g)
NVC	non-volatile compounds (g NVC in liquid phase per 100 g raw material, oven dry basis)
O + M	oligosaccharides + monomers (kg)
Qp_{EtMAX}	productivity calculated at maximal concentration of ethanol (g/L/h)
R^2	coefficient of determination (dimensionless)
R_0	severity factor (min)
S_0	severity (dimensionless)
SY	solid yield (g solid recovered/100 g raw material, oven dry basis)
t	time (h)
T(t)	temperature profile in the heating stage (°C)
T'(t)	temperature profile in the cooling stage (°C)
$t_{1/2}$	reaction time needed to reach 50% of Y_{GMAX} (h)
t_F	time needed for the whole heating–cooling period (min)
t_{MAX}	time needed to achieve the target temperature (min)
T_{MAX}	target temperature (°C)
T_{REF}	reference temperature (100 °C)
Y_{Et}	ethanol yield (%)
Y_G	glucose yield (%)
Y_{GMAX}	maximum glucose yield achievable at infinite reaction time (%)
Y_{Gt}	gluczose yield at time t (%)

1. Introduction

Currently, the search for alternative raw materials to be used as renewable sources for energy production is one of the most important challenges to achieve a sustainable growth based on a bioeconomy strategy [1]. In this context, lignocellulosic biomass is one of the most promising raw material for biofuel production considering its great availability and limited price [2]. Lignocellulosic materials (LCM) such as wood provide abundant and renewable feedstock that doesn't compete with food crops [3].

LCM structural composition includes a complex structure composed of cellulose (a linear polymer made from glucose structural units), hemicellulose (branched polymer made up of sugars and substituents) and lignin (polymer made up of oxygenated phenylpropane structural units) [4]. In order to produce bioethanol, the sugars forming polysaccharides can be hydrolyzed by cellulolytic enzymes and subsequently fermented by microorganisms such as *Saccharomyces cerevisiae* [5,6]. Nevertheless, the three-dimensional and recalcitrant structure of LCM hinders the enzymatic hydrolysis of cellulose and subsequent fermentation of glucose to ethanol [7].

Bioethanol from lignocellulosic feedstock remains on the verge of commercialization due to higher capital and operating costs [8]. Second generation bioethanol could be made cost-competitive by the development of biorefinery-based processes for the integral use of lignocellulosic biomass [9]. An effective pretreatment plays a key role in the success of the process since it critically influences the subsequent stages of biofuel production [10].

Pretreatment using water at high temperature (also known as autohydrolysis or liquid hot water) consists in an attractive hydrolyzing medium that enable a wide variety of reactions without catalyst [11,12]. The autohydrolysis reaction may be considered either as a fractionation process or as a pretreatment to enhance biomass susceptibility to enzymatic hydrolysis. Hemicellulose is solubilized selectively by autohydrolysis, yielding spent solids mainly composed of acid insoluble lignin and cellulose, more susceptible to enzyme action [13].

The production of bioethanol from pretreated lignocellulosic biomass can be carried out by consecutive stages of enzymatic hydrolysis and fermentation (method known as separate hydrolysis and fermentation, SHF) or by a single stage of saccharification and fermentation (known as simultaneous saccharification and fermentation, SSF) [4,14]. The main advantage of the SHF process is that both steps (saccharification and fermentation) can be carried out at their individual optimal process conditions. While in the SSF process, a compromise should be accomplished on the reaction conditions. The main advantage of the SSF process is that the glucose produced is simultaneously consumed by yeast. This consumption decreases the product inhibition of enzyme catalysis. In addition, the SSF process can be carried out in one process step [15], resulting in overall cost reduction from the use of only one reactor [16].

Paulownia tomentosa is a fast growing, short-rotation woody crop plant with high biomass production, 50 t/(ha year) [17], significantly higher than the production of other species (such as poplar, switchgrass, miscanthus or willow), with values of 6–17 t/(ha year) [18]. In addition, *P. tomentosa* presents an elevate degree of tolerance to different abiotic stress conditions (such as resistance to rooting, drought and poor soils) [19,20]. These features are of utmost importance to select *P. tomentosa* as feedstock to produce bioethanol [21].

In previous research, Paulownia biomass was evaluated for pulping paper manufacture and lignin applications using combined processes of autohydrolysis and delignification [22,23]. Moreover,

enzymatic saccharification assessment of pretreated *P. tomentosa* for glucose production (without bioethanol yielding) using acid and alkali processes was reported by Ye and Chen [24]. Nevertheless, this raw material has not been previously evaluated for bioethanol production within a biorefinery scheme using autohydrolysis as pretreatment.

Therefore, this study is the first work showing a suitable process for bioethanol production and hemicellulose recovery (as xylooligosaccharides) from fast growing, short-rotation *P. tomentosa* wood using autohydrolysis as first step of a biorefinery. Lignocellulose biomass was processed by autohydrolysis under a wide range of severities (S_0 = 3.31–5.16), in order to fractionate the biomass into its main components (hemicelluloses, cellulose and lignin) and to improve enzymatic susceptibility of cellulose. Experimental data from enzymatic hydrolysis allowed the interpretation and evaluation of the hydrolysis kinetics as a function of autohydrolysis conditions. Moreover, biomass pretreated under selected conditions was successfully converted into bioethanol, comparing two strategies of SSF and SHF. Finally, overall mass balance of proposed processes and energy recovery from main fractions were calculated, compared and discussed. Taking into account experimental data obtained in this work and the advantages of the use of a fast growing short-rotation species, the strategy followed in this study is shown as an interesting solution to meet the feedstock needs for bioenergy production.

2. Materials and methods

2.1. Raw material

The raw material used in this study was *P. tomentosa* wood and was provided by a local wood plantation, located in Foz (Lugo, NW Spain). *P. tomentosa* was milled to a size of a particle of 8 mm (using a portable sieve shaker, mesh 5/16 in) and stored in containers with aeration in a cool, dry and dark place until its use.

2.2. Analysis of raw material

Samples from the homogenized lot were milled to a particle size less than 0.5 mm and analyzed (composition shown in Table 1) using the following methods: extractives [25], moisture [26], ashes [27], and quantitative acid hydrolysis [28]. Fig. 1 shows the analytical methods used in this work and the scheme of the whole process.

The liquid phase from quantitative acid hydrolysis was analyzed by high performance liquid chromatography (HPLC) for sugars and acetic acid concentration (conditions: detector, refractive index; column, Aminex HPX-87H; mobile phase, 0.01 M H_2SO_4; flow rate, 0.6 mL/min; temperature of column 50 °C). The concentrations of glucose, xylose, arabinose and acetic acid were employed to calculate the content of glucan, xylan, arabinan and acetyl groups. The insoluble phase from the quantitative acid hydrolysis was gravimetrically measured and reported as Klason lignin. Uronic acids were determined using a colorimetric method [29]. Analyses were carried out in triplicate.

Table 1
Chemical composition of *Paulownia tomentosa* (expressed in g/100 g wood in oven-dry basis ± standard deviation based on three replicate determinations).

Cellulose (glucan)	39.7 ± 0.97
Xylan	14.7 ± 0.56
Acetyl groups	3.29 ± 0.01
Klason lignin	21.9 ± 0.50
Extractives	5.60 ± 0.004
Ashes	0.50 ± 0.05
Uronic acids (expressed in glucuronic acid)	1.30 ± 0.30

2.3. Non-Isothermal autohydrolysis treatment of P. tomentosa

Water and *P. tomentosa* were mixed at a liquid to solid ratio (LSR) of 8 kg of water/kg of oven-dry raw material in a Parr reactor (Parr Instruments Company, Moline, IL) of 1 gallon of internal volume, equipped with four blade turbine impellers, heated by an external fabric mantle, and cooled by flowing water through an internal stainless steel loop. The reaction media was stirred at 150 rpm and heated following the standard temperature profile to reach the target temperature (Fig. 2). The harshness of autohydrolysis treatments can be expressed in terms of severity (S_0) which was defined as $S_0 = \log R_0$ by Lavoie [30]. R_0 is calculated by the following equation as function of temperature and time of autohydrolysis:

$$S_0 = \log R_0 = \log(R_{0_{HEATING}} + R_{0_{COOLING}}) = \log\left[\int_0^{tMAX} \exp\left(\frac{T(t) - T_{REF}}{\omega}\right)\cdot dt\right] + \left[\int_{tMAX}^{tF} \exp\left(\frac{T'(t) - T_{REF}}{\omega}\right)\cdot dt\right] \quad (1)$$

According to this expression, R_0 is the severity factor, t_{MAX} (min) is the time needed to achieve the target temperature T_{MAX} (°C), t_F (min) is the time needed for the whole heating–cooling period, and T(t) and T′(t) represent the temperature profiles in the heating and cooling stages, respectively. Calculations were made using the values reported usually for ω and T_{REF} (14.75 °C and 100 °C, respectively).

The range of studied temperatures included in Table 2 (T_{MAX}: 182–240 °C corresponding to severities, S_0, of 3.31–5.16) was chosen in basis of previous experience with other raw materials as corn cob and *Eucalyptus globulus* [31,32]. This range was selected with the purpose of studying extensively the pretreatment process. Operational conditions were evaluated to maximize hemicellulose derived compounds concentration in liquid phase and to improve enzymatic susceptibility of glucan present in solid phase.

Solids from autohydrolysis treatment were recovered by filtration, washed with water and quantified for solid yield determination (SY, g solid recovered per 100 g raw material, oven dry basis) and analyzed for chemical composition as was described in Section 2.2. All the analyses were carried out in triplicate. One aliquot of liquid phase (liquors) from autohydrolysis treatment was filtered through 0.45 μm membranes and employed for HPLC quantitation of glucose, xylose, arabinose, acetic acid, hydroxymethylfurfural (HMF) and furfural (F). A second aliquot was subjected to quantitative acid posthydrolysis (4% w/w sulphuric acid at 121 °C for 40 min), filtered through 0.45 μm membranes and analyzed in HPLC for oligosaccharides quantification. A third aliquot of liquor was used for non-volatile compounds quantification (NVC, g non-volatile compounds in liquid phase per 100 g raw material, oven dry basis) in an oven at 105 °C for 48 h.

2.4. Enzymatic hydrolysis of autohydrolyzed P. tomentosa

Enzymatic hydrolysis (EH) assays were carried out at 48.5 °C and pH 4.85 (using 0.05 N citric acid–sodium citrate buffer) in 100 mL Erlenmeyer flasks with orbital agitation (150 rpm) using commercial enzymes (“Celluclast 1.5 L” cellulases from *Trichoderma reesei* and “Novozyme 188” β-glucosidase from *Aspergillus niger*), which were kindly provided by Novozymes (Madrid, Spain). The cellulase activity of “Celluclast 1.5 L” concentrates was measured by the Filter Paper assay, and the activity was expressed in terms of Filter Paper Units, FPU [33]. The β-glucosidase activity

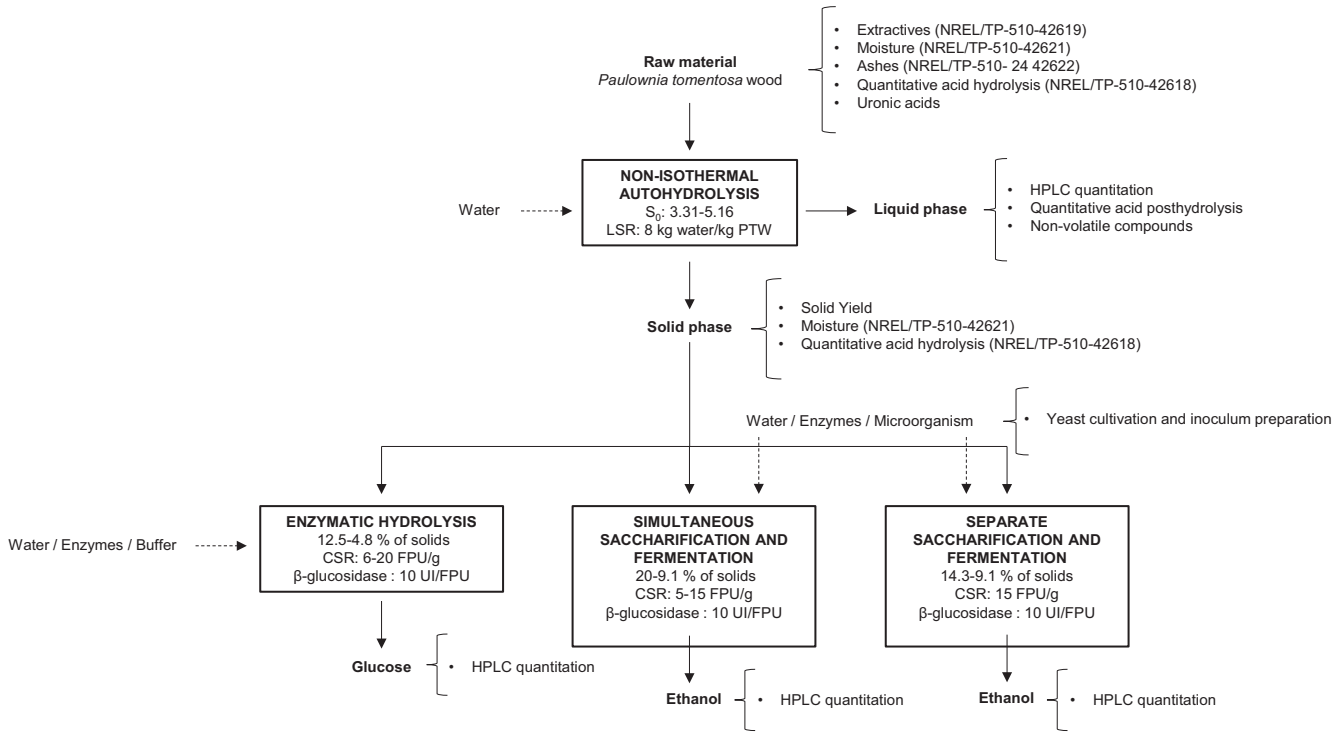

Fig. 1. Flow chart of whole process and analytical methods used in this work.

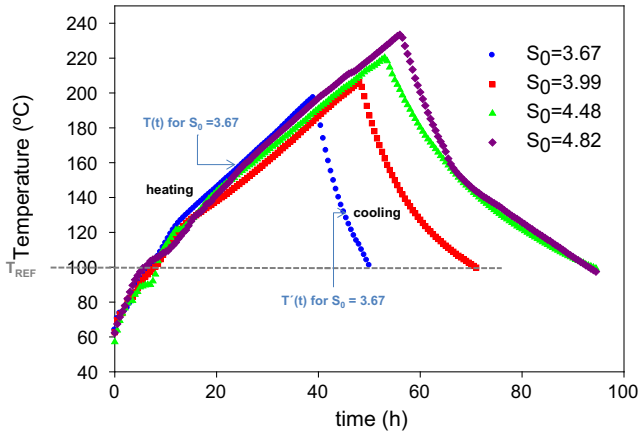

Fig. 2. Heating and cooling temperature profiles of autohydrolysis assay carried out at S_0 = 3.67, 3.99, 4.48 and 4.82. Temperature was controlled with a thermocouple inserted in a thermowell, which extends near the bottom of the reactor vessel (T (t): heating profile, T′ (t): cooling profile, T_{REF}: temperature of reference, 100 °C).

of "Novozyme 188" concentrates was measured in International Unit (IU) using *p*-nitrophenyl-β-d-glucopyranoside as substrate following the method described in Paquot and Thonart [34]. One unit of activity (IU) was defined as the release of 1 μmol of *p*-nitrophenol per minute. The enzyme activities were 70 FPU/mL for Celluclast 1.5 L (or 82.6 FPU/g) and 630 UI/mL for Novozyme 188 (or 743.4 UI/g). EH experiments were carried out in duplicate using a percentage of solids in the range of 4.8–12.5% at Cellulase to Substrate Ratio (CSR) in the range of 6–20 FPU/g of pretreated Paulownia. Novozyme 188 was added at ratio of 10 UI of Novozyme 188 per FPU of Celluclast 1.5 L. Samples from EH assays were withdrawn at desired times in the range 0–120 h, centrifuged (5000 rpm for 10 min), filtered through 0.2 μm membranes and analyzed by HPLC for monosaccharides, using the method cited in Section 2.2. The results of EH can be expressed in terms of glucose concentration (g/L) and in terms of glucose yield (Y_G) (%), calculated using the following equation [35].

$$\%Y_G = \frac{[Glucose] + 1.053[Cellobiose]}{1.111 f [Biomass]} \quad (2)$$

where [Glucose] is glucose concentration (g/L), [Cellobiose] is cellobiose concentration (g/L), [Biomass] is dry biomass (or LCM) concentration (g/L), *f* is cellulose fraction in dry biomass (g/g), the multiplication factor, 1.053, converts cellobiose to equivalent glucose. In all experiments, cellobiose was not detected.

2.5. Yeast cultivation and inoculum preparation

The strain *Saccharomyces cerevisiae* CECT-1170, obtained from the Spanish Collection of Type Cultures (Valencia, Spain), was employed for fermentation. Cells were grown at 30 °C for 24 h in a medium containing 10 g glucose/L, 5 g peptone/L, 3 g malt extract/L, and 3 g yeast extract/L.

After yeast grown, cells were recollected and inoculated to experiments of saccharification and fermentation with 2 g/L (dry weight basis).

2.6. Saccharification and fermentation of autohydrolyzed P. tomentosa

Pretreated *P. tomentosa* under selected conditions were used as substrates for bioethanol production following two strategies of saccharification and fermentation: simultaneously and separate (SSF and SHF).

SSF assays were carried out in 100 mL Erlenmeyer flasks (orbital shaking at 120 rpm, 35 °C, and pH 5). SSF media were prepared by mixing the appropriate amounts of substrate (pretreated *P. tomentosa*), water, buffer, nutrients and yeast inoculum. Suspensions containing water, buffer and solid substrates were autoclaved at 121 °C for 15 min separately from the nutrients, and thermostated at 35 °C. SSF experiments were carried out under

Table 2
Solid and liquid phases composition of pretreated *Paulownia tomentosa* wood (oligosaccharides are expressed as monosaccharides equivalent) ± standard deviation based on three replicate determinations.

T_{MAX} (°C)	182	197	203	206	209	210	212	218	220	230	232	240
So (dimensionless)	3.31	3.67	3.85	3.99	4.08	4.19	4.24	4.43	4.48	4.72	4.82	5.16
SY (g solid recovered per 100 g raw material, oven dry basis)	78.8	75.4	69.6	71.5	68.9	70.7	71.7	70.9	67.7	66.1	67.3	63.8
NVC (g non-volatile compounds in liquid phase per 100 g raw material, oven dry basis)	12.9 ± 0.3	22.1 ± 0.1	26.4 ± 0.01	22.7 ± 0.3	23.4 ± 0.3	22.2 ± 0.1	22.8 ± 0.2	15.6 ± 0.1	15.7 ± 0.1	9.98 ± 0.1	10.3 ± 0.1	9.01 ± 0.04
Solid Phase Composition (g/100 g pretreated material, oven dry basis)												
Cellulose	40.5 ± 0.9	47.8 ± 1.1	50.1 ± 0.6	54.5 ± 0.4	54.4 ± 1.4	56.3 ± 0.7	53.3 ± 0.1	53.0 ± 1.4	52.9 ± 0.6	53.1 ± 0.5	48.8 ± 1.7	53.2 ± 0.4
Xylan	11.0 ± 0.2	7.12 ± 0.13	5.33 ± 0.19	4.44 ± 0.21	3.73 ± 0.08	2.64 ± 0.87	3.28 ± 0.10	2.11 ± 0.39	0.95 ± 0.50	0.49 ± 0.05	0.20 ± 0.02	0.00 ± 0.00
Acetyl groups	2.60 ± 0.06	1.35 ± 0.04	1.08 ± 0.07	1.02 ± 0.02	0.86 ± 0.08	0.52 ± 0.21	0.49 ± 0.16	0.00 ± 0.00	0.19 ± 0.49	0.00 ± 0.00	0.02 ± 0.04	0.00 ± 0.00
Klason lignin	29.1 ± 0. 5	33.0 ± 0.3	34.4 ± 0.6	33.3 ± 0.8	34.7 ± 1.0	35.7 ± 0.4	36.6 ± 0.8	37.6 ± 1.0	41.5 ± 0.3	41.4 ± 0.7	45.4 ± 0.5	42.3 ± 0.3
Liquid Phase Composition (g/L)												
Glucose	0.49	0.60	0.61	0.54	0.48	0.96	0.45	0.42	1.07	1.40	0.51	1.24
Xylose	0.43	0.77	1.18	1.78	2.19	4.05	2.04	3.11	4.89	1.78	0.31	0.68
Arabinose	0.11	0.56	0.67	0.48	0.40	0.23	0.56	0.00	0.18	0.00	0.00	0.00
Acetic acid	0.61	1.01	1.50	2.05	2.51	2.72	2.77	4.68	4.93	6.34	5.93	7.16
Hydroxymethylfurfural	0.04	0.05	0.11	0.21	0.26	0.44	0.22	0.44	0.95	1.78	1.28	2.62
Furfural	0.07	0.12	0.31	0.67	1.00	1.26	0.92	1.90	3.00	4.58	2.80	4.70
Oligosaccharides*	6.21 ± 0.10	12.9 ± 1.9	17.3 ± 0.5	20.2 ± 0.2	19.4 ± 0.1	14.7 ± 0.2	14.7 ± 0.8	8.60 ± 0.38	5.06 ± 0.24	0.89 ± 0.18	1.11 ± 0.13	0.69 ± 0.29
Glucooligosaccharides	0.73 ± 0.03	0.47 ± 0.00	0.70 ± 0.07	1.25 ± 0.05	1.32 ± 0.01	1.36 ± 0.01	0.59 ± 0.06	1.10 ± 0.04	1.14 ± 0.01	0.89 ± 0.04	0.57 ± 0.04	0.69 ± 0.02
Xylooligosaccharides	4.66 ± 0.05	10.9 ± 0.1	13.9 ± 0.4	15.7 ± 0.1	15.3 ± 0.2	10.9 ± 0.0	12.3 ± 0.6	7.02 ± 0.14	3.25 ± 0.07	0.00 ± 0.05	0.55 ± 0.32	0.00 ± 0.02
Arabinooligosaccharides	0.27 ± 0.01	0.09 ± 0.01	0.00 ± 0.03	0.00 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.13	0.07 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Acetyl groups linked to oligosaccharides	0.54 ± 0.14	1.38 ± 1.85	2.77 ± 0.06	3.23 ± 0.14	2.81 ± 0.16	2.43 ± 0.24	1.82 ± 0.14	0.42 ± 0.16	0.67 ± 0.19	0.00 ± 0.21	0.00 ± 0.15	0.00 ± 0.25

* Oligosaccharides were measured as sum of xylooligosaccharides, glucooligosaccharides, arabinooligosaccharides and acetyl groups linked to oligosaccharides.

selected operational conditions using different solids and cellulase loadings (9–20% of solids and CSR: 5–15 FPU/g). At time 0, enzymes and yeast inoculum were added. For 100 mL of media, 10 mL of inoculum and 10 mL of nutrients (concentrations: 5 g peptone/L, 3 g yeast extract/L and 3 g malt extract/L) were added. All the SSF experiments were carried out in duplicate.

In SHF experiments, an EH was accomplished before fermentation. Suspensions containing water, buffer and solid substrates were autoclaved at 121 °C for 15 min and thermostated at 48.5 °C before EH stage. EH experiments were carried out at 48.5 °C and pH 4.85, in 100 mL Erlenmeyer flaks with orbital agitation (150 rpm) using cellulolytic enzymes. EH experiments were carried out under different operational conditions for the autohydrolyzed *P. tomentosa* substrates (9–14.3% of solids and CSR: 15 FPU/g). The reaction time of EH was varied in the range 16–72 h. Once the EH step was finished, cells were added to start the fermentation stage, using the same conditions for inoculum and nutrient loading as the SSF experiments. These volumes of inoculum and nutrients were taken into consideration in the initial calculation for solid liquid ratio. Fermentation was carried out in an orbital shaker at 120 rpm and 30 °C for 48 h. All the SHF experiments were carried out in duplicate.

At preset times, samples were withdrawn from the media, centrifuged at 5000 rpm for 10 min, and aliquots of supernatants were filtered through 0.2 μm membranes and assayed for monosaccharides, acetic acid and ethanol by HPLC using the method described in Section 2.2. The results of SSF and SHF can be expressed in terms of ethanol concentrations (g/L) and in terms of ethanol yield (Y_{Et}) (%), using the following equation [35].

$$\%ethanol\ yield = \frac{[EtOH]_f - [EtOH]_0}{0.5l(f[Biomass]1.111)} \times 100\% \quad (3)$$

where $[EtOH]_f$ is ethanol concentration at the end of the fermentation (g/L) minus any ethanol produced from the enzyme and medium, $[EtOH]_o$ is ethanol concentration at the beginning of the fermentation (g/L) which should be zero, [Biomass] is dry biomass concentration at the beginning of the fermentation (g/L), *f* is cellulose fraction of dry biomass (g/g), 0.51 is conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast. 1.111 is the stoichiometric factor that converts cellulose to equivalent glucose.

3. Results and discussion

3.1. Autohydrolysis fractionation of P. tomentosa wood

Table 1 shows the chemical composition of raw material used in this study. In addition, SY and chemical composition of spent solids or autohydrolyzed *P. tomentosa* and liquors from autohydrolysis treatment were also listed in Table 2.

The autohydrolysis SY varied in the range 63.8–78.8 g solid recovered per 100 g raw material, oven dry basis. These values are close to the weight percent of the raw material corresponding to the joint contributions of cellulose and lignin, suggesting that both fractions were not significantly affected by the treatment. About 90% of pretreated material composition corresponded to glucan and lignin, and the combined amounts of these fractions matched the ones contained in the raw material. Accordingly, glucan was recovered almost quantitatively in the solid phase with an average content of 90 g of glucan of pretreated wood/100 g of glucan in the raw material. Glucan content was higher than 50 g of glucan/100 g of pretreated biomass for $S_0 > 3.85$, except at $S_0 = 4.82$. Lignin content in pretreated samples was in the range of 29–45 g of lignin/100 g of pretreated *P. tomentosa*. The remaining solid composition corresponded to residual hemicelluloses as

xylan (that achieved a maximum value of 10.95 g/100 g pretreated *P. tomentosa* for the lowest severity) and other minor compounds. The removal of hemicelluloses from solid phase increased with severity, achieving complete solubilization in the harshest conditions of severity conducted at $S_0 = 5.16$. The results obtained in this work are in agreement with reported data using hardwoods as *Eucalyptus globulus* wood in which 98% of glucan and 80% of Klason lignin were recovered in solid phase and xylan was almost totally solubilized at $S_0 > 4.67$ [36].

As seen in Table 2, the main compounds present in the autohydrolysis liquor (or liquid phase) corresponded to hemicellulose-derived compounds, including oligosaccharides, monosaccharides and sugar degradation products [4]. Xylooligosaccharides were the majority hemicellulose-derived compound which achieved the maximal concentration (15.7 g/L or 13 kg/100 kg of raw material) at S_0 of 3.99 representing 60% of the compounds identified in the liquid phase and 83.1% of xylan solubilization in raw material into xylooligosaccharides. These results can be compared with reported data in literature using autohydrolysis treatment (also known as liquid hot water) in which 64.1% and 74.1% of hemicellulose from Brewer's spent grain and corn cob respectively, were solubilized at 190 °C for 30 min ($S_0 = 4.13$) [37]. The maximal concentration of xylose and xylooligosaccharides (17.5 g/L, measured as sum) was obtained in this work at $S_0 = 3.99$. At similar autohydrolysis conditions ($T_{MAX} = 200$ °C), maximal oligosaccharides extraction from *Paulownia fortunei* was also reported by Caparrós and co-workers [22]. High oligosaccharides extraction (14.7 kg/100 kg of olive stone) was also solubilized by autohydrolysis pretreatment of olive stones at milder severity conditions ($S_0 = 3.59$) [38]. On the other hand in this work, glucooligosaccharides, arabinooligosaccharides and acetyl groups linked to oligosaccharides represented a percentage lower than 30% of total identified oligosaccharides for $S_0 \leqslant 4.19$. From $S_0 \geqslant 4.19$, xylooligosaccharides started to degrade into xylose which reached up to 4.89 g/L at $S_0 = 4.48$. In consequence, the highest furfural concentration was 4.7 g/L at $S_0 = 5.16$. For the use of biomass for bioethanol production, it is important to highlight the presence of degradation compounds (such as furfural, HMF and acetic acid) since they are considered inhibitors of subsequent stage of saccharification and fermentation [39]. Glucose was also present in low amounts (with concentrations of 0.42–1.40 g/L) which represented <4% of glucan solubilization showing the reduced solubilization of glucan fraction in comparison with xylan solubilization.

The data described above indicate that autohydrolysis treatment under selected conditions is an appropriate process for the selective fractionation of Paulownia wood obtaining a solid fraction composed mainly by glucan and lignin and high solubilization of hemicelluloses in liquid phase.

3.2. Evaluation of enzymatic susceptibility of autohydrolyzed *P. tomentosa*

In a second step of the biorefinery scheme, *P. tomentosa* treated by autohydrolysis was used as substrate in assays of enzymatic hydrolysis in order to evaluate the susceptibility of pretreated biomass for glucose production. Fig. 3(a) and (b) displayed time course of glucose production and respective glucose yield comparing some selected conditions studied in this work (S_0: 3.31–4.82). For these preliminary assays of enzymatic susceptibility, favorable conditions of CSR = 20 FPU/g and solids loadings (4.8%) were

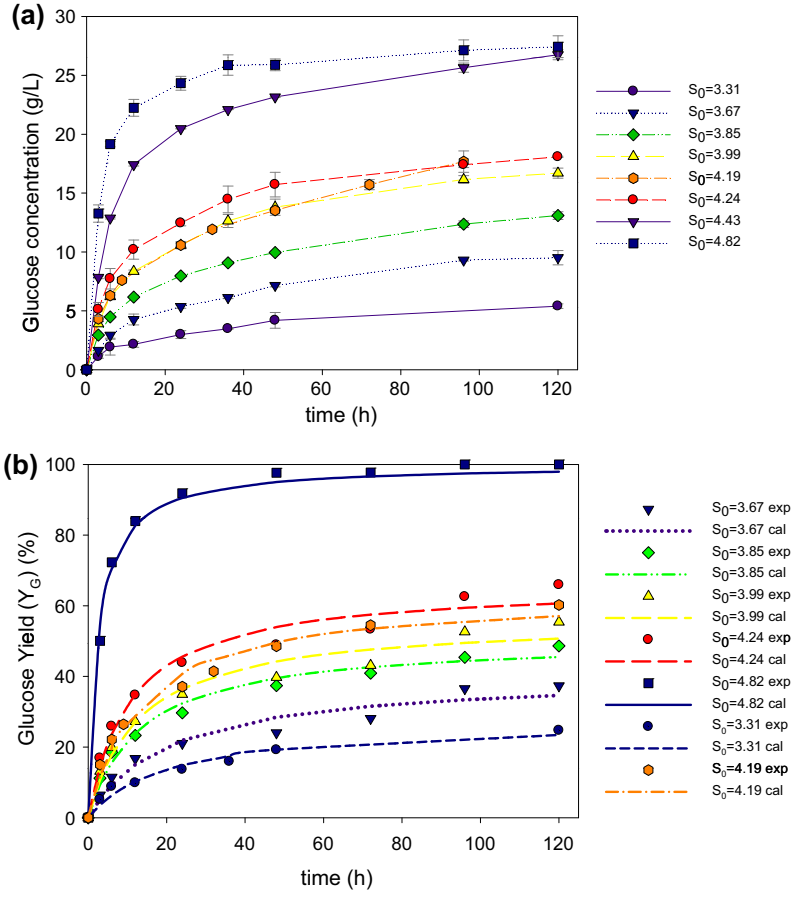

Fig. 3. (a) Time course of glucose concentration (g/L) of Paulownia wood treated at autohydrolysis conditions of S_0 in the range: 3.31–4.82; (b) Yield of glucose (%) at autohydrolysis conditions of S_0 in the range: 3.31–4.82. Experiments were carried out in duplicate, error bars represented standard deviation.

selected. As evident in Fig. 3, the harshness of pretreatment has a positive effect on the susceptibility of pretreated biomass to enzymatic hydrolysis. An increase in the autohydrolysis severity from S_0 3.31 to S_0 4.82 allowed glucose concentration to increase at 120 h from 5.4 g/L to 27.5 g/L. Autohydrolyzed solids pretreated at S_0 < 3.99 presented a low glucose yield, reaching values of Y_{G120} < 50% (Fig. 3b). Under this autohydrolysis condition (S_0 = 3.99) the highest recovery of xylan (as xylose and xylooligosaccharides) was obtained as was discussed above. On the other hand, higher harshness conditions of autohydrolysis were more appropriated to improve enzymatic saccharification. In fact, glucose yield reached up values of 100% when Paulownia wood was pretreated at S_0 of 4.82. High glucose yield (100%) was also achieved from autohydrolyzed *Eucalyptus globulus* wood at S_0 > 3.67 [40]. At similar severity condition (S_0 = 4.81), enzymatic hydrolysis of olive stone attained a lower glucose yield of 54.3% [38]. Moreover, autohydrolysis was also used for the enzymatic saccharification improvement of brewers's spent grain and corn husk at S_0 = 4.13 achieving a 76.08 and 63.30% of glucose yield, respectively [37]. Enzymatic saccharification obtained in this work can be positively compared with other pretreatments (such as dilute 1.1% of sulphuric acid at 145 °C for 40 min, 10% of NaOH alkali at 80 °C for 15 min and ultrasonic-assisted 10% NaOH alkali at 80 °C for 15 min and 60 W) using 2.5% of pretreated *P. tomentosa* and 40 FPU/ g of cellulase in which 89.3, 88.5 and 91.7% of glucose yield was reported, respectively [24,41].

The experimental data obtained from enzymatic hydrolysis in this set of experiments (Fig. 3b) followed typical patterns. Therefore, values of glucose yield were fitted to the following equation [42]:

$$Y_{Gt} = Y_{GMAX} \cdot \frac{t}{t + t_{1/2}} \quad (4)$$

where Y_{Gt} is the glucose yield at time *t*, Y_{GMAX} is the maximum glucose yield achievable at infinite reaction time, and $t_{1/2}$ (h) measures the reaction time needed to reach 50% of glucose yield.

The representation of calculated and experimental data (Fig. 3b) and the values of R^2 (see Table 3) showed the goodness of adjustment to the empirical model. Moreover, kinetics parameters from Eq. (4) (Y_{GMAX} and $t_{1/2}$) were represented as function of autohydrolysis conditions in Fig. 4 for an easier interpretation of autohydrolysis effect on glucan saccharification. As evident in Fig. 4, Y_{GMAX} significantly increased from S_0 = 4.19 in which the recovery of xylan was 78.9% as xylooligosaccharides and xylose (10.9 and 4.1 g/L, respectively). Therefore, this condition was more suitable for glucose and xylose recovery than severity of 3.99 in which maximal xylooligosaccharides concentration was achieved.

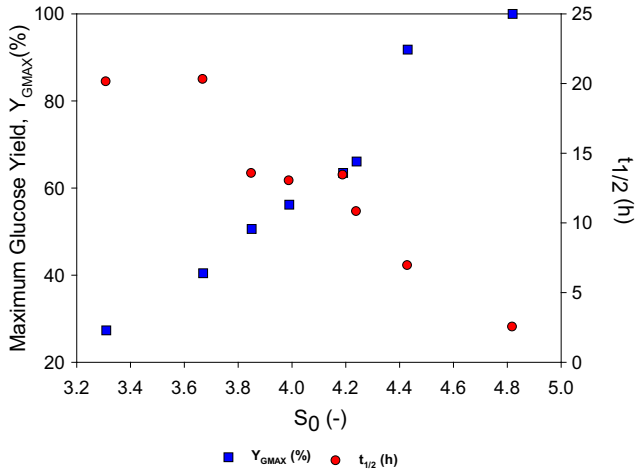

Fig. 4. Representation of kinetics parameters from enzymatic hydrolysis (Y_{GMAX} in% and $t_{1/2}$ in h) at autohydrolysis conditions of pretreatment (S_0).

Interestingly, $t_{1/2}$ started to decrease at S_0 > 3.67 achieving values of 13 h and lower of 6 h for S_0 > 4.24. These results showed that the severity of pretreatment increased the glucose yield and reduced the time of hydrolysis. Using EH data to formulate the kinetics parameters for Eq. (4), it is possible to predict glucose yield and glucose concentration at time t for a wide range of conditions of the autohydrolysis process (Fig. 3b).

Nevertheless, to attain a cost-effective lignocellulosic ethanol process is necessary to achieve glucose concentration more competitive (ethanol concentration higher than 40 g/L) [36]. High solid loading leads to elevated ethanol concentration and lower distillation cost. Nevertheless, to operate at high solid concentrations involves operational limitations due to low water availability and poor mass and heat transfer which reduce the ethanol yield [39]. In order to attain this aim, other set of experiments were proposed (increasing the solid loading and varying the CSR). The conditions were listed in Table 3. As general trend, the solid loading increment and the reduction of CSR (up to 12.5% and 6 FPU/g, respectively) decreased glucose yield for selected autohydrolysis conditions (S_0: 4.08, 4.24 and 4.43). Operating at 12.5% of solid loading and CSR of 10 FPU/g, Y_{GMAX} was lower than 30% for S_0 < 4.43. This yield was improved (Y_{GMAX} > 60%) with reduction of solid loading up to 9%, achieving Y_{GMAX} of 86% for S_0 = 4.43. Enzyme loading of 6 FPU/g was suitable for Paulownia treated at S_0 4.43 and 6.3% of solids achieving Y_{GMAX} > 78%.

Table 3
Operational conditions (percentage of solids and CSR) and main results (glucose yield at 48 h, Y_{G48}; maximal Glucose Yield, Y_{GMAX}, time needed to achieved ½ of Y_{GMAX}, $t_{1/2}$ and coefficient of determination R^2) obtained from enzymatic hydrolysis of selected autohydrolyzed Paulownia wood.

Run	S_0	Conditions of enzymatic hydrolysis		Main results			R^2
		Solids loading (%)	CSR (FPU/g)	Y_{G48} (%)	Y_{GMAX} (%)	$t_{½}$ (h)	
1	4.08	12.5	10	18	24	9.77	0.981
2		9	10	54	66	11.1	0.943
3		6.3	6	41	55	16.3	0.950
4	4.24	12.5	10	19	27	17.2	0.991
5		9	10	61	76	13.7	0.967
6		6.3	6	48	69	20.8	0.972
7	4.43	12.5	10	36	63	30.8	0.988
8		9	10	72	86	11.9	0.962
9		6.3	6	58	78	16.3	0.978
10	5.16	9	15	79	79	5.71	0.994

3.3. Comparison of simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) processes for bioethanol production

Two strategies of saccharification and fermentation separately and simultaneously for ethanol production from autohydrolyzed *P. tomentosa* were evaluated.

3.3.1. Effect of autohydrolysis severity on ethanol production

In view of the results obtained from enzymatic susceptibility, conditions of autohydrolysis treatment and solids and enzyme loadings were selected and listed in Table 4. Fig. 5a and b shows time course of ethanol and glucose concentration of SSF and SHF, respectively. As seen in SSF, accumulated glucose was rapidly consumed before 12 h. For S_0 4.48–4.72, 25.5–27.5 g/L of ethanol concentration (corresponding to ethanol yield of 89–95%) were achieved at 48 h of fermentation. At S_0 = 4.19 (Fig. 5a), lower ethanol concentration (16.3 g/L) was obtained. These results showed a clear effect of autohydrolysis treatment on saccharification and fermentation performance. Severity of pretreatment higher than 4.19 was necessary to obtain an ethanol yield > 54% in SSF.

Comparatively in SHF, ethanol production was clearly improved achieving ethanol concentrations in the range of 23.9–31.5 g/L for S_0 of 4.19 and 4.72, respectively. This improvement meant an increase of 47% ethanol concentration by SHF in comparison with SSF at S_0 = 4.19. Thus, ethanol yield was also enhanced and achieved values that varied in the range of 79–100%. Ethanol productivities for SSF and SHF were calculated at stationary phase (when ethanol concentration was maximum) and listed in Table 4, showing higher productivities by SHF strategy. The production of glucose was improved due to optimal temperature at the saccharification stage. Therefore, ethanol productivity was higher. In addition, glucose release was improved with increase of treatment severity as was discussed above in the evaluation of enzymatic susceptibility (Fig. 4). In this sense, the duration of enzymatic hydrolysis was evaluated depending on enzymatic susceptibility of pretreated *P. tomentosa*. The duration of enzymatic hydrolysis was chosen in basis of glucose production profiles (Fig. 5b) in which can be observed that the production of glucose concentration increased less than 5 g/L in the last 24 h for each experiment. Therefore in SHF experiments (Fig. 5b), the duration of enzymatic hydrolysis was: 72 h for pretreated *P. tomentosa* at S_0 4.19 and 4.48; 36 h for S_0 = 4.72; and 24 h for autohydrolysis severity at 5.16. The glucose achieved a concentration between 38.6 and 56.0 g/L that was consumed by the yeast in less than 12 h.

Direct comparison with reported data in literature is not straightforward since the process variables (selected raw material, pretreatment and the conditions of solid and enzyme loadings used in the saccharification and fermentation) may vary substantially. Nevertheless, literature reported works comparing strategies of saccharification and fermentation [43–45]. Ethanol concentration (39.9 g/L) from acid treated rapeseed (180 °C for 20 min and 0.5% H_2SO_4) by SHF was higher than results obtained by SSF and pre-saccharification and simultaneous saccharification and fermentation (PSSF) at high solid loadings, 20% of solids [43]. Higher ethanol concentration (with an ethanol yield of 92%) was also obtained by SHF than SSF using steam-exploded poplar wood (S_0 = 4.13) at 100 g of dry biomass/L (10% of solids) and 0.06 g of enzyme/g of dry biomass [45].

From results of Fig. 5, it was concluded that the highest ethanol concentrations in SSF and SHF using 9% of solids were obtained from Paulownia wood treated at 4.72, achieving 27.5 g/L (corresponding to 95% of conversion at 48 h) and 31.5 g/L (with 100% of conversion at 54 h), respectively.

Table 4
Operational conditions (percentage of solids and CSR) and main results obtained from SHF and SSF of autohydrolyzed Paulownia tomentosa: maximal ethanol concentration (C_{EtMAX}); Ethanol Yield (Y_{Et}) and productivity calculated at maximal concentration of ethanol (Qp_{EtMAX}).

Operational conditions			Main results					
			SSF			SHF		
S_0	Solids loading (%)	CSR (FPU/g)	C_{EtMAX} (g/L)	Y_{Et} (%)	Qp_{EtMAX} (g/Lh)	C_{EtMAX} (g/L)	Y_{Et} (%)	Qp_{EtMAX} (g/Lh)
4.19	9	15	16.3	54	0.31	23.9	79	0.27
4.48			25.5	89	0.50	30.7	100	0.36
4.72			27.5	95	0.54	31.5	100	0.55
5.16			27.0	94	0.53	30.3	100	0.64

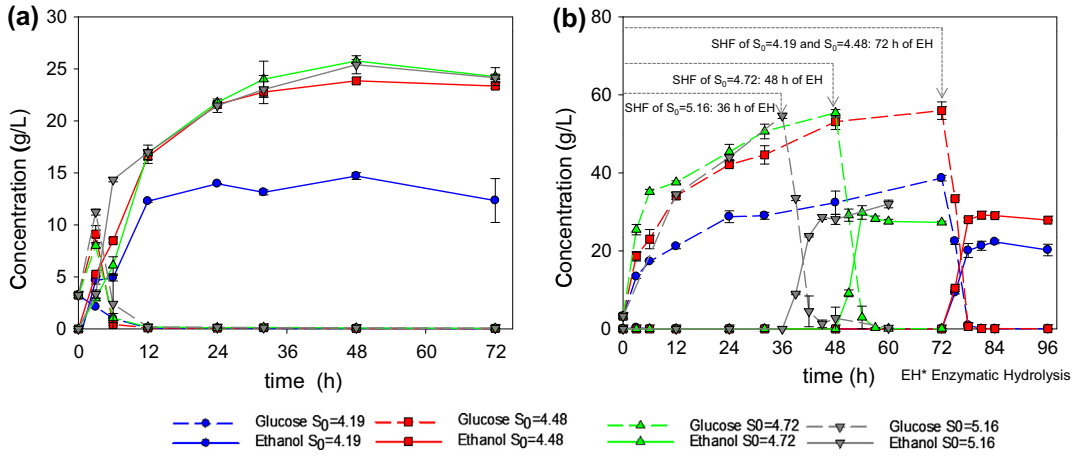

Fig. 5. Ethanol and glucose concentration (g/L) obtained from Paulownia (treated at S_0:4.19–5.16) by: (a) SSF process and (b) SHF process. Experiments were carried out in duplicate, error bars represented standard deviation.

3.3.2. Effect of operational SHF and SSF conditions on ethanol production

At autohydrolysis condition of S_0 = 4.72, the results showed that autohydrolyzed Paulownia wood was a suitable substrate for enzymatic hydrolysis of glucan and subsequence fermentation to ethanol. Taking into account the improved results from SHF strategy, a set of experiments was also studied varying the time of enzymatic hydrolysis (Table 5) in order to evaluate and optimize the saccharification time on ethanol production with 14.3% of solids and CSR = 15 FPU/g. Under these conditions, SHF strategy was carried out and ethanol and glucose concentration were displayed in Fig. 6a. Times of enzymatic hydrolysis (or time of inoculation) were carried out within 16–48 h in which glucose concentration achieved 56.0–85.9 g/L (corresponding to 73–95% of glucose yield). Fermentation stage for all of SHF experiments was performed for 48 h in which glucose was completely consumed. The highest ethanol concentration (37.2 g/L corresponding to 81% of conversion) was achieved at 48 h of enzymatic hydrolysis (SHF-4). Nevertheless, comparing ethanol productivities for SHF experiments, SHF-1 inoculated at 16 h of hydrolysis yielded 80% of ethanol with 1.77-fold higher productivity than SHF-4.

On the other hand, Fig. 6b shows the time course of ethanol production from Paulownia treated at S_0 = 4.72 by SSF process. Comparatively, results obtained from SSF-2 showed higher ethanol concentration than SHF 1–4 assays. The SSF strategy improved ethanol yield using 14.3% of solids which is attributed to minimal accumulation of glucose in the SSF medium, compared with the SHF strategy. In SHF, glucose accumulates in the enzymatic hydrolysis medium, promoting end-product inhibition of cellulase enzymes [43], which leads to less optimal glucose yield and subsequently lower ethanol yield. In order to improve the overall process of ethanol production from Paulownia wood, percentage of

Table 5
Experimental conditions (solid loading and CSR) and main results (maximal ethanol concentration, $C_{EtMAX;}$ Ethanol yield, Y_{Et} and productivity calculated at maximal concentration of ethanol, $QpEt_{MAX}$) of SSF and SHF assays carried out with autohydrolyzed Paulownia wood at S_0 = 4.72.

Run	Solids loading (%)	CSR (FPU/g)	Time of inoculum or enzymatic hydrolysis (h)	C_{EtMAX} (g/L)	Y_{Et} (%)	Qp_{EtMAX}
SHF-1	14.3	15	16	36.7	80	0.92
SHF-2	14.3	15	24	34.0	74	0.94
SHF-3	14.3	15	36	36.2	78	0.60
SHF-4	14.3	15	48	37.2	81	0.52
SSF-1	20	15	–	52.7	80	0.73
SSF-2	14.3	15	–	40.4	88	0.84
SSF-3	11.1	15	–	29.5	83	0.62
SSF-4	14.3	10	–	33.8	73	0.70
SSF-5	14.3	5	–	22.7	49	0.47

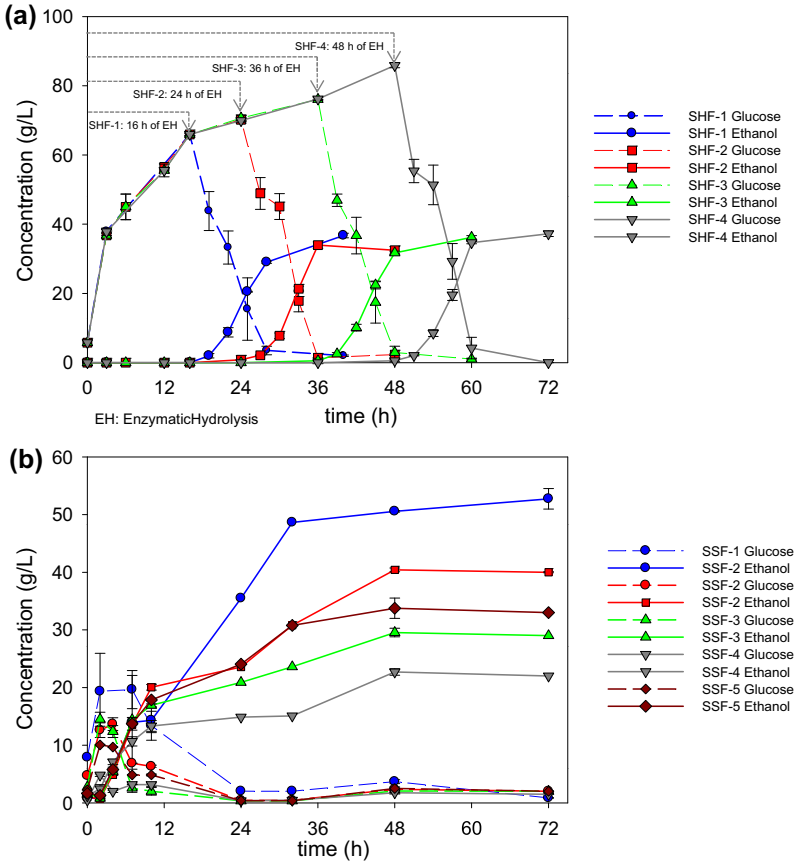

Fig. 6. a) Time course of ethanol concentration (g/L) from Paulownia wood treated at S_0 = 4.72 (a) by SHF process varying the time of enzymatic hydrolysis step and (b) by SSF process. Experiments were carried out in duplicate, error bars represented standard deviation.

solids was increased up to 20% (to increase the ethanol concentration) and CSR was decreased up to 5 FPU/g (to reduce enzyme loading) in SSF process. Operational conditions were also listed in Table 5. The highest ethanol concentration (52.7 g/L) was obtained with 20% of solids. It is important to highlight that ethanol yield was reduced using 20% of solids (achieving 80%) in comparison with 83 and 88% obtained with solids loading of 11.1% and 14.3%, respectively. Nevertheless, this decrease in ethanol yield was not very significant showing the feasibility to operate at high solid loadings under this condition of autohydrolysis (S_0 = 4.72). On the other hand, the reduction of enzyme loading up to 5 FPU/g was not suitable to ethanol production achieving yield lower than 50%. An ethanol yield of 73% was achieved using an enzyme loading of 10 FPU/g of substrate. The reduction of enzyme dosage could be improved by applying an enzyme recycling strategy being possible its use in successive batches [46].

3.4. Overall balance of autohydrolyzed P. tomentosa wood

Considering the results obtained in this study, Fig. 7 summarizes the fractionation process approached using autohydrolysis treatment as first stage of biorefinery in which ethanol production can be compared considering different severities of treatment (4.19 and 4.72). These selected conditions of treatment were chosen taking into account the highest ethanol yield from saccharification and fermentation experiments (S_0 = 4.72) and the recovery of hemicellulose-derived compounds from autohydrolysis liquors and feasible enzymatic hydrolysis of glucan (S_0 = 4.19). Thus, Fig. 7 shows two suitable process configurations for ethanol production by SHF and SSF that allows an easier and direct comparison between alternative process proposals. SHF strategy was more appropriate than SSF for ethanol production at milder (S_0 = 4.19) and higher (S_0 = 4.72) conditions of treatment. An increase of 24.2% and 14.6% of ethanol yield was obtained at these conditions, respectively (Fig. 7). Regarding enzyme spending comparison, 0.93 kg of ethanol (at S_0 = 4.19 and SHF, Fig. 7a) and 1.23 kg of ethanol (at S_0 = 4.72 and SHF, Fig. 7b) per kg of cellulase employed were obtained meaning a 32% higher ethanol production for the same enzyme loading. This fact implies the possibility to reduce the enzyme loading at S_0 = 4.72.

Considering an overall balance of process, per 100 kg of *P. tomentosa*: 18.86 kg of hemicellulose-derived compounds (11.38 kg as oligosaccharides) and 17.01 kg of bioethanol were recovered and produced from autohydrolysis liquor and by SHF, respectively at S_0 = 4.19. On the other hand, 21 kg of ethanol per 100 kg of *P. tomentosa* (corresponding to 92% of ethanol yield) and low recovery of hemicellulosic fraction were obtained at S_0 = 4.72 by SHF. The ethanol obtained in this work can be

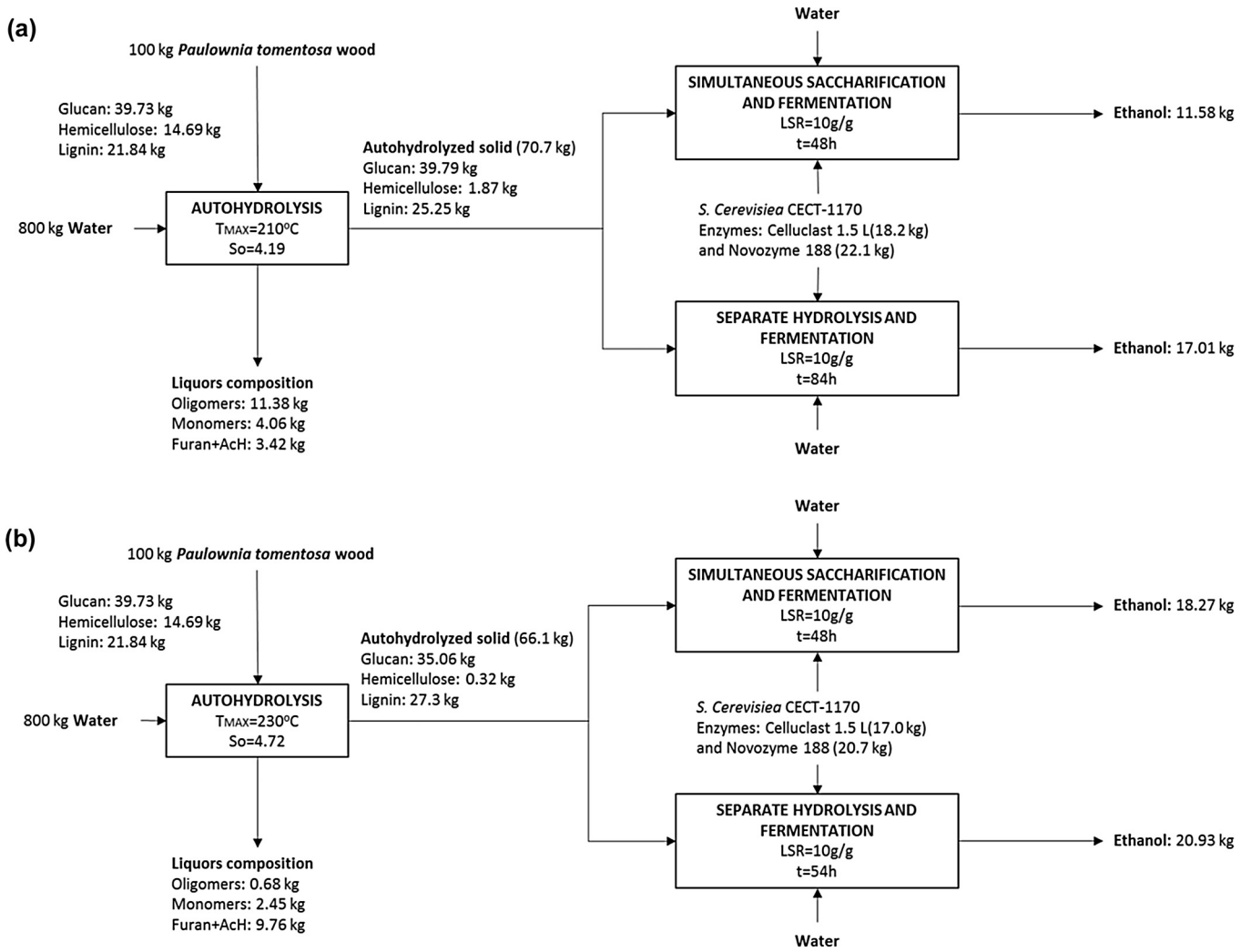

Fig. 7. Overall balance of Paulownia wood processing by autohydrolysis and saccharification and fermentation simultaneously and separately at severity conditions of pretreatment: (a) S0 = 4.19 and (b) S0 = 4.72 (results expressed in kg/100 kg raw material, oven dry basis).

Table 6
Energy analysis for 100 kg of raw *Paulownia tomentosa* (HHV = 1556 MJ) obtained from data presented in Fig. 7 (O + M: oligosaccharides + monomers; E_{LIGNIN}: energy obtained from the combustion of residual lignin; E_{EG}: energy obtained from ethanol from glucan; E_{ELP}: energy obtained from the ethanol from xylose fermentation).

Configuration (severity/fermentation mode)	Output energy and/or chemicals				Energy recovery (%)
	O + M (kg)	E_{LIGNIN} (MJ)	E_{EG} (MJ)	E_{ELP} (MJ)	
Scenario 1: O + M for other industries					
S_0 = 4.19/SSF	15.44	589	343	–	59.9
S_0 = 4.19/SHF	15.44	589	504	–	70.2
S_0 = 4.72/SSF	3.13	637	541	–	75.7
S_0 = 4.72/SHF	3.13	637	620	–	80.7
Scenario 2: O + M for ethanol production					
S_0 = 4.19/SSF	–	589	343	199	72.6
S_0 = 4.19/SHF	–	589	504	199	83.0
S_0 = 4.72/SSF	–	637	541	40	78.2
S_0 = 4.72/SHF	–	637	620	40	83.3

favorably compared with data reported by other authors who obtained 23.3 kg of ethanol/100 kg of *Eucalyptus globulus* after an autohydrolysis treatment at S_0 = 4.67 [40] and 12 kg of ethanol/100 kg of rapeseed straw after autohydrolysis at 217 °C for 42 min [43]. Evaluation of pretreatment biomass in order to maximize the ethanol production is a relevant issue for the scale-up of process. Recently, ethanol obtained from *Eucalyptus grandis* in pilot and laboratory scales using acid-pretreatment followed by steam explosion was compared, obtaining 82.5 and 113 kg/ton dry biomass, respectively [47]. The ethanol produced from two configurations evaluated in this work at laboratory scale (170–210 kg/ton of Paulownia) can be positively compared with these reported data. In basis of these results and Paulownia biomass production per hectare, 10,779–13,300 L ethanol/(ha·year) could be produced.

3.5. Energy analysis of fractionated P. tomentosa wood

For a better comparison of process configurations proposed in this work, an energy analysis of the data shown in Fig. 7 was carried out. The Higher Heating Value (HHV) of *P. tomentosa* as raw material was calculated using the chemical composition of raw material (Table 1) and the corresponding HHV of 17.80, 17.62 and 23.32 MJ/kg for glucan, hemicellulose and lignin, respectively [48,49]. Considering these main structural fractions, the calculated HHV for *P. tomentosa* was 15.56 MJ/kg. This data was comparable with the value experimentally determined by Lopez and co-workers [50] for *P. fortunei* (HHV = 17.83 MJ/kg). The small differences observed could be due to the contribution of non-structural components.

As presented in Fig. 7, four alternative configurations of Paulownia biorefinery process were proposed, attending to biomass fractionation (conditions to maximize the oligosaccharides concentration from hemicellulose in liquid phase at S_0 = 4.19 and to obtain the maximum enzymatic hydrolysis yield from solid phase at S_0 = 4.72) and the strategy used for bioethanol production by SSF or SHF. Table 6 shows the energy production from these four alternatives, comparing the energy obtained from: (i) ethanol (using HHV = 29.6 MJ/kg for ethanol) produced from glucan (E_{EG}), (ii) combustion of solid after SHF or SSF, composed mainly by residual lignin (E_{LIGNIN}) and (iii) ethanol from liquid phase (E_{ELP}) containing C5 sugars (an ethanol yield of 85% was considered for C5 fermentation according to previous work [51]).

Considering the overall balance shown in Fig. 7 and the HHVs, energy recovery from ethanol or lignin combustion was calculated and included in Table 6. Energy recovery was calculated considering these four biorefinery configurations and two scenarios for each configuration: scenario (1) oligosaccharides and monomers from liquid phase for other industrial uses (such as chemical, pharmaceutical and food products), increasing the versatility of biorefinery, or scenario (2) use the ooligosaccharides and monomers from liquid phase for ethanol production. In all cases, energy from combustion of cellulosic ethanol and lignin was considered. The energy recovery varied in the range from 59.9–70.2% at lowest severity (S_0 = 4.19) and 75.7–80.7% at highest severity (S_0 = 4.72). It is noteworthy that similar energy recoveries (around 83% of energy contained in raw material) were achieved in all configurations evaluated including or not ethanol from hemicellulosic fraction (C5). Moreover, energy obtained from lignin combustion was similar to the energy obtained from ethanol which confirms the need to employ lignin to improve the economy of the biorefinery process. On the other hand, oligosaccharides fraction from liquid phase can be addressed to several industries (such as chemical and food) or employed for energy production, depending on the economic benefit or the biofuels needs which improves the versatility of the process.

Annually in terms of energy produced per hectare, the Paulownia biorefinery proposed in this work would be able to obtain 13,300 L of cellulosic ethanol or 15,070 L including hemicellulosic ethanol which would be equivalent to 310,000–350,000 MJ. Additionally, taking into account the lignin combustion obtained from Paulownia-to-ethanol process, 294,000–318,000 MJ could be produced. These data imply an overall production of 628,000 MJ/(ha year) or 648,000 MJ/(ha year) including C5 bioethanol.

4. Conclusions

In this work, an evaluation of autohydrolysis pretreatment for bioethanol production from *P. tomentosa* wood was carried out following the biorefinery concept. High biomass production and tolerance of abiotic stress conditions make *P. tomentosa* wood an interesting biomass for energy purposes. This work provides experimental data of *P. tomentosa* fractionation, fermentation strategies to increase ethanol yield and an energy analysis of whole process showing the feasibility of this biomass as energy crop. Nevertheless, one single condition of autohydrolysis was not satisfactory to maximize the recovery of all fractions. High recovery of hemicellulose-derived compounds (as xylooligosacchairdes) was obtained at milder conditions of pretreatment (S_0 = 3.99).On the other hand, glucose yield of enzymatic hydrolysis was enhanced with increase of pretreatment harshness (S_0 > 4.43) achieving Y_G > 70%. After this fractionation evaluation, two severities were selected to compare ethanol production by SHF and SSF, attending to hemicellulose-derived compounds recovery (S_0 = 4.19) and ethanol yield (S_0 = 4.72). Overall mass balance of these configurations showed that SHF strategy was more appropriate for ethanol production than SSF, increasing up to 24.2% the ethanol production. Nevertheless at S_0 = 4.72, SSF strategy was more suitable than SHF to operate at high solid loadings (20%), achieving 52.7 g/L of

ethanol. Energy analysis of these two configurations showed that lignin combustion was necessary to improve the energy recovery. Comparing two strategies, C5 fermentation into ethanol could increase the energy recovery up to 83% at $S_0 = 4.19$ by SHF. This study showed that autohydrolysis is an appropriate pretreatment for the fractionation of Paulownia wood. In addition, these results provide data for further techno-economic analysis in order to compare the two configurations and select the optimal operational conditions.

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